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Liver Micro-Circulator Back After Its Partial Resection For 15 Day Of Life

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ABSTRACT: In an experiment on 180 white mongrel male rats, 31-34% (left lateral lobe) of the liver was resected on the 15th day of life. We found that after resection of the liver, constantly occurring liver complexes were detected, which consist of 3 types of lobules. The initial link of blood outflow from the hepatic lobes is the initial hepatic venules, which are formed from the fusion of sinusoids in the subcapsular zone of the liver. After resection, the main process is the formation of new structural and functional units – liver lobes in the subcapsular area of the organ.
KEYWORDS: liver, regeneration, the lobule, the hepatic venules, sinusoidal vessels

1. INTRODUCTION

Currently, the study of the formation of the liver and the formation of its functional structural units (1,3,6,11), as well as their compensatory capabilities are widely represented in the literature (1,2,5,7,10). Moreover, various authors studied the formation of cell structures and their features (4,9,12), sinusoid lining cells (1,4,11), bile ducts (1,7,8) and parenchymal components in various periods of postnatal ontogenesis. The questions of hepatocyte proliferation (3,4,9) in the developing liver and their various parameters both after birth and at different periods of postnatal life have been well studied (8, 10, 12).

On the other hand, a large number of experimental and clinical works are devoted to the study of pathologically altered liver, which occur with an increasing frequency and lead to serious consequences (1,6,7,9). Great interest in liver regeneration is caused primarily by the fact that an understanding of the mechanisms of reparative processes is of great importance for clinical hepatology (1,2,9), for which the restoration of the impaired structure and function of the organ is a paramount and still unsolved problem (7,10,11)

The aim of the study was to study the dynamics of changes in the microvasculature of the liver and to trace the features of the recovery processes after resection on the 15th day of postnatal development.

2. MATERIALS AND METHODS

In accordance with the objectives, the studies were performed on 180 white outbred rats males on the 15th day of life produced a resection of 31-34% (left side lobe) of the liver. The second group consisted of 60 animals that served as control. In the first series of experiments in the morning, on an empty stomach, 15-day-old rat pups were operated under aseptic conditions under general ether anesthesia. The abdominal cavity was opened along the white line of the abdomen, having previously revised the abdominal organs, then ligated the silk thread No. 003 on the base of the left lateral lobe. The lobes were cut off distal from the ligature, with further removal of the cut off left lateral lobe. The abdominal cavity was

irrigated with a solution of penicillin at the rate of 50 units. 1 gram of weight and sewn tightly.

After the operation, the rat pups were kept separate from the mother for 2 hours, as some females ate their cubs having noticed a strange smell on them (ether, alcohol, blood, etc.). Then the cubs were fed breast milk. 5-6 hours after resection, the operated rats showed approximately the same motor activity as before the resection. On the 4-6th day after the operation, the animals, as in group 2, independently switched to mixed nutrition. From one month old, rats, as in other groups, switched to definitive nutrition.

The operated animals were slaughtered by decapitation in the morning hours in the periods: 3, 5, 7, 10, 15 days and 1 month after the operation. Control animals were killed at the appropriate time after resection.

To make serial sections, the pieces were soaked in paraffin and poured into a mixture of paraffin with 5% wax. Slices were prepared in parallel with the liver capsule on a MS-21 sledge microtome; for this, slices were placed horizontally on the microtome. The thickness of each slice c was 30–40 μm — after pouring with a mass of Herot, and 20–30 μm — after pouring a mixture of mascara with 2% gelatin. After dewaxing of the slices, they were enclosed in a balm and numbered; viewing and description of the preparations were performed according to increasing numbering. Morphometry was performed using an MOV-15X eyepiece micrometer using a P-2 binocular microscope. The internal lumen of sinusoids and the initial hepatic venules was measured in the middle third of their length, the hepatic, septal (round lobular) and interlobular veins at the level of their detection.

The area of the lobules was calculated on serial sections according to their true sizes, by sketching their contours with recording their parameters in the description protocols. Most lobules had a polygonal shape, we divided the latter into triangles and determined the area of the triangles by their true size by the formula:

$$S1 = a1 \times h1 / 2$$

al is the base of the first triangle, h1 is the height of the first triangle. Summing up the indicators of all geometric figures inside one lobule according to the formula: $S = S1 + S2 + S3 + ... + Sn$

found the true dimensions of the cross-sectional area of the lobules. In this case, S is the area of the lobules, S1, S2, S3, Sn is the area of the first, second, third, n-th triangle inside one lobule. The parameters of the lobules were calculated by us at the level of detection of around lobular (septal) veins.

Based on the obtained serial sections, volume reconstruction of the liver complexes was performed. Liver microvessels were measured using an MOV-15X eyepiece micrometer and an ocular ruler. Liver mass was measured using an VT-500 analytical and torsion balance. The volume of the liver was determined by a special device developed by us (rat. Proposal No. 1024 1991).

Pieces of liver for histological studies were fixed in Carnoy fluids, 12% neutral formalin, for quantitative studies in FSU. The general histological picture of the liver was studied on sections with a thickness of 5 μm stained with hematoxylin and eosin.

To fully characterize the vascular bed of the liver, scanning electron microscopy of corrosive preparations was used. After perfusion through the thoracic aorta and portal vein, prepolymerized methyl methacrylate was injected into the vascular system with the addition of 2% benzoyl peroxide and 2% dimethylalanine. Then, the hardened organ was corroded in a 30% KOH solution, the preparations were washed and dried in air (Karaganov Ya.L., 1981). After microdissection and gold sputtering in the Gika IB-3, corrosion replicas were viewed in Hitachi S-405A SEM and photographed at the V.V. Vakhidova.

Digital data was processed by the method of variation statistics using a table to calculate the root mean square (standard) error and confidence intervals of arithmetic mean

values according to B. Strelkov. (1965), on a computer using the programs STATGRAPHICS 2.1. Differences satisfying P <0.05 were considered significant.

3. RESULTS AND DISCUSSION

When opening the abdominal cavity of animals, 3 days after removal of the left lateral lobe of the liver, it is enlarged, full-blooded. The stump of the left lateral lobe is shrouded in an omentum. The absolute mass of the liver is 0.819 ± 0.026 g (in the control - 0.962 ± 0.042 g), the relative - 3.44 ± 0.16% of body weight (in the control - 3.92 ± 0.017%) and exceeds the mass remaining after resection by 73 ,9 %. When studying serial sections, it was found that immediately under the organ capsule there is an area where the lobular structure of the liver is not determined. Interlobular and around lobular vessels are not detected here. Sinusoids form a network with a large number of anastomoses. Their radar orientation is not clearly expressed. Among the sinusoidal network are avascular zones, on the edge of which the sinusoids deepen deep into the organ. The internal lumen of the sinusoids is expanded and ranges from 9 μm to 15 μm 13.09 ± 0.12 μm), while in the control it is 8.86 ± 0.14 μm.

When opening animals 5 days after partial hepatectomy, the remaining lobes of the liver are increased in size. The base of the resected left lobe is shrouded in an omentum. The lobes of the liver left after the operation are increased, their mass is 1.091 ± 0.031 g, and corresponds to 131.6% of the resection day. Compared with the previous period, it grows by 33.2% (in the control by 18.3%). On day 5, compared to the day of surgery, the liver mass increases 2.5 times.

When studying serial sections, it was found that microvessels differ in their angioarchitectonics both from the control and from the previous term. The vascular architectonics of the lobules and all areas of the liver complex are also changing. 5 days after the operation, a single sinusoidal network is revealed under the fibrous capsule of the liver. Compared with the previous period, the diameter of the sinusoids (13.03 ± 0.12 μm) decreases (10.32 ± 0.26 μm), although it remains significantly larger than in the control (8.36 ± 0.17 μm). The same changes occur with the length of sinusoidal hemocapillaries (162.68 ± 1.67 and 146.33 ± 1.98 μm, respectively, in terms of time). From the fusion of several sinusoidal vessels, the initial hepatic venules are formed, the diameter of which varies from 16.8 microns to 21 microns and averages 19.59 ± 0.15 microns.

On the 7th day after the operation, the remaining lobes of the liver are enlarged. It is noted that the omentum grows into the operated stump and envelops the resected left lateral lobe. The absolute mass of the liver is 1.34 ± 0.03 g (in the control - 1.341 ± 0.047 g), and reaches the mass of control animals. Relative to the previous period, it increases by 22.8% (in the control - 17.8%), the relative weight is 4.37 ± 0.21% (in the control - 4.29 ± 0.20%) of the animal's body weight .

At a depth of 100-120 μm, 2-4 initial hepatic venules merge to form hepatic veins of the first order. They significantly decrease in transverse dimensions, amounting to 24.91 ± 0.25 μm. These indicators are less than 5 days after resection (26.63 ± 0.40 μm), and from control indicators (29.99 ± 0.38 μm).

In the depth of 120-140 μm, interlobular veins are determined, which have an internal lumen of 27.27 ± 0.25 μm. It should be emphasized that their increase compared to the 5th day after the operation (25.61: 26.16 μm) and a significant decrease than in the control (28.04: 29.02 μm). To a depth of 320-380 microns from the fibrous capsule, lobules with 2-3 hepatic veins of the first order are preserved, which are located within the "elongated" lobules. To this level, the distance between adjacent hepatic veins of the first order decreases progressively within the wide lobules.

At a distance of 380-440 μm from the capsule of the liver, a connection occurs at an acute angle of 2-3 hepatic veins of the first order, with the formation of one hepatic vein of the second order (central vein) inside a single connective tissue framework. The internal lumen of the hepatic veins of the first order ranges from 25.2 μm to 32.2 μm and averages 24.91 ± 0.25 μm. Vascular architectonics is formed from type 1 lobules, and the section zone resembles the IV zone of the normal liver complex.

Comparing the indices of the liver complexes on the 7th day after resection with the control, it was found that the depth of the I zone of the liver complex in both periods of the study has approximately the same depth.

But it should be emphasized that if in the control with 80-100 μm lobules of the 1st type with one hepatic vein of the first order are detected, then 7 days after the operation they are absent. At the level of 100-120 microns to 320 microns, there are 2 types of lobules (in the control with 220-240 microns) and 3 types of lobules with 340 microns. At a level of 380-440 μm from the capsule of the liver, 2-3 hepatic veins of the first order merge at an acute angle, with the formation of one hepatic vein of the second order (central vein) inside the lobules. Vascular architectonics is formed from lobules of type 1, and the zone resembles the IV zone of the normal liver complex. Structures appearing in this zone are the base of the liver complex.

Thus, on the 7th day after liver resection, the increase in the size of the liver complexes and lobules continues, which is expressed in an increase in the levels of fusion of the hepatic veins of the first and second order and involvement of the outflow of the hepatic veins of the third order in the vessels. The sequence of identification of the zones of the complex and the main features of their vascular architectonics undergoes significant changes. Although the caliber of sinusoids remains virtually unchanged, their length increases dramatically. The same dynamics is noted in the parameters of the initial hepatic venules. Lobules also hypertrophy, unlike the control, where in the second zone there are small “classical” lobules of the 1st type with one hepatic vein of the 1st order in the center, then on the 7th day after the operation, lobules of the 2nd type, polygonal (usually 7-9) shape with 2 - 3 hepatic veins of the first order. The distance between them increases significantly more than 5 days after resection and in the deeper sections (380-440 μm) than in the previous period (320-340 μm), 2-3 hepatic veins of the first order merge with the formation of hepatic veins of the second order (central veins).

On the 10th day after resection of the left lateral lobe of the liver, regrowth of new lobes from the base of the resected lobe was not detected. A significant increase in the lobes of the liver left after the operation is noted. The absolute mass of the liver is 1.624 ± 0.048 g (in the control - 1.699 ± 0.051 g), relative - 4.54 ± 0.19% (in the control - 4.63 ± 0.20%) of body weight. Compared to the mass of the liver left after resection, it increases by 3 times, while in the control it is 2.35 times.

When studying serial contrasted preparations, a sinusoidal network without a lobular structure characteristic of the liver is found under the fibrous capsule of the liver. Sinusoids, merging, form the initial hepatic venules having the shape of a truncated cone with a diameter of 22.10 ± 0.30 μm, which exceeds the control indicators (19.87 ± 0.26 μm), as well as indicators of the previous term (17.83 ± 0 , 20 μm). Their length also increases and corresponds to 180.26 ± 2.97 μm (in the control 129.85 ± 2.05 μm). Interlobular and around lobular vessels at this level are not detected. The initial hepatic venules, merging in 2 or 3, in a depth of 80 - 100 μm, form the first order hepatic veins, the diameter of the internal lumen of which corresponds to 31.14 ± 0.33 μm, in the control 27.38 ± 0.33 μm. In the depths of 120-140 (in the control of 60-80 microns), round-lobed vessels that surround the "classical" type 1 lobules are detected, and in the depths of 160-200 microns, interlobular veins are detected (in the control of 100-120 microns). The cross-sectional area of the lobules at this

level ranges from 0.078 mm mm2 to 0.214 mm2 and averages 0.124 ± 0.004 mm2, which is significantly lower than the previous period (0.164 ± 0.004 mm2) and is associated with the appearance of small lobules, the sizes of which can be even smaller normal. Along with frequently occurring lobules with one hepatic vein of the first order of type 1, occasionally lobules of an "elongated" shape were found in which 2-3 hepatic veins of the first order were determined where sinusoidal hemocapillaries flowed. Slices have different sizes and dividing them into classes you can distinguish 4 varieties of wedges in a given period. Some large, contain 1 hepatic vein of the first order into which the short initial hepatic venule flows, have an area of from 0.201 mm2 to 0.214 mm2 (3.2%), others are also large, but have only one hepatic vein of the first order, and the area the cross section corresponds to from 0.151 mm 52 0 to 0.200 mm 52 0 (16.1%). The third group is the largest in number, small, not always clearly delimited from neighboring larger lobules, which are often limited by transverse sections of sinusoids. They contain one hepatic vein of the first order and have an area of 0.101 mm2 to 0.150 mm2 (61.3%). And the fourth group has the smallest segments with an area of 0.078 mm2 to 0.100 mm2 (19.4%).

The length of the sinusoids is 159.18 ± 1.95 μm, which still remains longer than in the control (140.08 ± 2.23 μm) but significantly decreases than in the previous period (173.60 ± 1.67 μm).

At a depth of 160-180 microns, the adjacent boundaries of the lobules disappear and lobules of elongated elongated shape with 2 to 3 hepatic veins of the first order within one structural unit appear. Lobules at this level acquire a polygonal shape, in the center of which are 2-3 hepatic veins of the first order. Their internal clearance is 31.14 ± 0.33 microns and varies from 25 microns to 35 microns., And the distance between them ranges from 154 microns to 280 microns. In such segments, the fusion of 2-3 hepatic veins of the first order occurs at a depth of 300-320 microns from the surface of the liver.

The second order hepatic vein resulting from this fusion is located in the center of the lobule. At this level is the base of the "newly formed" liver complex. At a depth of 380-400 microns from the capsule of the organ, the borders of adjacent lobules are smoothed, they are combined in pairs. Vascular architectonics begins to resemble the III zone of a normal complex. Lobules of type 2 are formed of an "elongated" form and, in contrast to normal liver complexes, in this case outflow vessels are hepatic veins of the II, rather than I order, in intact animals. They approach each other, sinusoidal vessels gradually disappear between them, and type 3 lobules are formed at a depth of 500-520 μm from the surface of the liver. In the deeper sections, at the level of 540-560 microns, they merge to form hepatic veins of the third order.

Thus, on the 10th day after the operation, there is not only a quantitative, but also a qualitative change in the elements of the microvasculature of the liver lobules and complexes. The emergence of new structural units causes a change in the vascular architectonics of the zones of the complex. The increase in comparison with the previous period of the level of confluence of the central veins of adjacent lobes indicates the formation of new complexes that push the "old" into deeper layers.

Table 1 Indicators of the liver complexes of the liver of rats after its resection on the 15th day of life

RESEARCH TERMS	NUMBER OF CASES	LEVEL OF IDENTIFICATION AROUND SANDED VEINS	LEVEL OF DISAPPEARANCE OF RELATED BOILERS	HEPATIC VEIN FUSION LEVEL I ORDER

	EXPERIMENT	CONTROL	EXPERIMENT min max		CONTROL min max		EXPERIMENT min max		CONTROL min max		EXPERIMENT min max		CONTROL min max	
3 days	15	15	140	160	80	100	180	200	140	160	340	360	280	300
5 days	15	15	40	60	160	180	180	220	160	180	340	360	300	320
7 days	15	15	80	100	120	140	240	280	160	180	400	440	320	340
10 days	15	15	140	160	100	120	280	300	160	180	440	460	320	340
15 days	15	15	160	180	60	90	340	360	180	210	480	500	330	360
30 days	15	15	60	100	60	80	260	300	180	220	460	500	360	380

Measurements were taken with respect to the fibrous capsule of the liver.

On the 15th day after the operation, the absolute mass of the liver is 2.284 ± 0.099 g (in the control, 2.267 ± 0.107 g). In relation to the liver left after resection, the mass increases by 484.9% (in the control 313.5%). Relative liver mass averages 5.07 ± 0.17% (in the control 4.94 ± 0.21%).

30 days after liver resection, omentum ingrowth into the surgical wound is detected. The organ lobes left after surgery are hypertrophied; its absolute mass by this time is 3.529 ± 0.109 g. In relation to the mass left after resection, it increases by 7.49 times (in the control 4.56), and in relation to the previous period it increases by 54, 5% (in the control by 45.5%), which indicates a relatively rapid growth of the liver in comparison with control animals.

When studying serial sections, it was found that the angioarchitectonics of the vascular bed of the subcapsular part of the regenerating liver differs both from identical sections of control animals and from data obtained 15 days after resection. So, 1 month after removal of the left lateral lobe, the lobular structure in subcapsular sections is not determined. In this zone, a continuous network of sinusoidal vessels is detected, which is formed from often anastomosing sinusoidal hemocapillaries. From the first sections, sinusoids emerging from the depths are determined, which are more or less radially directed to the avascular zones. If in the previous period the initial hepatic venules were determined from the first sections, then in this period they are detected from a depth of 20-40 microns from the capsule. The initial hepatic venules have a more arcuate course and their length (185.25 ± 3.08 μm) is significantly less than in the previous period (206.50 ± 2.56 μm), but remains larger than in the control (159.25 ± 3, 08). If 15 days after the resection from the fusion of sinusoids from the first sections, several adjacent initial hepatic venules were detected, then after 1 month they appear in the deeper sections, less both in length and in diameter (18.37 ± 0.30 μm) .

At a depth of 80-120 μm from the fibrous capsule of the liver, round lobular vessels appear, delimiting the lobules with one hepatic vein of the first order. These structures belong to the 1st type of lobules, and have a hexagonal shape. The average cross-sectional area of the lobules varies from 0.130 mm2 to 0.308 mm2 and averages 0.182 ± 0.008 mm2. It should be noted that the area of lobules compared with the previous period after resection (0.269 ± 0.011 mm2) is significantly reduced, and compared with the control (0.165 ± 0.010 mm2), it remains somewhat larger. Analysis of the area of the lobules of the liver show their different variability. Considering their distribution by classes, it was determined that the main part of the lobules have an area ranging from 0.151 mm2 to 0.200 mm2 (44.9%) and from 0.130 mm2 to 0.151 mm2 (24.1%). Slices with an area ranging from 0.201 mm2 to 0.250 mm2 (24.1%) and from 0.251 mm2 to 0.300 mm2 (6.9%) are much less defined. Comparing these results with the control, where the bulk of the lobules have an area in the range of 0.101-0.150 mm2 (47.6%) and 0.151-0.200 mm2 (28.6%), we can conclude that on the 30th day after the resection, new lobules are formed with a smaller area.

Around lobular venules are detected at a depth of 80-120 microns, and interlobular veins at a depth of 160-180 microns from the capsule of the liver. The diameter of the round lobular veins is 14.17 ± 0.20 μm of the lobular veins 31.07 ± 0.4 μm (in the control - 27.77 ± 0.35 μm).

The merging of adjacent lobules with the formation of "elongated" lobules with 2-3 hepatic veins of the first order occurs at a level of 180-200 microns from the capsule of the liver. At this level, segments of type 2 are determined. In a depth of 300-320 μm, 3 type lobules appear. In lobes with 2-3 hepatic veins of the first order, their fusion occurs at a depth of 340-360 microns. The second order hepatic veins formed in this case have a diameter of 35.70 ± 0.25 μm and the vascular architectonics here become similar to those of the IV zone of the normal complex (Table 1). At this depth is the base of the newly formed liver complex (in the control 360-400 microns).

Thus, on the 30th day after liver resection, significant changes occur in both individual zones of the newly formed liver complex and its individual components. The diameters of the interlobular and around lobular veins, as well as the hepatic veins of the second order, are increasing. Despite a significant decrease in the average cross-sectional area of the lobules, the level of their detection is slightly increased, which indicates the growth processes of the tops of the lobules in the subcapsular direction. At the same time, the tops of new liver complexes are formed due to the separation of larger structures into small ones.

4. CONCLUSION

1. The initial link in the outflow of blood from the hepatic lobules is the initial hepatic venules, which are formed from the fusion of sinusoids in the subcapsular zone of the liver. From the merger of these venules, hepatic veins of the first order are formed (normal - the central veins).

2. In the liver, along with lobules, it is possible to distinguish constantly occurring liver complexes, which are higher than the lobule level of the structural organization of the hepatic parenchyma.

3. The parameters of the microvasculature of the lobes of the regenerating liver in all periods exceeded those of growing animals, but lagged far behind the multiplicity of the increase in liver mass.

4. The basis of the regeneration process in the liver of young animals is an increase in the number of structural and functional units.

5. The wave-like change in the average cross-sectional area of the lobules and the height of the liver complex indicates the formation of new lobules and complexes.

6. The processes of formation of microvascular organ architectonics and compensatory-adaptive processes in the microvasculature of the regenerating liver have common features. In both cases, the main process is the formation of new structural and functional units in the subcapsular zone of the liver.

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