REGENERATIVE PROCESSES IN THE LIVER OF PIGS FOLLOWING PARTIAL HEPATECTOMY

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The question of morphological changes in the liver of mammals that have undergone regeneration hypertrophy following hepatectomy is far from resolution. This pertains, in the first order, to changes in the large structural components of the organ – the hepatic lobules and the vascular network.

It is known that, in pigs, the liver is clearly constructed: due to the presence of marked connective tissue partitions between the individual hepatic lobules, the latter are easily distinguished.

We considered it of interest to elucidate the character of the changes in the hepatic lobules in the process of regeneration of the organ, to determine whether or not new lobules are formed, and to determine the histogenetic characteristics of the process of liver regeneration in pigs.

EXPERIMENTAL METHOD

In the experiment we used 10 young pigs from a single litter, weighing 6-7 kg, 1½ months in age. In 6 of the animals, under local, novocaine anaesthesia, we performed partial hepatectomies: through an incision in the skin and muscle under the left subcostal margin, we partly externalized the left lateral and central lobes of the liver, and then removed the distal portions of both lobes. Prior to the partial hepatectomy, silk literatures were placed in the parenchyma of the lobes being traumatized, near the site of amputation. The removed portion of the liver weighed an average of 29 grams, with a total weight of the organ of 200-250 grams. During the operation, and over the 2 days immediately following, each animal was injected with approximately 1,000,000 units of penicillin. Two animals were sacrificed on the 3rd, 14th, and 63rd day after the operation (at 10-11:00 A.M.), as well as one control pig at each of these intervals. After slaughtering, we weighed the animals and the extracted liver.

For histological investigation, the liver was fixed in Zenker's solution with formol and in 10% formalin. Paraffin sections, 6-8 micra in thickness, were stained with hematoxylin and eosin, according to Mallory. Using the preparations, stained according to Mallory, we drew 40-50 lobules in each case, calculated their area, then calculated the mean cross sectional area of one lobule. We determined the area of the lobules in a traumatized lobe – near and distant from the wound surface, and in a non-traumatized lobe. At the same time, we measured the area of the lobules in the liver of a control animal. In the early periods of regeneration (3-14 days after the hepatectomy) we determined the mitotic activity of the liver cells in the experimental and control animals.

EXPERIMENTAL RESULTS

In all 6 of the pigs that underwent the operation, the postoperative period passed smoothly. On autopsy of the animals, 3 days after the partial hepatectomy, the stumps of the operated lobes of the liver showed normal coloring, and a well manifested lobular appearance. At the wound surface, in the areas where the ligatures were placed, we observed minimal necrosis of the parenchyma; the free borders of the wound surface were clean and without signs of necrosis. By relative weight, the liver of the experimental animals was close to the weight of the liver in the controls (in the experimental group – 3.1%, in the control – 3.8%; mean absolute weight of the liver in the experimental group was 199 grams, in the control – 250 grams).

TABLE 1. Character of the Distribution of Mitoses in the Liver Lobule on the 3rd Day After Partial Hepatectomy

Index	Localization of mitoses		
	at the central vein	middle of the lobule	periphery of the lobule
Number of mitoses in 50 liver lobules	12	33	70
Average number per lobule	0.2	0.6	1.4

TABLE 2. Dimensions of the Liver Lobules in Experimental and Control Animals at Different Periods of Regeneration

Group of animals	Localization of the lobules	Time after operation (in days)		
		3	14	63
		dimensions of the lobules (in mm^2)		
Experimental	In the traumatized lobe, near the wound surface	Borders of the lobules undistinguishable	181	403
	In the traumatized lobe, distant from the wound surface	328	365	631
	In the non-traumatized lobe	312	336	677
Control	In the right lobe	248	369	894

On histological investigation of the liver from experimental animals, in the areas of parenchyma adjacent to the wound surface we observed dilatation of the large and small vessels and sinusoids. At the very edge of the wound surface, along with resorption of escaped blood, there occurred a pronounced widening of the biliary ducts and the formation of a scar. Above this zone, there appeared groups of hepatic cells that were distinguished by markedly eosinophilic and homogeneous cytoplasm. The remaining portion of the parenchyma in the traumatized lobe was normal.

In the liver of the operated and control animals, we encountered mitotically dividing hepatic cells. In the first case, they were significantly more frequent. In the regenerating liver, the mitotic coefficient was equal to an average of 2.5%, while in the control – 1%. In the traumatized lobe, within the zone of liver cells adjacent to the wound surface, the mitotic index was as high as 4.5%. We observed more frequent localization of the mitoses in the middle portion of the lobule and at its periphery. Significantly fewer mitoses were seen near the central vein (Table 1).

On the 3rd day after partial hepatectomy, the dimensions of the lobules in the traumatized and non-traumatized lobes of the liver somewhat exceeded those in the liver of the control animal (Table 2). Thus, in the experimental animals, the mean measurement of the cross sectional area for the lobule in the traumatized lobe was equal to 328 mm^2 (magnified by 20 times), while in the control – 248 mm^2, i.e., in the experimental animals the lobules were markedly hypertrophied. The difference between the dimensions of the lobules in the experimental group and the control was statistically significant ($P = 0.0001$). Near the wound surface, at this interval, the lobules were intensely disrupted, and their borders were poorly distinguished.

Investigation of the regenerating liver in the young pigs on the 14th day after partial hepatectomy showed that the fundamental mass of the liver parenchyma, including the traumatized lobes, possessed normal histoarchitectonics. The mitotic activity of the liver cells was markedly reduced. The mitotic coefficient in the experimental and control groups was equal to 0.8-1%. At the wound surface of the traumatized lobes there was scar formation. The dilated biliary ducts at the edge of the wound underwent gradual degeneration.

The zone of parenchyma, 3-7 mm in width, adjacent to the wound surface, was ligher in color, which was caused partly by dilatation of the vessels and sinusoids and partly by the less homogeneous and granular protoplasm of the liver cells in this area. The lighter zone at the wound surface consisted of small liver lobules (Fig. 1), which seem to surround the entire edge of the traumatized region. In the more proximal portion of the lobe, these lobules were rarely encountered. According to our measurements (see Table 2), the dimensions of the liver lobules at the wound surface were almost half as large as in the remaining lobes of the traumatized organ or in the control. The remaining portion of the parenchyma in the traumatized and non-traumatized lobes consisted of large hepatic lobules,

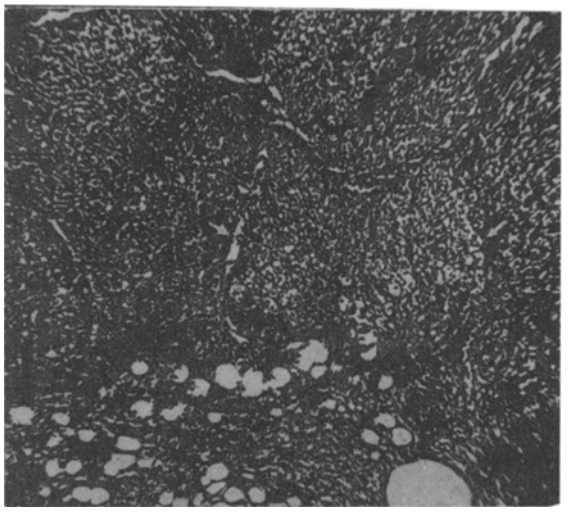

Fig. 1. Small liver lobules at the wound surface on the 14th day after partial hepatectomy (indicated by arrows). Magnification × 36.

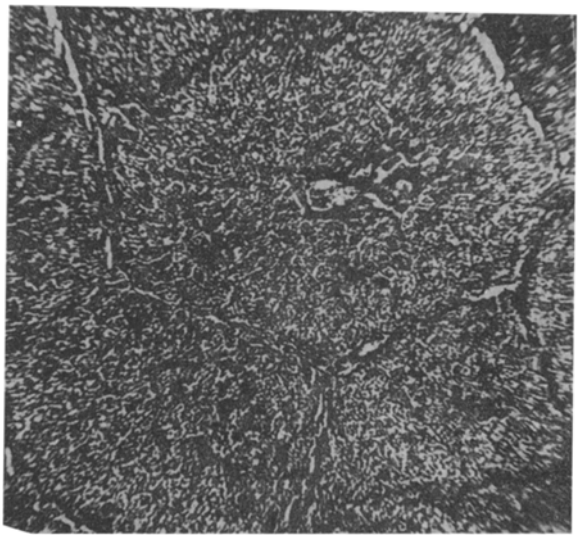

Fig. 2. Large liver lobules in the traumatized lobe, distant from the wound surface, on the 14th day after partial hepatectomy. Magnification × 36.

equal in size to the liver lobules of the control (Fig. 2). By comparing the dimensions of the liver lobules, it can be seen that after 14 days the dimensions of the basic mass of lobules in the experimental group were practically unchanged, while in the control they increased markedly. This fact can be explained by the evidence that small changes in the weight of the liver during growth are not necessarily accompanied by a marked increase in the dimensions of the lobules [10, 14]. Also, individual variation is not excluded. In our case, the weight of the liver in the experimental animals had increased by an average of 70 grams after 2 weeks, while in the control – by more than 100 grams (mean absolute weight of the liver in the experimental animals was 287 grams, in the control – 353 grams).

Due to the death of one of the experimental animals, on the 63rd day after partial hepatectomy we carried out a histological investigation on one experimental and 2 control animals. In the experimental pig, and in one of the control pigs, the liver was partly infected by echinococcus, as evidenced by macroscopically striking parenchymal changes in certain areas, as well as an elevated eosinophile count in the peripheral blood.

Despite the above facts, we investigated the area of the wound surface and the healthy regions of the liver in the experimental animal, as well as the liver of the control animals. We observed a thick scar on the wound surface. The liver lobules at the wound surface often appeared abnormally shaped, and were surrounded by thick connective tissue walls (their dimensions increased in comparison with the previous interval of the investigation, but still differed markedly from the measurements of the lobules in the remaining portions of the traumatized hepatic lobes). Statistical analysis of the numerical data showed a significant difference between the measurements of the lobules at the wound surface and the lobules in the remaining areas of the traumatized lobe ($P = 0.0002$). In a number of sections, the division of the liver into individual lobules was very poorly manifested at the wound surface. In the total mass, the measurements of all the liver lobules, both in the experimental and control organs, increased in connection with growth of the animals.

Although in investigating liver regeneration in young pigs we used a small amount of data, we were able to note a number of interesting facts. The distinguishing characteristics in the liver structure of the pig did not affect the nature of the response to trauma, i.e., the changes caused by removal of a portion of the liver were analogous to those which we observed in other species of animals [1, 4, 5, 6]. Soon after the operation, we noted an increase in the nitotic coefficient for the liver cells. Because of the clearly manifested lobular structure of the liver, it was possible to demonstrate the uneven distribution of mitoses in the borders of the lobule, specifically; mitotically dividing cells were localized in large numbers in the middle and peripheral zones of the liver lobule, but rarely around the central vein. This fact was also pointed out by Meister [3], who studied the regenerating liver of the rabbit. However, ultimately the question of the localization of mitoses in the lobule was inadequately investigated, since the liver of the majority of mammals has a poorly manifested lobular structure, and is not very suitable for this plan of study [2]. The increase in the number of mitoses toward the direction of the periphery of the lobule is obviously caused

both by a number of known [7-13] physiological characteristics of the liver cells located at the marginal zone of the lobule, and by the more favorable conditions for the supply of their arterial blood.

Concerning the characterization of the state of the liver lobules in the process of hepatic regeneration in pigs, it should be noted that, at the early stages of regeneration (3 days after partial hepatectomy), in the basic mass of the liver they are hypertrophied.

It is true that the hypertrophy of the lobules in the experiments presented was not as marked as in the experiments on rats [4]. This is due to the fact that in the pigs we removed a relatively small portion of the liver, consisting of 1/8th of the weight of the organ. The presence of smaller liver lobules at the wound surface than in the remaining portion of the organ, on the 14th day after the operation, brings to mind the possibility that they are newly formed in this area of the traumatized organ. In addition, it is completely possible that the small lobules at the wound surface are areas of the parenchyma of old lobules that were retained after the trauma and have undergone reconstruction. We observed similar phenomena in the experiments on rats, associated with peripheral wounds of the liver [5].

Special supplementary investigations are needed for a more exact answer to the question of whether new lobules can be formed in the liver of mammals during hepatic regeneration.

SUMMARY

About 1/8 of the liver (distal portions of the left lateral and central lobes) were removed in 6 young pigs aged 1.5 months. Regenerating liver was investigated on the 3rd, 14th and 63rd postoperative days. As demonstrated, regeneration of the liver at the early periods was accompanied by the rise of mitotic activity of hepatic cells and hypertrophy of hepatic lobules. Mitoses within the lobule were unevenly distributed – their greatest number was localized in the middle and peripheral zones of the lobule. On the 14th-63rd days of regeneration smaller hepatic lobules were revealed at the wound surface; the possibility of their formation anew was considered. Perhaps, however, the small lobules represented the retained and reconstructed areas of old lobules.

LITERATURE CITED

1. L. D. Liozner, N. S. Artem'eva, I. V. Markelova, et al., in the book: Regenerative Processes in Vertebrate Animals [in Russian]. Moscow, (1959) p. 240.
2. L. D. Liozner, (Edit.), Regeneration of Organs in Mammals [in Russian]. Moscow, (1960) p. 199.
3. V. É. Meister, Regeneration of Liver Tissue Following the Removal of Entire Lobes of the Organ [in Russian]. SPb. (1894).
4. V. F. Sidorova, Byull. eksper. biol., No. 8,(1959) p. 99.
5. V. F. Sidorova, Byull. eksper. biol., No. 3, (1961) p. 97.
6. V. F. Sidorova, Byull. eksper. biol., No. 12, (1961) p. 88.
7. B. P. Solopaev, in the book: Questions in Reparative and Physiological Regeneration [in Russian]. Moscow, p. 64 (1960).
8. W. Eger and H. Ottensmeier, Arch. path. Anat. Bd. 322, S. 175 (1952).
9. S. Lagerstedt, Acta anat. (Basel), Suppl. 9, (1949) p. 1.
10. M. McKellar, Am. J. Anat., Vol. 85, (1949) p. 263.
11. R. Noël, Arch. Anat. micr. Morph. exp., Vol. 19, (1923) p. 1.
12. A. Novikoff, Science, Vol. 113, (1951) p. 320.
13. A. Novikoff, Exp. Cell. Res., Suppl. 2, (1952) p. 123.
14. E. G. White, J. Anat. (Lond.), Vol. 73, (1939) p. 365.