HISTOGENETIC AND STRUCTURAL REGENERATIVE CHANGES IN THE LIVER OF RATS FOLLOWING INFLICTION OF PERFORATING AND MARGINAL WOUNDS

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Despite the large number of investigations devoted to studying the regeneration of the liver in mammals, the question of its structural characteristics during regeneration following various forms of injury remains inadequately examined.

Study of the structural characteristics of the regenerating organ presents dual interest: on the one hand, these characteristics cannot help but reflect the organ's functional activity, and, on the other, it is important to establish to what extent new structures or functional units can arise in the organs of mammals. The formation of new structural components has already been shown possible for a number of mammalian internal organs, in association with their regeneration (acini of the salivary gland [1], villi of the intestine [2], excretory and endocrine portions of the pancreas [7, 8, 10], follicles of the thyroid gland [5], etc.).

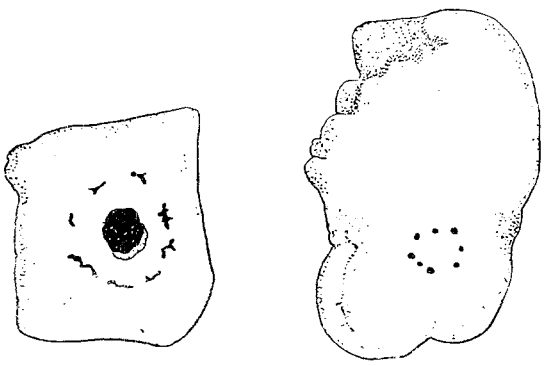
The data in the literature pertaining to the possibility of liver regeneration including the formation of new structural units – lobules – is scanty and extremely contradictory. In a previous investigation we showed [13] that after lobes of the liver were completely removed in rats there ensued hypertrophy of the hepatic lobules in the remaining lobes, accompanied by an increase in the distance between the central veins. Formation of new lobules was not observed in association with this type of operative intervention. Nevertheless, individual authors [6, 9] indicate that the formation of new liver lobules is possible, particularly when the liver is regenerating following the infliction of a perforating injury in one of the lobes of that organ.

In this work we continued the study of the structural properties in the regenerating liver; we investigated the character of the regenerative reaction and of the reconstituting processes caused by the presence of a damaged surface. In this case, the injury was created by inflicting perforating and marginal wounds in the organ.

EXPERIMENTAL METHOD

In the experiment we used white male rats, 200-250 g in weight. In the first series 60 rats were employed, a continuous perforation being applied to the distal portion of the left lateral lobe of their livers; a surgical punch was used, leaving a wound 4 mm in diameter (the portion removed weighed 32 mg). In the second series we removed the inferior margin of the left lateral and central lobes of the liver in 60 rats. The portion removed weighed approximately 1/3 of the total mass of the organ (2,230 mg, with a total liver weight of 9,850 mg).

In both series of experiments the injured surface was not treated in any way. The regenerating liver was investigated on the 8th, 15th, 30th, 45th, 60th, 75th, 90th and 180th day following the operation. For histological investigations the liver was fixed in a 10% solution of formalin, and paraffin sections were stained with hema-



a b

Fig. 1. Arrangement of the marks with India ink about the round opening in the left lateral lobe of the liver. a) At time of operation; b) 2 weeks after operation.

toxylin-eosin, Mallory, and Van Guisen stains. To study the structural changes and the appearance of the contours to the hepatic lobules, we injected the hepatic vessels with an India ink suspension containing gelatin, following a method previously described [13]. Treatment of the preparations and the drawing and measurement of the individual lobules was carried out in the same manner as in the foregoing investigation.

EXPERIMENTAL RESULTS

Inasmuch as the histogenetic processes occurring in regeneration of the liver have been described in detail [4, 6, 9, 12], we are only concerning ourselves with certain moments, which, as will be seen, engender the structural changes in the organ.

The outcome of the operative procedure in the first series of experiments was not uniform. In some cases we observed a small zone of necrosis around the wound aperture, while in others there arose a coniform infarct of the liver parenchyma from the aperture down to the distal edge of the lobe, a result of significant trauma to the vessels supplying the periphery of the lobe. In both cases, a scar formed at the site of the injury by the 30th day, arranged in the form of a small spider-like growth at the location of the former aperture or extending like a band along the original wedge-shaped infarct. Mitoses were only observed in the liver cells in the early periods of regeneration and were in small number, this being related to the minimal volume of parenchyma removed during the operation. They were usually localized near the site of the trauma. In the late observation periods marked histogenetic changes did not occur at the site of the defect, with the exception of a decrease in the dimensions of the scar and degeneration of the growing bile ducts.

If healing of the wound occurred without the formation of a coniform infarct, then in the beginning stages of hepatic regeneration the impression was created that the parenchyma around the margins of the wound was contracted by the forming scar. As a result of this, division of the parenchyma into lobules was virtually absent at this time. We set up special experiments in order to clarify whether the liver tissue grew out from the wound edges or whether it was actually pulled out by the contracting defect. It was observed that the marks made on the parenchyma with India ink during the operation, arranged in a circle around the wound opening, were still clearly seen after 2 weeks, but were located considerably nearer to each other (Fig. 1). While at the beginning of the experiment the area of the opening and the parenchyma enclosed by the marks was equal to 78.50 mm^2, after 2 weeks it was reduced to only 11.35 mm^2 (these averages are taken from the measurements in three cases). Beginning with the 30th post-operative day and later it became apparent that the lobules surrounding the site of the original opening were deformed and markedly hypertrophied (Fig. 2,a) while they possessed normal size and form at a distance from the site of trauma (Fig. 2,b). Special measurements of the size of the lobules near the site of the original opening and at a distance from it, performed at all periods of the regeneration up to 6 months, supported our observation (Table 1).

In Table 1 we find an increase of 1.9 times in the average area of the lobule for 2-3 animals. In each of the cases we counted 6-14 lobules.

Deformation of the lobules near the original defect was caused by stretching of the parenchyma by the forming scar, and their hypertrophy ensued only near the site of injury, this being related to the removal of a small amount of liver tissue. This, the processes of proliferation only involved the area of parenchyma surrounding the site of trauma, not extending the distance to the uninjured portion. Along with this, the dimensions and form of the lobules in the uninjured part of the lobe were not altered. We were unable to detect processes involving the formation of new lobules in this series of experiments.

In the early stages of observation, in the second series of experiments, we noted a small zone of liver parenchyma near the wound surface which was undergoing necrotic changes.

Proximal to this zone we observed a leuko-histocytic wall, sharply dividing the zone that was undergoing degeneration from the uninjured portion of the organ.

TABLE 1

Dimensions of the Hepatic Lobules in Association with Regeneration of the Liver Following the Infliction of Perforating and Marginal Wounds

Time elapsed after operation (in days)	Nature of the operative procedure				
	infliction of a perforating wound		removal of liver edge		control
	area of a section of liver lobule in the untraumatized portion (in mm^2)	area of a section of lobule at the wound surface (in mm^2)	area of a section of lobule in the untraumatized portion of the lobe (in mm^2)	area of a section of lobule at the wound surface (in mm^2)	area of a section of lobule (in mm^2)
6-8	135	Contours of the lobules poorly defined	326	Contours of the lobules poorly defined	165
14	–	The same	234	The same	–
30-45	128	249	323	147	–
60	–	259	313	182	–
90	178	292	245	148	–
180	186	284	222	148	177

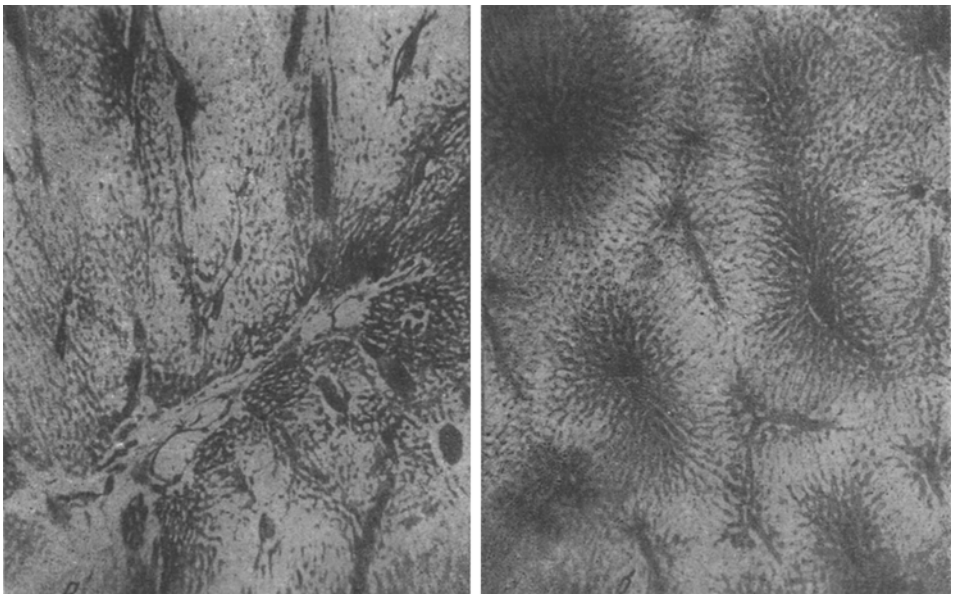


Fig. 2. Deformation of the hepatic lobules at the scar. a) On the 30th day following infliction of a round perforation in the left lobe of the liver; b) liver lobules of normal form and size on the upper region in the untraumatized portion of the same lobe.

On the 2nd-3rd post-operative day we observed a large number of dividing liver cells. Mitoses were encountered both in the traumatized lobes as well as the hepatic lobes not subjected to injury. We were unable to note a principle localization of the mitoses at the wound surface (Table 2).

In subsequent intervals (10th-14th day) we observed only bile duct and vascular growth, resorption of the products of necrosis, and formation of young scar tissue, at the wound surface. The latter subsequently grew along the pathway of the vessels in the parenchyma by small extensions, compressing to a slight degree the liver parenchyma growing within. As a result, small projections formed on the wound surface, in the form of festoons. At this point it should be noted that no growth of the liver parenchyma from the wound edge occured in this case.

TABLE 2

Mitotic Activity of the Liver Cells Following Removal of 1/3 of the Liver

Time elapsed after operation (in days)	No. of the animal	Number of mitoses per 6,000 liver cells	
		traumatized lobe	untraumatized lobe
2	1	3	7
	2	87	62
	3	28	56
	4	46	54
	5	8	7
3	1	32	40
	2	20	23

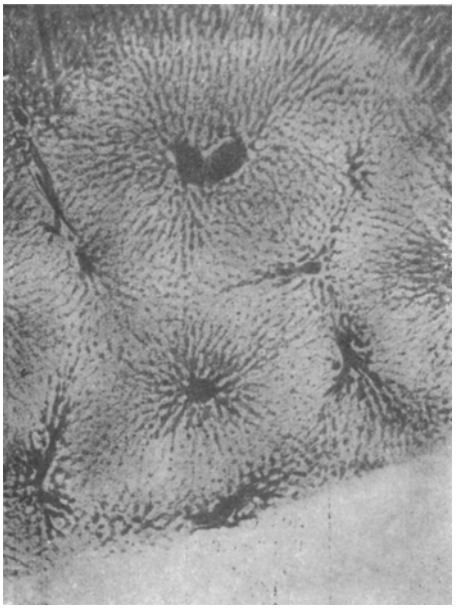


Fig. 3. Newly formed liver lobules at the wound surface on the 60th day after marginal removal of a portion of the liver parenchyma.

The liver regeneration that ensued was of the regeneration hypertrophy type, i.e., as a result of proliferative processes throughout the entire organ. By the 30th day and beyond the wound surface of the traumatized lobes was covered by a compact thin scar, and the large portion of the bile ducts growing at the wound surface had degenerated.

The results of studying the structural changes were the following. Initially the lobular structure was poorly defined in the traumatized lobes. After the 14th day the structure of the individual lobes became clearly demarcated, especially at a distance from the site of injury. Here the lobules were observed to be hypertrophied. At the very edge of the wound surface during the early periods, observation of the lobules showed them to be generally indistinct, or so deformed that nothing could be said about their form or magnitude. However, beginning with the 30th day we could distinguish a clearer portion of the parenchyma at the wound surface, often rather well structured. In this portion, along with enlarged lobules, we encountered lobules of small dimensions and normal form. At later intervals it was also possible to frequently observe fine lobules in the sections, single or in groups (Fig. 3).

Based on measurements of the dimensions of the individual lobules, we are of the impression that in association with this type of operative procedure it is possible for new lobules to be formed at the wound surface in addition to the hypertrophy of hepatic lobules which occurs throughout the fundamental mass of the organ. The dimensions of the newly formed lobules are smaller than normal or are close to the dimensions of the lobules in the control animals (see Table 1).

It should be emphasized that the number of newly formed lobules at the wound surface is rather small, and the lobules are not always clearly defined. They can probably only arise under certain conditions, created at the wound site. The very fact that it is possible for liver lobules to be newly formed in the postnatal period during regeneration is of unquestionable interest. In all probability, the means by which the new lobules are formed is analogous to what is encountered in the liver of newborn rats [14]. It is interesting to note that the times of appearance of the newly formed structural units of the liver – lobules (25-30th day), coincide with those associated with the arisal of new glomeruli in the kidney [11] and alveoli in the lung [3] during regeneration of these organs following removal of an appreciable portion.

The general reaction of the liver to removal of a major portion of it (1/3) is reflected by hypertrophy of the hepatic lobules throughout the remaining mass of the organ. This is a result of proliferative processes involving the entire organ in the early stages of regeneration (see Table 2). Basically, the processes which occur here are similar to those which take place in association with the removal of entire lobes of the organ.

SUMMARY

Experiments were staged on male albino rats weighing 200-250 gm. In the first series of experiments a perforating wound of the liver (4 mm in diameter) was inflicted with a metal instrument. In the second series of experiments distal portions of the left lateral and central lobes of the liver (constituting in all about 1/3 of the organ) were removed. A scar formed in both series of experiments; there was no parenchyma growth on the wound surface. In the first series of experiments hepatic lobules were deformed and hypertrophied during restoration only in the area adjacent to the wound. Characteristic of the second series of experiments was hypertrophy of the lobules in the whole of the organ. In a number of cases close to the wound surface newly formed hepatic lobules were noted in the second series of experiments.

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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.
