MECHANISMS OF REPARATIVE REGENERATION OF THE PATHOLOGICALLY CHANGED LIVER

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Restoration of the normal parenchyma during reparative regeneration of the liver was studied by histological, cytological, biochemical, and immunochemical methods in rats with experimental cirrhosis and hepatitis. Analysis of the mitotic activity of the hepatocytes, the dynamics of the change in the number of binuclear cells, and the change in size and ploidy of the mononuclear hepatocytes revealed some general and specific rules governing reparative regeneration and the reversibility of the pathological changes in these conditions. Somatic polyploidization is suggested as playing an essential role in the reparative regeneration of the pathologically changed liver.

After reversibility of pathological changes during the reparative regeneration of organs had been shown to be possible in some forms of pathology [4-6, 9], the investigation of mechanisms of regeneration and the laws governing the reversibility of pathological changes in various forms of disease acquired special importance. A particular role in reparative regeneration and in the reversibility of pathological states is played by normalization of the parenchyma responsible for the restoration of function of the damaged organ.

This paper describes a study of mechanisms of normalization of the parenchyma during regeneration of the liver when damaged by potassium permanganate and carbon tetrachloride.

EXPERIMENTAL METHOD

Experiments were carried out on 240 male albino rats weighing 150-180 g with experimental hepatitis and cirrhosis of the liver. Cirrhosis of the liver was induced by subcutaneous injection of carbon tetrachloride, and hepatitis by subcutaneous injections of potassium permanganate. Of the total liver tissue 50% was removed from rats with cirrhosis of the liver and 30% from rats with hepatitis. The experimental animals were killed at 8 a.m., 1, 2, 3, 7, and 30 days after the operation. Pieces of liver were fixed in 10% neutral formalin. Paraffin sections, 7 μ in thickness, were stained with hematoxylin-eosin. The mitotic index of the hepatocytes was determined and the number of binuclear cells counted in 6000-7000 liver cells. The volume of 500 nuclei of mononuclear hepatocytes was determined in each animal by means of the MOV = 1-15 ocular micrometer. The numerical results were classified by ploidy [7]. The DNA content in the hepatocyte nuclei was determined cytophotometrically in preparations of isolated cells [8] stained by Feulgen's method. Photometry was carried out by the single-wave method in monochromatic light with wave length 488 nm [2]. The RNA content (by Meibaum's orcine method) and the DNA content (by Barton's diphenylamine method) were determined biochemically in the liver tissue. The state of the liver function was assessed from the histidase and urocainase activity (expressed in units of activity) in the blood serum [3, 10]. The serum and tissue proteins of the liver were analyzed by immunoelectrophoresis [1].

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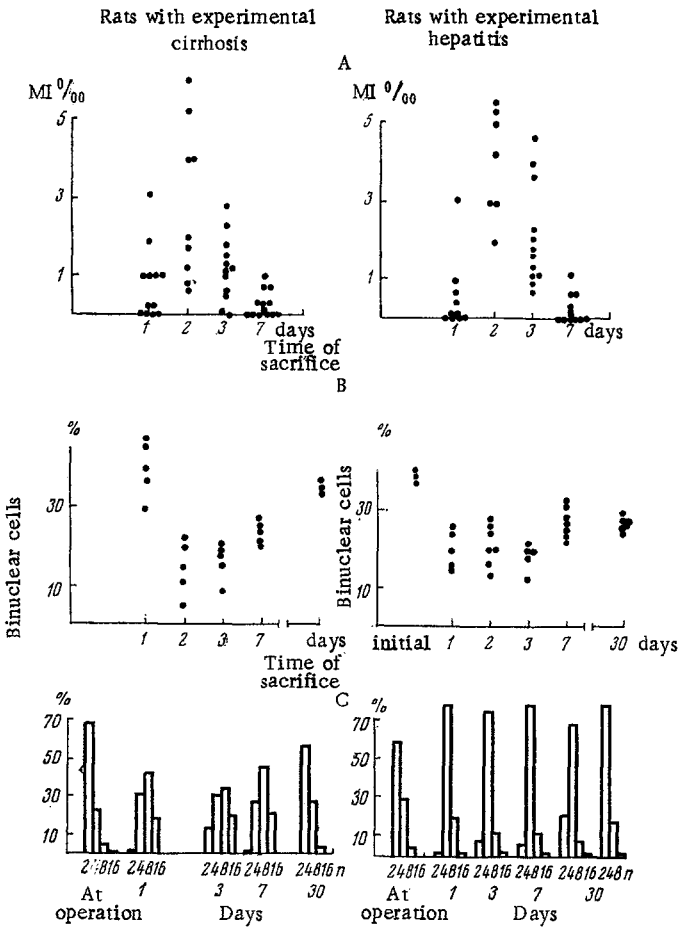

Fig. 1. Mitotic activity (A), changes in number of binuclear hepatocytes (B), and distribution of hepatocyte nuclei by ploidy classes (C) in regenerating liver of rats with experimental cirrhosis (I) and hepatitis (II). Abscissa: A and B) time of sacrifice (in days); C) time of determination (in days); ordinate: A) mitotic activity (in $^0/_{00}$); B) number of binuclear hepatocytes (in %); C) percentages of hepatocyte nuclei of different ploidy classes.

EXPERIMENTAL RESULTS

A histological study showed that changes of an inflammatory and destructive character resembling hepatitis developed in the rat liver as a result of injection of potassium permanganate. Carbon tetrachloride led to the development of cirrhosis of the liver in the rats.

During the first month after resection of part of the pathologically changed liver restoration of the normal structure of the parenchyma of the organ was observed in the rats of both experimental groups. In the rats with experimental hepatitis, by the second half of the month after resection there was a considerable improvement in the state of the organ, but in the animals with cirrhosis normalization of the parenchyma preceded a sharp decrease in the amount of connective tissue proliferating superfluously, which took place much later.

Activity of organ-specific enzymes (histidase and urocainase), which is normally absent, was high in the animals with experimental hepatitis killed before the operation (histidase 6 units, urocainase 1.5 units), and it fell during reparative regeneration of the liver to reach normal by the seventh day.

Analysis of the mitotic activity of the hepatocytes showed that in the pathological conditions studied mitoses appeared in the regenerating liver after 24 h, reached their maximum on the second day, and became solitary in number 7 days after the operation. At the time of its maximum, mitotic activity reached

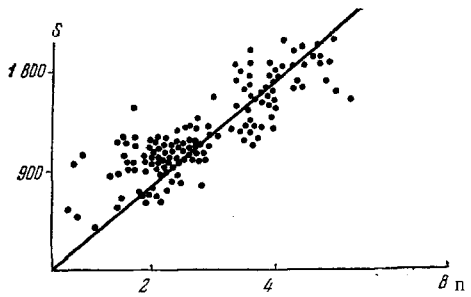

Fig. 2. DNA content (n) plotted against area (S) of hepatocyte nuclei of regenerating pathologically changed rat liver.

reached 5.5 ‰ in the liver of rats poisoned with potassium permanganate and 6.4 ‰ in the liver of rats with cirrhosis (Fig. 1A)

Resection led to a decrease in the number of binuclear cells on the first (in the rats with hepatitis), second, and third days. On the seventh day their number was increased, but one month after the operation the number of binuclear hepatocytes returned to normal (Fig. 1B).

The cytophotometric study of ploidy of the hepatocytes showed direct positive correlation between the size of the nuclei and their DNA content in the regenerating pathologically changed rat liver (Fig. 2).

Diploid and tetraploid mononuclear hepatocytes predominated in the pathologically changed rat liver (Fig. 1C). An increase in the number of tetraploid and octaploid cells was observed 24 h after resection in the liver of all the experimental animals. This ratio between the ploidy classes continued for a week in rats with both forms of pathology. The number of diploid hepatocytes was considerably increased 30 days after resection. It must be noted that the general level of ploidy of the mononuclear hepatocytes was considerably lower in the liver of rats with experimental hepatitis.

The maximal DNA content in the liver damaged by potassium permanganate was demonstrated biochemically on the second day after resection (1.4 mg/g fresh weight compared with a normal value of 0.95 mg; $P < 0.001$). The changes in the RNA content in the initial period of regeneration were similar in the liver tissue of the rats with both pathological conditions. An increase in the RNA content was observed 24 h after resection (in the rats with hepatitis to 9.5 mg/g, compared with a normal level of 5.00 mg/g; $P < 0.001$ and $P = 0.02$, respectively). After some decrease on the second day the RNA content rose again on the third day after resection (9.3 mg/g in the liver of rats with hepatitis and 6.8 mg/g in the rats with cirrhosis; $P < 0.001$ in both groups).

The immunochemical study of the blood serum and liver tissue proteins revealed no new proteins specific for the particular disease or proteins with a modified immunological structure. Embryo-specific α_2-globulin was detected in the blood serum in both these pathological forms, and its titer increased in the early stages but decreased in the later stages of regeneration.

These results showed that a series of similar processes takes place in the regenerating liver of rats poisoned with potassium permanganate and carbon tetrachloride: 1) a simultaneous but slight increase in the mitotic activity of the hepatocytes; 2) similar dynamics of the changes in the number of binuclear cells; 3) hypertrophy of the nuclei of the mononuclear hepatocytes connected with their polyploidization, which coincided in time with an increase in the mitotic activity and persisted after mitotic activity decreased; 4) similarity between the immunochemical properties of the blood serum and liver tissue.

However, during reparative regeneration of the liver of rats with the different types of pathology, certain specific features were observed. During regeneration of the liver damaged by potassium permanganate an increase in the number of diploid cells was observed on the seventh day after resection. This same phenomenon was found in the regenerating liver with experimental cirrhosis, one month after resection.

These results confirm the hypothesis that somatic polyploidization plays a special role in reparative regeneration of the pathologically changed liver.

The course of these processes in the regenerating pathologically changed liver evidently run in the direction of an increase in the function of the residual cells as a result of somatic polyploidization, which enables the organ to survive the unfavorable period (the first days after resection) under the most advantageous conditions for function. In the next stage of regeneration polyploid cells may act as a reserve for cell division.

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