STRUCTURAL CHANGES IN RATS' LIVER DURING THE FIRST 2 WEEKS FOLLOWING 2/3 PARTIAL HEPATECTOMY

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Liver regeneration followed by liver resection is one of the most frequently studied processes, both in the clinic and in the experiment [1,3,6,7,23,25,26]. Dramatically is increased frequency of liver resection in recent years. This is due, on the one hand, to increased cases of space occupying liver pathologies which were previously considered as inoperative, including through endoscopic intervention [14] and on the other hand, the increase in the frequency of liver resections is associated with widespread transplantations of half liver from a living donor.

According to the data of Eurotransplant International Foundation in 2019 in Europe 116 cases of liver transplantation were performed from living donor. The United Network for Organ Sharing reports 524 liver transplantation from living donor. In total, between 1988 and October 31, 202 7715 liver transplantation were performed in the United States from a living donor.

One of the important characteristics of liver transplantation from a living donor is that, after the operation both, the remnant liver in the donor and half liver transplanted into the recipient are regenerated - by restoring the initial mass and volume of the liver. This characteristic led to the revision of regeneration processes, including given that the regeneration of the transplanted liver takes place under conditions of denervation and delymphatization [2,13,19].

Despite that several hundred papers on liver regeneration are published each year, important questions for regenerative medicine remain unanswered, including such simple question as: Does normal liver differ from regenerated one, and if so, how it differs.

It is known that postanal period of ontogenesis involves the proliferation of both liver lobules and cells, while the liver regeneration happens due to cell proliferation without increasing the number of lobules [18,24].

Our study of both the specimens of the liver and the three-dimensional architecture of its tubular structures and connective tissue spaces has shown that the process of liver regeneration takes place due to/is accompanied by complex morphological changes. The changes concerns both the parenchymal and stromal components of the liver [20,27,28]. In this study comparison of normal and regenerated liver morphologies allowed us to conclude that regeneration of liver mass after resection is due to hepatocyte hypertrophy, changes in their shape and size, sinusoidal dilatation and proliferation, as well as their prolongation, and multiplication of interlobular connective tissue, which causes changing the structure of the lobul - remodeling. Remodeling is also indicated by the difference in the shape and size of hepatocytes from normal in the hepatic acinus zones according to the Rappaport [5,21].

In addition, we have also shown the formation of "megalobules" by the union of adjacent lobules after 2/3 resection in 9-month-regenerated liver, as well as smaller lobules than in normal one. The description of the megalobules is similar with the data of Papp et al., who showed the formation of big surface lobules [4,18]. As for the presence of small lobules in the regenerated liver, similar data could not be found by us. The presence of small lobules requires additional studies to confirm whether, in addition to lobule enlargement and remodeling, they also multiply in the regeneration process.

It has been proven that the increase in rodent liver size and weight after resection ends 7-10 days after surgery, and the recovery of lobules architecture - in 10-14 days [11,17,21]. Although most studies of liver regeneration focus on these dates, even in the acute period after hemihepectomy, the questions we have highlighted above concerning the structural difference between normal and regenerated livers remain unanswered.

Considering the above, we aimed to investigate changes the hepatocyte size and shape and the architecture of the sinusoidal network in the 2-week dynamics after resection 2/3 of the liver.

Material and methods. The experiments were performed on 16 adult male Wistar rats weighing 190-200 grams, who underwent 2/3 hepatectomy. We examined their liver tissue by histological, immunohistochemical, morphometric methods, and the spatial architecture of the sinusoidal capillary network by electron microscopy of the corrosion casts. The study was conducted in 24 hours, 48 hours, 96 hours, 1 week, and 2 weeks after surgery. The resected part of the liver of the same rat was used as a control. Corrosion casts of normal animal liver were taken from the archives left from previous studies [27,28]. The sex and weight of the rats in these studies were similar.

The number and distribution of animals according to the research terms and methods are given in Table 1.

Table 1. Number and distribution of animals in the study group by term and research methods

Groups and terms / Research Methods	Research Group (PH)				
	24 H	**48 H**	**96 H**	**1 week**	**2 weeks**
Histology*	2	2	2	2	2
Immunohistochemistry*	2	2	2	2	2
Morphometry*	2	2	2	2	2
Corrosion Casts	1	1	1	2	1
Sum of animals	**3**	**3**	**3**	**4**	**3**

** - the same animal was used for these methods*

Surgery models. **2/3 Hepatectomy:** The Animal Care Protocol incorporated the recommendations of the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals to minimize animal pain and / or discomfort, both during and after surgery [9]. Before and after the experiments the animals were kept in comfortable laboratory conditions (22° C, 12 h/12 h, light /dark, 60% humidity, free access to food and water). They had restricted access to the food only on the day before the operation. The operation was performed in fasting state, with general anesthesia with a mask of diethyl ether.

2/3 hepatectomy was performed according to the protocol of Claudia Mitchell & Holger Willenbring, by using double knot method. After opening the abdominal cavity, the liver was mobilized by crossing the liver ligaments. Left lateral lobe (26% of liver) was resected after the first knot which was followed by the second knot and resection of median lobe (38-42% of liver) [16]. Resected parts of liver were examined macromorphological for the exclusion any pathology.

Histology. Liver tissue sections of 3-μm were stained by standard H&E method and studied microscopically with different magnification. (Primo star ZEISS, Jena, Germany) equipped with a digital video camera (ZEN 2.3 SP1).

Immunohistochemistry. For immunohistochemical investigation Keratin-8 antibody (MyBiosourse) was used. Diluted rate 1:200 in 0,01M Phosphate Buffer Saline (PBS) pH7.4 (Sigma Aldrich). The tissue was formaldehyde fixed and a heat mediated antigen retrieval step in citrate buffer was performed. The tissue was then blocked and incubated with the antibody for 2 hours at 22°C. An HRP (расшифровать) conjugated goat anti-rabbit antibody was used as the secondary. Slides were observed and imaged under a light microscope (Primo star ZEISS, Jena, Germany) equipped with a digital video camera (ZEN 2.3 SP1).

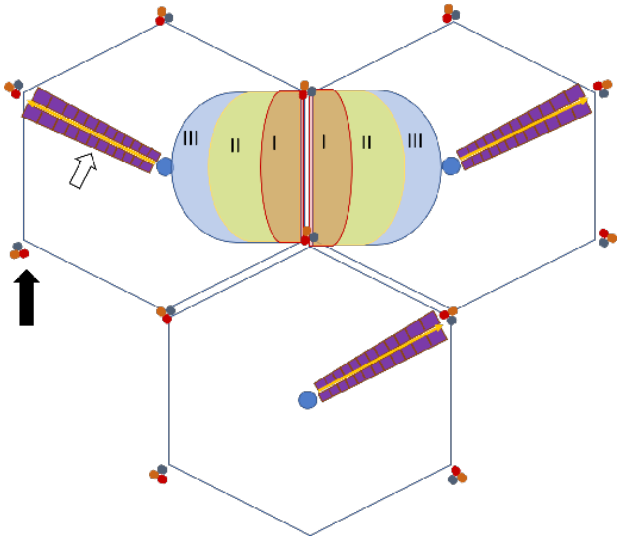

Fig. 1. Liver lobules and acinus. I, II,III – the zones of acinus; White arrow – hepatocytes' plate; Black arrow – portal triad; White arrowhead – central vein

Morphometry. For morphometric analysis, we selected areas similar to those previously described by us (Fig. 1) [27,28], namely:

a) the hepatocytes of the first zone of the hepatic acinus, located near the line connecting the adjacent portal triads (on both sides), corresponding to the perpendicular line from the portal area to the axis connecting the central veins of the adjacent liver lobules. This is the zone that first receives oxygen and nutrients, the dominant process in this area is oxidative metabolism (gluconeogenesis, proteosynthesis) [8,12].

b) the hepatocytes of the third zone of hepatic acinus, located around the central vein, corresponding the top part of portal triad. This is the zone which receive the least amount of oxygen. The reduction processes are predominant in this location – e.g. detoxication [12].

Slides stained with CK8 marker were used for morphometric analysis. CK8 provided good visualization of the hepatocyte membrane and ensured a high accuracy of marking the measurable area.

Hepatocyte area and perimeter measurement were performed on the right lobes of the liver of both the control and study group.

Histological slides were scanned on Motic Digital Slide scanner and morphometry was performed using Motic digital scanner assistant software Motic VM 3.0. The working area was magnified 40 times and the cell membranes were lined up manually because the shape of the hepatocytes did not normally exactly match any of the geometric figures. For morphometric analysis were selected cells with fully visualized membrane and nucleus. 3 samples of I and III zones were selected from each animal. 100 cells in each zone were measured.

Scanning Electron Microscopy (SEM) of corrosion casts

All the conditions and sequences were done as previously described by us [27,28]. To make corrosion casts we used a mixture of benzoyl peroxide, MAYCRYL C.C., powder and Protacryl-M as described in our article. Injectable solidifying mass was injected into rats via portal vein under anesthesia with diethyl ether, which was followed by pre-rinsing of the blood bed with 0.9% saline (rinsing time in 20 ml/min).

We examined corrosion casts with electron microscope JEOL-JSM-6510LV, which allowed the sample to be visualized by analyzing both direct and reflected electron flows in both high and low vacuum conditions. For investigation under high vacuum conditions, corrosion casts were coated with a layer of gold atoms, JEC-3000FC (using Tokyo BOEKI Group, Japan apparatus (vacuum=3.2 Pa, coverage time=180 sec).

Continuous variables are presented in average (min, max, standard deviation). Two-sample t-test was used for comparison of continuous variables. These tests were 2-sided. The P values <0.05 was considered statistically significant. Analysis was performed with SAS version 9.3 software (SAS Institute, Inc., Cary, NC, USA).

Results and discussion. Tables 2 and 3presents perimeter and area of hepatocytes on study and control groups.

24, 48, and 96 hours after liver resection, the area and perimeter of hepatocytes increased in the first and third zones of the acinus compared to normal ($p<0.001$). 1 week after resection, the area and perimeter of hepatocytes in the third zone of the acinus were smaller than normal ($p<0.05$), and the perimeter and area of hepatocytes in the first zone exceeded the normal values ($p=0.05$). In addition, the perimeter and area of hepatocytes in the first and third zones of the acinus were smaller compared to similar data for previous regeneration terms ($p<0.05$). 2 weeks after resection, the area and perimeter of the regenerated liver hepatocytes in the first and third zones of the acinus exceeded the normal values obtained one week after resection ($p<0.001$).

Table 2. Hepatocytes area and perimeter in the I zone of acinus

Terms and zone / Data	Area of I zone (μm²)						Perimeter of I zone (μm)					
	Norma	24 H	48 H	96 H	1 week	2 weeks	Norma	24 H	48 H	96H	1 Week	2 Weeks
Average	255	349	479	315	275	389	62	76	93	74	63	77
Minimum	128	203	228	135	132	219	44	52	65	47	46	53
Maximum	418	780	982	863	734	737	88	106	150	108	92	105
St. Deviation	66	97	160	117	88	120	8	11	16	13	11	12

Table 3. Hepatocytes area and perimeter in the III zone of acinus

Terms and zone / Term data	Area of III zone (μm²)						Perimeter of III zone (μm)					
	Norma	24 H	48 H	96 H	1 week	2 weeks	Norma	24 H	48 H	96H	1 Week	2 Weeks
Average	283	388	400	347	244	379	64	76	77	75	61	74
Minimum	119	224	123	34	34	160	47	54	50	49	42	51
Maximum	523	665	897	654	506	715	90	105	111	103	83	104
St. Deviation	88	95	131	97	76	118	10	11	13	12	9	13

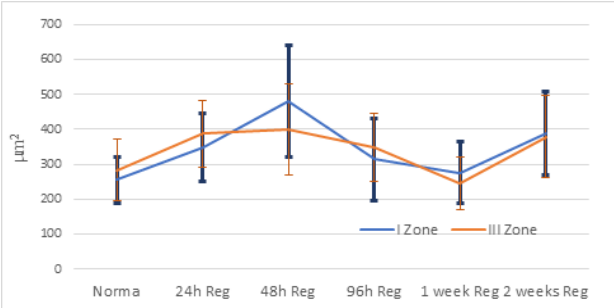

Diagram 1. Area of hepatocytes of I and III zones of acinus

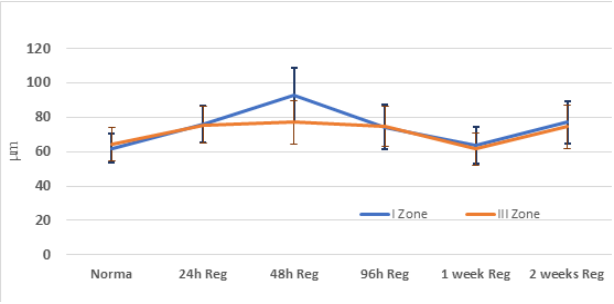

Diagram 2. Perimeter of hepatocytes I and III zones of acinus

Comparison of hepatocyte areas and perimeters by zones at the same terms shows that, at normal and at 24 h of regeneration, the area of hepatocytes in the third zone exceeds the area of hepatocytes in the first zone (p=0.01; p=0.005, respectively), and no significant difference is observed between the perimeters of the hepatocytes (respectively p=0.06; p> 0.9).

The situation is changed at 48 and 96 hours after resection, when the area and perimeter of hepatocytes in the first zone of the acinus exceeded the area and perimeter of hepatocytes in the third zone (p <0.05).

One week after 2/3 liver resection, the area of hepatocytes in the first zone was significant larger than the area of hepatocytes in the third zone (p=0.009), and the difference between perimeters was not significant (p=0.1). However, two weeks later, the area and perimeter of hepatocytes in the first zone of acinus do not differ from the area and perimeter of hepatocytes in the third zone (p=0.5; p=0.2). All data are presented graphically in Diagrams 1 and 2.

By the histological examination of the normal rat liver can often identify lobules where the hepatocytes are arranged radially, in the form of plates of one or two hepatocytes, between which the sinusoids are more or less equal in size (Fig. 2A, 3A).

On the 24th and 48th hours after 2/3 hepatectomy, it is difficult to identify the lobules, and the lobules whose outline can be identified are increased. The radial arrangement and architecture of the hepatocyte plates are disordered. They are replaced by conglomerates of liver cells and sinusoidal capillaries. In some areas of these conglomerates, zones without sinusoids, as well as with sinusoids of different sizes and shapes, are identified. In addition, some sinusoids are sharply widened. The typical configuration of the cytoplasmic membrane of hepatocytes are changed, cytoplasmic protrusions (procesus) appear on some hepatocytes. Multiple mitotic figures (Fig. 2B, C, 3B, C) are observed On the 48th h of regeneration. On the 96th h of regeneration mitotic figures are found in unit quantities. Part of hepatocytes undergo destructive-necrotic changes. Such necrotic hepatocytes are often bordered by binuclated, large, or mitotic hepatocytes. Necrotic hepatocytes are also often found in so-called, bloodless areas (Fig.s 2D; 3D). 1 week after regeneration, the liver tissue returns to a more or less typical architecture, and the size of the lobules that can be identified on histological preparations is larger comparing to normal. Part of the sinusoids is dilated, and in some areas there are markedly irregular sinusoids with branching. The plasma membrane of hepatocytes surrounding such sinusoids is also abruptly irregular, sometimes so much that the liver cells have a star-like shape (Fig. 2E; 3E; Fig. 5). On the 24th , 48th , and 96th hours of regeneration, hepatocytes differ markedly in size from normal hepatocytes. Typical form of the normal hepatocyte (Fig. 2A, 3A) are replaced by hepatocytes with dramatically different shapes and sizes, which are connected to each other by an unusually shaped

plasma membrane protrusion so that the histological picture of whole section resembles the puzzle construction (Fig. 2B, C, D, E, F; 3B, C, D, E, F). Connection of hepatocytes through these new "growths" (protrusions) indicates formations of new connections, and remodeling of hepatocyte cords, as it is shown in the case of changes in aortic endothelial cell under pressure [10].

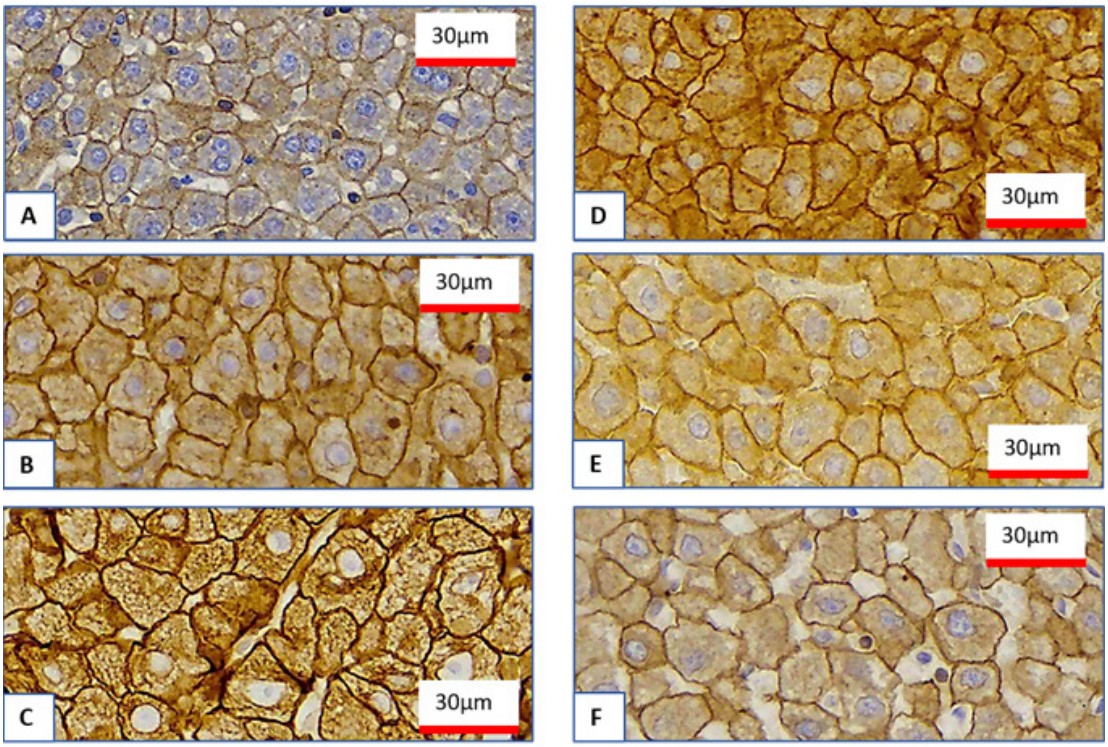

Fig. 2. Hepatocytes in the I zone of acinus; A - Control; B – 24h after PH; C – 48h after PH; D – 96h after PH; E – 1week after PH; F – 2 weeks after PH; Marked with CK8; X1000

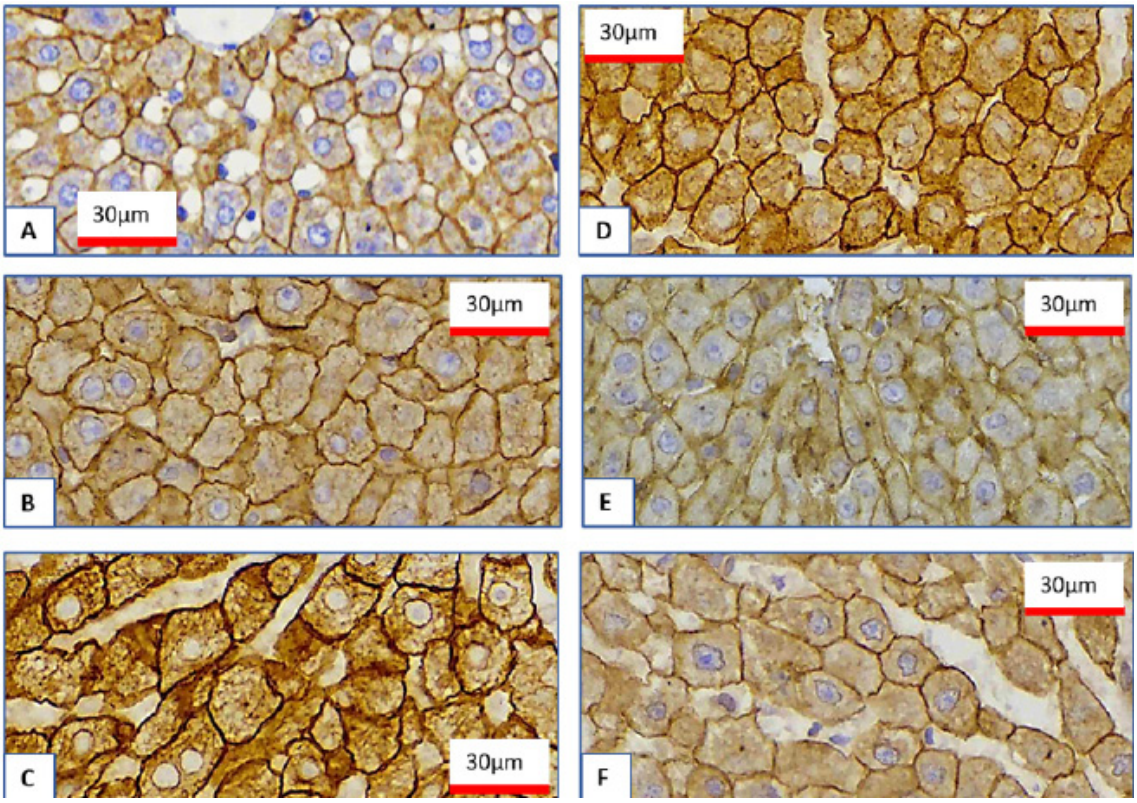

Fig. 3. Hepatocytes in the III zone of acinus; A - Normal Liver; B – 24h after PH; C – 48h after PH; D – 96h after PH; E – 1week after PH; F – 2 weeks after PH; Marked with CK8; X1000

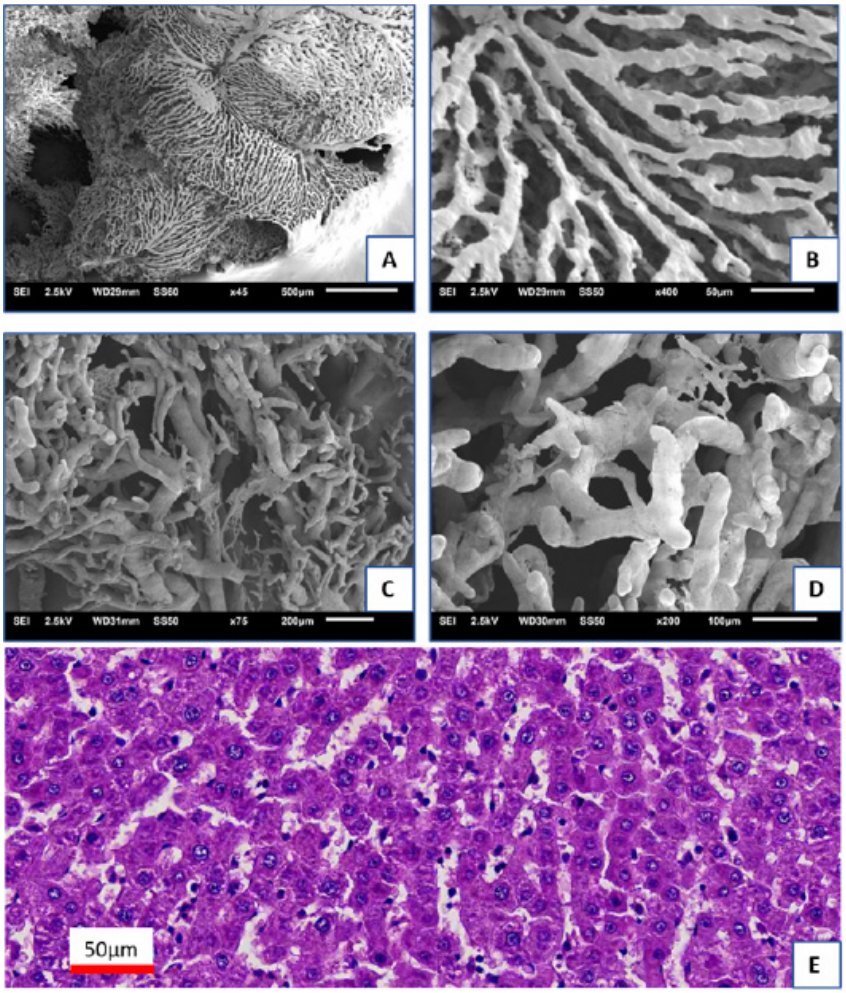

Fig. 4. SEM of Corrosion cast of liver regeneration after 1 week of PH;
A – sinusoidal network of adjusted lobules. Injection replicas of the sinusoids with different forms and diameters.
B- Rough surface injection replicas of thin, zigzag-like form sinusoids.
C,D – sprouting of hepatic vein tributaries and large sinusoids. E – H&E stain; Zigzag-like form sinusoids (corresponds with B)

It is noteworthy that within a week after regeneration in some areas there is intense formation of sinusoid capillaries and an abundance of small tributaries of the hepatic veins (central and sublobular veins) against the background of the small amount of portal triads.

SEM examination of corrosion cats of the same term reveals a network of sinusoids that spatially lines lobules of different shapes and sizes, including those that appear to be a combination of two "normal" lobules (megalobules) (Fig. 4A). Sometimes the diameter of sinusoids is markedly different. Particularly the superficial sinusoids. In some areas, small (narrow) diameter casts of sinusoids are observed, which have an irregular rough surface with small bud protrusions, which gives the contour of these casts a zigzag shape (Fig. 4B). These casts correspond to the sinusoids observed in some slides prepared on the same term and stained by H&E. SEM of corrosion casts reveals the replicas of the hepatic vein tributaries and associated with them large sinusoids which with the typical features of vascular sprouting. Such sprouting casts sometimes anastomose to each other (Fig. 4 C, D).

After 2 weeks of liver resection, the number of areas whose construction looks like normal increases. In addition, areas with hepatocytes with cytoplasmic growths (protrusion) and sinusoids of different diameters are still detected. Regenerative nodules without sinusoids indicating that the sinusoidalization process is not complete.

Changes the data of hepatocyte area and perimeter obtained by us are not characterized by a single common tendency. Taking into account, that hepatocytes of a strange (non-standard) shape appear with a kind of cytoplasmic protrusions (Fig. 5), we must assume that not only the vascular network and, consequently, the shapes and sizes of the lobules, but also the population of hepatocytes are subject to transformation. Comparing the current data obtained by morphometry of hepatocytes after 2/3 hepatectomy, with the similar data obtained 9 months after 2/3 hepatectomy, previously published by us, it turns out that they are also different from each other [28]. This gives us reason to assume that structural transformation of the liver is a prolonged process after 2/3 resection. These data corrects the statement that liver regeneration processes in rodents after resection of 2/3 ends in 7 – 10 [11,17,22].

Based on the results of our research, we consider that such formulation is more correct: after 2/3 resection, the liver regenerates and regains its mass and volume in 7-10 days, although the transformation of both, its cellular and vascular structures, which in turn leads to spatial transformation of liver cells, continues for long periods from resection.

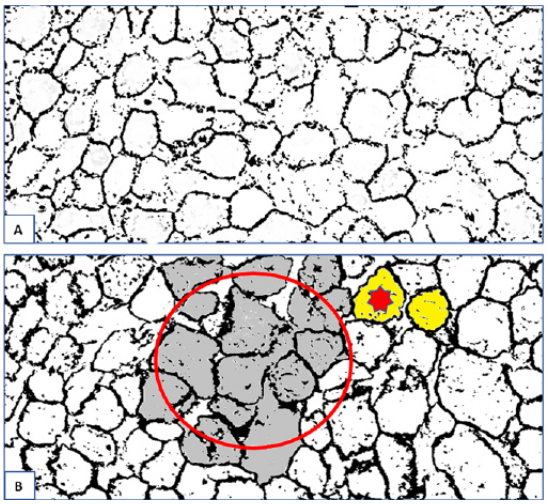

Fig. 5. Hepatocyte forms changing.

A) Normal Liver. Scanned image. Marked with CK8; edited by ImageJ software X1000

B) Liver after 1 week Regeneration. Marked with CK8; edited by ImageJ software X1000. Hepatocytes with dramatically different shapes and sizes, which are connected to each other by an unusually shaped plasma membrane protrusion so that the histological picture of whole section resembles the puzzle construction (bordered by red circle); Hepatocytes with zigzag-like membrane (asterisk)

It is under the question, whether the permanent transformation of liver architecture is caused only by 2/3 hepatectomy or it is a typical phenomenon for the liver that occurs throughout ontogenesis. During the period of ontogenesis, the liver (as well as the whole organism) increases in volume and weight, and this increase is associated with the proliferation of liver lobules [18]. After completion of postnatal growth, in the dynamics of ontogenesis, confirmation or rejection of possible changes in liver architecture would be important for the study of liver structure, as a whole, and as an individual component of the organ.

Sprouting of the hepatic vein tributaries (Fig. 4C, D) detected on corrosion casts confirms that the proliferative processes are not complete and therefore the liver/lobules remodeling process continues. The sprouting of the above-mentioned venules corresponds to areas quite commonly found on histological slides, where exists a lot of central veins and sublobular veins without a corresponding number of triads, this does not fit with the classical description of rat liver. In addition, a similar proliferation of veins may be some indication of the formation of new lobules.

Conclusion. The process of regeneration of rat liver does not end in one or two weeks. Despite the recovery of liver volume and mass, which is mainly based on hepatocyte mitoses, the regenerated liver undergoes a permanent process of transformation of hepatocyte shape and size, as well as the transformation of the vascular network. New intercellular connections are formed, including with the involvement of atypical membrane protrusions of deformed neighboring hepatocytes. The vascular network also undergoes transformation - by changing the shape and size of existing structures and by forming new sinusoidal capillaries and venules.

These transformations underlie changes in the spatial architecture of the liver lobules.

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SUMMARY

STRUCTURAL CHANGES IN RATS' LIVER DURING THE FIRST 2 WEEKS FOLLOWING 2/3 PARTIAL HEPATECTOMY

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Aim of study - Investigation of changes in hepatocyte size and shape and architecture of the sinusoidal network in 2-week dynamics after resection 2/3 of the liver.

The experiments were performed on 16 adult male Wistar rats weighing 190-200 grams who underwent 2/3 resection of liver, while a resected portion of the liver of the same rat was considered as a control. We examined liver tissue by histological, immunohistochemical, morphometrical methods, and the architecture of the sinusoidal capillary network by electron microscopy of corrosion casts. The study was conducted in 24 hours, 48 hours, 96 hours, 1 week, and 2 weeks after surgery.

The shape and size of the hepatocytes in the first and third zones of the liver acinus change with the term of the experiment. With changes in the shape and size of hepatocytes, new intercellular connections are formed, including with the involvement of atypical membrane protrusions of deformed neighboring hepatocytes.

One week after regeneration, electron microscopic examination of corrosion casts reveals a network of sinusoids that spatially define lobules of different shapes and sizes, including those that appear to be a combination of two "normal" lobules. Superficial sinusoids are often markedly dilated (up to 25 μm). In addition, small-diameter (6-7μm) sinusoidal casts with a rough surface and small bud-shaped protrusions are observed in some areas, giving the line of this a zigzag shape. The existence of hepatic vein tributaries and associated with them large sinusoids, found In single areas, reveals the characteristic feature of vascular sprouting.

Based on the data obtained, it can be assumed that despite the recovery of liver mass, the regeneration process is not complete. Regenerated liver undergoes a permanent process of transformation of hepatocytes' shape and size, as well as the transformation of the vascular network, which is the basis for changes in the spatial architecture of the liver lobules.

Keywords: 2/3 liver resection; Liver regeneration; Corrosion casts; Sinusoidal network transformation; Hepatocytes morphometry.

РЕЗЮМЕ

СТРУКТУРНЫЕ ИЗМЕНЕНИЯ ПЕЧЕНИ КРЫС В ТЕЧЕНИЕ ПЕРВЫХ 2-НЕДЕЛЬ ПОСЛЕ 2/3 ГЕПАТЭКТОМИИ

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Цель исследования - изучение изменений размеров и формы гепатоцитов и архитектоники сети синусоидов в течение 2-недель после резекции 2/3 печени.

Эксперименты выполнены на 16 крысах самцах линии Wistar весом 190-200 грамм, у которых выполнена частичная гепатэктомия. Удаленная часть печени проанализирована в качестве контроля для каждого животного. Ткань печени спустя 24, 48, 96 часов и через 1 и 2 недели изучена с помощью гистологических, иммуногистологических и морфометрических методов; архитектоника синусоидных капилляров исследована с помощью сканирующей электронной микроскопии коррозионных препаратов.

Форма и размеры гепатоцитов первой и третьей зоны печеночных ацинусов изменяются в течение всего срока наблюдений. Изменение формы и размеров гепатоцитов приводит к формированию новых межклеточных контактов, которые в ряде случаев образуются благодаря атипичным отросткам деформированных соседних клеток. Спустя неделю после гепатэктомии электронно-микроскопическое исследование коррозионных препаратов выявило сеть синусоидов, которые располагаются внутри долек различной формы и размеров. В некоторых случаях создается впечатление, что дольки аномальной формы и размеров образованы комбинацией двух «нормальных» долек. Поверхностные синусоиды часто заметно расширены (до 25 мкм). На некоторых участках наблюдаются слепки синусоидов малого диаметра (6-7 мкм) с шероховатой поверхностью и небольшими выступами в форме бутона, что придает им зигзагообразную форму. В ряде зон обнаруживается наличие притоков печеночных вен и связанных с ними синусоидов большого диаметра, что является признаком сосудистого разрастания.

Полученные данные позволяют предположить, что, несмотря на восстановление массы печени, процессы регенерации не завершаются. В регенерирующей печени продолжаются перманентные процессы трансформации формы и размеров гепатоцитов, а также перестройки сети сосудов, которые лежат в основе изменений пространственной архитектуры долек печени.

რეზიუმე

ვირთაგვის ღვიძლის სტრუქტურული ცვლილებები 2/3 ჰეპატექტომიიდან პირველი 2 კვირის განმავლობაში

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[2] თსუ, ალექსანდრე ნათიშვილის სახ. მორფოლოგიის ინსტიტუტი; [3]ფედერალური სამედიცინო ბიოლოგიური სააგენტო, ფიზიკურ-ქიმიური მედიცინის ფედერალური კვლევითი და კლინიკური ცენტრი, მოსკოვი, რუსეთი

კვლევის მიზანს წარმოადგენდა ღვიძლის 2/3-ის რეზექციის შემდეგ ღვიძლის რეგენერაციის პროცესში ჰეპატოციტთა ზომის, ფორმის და სინუსოიდთა ქსელის სივრცული არქიტექტონიკის ცვლილებების გამოკვლევა 2-კვირიან დინამიკაში.

ექსპერიმენტები ჩატარდა Wistar-ის ჯიშის 16 ზრდასრულ მამრ ვირთაგვაზე, წონით 190-200 გრამი, რომელთაც ჩაუტარდა პარციალური ჰეპატექტომია; მათ კონტროლად აღებული იყო იმავე ვირთაგვას ღვიძლის რეზეცირებული ნაწილი. ღვიძლის ქსოვილის გამოკვლევა ჩატარდა ჰისტოლოგიური, იმუნოჰისტოქიმიური, მორფომეტრიული მეთოდებით; სინუსოიდური კაპილარების ქსელის სივრცული არქიტექტონიკა - კოროზიული ტვიფრების მასკანირებელი ელექტრონული მიკროსკოპიით. კვლევა ჩატარდა ოპერაციიდან 24, 48 და 96 საათის, 1 და 2 კვირის შემდეგ.

ექსპერიმენტის ვადებთან ერთად ცვალებადობა განიცადა ღვიძლის აცინუსის პირველი და მესამე ზონების ჰეპატოციტების ფორმისა და ზომის ურთიერთშეფარდებამ. ჰეპატოციტების ფორმისა და ზომის ცვლილებებთან ერთად ჩამოყალიბდა ახალი უჯრედშორისი კავშირები, მათ შორის ფორმაშეცვლილი მეზობელი ჰეპატოციტების ატიპიური მემბრანული მორჩების ჩართულობით.

რეგენერაციის 1 კვირის შემდეგ კოროზიული პრეპარატების მასკანირებელი ელექტრონული მიკროსკოპით გამოკვლევისას გამოვლინდა სინუსოიდთა ქსელი, რომელიც სივრცულად საზღვრავდა სხვადასხვა ფორმისა და ზომის წილაკებს, მათ შორის ისეთებსაც, რომლებიც, თითქოს შექმნილია ორი „ნორმული“ წილაკის გაერთიანებით. ზედაპირულად მდებარე სინოსოიდები ხშირად იყო მკვეთრად გაგანიერებული (25 მკმ-მდე). ამასთანავე, ცალკეულ უბნებში აღინიშნებოდა მცირე დიამეტრის (6-7 მკმ) სინუსოიდთა ტვიფრები ხორკლიანი ზედაპირით და მცირე ზომის კვირტისებური წანაზარდებით, რაც ამ ტვიფრების კონტურს ზიგზაგისებურ ფორმას აძლევს. ცალკეულ უბნებში აღინიშნა ღვიძლის ვენების შენაკადებისა და მათთან დაკავშირებული მსხვილი სინუსოიდების ისეთი ტვიფრები, როგორებიც დამახასიათებელია სისხლძარღვთა სპრუტინგისთვის. მიღებული მონაცემების საფუძველზე ავტორები გამოთქვამენ ვარაუდს, რომ მიუხედავად ღვიძლის მასის აღდგენისა, რეგენერაციის პროცესი არ სრულდება. რეგენერირებულ ღვიძლში მიმდინარეობს ჰეპატოციტთა ფორმისა და ზომის, ასევე სისხლძარღვოვანი ქსელის ტრანსფორმაციის პერმანენტული პროცესი, რაც საფუძვლად უდევს ღვიძლის წილაკების სივრცული არქიტექტონიკის ცვლილებებს.