REGENERATION OF THE LIVER AFTER REPEATED RESECTION

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The high power of regeneration of the liver after eight successive resections of the organ was demonstrated by histological, histochemical, and karyometric methods. After eight partial hepatectomies there was a less marked increase in mitotic activity of the hepatocytes than after the first operation. Differences in polyploidization of the hepatocytes were discovered after the first and eighth partial hepatectomies: after the first operation in the early stages there was an increase in the number of hepatocyte nuclei with a high degree of ploidy, and a tendency toward restoration of the hepatocyte nuclei appeared only after 1.5 months, while after the eighth operation the division of polyploid cells was observed at once, with a consequent increase in the number of cells with small nuclei. The ratio between the various classes of nuclei after 1.5 months was close to the control figure for the same age.

Regeneration of the liver after repeated resection is beginning to attract attention as a subject for research because of the striking powers of recovery possessed by the liver [7-10, 12]. However, many aspects of regeneration of the liver after repeated resection remain incompletely studied.

For some years now the writers have studied the liver after repeated resection [2, 4, 6]. This paper deals with the special features of regeneration of the liver after eight successive resections of that organ.

EXPERIMENTAL METHOD

Regeneration of the liver after repeated resection was studied in 300 noninbred albino rats weighing 165 ± 1.8 g. The experimental animals were kept under conditions of permanent artificial lighting. The liver was resected in the morning (8-11 A.M.) at intervals of 1.5 months (6-7 weeks). Hepatectomized animals were sacrificed on the 2nd and 7th day and 1.5 months after each operation; intact animals of the same age were sacrificed at the same times.

At the first operation the left lateral lobe of the liver, accounting for 35.4% of the initial weight of the liver, was removed.

At the second operation the central lobe, accounting on the average for 48.6% of the residual liver tissue after the first hepatectomy, was removed.

At the third operation the right lateral lobe of the liver, accounting on the average for 35.25% of the residual tissue of the liver after the second hepatectomy, was removed. At the fourth operation the cordate lobe, accounting for 33.61% of the residual liver tissue after the third hepatectomy was removed. At the fifth operation the accessory lobe of the liver, accounting on the average for 29.59% of the regenerating liver, was removed. At the sixth operation 1.16 g of liver tissue, on the average 18.83% of the residual tissue of the liver after the fifth hepatectomy, was removed. During the seventh operation 1.17 g of liver tissue, or on the average 13.2% of the liver tissue after the sixth resection, was removed. At the eighth operation 1.23 g liver tissue, on the average 19.4% of the tissue of the regenerating liver after the seventh operation, was removed.

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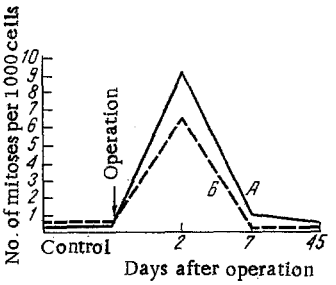

Fig. 1

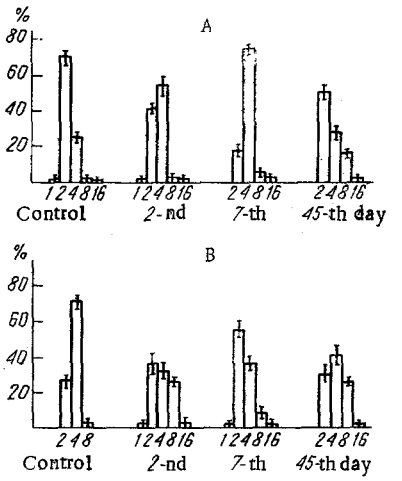

Fig. 2

Fig. 1. Mitotic activity of hepatocytes in control and regenerated liver of rats at different times after resection: A) mitotic activity of hepatocytes after single resection of liver; B) mitotic activity of hepatocytes after eight successive resections of liver.

Fig. 2. Distribution of nuclei of hepatocytes by classes of ploidy in control and regenerating rat liver: A) after first operation; B) after eighth operation. Numbers beneath columns denote classes of ploidy.

Pieces of liver were fixed in neutral 10% formalin and Carnoy's fluid and embedded in paraffin wax. Depending on the object of the investigation, sections were cut to different thicknesses (5 and 7 μ). Some sections were stained with hematoxylin-eosin, by Van Gieson's method, and with aqueous blue. The PAS reaction was carried out to detect glycogen, the tetrazolium reaction for total protein, the Feulgen reaction for DNA, and staining with Sudan III to reveal neutral lipids.

The mitotic index of the hepatocytes was determined by counting the number of mitoses per 7000 cells (ocular 10, objective 90).

Previous investigations using karyometric and cytophotometric analysis have shown that the nuclear volumes of the hepatocytes in the regenerating liver of rats correlate with their ploidy. On this basis, the ploidy of the hepatocytes was studied by karyometric analysis of their nuclei using a screw-adjusted ocular micrometer (ocular 15, objective 60). The nuclei of 500 normal mononuclear hepatocytes were measured in each specimen.

EXPERIMENTAL RESULTS

As a result of the eight partial hepatectomies, the quantity of liver tissue removed was 2.5 times greater in weight than the normal liver, and in some animals the total weight of resected liver tissue was three times the weight of the intact liver.

The relative weight of the liver 1.5 months after the eighth operation (3.63%) was not significantly different from its relative weight in intact animals of the same age (3.73%).

A study of histological preparations of the liver of the animals on the 2nd day after the eighth operation revealed polymorphism of the hepatocytes and their nuclei. The liver cells were infiltrated with large droplets of fat which contrasted with the tiny droplets after the first resection of the organ. The cytoplasm of the hepatocytes was strongly vacuolated. Cloudy-swelling degeneration in the cytoplasm of the hepatocytes was revealed by staining for protein. The glycogen content of the liver was sharply reduced. The mitotic index was 6.5 $^0/_{00}$ (Fig. 1).

A study of the volume of the hepatocyte nuclei showed that the number of diploid nuclei was almost doubled, while the number of tetraploid nuclei was reduced by almost half, and at the same time there was a considerable increase in the number of octaploid and 16-ploid nuclei.

The increase in the number of diploid hepatocyte nuclei was due to division of tetraploid nuclei (Fig. 2).

On the 7th day after the eighth operation the fatty infiltration of the hepatocytes had changed in character to the small droplet type. The liver glycogen concentration was higher than on the 2nd day. Mitotic division among the hepatocytes was considerably less frequent. The mitotic index was 0.3 $^0/_{00}$. On the 7th day there was a further increase in the number of diploid hepatocyte nuclei. The number of tetraploid nuclei was almost unchanged. The number of octaploid and 16-ploid nuclei showed a sharp decrease. The increase in the number of cells with small nuclei at this period can be explained by division of the octaploid and 16-ploid nuclei.

No fatty infiltration of the hepatocytes could be detected 1.5 months after the eighth operation. The glycogen concentration in the liver cells was almost normal. The cytoplasm was finely granular. Mitotic division among the hepatocytes was virtually absent.

The study of the volume of the hepatocyte nuclei showed a tendency toward a decrease in the number of diploid nuclei and an increase in the number of tetraploid and octaploid nuclei. The number of 16-ploid nuclei was unchanged. The relative proportions of the nuclear classes were close to those in the control animals of the same age.

It can be concluded from these results that the liver possesses high regenerative power after frequent resection of the organ. The absolute and relative weight of the liver after the eighth resection were not significantly different from those after the first resection or of the liver of intact animals of the same age. A histological investigation showed that the regenerating liver 1.5 months after the eighth operation is very little different from the liver regenerating after the first resection, or from the liver of intact animals. However, some special features of the liver regenerating after the eighth operation must be pointed out. Fatty infiltration and cloudy swelling were more marked after the eighth operation than at the same time after the first operation. Bands of hepatocytes with homogeneous cytoplasm were seen in the periportal zones. After the eighth operation the same pattern of change in the dynamics of the mitotically dividing hepatocytes was observed, but the maximal mitotic activity of the hepatocytes found on the 2nd day after the eighth operation was somewhat lower than at the same time after the first operation. The study of the volume of the hepatocyte nuclei after the eighth hepatectomy showed differences in the level of polyploidization of the hepatocytes compared with that after a single resection.

Whereas on the 2nd and 7th days after the first hepatectomy an increase in the number of hepatocyte nuclei with a higher level of ploidy (4 n-8 n) was accompanied by a sharp decrease in the number of diploid nuclei, and a tendency for restoration of the normal number of diploid nuclei, evidently by division of nuclei with higher ploidy, was not observed until 1.5 months later, on the 2nd and 7th days after the eighth operation there was a growing increase in the number of diploid nuclei (on account of division of tetraploid nuclei on the 2nd day, and of division of octaploid and 16-ploid nuclei on the 7th day). The ratio between the classes of nuclei 1.5 months after the eighth resection of the organ was similar to that in the control animals of the same age.

The results showed that, besides mitotic division of the hepatocytes, polyploidization of these cells plays an important role in reparative regeneration of the liver.

Polyploidization of the hepatocytes helps them to carry out their increased functions and may also act as a reserve of new hepatocytes as a result of division of the polyploid cells, as was seen particularly clearly after the eighth hepatectomy, which was performed on aging rats, in which the principal classes of hepatic nuclei are those with high ploidy.

After the eighth resection the appearance of new hepatocytes is due principally to division of polyploid cells.

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