HYPERTROPHY OF LIVER CELLS AND THEIR NUCLEI IN THE REGENERATING LIVER OF RATS OF DIFFERENT AGES

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Measurement of the size of mononuclear hepatocytes and the size of their nuclei in the regenerating liver of noninbred albino rats showed considerable hypertrophy of these elements. Hypertrophy of the nuclei is due mainly to the development of polyploidy.

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Restoration of the mass of the liver, observed in the first 2-3 weeks after resection of $\frac{2}{3}$ of the organ, is due to rapid proliferation [5, 7] and hypertrophy [4, 8, 10] of the liver cells. In the opinion of some workers, an increase in the volume of the cytoplasm and nucleus of the hepatocytes in the regenerating liver can be observed in the first 24-72 h after partial hepatectomy [3, 4, 8], while according to others, hypertrophy of the liver cells in the regenerating organ continues until the 12th [2] and 28th [9] days, while according to our observations it continued for two months [1].

In the present investigation changes in the size of the hepatocytes and of their nuclei during regeneration of the liver were studied in animals of different ages, because some aspects of this problem are incompletely understood.

EXPERIMENTAL METHOD

Experiments were carried out on 113 female rats of different ages. The animals were divided into three groups: group 1 included sexually immature rats (aged one month), group 2 consisted of young, sexually mature animals (aged 4-5 months), and group 3 of aging animals (aged 12 months). Two-thirds of the liver was removed from the experimental rats of all three groups by the method of Higgins and Anderson [5], while a mock operation was performed on the control animals. The experimental and control animals were sacrificed in groups of 5 or 6 at various times after the operation. The regenerating and intact liver was weighed and material fixed in Carnoy's fluid. By means of a screw-adjusted ocular micrometer, two perpendicular diameters of the mononuclear hepatocytes and one diameter of their nuclei were measured in histological sections 7 μ in thickness, stained with hematoxylin-eosin. Altogether 100 cells and nuclei were measured in the liver of each animal. The area of the mononuclear hepatocytes was calculated by multiplying the two measured diameters, and the area of the nucleus from the formula for the area of a circle πr^2. The ploidy of the hepatocytes in the regenerating and intact liver was judged from the distribution of nuclear volumes. In the liver of each animal 500 nuclei of the mononuclear hepatocytes were measured. The volume of the nuclei was calculated from the formula for the volume of a sphere $\frac{4}{3}\pi r^3$. Nuclei with a volume of 90 μ^3 were conventionally taken as diploid nuclei (2n), nuclei with a volume of 215 μ^3 as tetraploid (4n), nuclei with a volume of 421 μ^3 as octaploid (8n), and so on. The numerical results were subjected to statistical analysis by the Fisher-Student method.

EXPERIMENTAL RESULTS

The absolute weight of the regenerating liver was restored in the sexually immature rats (group 1) on the 3rd day after the operation and it continued to increase up to 5.5 g on the 30th day. The relative weight of the regenerating liver showed little change and amounted to 4.8-5.1% (Table 1).

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TABLE 1. Changes in Absolute and Relative Weight of Liver of Rats of Different Ages

Duration of experiment (in days)	Control			Experiment		
	weight of rat (in g)	weight of liver		weight of rat (in g)	weight of liver	
		absolute (in g; M ± m)	relative (in percent)		absolute (in g; M ± m)	relative (in percent)
		Group 1				
3	32	1,3±0,05	4,1	31	1,5±0,18	4,8
7	36	1,6±0,10	4,4	29	1,5±0,08	5,1
14	46	2,4±0,22	5,2	44	2,2±0,10	5,0
30	100	4,9±0,52	4,9	109	5,5±0,24	5,1
		Group 2				
3	192	6,9±0,20	3,6	175	5,1±0,07	2,9
5	205	7,0±0,25	3,4	192	5,8±0,21	3,0
14	225	7,8±0,18	3,4	210	8,0±0,20	3,8
30	236	8,5±0,27	3,6	225	8,2±0,24	3,6
		Group 3				
3	296	10,1±0,20	3,4	287	6,1±0,21	2,1
7	311	11,2±0,54	3,6	286	7,3±0,40	2,5
30	306	10,4±0,20	3,4	300	9,4±0,35	3,1

Note. At each period of investigation 5 or 6 rats were used.

The control sexually mature animals of group 2 grew normally, as shown by the gradual increase in body weight and weight of the liver. The relative weight of the liver during the period of observation changed only very slightly, and amounted to 3.4-3.6%. The absolute and relative weight of the regenerating liver in the rats of group 2 on the 3rd-5th day remained somewhat below the corresponding values for the control animals. Complete restoration of the weight of the regenerating liver of the young sexually mature rats was not observed until two weeks after the operation (Table 1).

In the control rats of group 3 the body weight and weight of the liver showed little change during the period of investigation. Restoration of the absolute and relative weight of the regenerating liver in the aging animals was not observed until one month after the operation (Table 1).

During the period of investigation (3-30 days) growth of the mononuclear hepatocytes and of their nuclei was observed in the control liver of the sexually immature rats: the area of the cell increased from 163 to 335 μ^2 (P = 0.001), and the area of the nucleus from 25 to 44 μ^2 (P = 0.001). The mononuclear hepatocytes and their nuclei were larger in area in the regenerating liver on the 3rd, 7th, and 14th days than in the liver of the control rats of the same age. Hypertrophy of the cells and nuclei was most clearly seen on the 7th day after the operation: at this time the area of the cell was 306 μ^2 and that of its nucleus 50 μ^2 (163 and 25 μ^2, respectively, in the control). The differences are not statistically significant. On the 14th day after operation, the hepatocytes and their nuclei increased in size, and after one month they were the same size as the controls: the mean area of the cell in the regenerating liver was 322 μ^2, and of its nucleus 44 μ^2, while in the control the figures were 335 and 45 μ^2, respectively (Fig. 1, I).

No significant changes in the dimensions of the mononuclear hepatocytes took place in the liver of the sexually mature rats (group 2) during the period of investigation: their area varied from 271 to 300 μ^2, while the area of their nuclei increased gradually from 35 to 45 μ^2 (after one month). At all periods of the investigation the dimensions of the hepatocytes and their nuclei in the regenerating liver exceeded the corresponding control values. Hypertrophy of the cells and nuclei was most obvious on the 14th day after hepatectomy: the area of the cell at this time was 370 μ^2 and the area of its nucleus 57 μ^2; the corresponding control values were 300 and 39 μ^2. The differences are statistically significant. After 30 days, although the hypertrophy of the cells and nuclei had diminished, they were still larger than the controls (Fig. 1, II).

During the experiment no changes were observed in the dimensions of the hepatocytes and their nuclei in the intact liver of aging rats (group 3). The mean area of the cell was 286-302 μ^2, and of its nucleus 42-45 μ^2. In the regenerating liver of the aging rats, definite hypertrophy of the cells and their nuclei

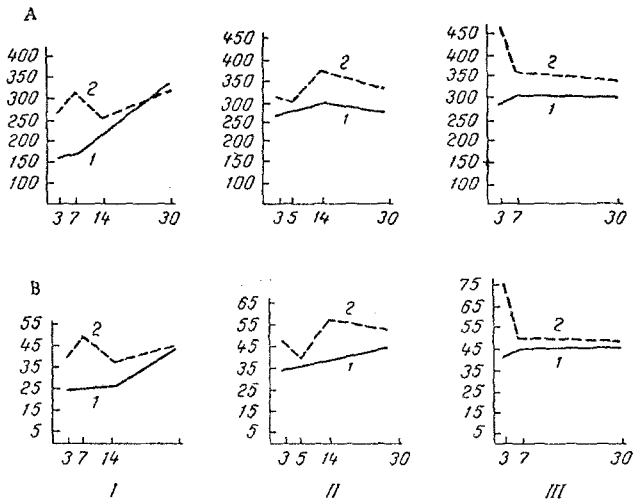

Fig. 1. Area of mononuclear hepatocytes and their nuclei. I) Sexually immature rats; II) young, sexually mature rats; III) aging rats. Abscissa: duration of experiment (in days); ordinate: area of cell (A) and nucleus (B) in μ^2; 1) control; 2) experiment.

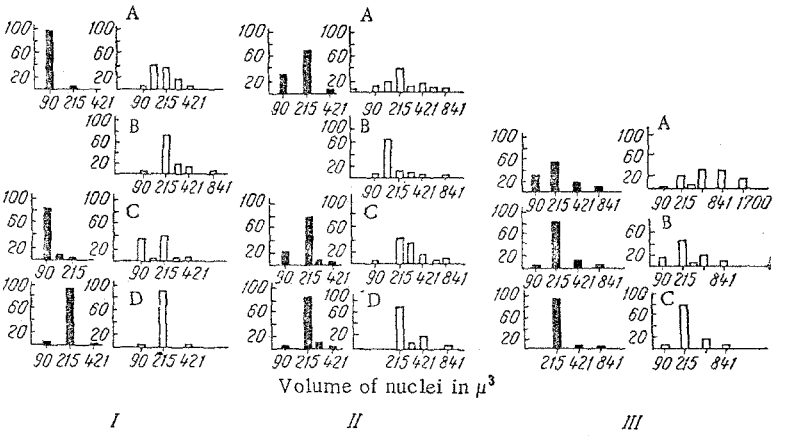

Fig. 2. Percentage distribution of nuclei in mononuclear hepatocytes by size. I) Sexually immature rats: A) 3 days, B) 7 days, C) 14 days, and D) 30 days after operation; II) young, sexually mature rats: A) 3 days, B) 5 days, C) 14 days, and D) 30 days after operation; III) aging rats: A) 3 days, B) 7 days, and C) 30 days after operation. Abscissa: volume of nuclei (in μ^3); ordinate: number of nuclei (in percent); shaded columns represent control, unshaded represent experiment.

developed on the 3rd day after resection: at this period the area of the hepatocyte was 482 μ^2 and of its nucleus 82 μ^2 (286 and 42 μ^2, respectively in the control; $P < 0.001$). Thereafter the dimensions of the cells and nuclei gradually decreased, approaching the control values (Fig. 1, III).

The results of calculation of the nuclear volumes of the liver cells and their relative percentages showed that in the intact liver of the group 1 rats a gradual increase in the number of tetraploid nuclei with a volume of 215 μ^2 and a decrease in the percentage of diploid nuclei with a volume of 90 μ^3 took place during the period of investigation. In the regenerating liver on the 3rd-14th day after operation the percentage of tetraploid nuclei increased, while after the 7th day, in addition, about 12% of octaploid nuclei appeared and the percentage of diploid nuclei fell. After 30 days, the nuclear composition of the regenerating liver of the sexually immature rats differed only slightly from that of the control animals, and in both cases the nuclei were tetraploid (Fig. 2, I).

The nuclear composition of the intact liver of the young, sexually mature rats (group 2) was mainly tetraploid. Diploid nuclei also were found, but their number fell gradually from 30 (on the 3rd day after the experiment began) to 3% (on the 30th day). The number of octaploid nuclei did not exceed 2-3%. In the regenerating liver of the sexually mature rats the number of octaploid nuclei increased at all times, and in addition, 16 n-ploid nuclei with a volume of 841 μ^3 appeared and accounted for 1-7% of the total; no such nuclei were found in the liver of the control rats. With respect to its nuclear composition, the regenerating liver of the sexually mature rats most closely resembled the liver of the control animals on the 30th day after resection, but no complete analogy was observed even at this time of the investigation, because up to 20% of octaploid nuclei could still be seen in the regenerating liver compared with only 2% in the control (Fig. 2, II).

At all times of the investigation, most nuclei in the liver of the control rats of group 3 were tetraploid, but from 3 to 12% of octaploid nuclei were found, together with up to 32% of diploid nuclei, but only in the first days after the beginning of the experiment (3 days). In the regenerating liver of the group 3 rats, the percentage of octaploid and 16 n-ploid nuclei was increased on the 3rd-7th days after the operation, and in addition, on the 3rd day after resection up to 9% of very large nuclei with a volume of about 1700 μ^3 was found; no such nuclei were present in the control. The distribution of the nuclear composition in the regenerating liver of the aging rats came close to the control value on the 30th day after operation, although it was not completely identical (Fig. 2, III).

Hence, hypertrophy of the mononuclear hepatocytes in the regenerating liver of rats of all three age groups was characterized by the appearance of numerous (more than normally) large nuclei. In addition, nuclei occupying an intermediate position by volume between the main nuclear classes were found more commonly in the regenerating liver of the rats of groups 1, 2, and 3 at all times than in the liver of the control animals. It is difficult to say whether the hypertrophy of the nuclei and the presence of intermediate classes of nuclei in the regenerating liver are connected with polyploidization or whether, in some cases, they result purely from functional changes in the resected organ, unaccompanied by changes in DNA content. Appropriate cytophotometric investigations are necessary before this problem can be finally solved.

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