REPARATIVE REGENERATION OF LIVER WITH EXPERIMENTALLY INDUCED CIRRHOSIS

B. P. Solopaev, Yu. P. Butnev, and G. G. Kuznetsova

Laboratory of Biology and Biochemistry of the Institute of Experimental Pathology and Therapy (Director – Candidate Med. Sci. B. A. Lapin) AMN SSSR, Sukhumi
(Presented by Active Member AMN SSSR, N. N. Zhukov-Berezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 1, pp. 74-79, January, 1961
Original article submitted January 22, 1960

In clinical practice it is often necessary to deal with a pathologically altered liver (cirrhosis, hepatitis, etc.). Hence, it is of considerable interest to investigate the reparative regenerative capacity of a liver affected by a particular pathological process. In this plan of work, a study of the regeneration of a cirrhotically altered liver is of particular importance. Several authors [1,2,3] have observed a return to normal of the liver structure in rats with experimentally induced cirrhosis in the case where the animals were partially hepatotectomized.

We also carried out experiments of this kind, but on rabbits. Another important difference in our investigations was that they were of a morphophysiological nature, i.e., we not only demonstrated the morphological changes in the liver, but we also conducted biochemical analysis of the liver tissue, bile, and blood at different stages of regeneration. In this paper, we give a brief account of the general results of our experiments.

Experimental Method

Cirrhosis was induced in male rabbits by a daily subcutaneous injection of 0.3 ml of carbon tetrachloride over a period of 42 days.

During this period, six rabbits died. The 25 remaining animals developed a pronounced cirrhosis accompanied by ascites. A month after the course of carbon tetrachloride injections was completed, the control and experimental (with experimental cirrhosis) animals were subjected to partial resection of the liver – the left lobes (comprising a little more than half of the entire organ) were removed. Eight of the operated rabbits were sacrificed 2-30 days after the operation; 13 rabbits with experimentally induced cirrhosis, but not subjected to resection of the liver, perished during the 10 days after the start of the operations.

When the animals were sacrificed, their weight and the weight of the liver were determined. In the blood we determined the sugar content, total residual reduction, residual nitrogen, proteins, and cholesterol. Pieces of liver were taken for a quantitative determination of glycogen, ATP, cholesterol, fatty acids, the dry weight of the liver, and the respiration of liver slices in a Warburg apparatus.

The material for histological examination was fixed in 10% formalin and Bouin's fluid. Some of the pieces were embedded in paraffin, and some were cut on a freezing microtome. The sections were stained in hematoxylin-eosin, van Gieson, and Suadn III.

The number of mitoses were counted in 100 microscope fields (32 mm^2 slit in eyepiece diaphragm, eyepiece 7, objective 90). Sugar in the blood and bile was determined by the Hagedorn-Jensen method, the total residual reduction of the blood and bile by Stepun's method, blood proteins by the Robinson-Hogden technique, and the cholesterol in the blood and bile by Levchenko's method. The amount of glycogen was determined by

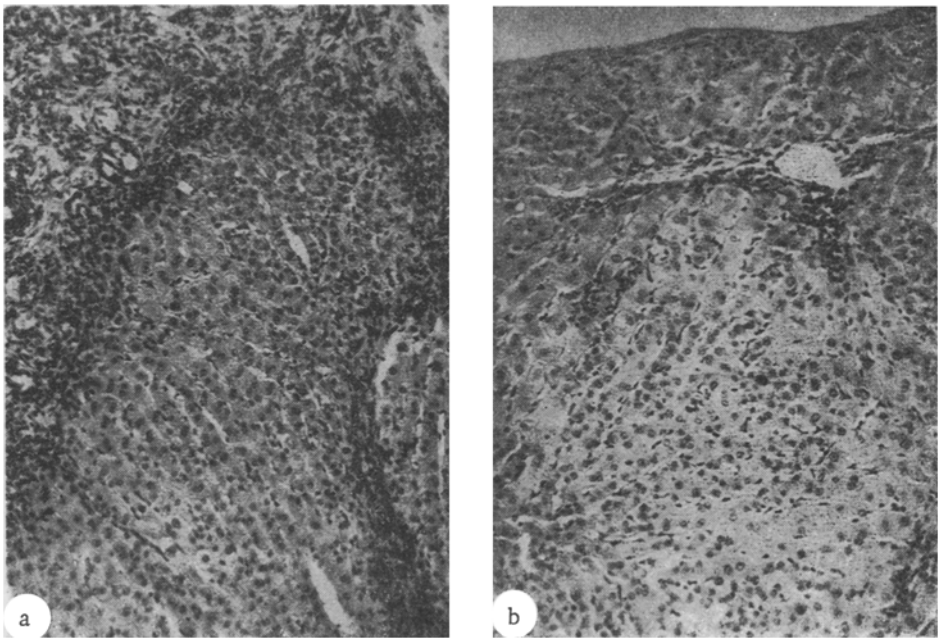

Fig. 1. Liver of rabbit with experimentally induced cirrhosis: a) during operation of partial resection of liver; b) 30 days after operation.

the weighing method after extraction from the liver by trichloroacetic acid and precipitation by alcohol; the ATP content was found by precipitating it from a protein-free filtrate with mercuric acetate, and subsequent hydrolisis in hydrochloric acid. The cholesterol content of the liver was determined by the Engel'gardt-Smirnova method. Respiration of the liver tissue was determined in a Warburg apparatus by the conventional method. The total fatty acid content of the liver tissue was determined by the weighing method after hydrolysis of the wet liver tissue in NaOH solution with subsequent acidification by an excess of sulfuric acid solution, extraction of fatty acids with ether, and subsequent washing of the ether with water, and evaporation.

Experimental Results

On microscopic examination of pieces of liver taken from animals with experimentally induced cirrhosis (on sacrifice, death, and partial resection operations) we found an abundant proliferation of connective tissue (Fig. 1a). Most of the liver lobes were surrounded by a considerable layer of connective tissue; some of the lobes appeared immured in it. The cytoplasm of cells in the center of such lobes was vacuolated, and the cytoplasm of cells on the periphery of the lobes adjoining the connective tissue strands appeared homogeneous. We observed degeneration and pycnosis in scattered nuclei of the liver cells. In places we found an abundant proliferation of bile ducts among the connective tissue. The remaining liver tissue in many spots was considerably modified; in places we noted discomplexation of the hepatic cylinders, absence of the typical radial arrangement around the central veins, and enlargement of Disse's spaces.

In experimental animals with developed cirrhosis, which had been operated on, we observed a considerable decrease in the amount of connective tissue and an approach of the liver structure towards normal from the end of the second week after operation (Fig. 1b).

Thus, partial hepatectomy suppressed the development of connective tissue in the cirrhotic liver.

As was shown by a study of the mitotic activity of liver cells (Fig. 2), its most pronounced rise in the control animals was observed in the first 5 days after operation; from the 7th day onwards, the mitotic activity greatly diminished. In animals with experimental cirrhosis, an increase in mitotic activity in the liver cells was also observed in the first 5 days after operation. The mitotic activity was not so high as in the control animals. However, when the great number of fields of view occupied by connective tissue was taken into account, the mitotic activity of the liver cells per unit area of remaining liver parenchyma in the experimental animals could be regarded as just as high as in the controls.

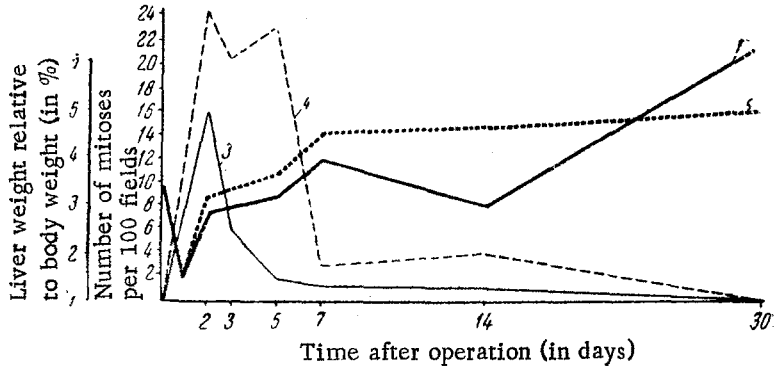

Fig. 2. Change in liver weight relative to body weight, and change in mitotic activity of liver cells during regeneration of liver with experimentally induced cirrhosis. 1) Relative weight of liver of rabbits with experimental cirrhosis; 2) relative weight of liver of control animals; 3) mitotic activity of liver cells of rabbits with experimental cirrhosis; 4) mitotic activity of liver cells of control rabbits.

The part of the liver left after operation both in the experimental and in the control animals considerably increased in weight and size (see Fig. 2). The liver of animals with experimentally induced cirrhosis recovered its relative weight more slowly in the first two weeks than the liver of the control animals; however, by the end of the month the relative weight of the liver of animals with experimentally induced cirrhosis exceeded the relative weight of a normal liver.

The dry weight of the liver (as a percentage per 100 g of wet tissue) in the control rabbits, which increased on the 3rd-5th day after operation, became normal by the end of the first week, and later remained at this level.

The dry weight of the cirrhotic liver was increased on the 2nd day, and then gradually decreased in the course of a whole month. The greater amount of liquid in the regenerated liver of the experimental animals was probably due either to the increased hydrophilia of its tissues, or to the greater amount of deposited blood. An interesting feature was that, when the ratio of the dry weight of the whole liver to the body weight was calculated, we found that not only was the preoperative relative dry weight restored by the 30th day after operation, but it even exceeded the initial level of the relative weight of the liver in normal rabbits.

Among the various indices characterizing the functional state of the liver, we can note three groups: the first group includes indices which return to normal; the second includes those which do not return to normal; and the third includes indices which undergo relatively slight variation. We found that such indices as the respiration rate of slices and the cholesterol content of the bile belonged to the first group. Slices of cirrhotic liver before operation absorbed more oxygen than slices of normal liver. During regeneration of the cirrhotic liver and the liver of control rabbits, we observed an increase in O_2 consumption from the 2nd day after operation, the greatest values being found after 1-2 weeks in the case of the liver of the controls, and after 2 weeks for the liver of experimental animals. By the end of the month after operation, the respiration rate of liver slices from both experimental and control rabbits reached normal.

The cholesterol content of the bile of the experimental and control animals varied in two stages: the peaks of the maximum cholesterol content were observed on the 2nd-3rd day and on the 7th day after operation.

The first increase in the cholesterol content of the bile was much more pronounced in control rabbits, and the second peak was more pronounced in the experimental animals. By the 30th day after operation, the cholesterol content of the bile of the control and experimental rabbits became normal.

The sugar and cholesterol content, and the total residual reduction of the blood of the control and experimental animals did not vary significantly during regeneration of the liver.

The quantity of residual nitrogen in the blood fell on the 5th day after operation in the experimental animals; on the 7th day in the controls; and then returned to its initial value.

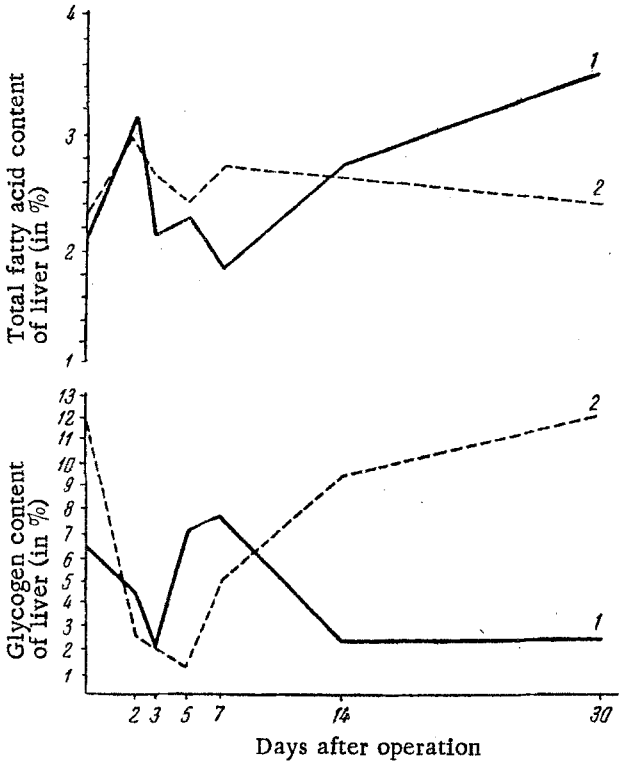

Fig. 3. Change in fatty acid and glycogen content of liver during regeneration of liver with experimentally induced cirrhosis: 1) in experimental rabbits; 2) in control rabbits.

We did not observe a return to normal of such indices as the glycogen content, cholesterol content, and total fatty acid content of the liver. The glycogen content of the cirrhotic liver was slightly more than half of that of the normal liver (Fig. 3). In the first few days after operation, the glycogen content of the liver in the control and experimental animals, dropped sharply, reaching a minimum on the 5th day in the control animals and on the 3rd day in the experimental animals. The subsequent course of the curves for the glycogen content of the liver in the experimental and control animals was different. In the liver of the control rabbits the glycogen content gradually increased and had returned to normal level by the end of a month; in the experimental animals, the increase in glycogen content by the 5th-7th day after operation gave way to a considerable fall by the 14th day, and by the end of a month the glycogen content of the liver was not only lower than in the control animals at this time, but was even lower than the initial glycogen content of the cirrhotic liver.

The total fatty acid content of the liver in the control animals, which increased by the 2nd day after operation, gradually fell, and by the end of a month reached the normal level (see Fig. 3). In the liver of the experimental animals we also observed a rise in the total fatty acid content on the 2nd day after operation; however, following the fall on the 3rd-7th day, a considerable rise took place, so that, by the 30th day, the initial preoperative background was exceeded. Thus, in contrast to the controls, the fatty acid content of the liver of the experimental animals had not returned to normal a month after operation.

The fat content revealed by Sudan III staining in the liver of the experimental animals varied in the same way as in the control animals during the first 2 weeks after operation. By the end of the first week, infiltration by large fat droplets gave way to infiltration by small fat droplets, and the fat content of the liver returned to normal by the end of the 2nd week. However, by the end of a month the fat content of the liver of the experimental animals was much higher and was manifested in the form of fine-droplet infiltration of the individual liver cells. The fat content of the liver of the experimental animals was comparably higher in the first few days after operation as compared with the 30th day, whereas the biochemical determination of the total fatty acid content of the liver showed higher figures on the 30th day. The increase of the total fatty acid content at this time was apparently due to bound forms of lipids (possibly, lipoprotein) which are not revealed by Sudan III staining.

The cholesterol content of the liver of the experimental animals by the end of a month after operation greatly exceeded the preoperative level, whereas, in the controls, it had returned to normal.

The sugar content of the bile of the experimental and control rabbits in the first few days after operation varied in parallel, increasing on the 3rd-5th day. The sugar content of the bile of the control rabbits then fell to normal (14th day), and was later maintained at this level; in the bile of the experimental animals it was a little lower by the 30th day than in the control animals. The total residual reduction of the bile of the control and experimental animals varied insignificantly. Preliminary data on the ATP content of liver indicated lower levels in the liver of experimental animals as compared with controls during the whole postoperative period.

An analysis of the biochemical changes at different times during regeneration showed the following. In the control animals on the second postoperative day, when the most pronounced histological changes in the liver

were observed (degenerative changes in liver cells, fat infiltration, mitotic division of liver cells), the glycogen content of the liver fell sharply, the fatty acid and cholesterol content of the liver increased, and the respiration of the liver tissue was enhanced. On the 5th-7th postoperative days, in the period when the weight and size of the liver increased most rapidly and the mitotic activity of the liver cells was most pronounced, we observed a reduction in the dry weight of the liver, an increase in glycogen, and return to normal of the fatty acid content of the liver, a reduction of the cholesterol content of the liver, bile, and blood, and the maximum respiration of liver slices. In the period during which liver regeneration was completed (14th-30th day), the dry weight of the liver, the glycogen, fatty acid and cholesterol content of the liver, blood, and bile, the residual nitrogen in the blood, and the respiration of liver tissue slices, became normal.

In contrast to the control animals, the dry weight of 100 g of liver in the animals with experimentally induced cirrhosis increased on the 2nd-3rd day after operation. The glycogen content of the liver fell, its fatty acid and cholesterol content increased, and the cholesterol content of the blood (above normal) and bile (four times initial level) increased. From the 3rd day, the residual nitrogen of the blood decreased considerably.

On the 5th-7th day after operation, the total dry weight of the liver increased with a drop in the dry weight of 100 g of liver; the total fatty acid content was reduced almost to the initial level; the glycogen and cholesterol content of the liver reached the normal level; the respiration of liver slices returned to normal; the residual nitrogen and cholesterol content of the blood became a little less than the initial values, and there was a considerable increase in the cholesterol content of the bile. The fat and carbohydrate metabolism appeared to be normal.

During the 14th-30th day, the total dry weight of the liver became normal with a reduction in the dry weight of 100 g of liver; the fatty acid and cholesterol content of the liver increased considerably; the cholesterol content of the blood and liver returned to normal; the residual nitrogen of the blood lay within normal values. Respiration of liver slices, which was enhanced on the 12th day, became normal by the 30th day. The glycogen content of the liver by the 30th day was still much lower than the initial level.

Thus, the most pronounced changes in the biochemical indices were observed in the first few days after operation. By the end of the first week, the majority of indices had become normal both in the experimental and in the control animals.

However, in the experimental animals after the 2nd week, in contrast to the controls, we noted an increase in the fatty acid and cholesterol content of the liver, and a considerable fall in the glycogen content of the liver.

The obtained results showed that liver with experimentally induced cirrhosis is capable of reparative regeneration. By the end of a month after the partial resection operation, the liver not only reached its initial weight, but even surpassed it. In the regeneration of liver with experimentally induced cirrhosis, we observed a distinct reduction in the amount of connective tissue and an approach of the liver structure to normal. A fact of great importance was the death of all the animals with experimentally induced cirrhosis which were not subjected to the operation of partial resection of the liver. It would appear that when half the cirrhotic liver is removed, the regeneration of the remaining parenchyma is stimulated to a greater extent than the multiplication of the connective tissue, and the amount of the latter progressively diminishes during regeneration of the liver.

The results of the biochemical analysis, conducted at different stages of the regenerative process, indicate that during regeneration there is a change in several biochemical indices, some of which later approach normal values, while others (glycogen, fatty acid, and cholesterol content of liver) do not become normal by the end of a month. Thus, though the liver structure approached that of a normal liver, and the amount of connective tissue in it was greatly reduced, there was still no return to normal of all the biochemical indices a month after operation.

SUMMARY

Cirrhosis of the liver, accompanied by ascites, was induced in rabbits by subcutaneous administration of carbon tetrachloride, and one-half of the liver was subsequently removed. A marked reduction in the amount of connective tissue, with the structure of the liver approaching normal, was observed during regeneration of the cirrhotic liver. The weight of the liver was restored to normal.

Animals with experimentally induced cirrhosis of the liver, and not subjected to partial resection, perished. At various stages of the regenerative process, biochemical analysis of liver tissue, blood, and bile were carried out. Some of the biochemical indices became normal, whereas the glycogen, fatty acid, and cholesterol content

of the liver was not restored to the normal level even by the end of the month. After excision of one-half of the cirrhotic liver, regeneration of hepatic parenchyma is evidently greater than the connective tissue proliferation.

LITERATURE CITED

1. G. R. Cameron and W. A. E. Karunaratne, J. Path. and Bact. 42, 1 (1936).
2. A. H. Islami, G. T. Pack, and J. C. Hubbard, Cancer 11, 663 (1958).
3. N. Rabinovici and E. Wiener, Israel Med. J. 17, 169 (1958).