THE SIZE OF UNINUCLEATE LIVER CELLS AND THE GLYCOGEN CONTENT OF NORMAL AND REGENERATING RAT LIVER

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The rapid recovery of liver weight observed during the 2-3 weeks after removal of two-thirds of it is associated with the processes of growth and differentiation of its component elements [2, 14, 16, 20]. Shortly after the damage, the number and size of the hepatic cells increase rapidly. According to many authors, the increase in the volume of the cytoplasm and nucleus of these cells may be observed in the first 24-72 hours after the operation [5, 8, 9, 13, 18]; others hold that the hypertrophy takes place within 12 days [7] or 28 days [19].

In the early regenerative periods, there are also many biochemical changes. In the first day after the operation, glycogen disappears almost entirely from the cytoplasm of the hepatic cells, and the original quantity is not restored until the third day [6, 11, 17].

At the present time, the condition of the hepatic tissue appears to have been quite well studied at the early stages of regeneration, but insufficient work has been done at more remote dates. We know only of a single report, that of Forti [10], which deals with this problem. He worked on rats, and found that 1-2 years after the removal of large amounts of liver, the remaining part, although it had increased in size, was nevertheless almost always smaller than the liver of the control animals. Also, he found that the operated liver was less active.

Because so little work has been done on liver regeneration long after removal of a portion, it seemed worthwhile to make a cytological study of this problem. It is of considerable theoretical and practical importance to determine to what extent the recovery of organs after trauma is complete and permanent, and whether the regenerated organ begins to differ from the normal after a certain time.

METHOD

By the method of Higgins and Anderson [15] female rats weighing 130-150 g, under ether anesthesia the left lateral and central lobes of the liver, comprizing about 70% of the organ, were removed. The experimental and control animals were killed 2, 5, 11, and 17 months after the operation. The liver was weighed and fixed in 80% spirit or in Shabadash, and embedded in the usual way in paraffin. Serial sections were stained in Gomori's alkaline phosphatase [12], and the measurements of the mononuclear hepatic cells and their nuclei were measured with an ocular micrometer. The area of the cells was calculated in two ways: 1) by multiplying two perpendicular diameters, i.e., the shape of the cell was arbitrarily assumed to be rectangular; 2) by drawing the cell with a drawing apparatus, cutting out, and weighing. The figures obtained by these two methods were very close, and the difference between them was not statistically significant. The area of the nuclei was calculated from the formula for the area of a circle. In the liver of each animal 100 uninucleate hepatic cells and their nuclei were measured, and the nucleus cytoplasm ratio calculated. The results were treated statistically by the Fisher-Student method. The method of Hotchkiss was used to reveal glycogen [19].

RESULTS

In the control (unoperated) rats, the increase in the mass of the liver occurred discontinuously; by the 17th month it weighed 11.8 g, whereas the relative weight of the liver during the whole period of investigation remained more or less constant at 3.4-3.8% of the body weight.

For 5 months after the operation, the absolute weight of the regenerating liver increased, then it became stabilized, and by the 17th month it was less than the weight of the livers of the control group of the same age. The

relative weight of the regenerating liver fell at the 17th month, when it was 2.7% of the body weight, i.e., 1½ times less than the previous regenerating weight at two months, when it was 4.1%; the relative weight was also less than the 3.7% value of the control animals (Table 1).

TABLE 1. Change in the Absolute and Relative Weight of Unoperated and Regenerating Livers

Duration of the expt. (months)	Group of animals	Number of animals	Weight of animals (in g)	Weight of liver	
				Absolute (in g)	Relative (in %)
2	Experimental	12	190	7.690	4.1
	Control	7	220	7.550	3.4
5	Experimental	13	237	7.960	3.4
	Control	6	230	8.600	3.8
17	Experimental	14	277	7.600	2.7
	Control	8	322	11.762	3.7

By measuring the uninucleate hepatic cells and their nuclei we found that the growth of their cytoplasm and nucleus in the nonoperated animals continued for 5 months, and by this time the area of the cells and of their nuclei were 123.3 and 133.3%. The value of 100% referred to the size of the hepatic cell and nucleus from the liver of an unoperated rat two months after the beginning of the experiment. Next we observed the reverse phenomenon: there was a gradual slow reduction in the size of the hepatic cells and of their nuclei. By the 17th month the area of the cells was only 113.2%, and that of the nuclei 87.9% (Figs. 1, 2; Table 2).

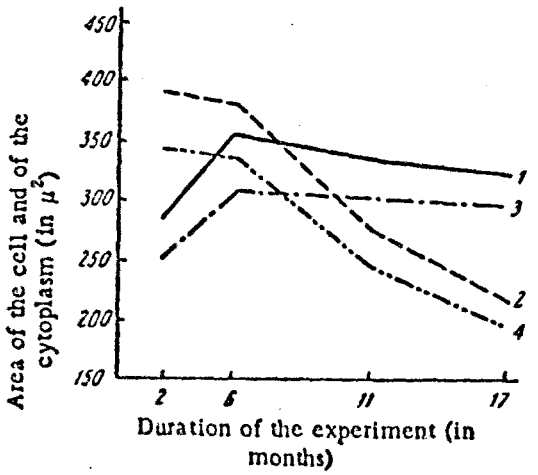

Fig. 1. Change in the area of the uninucleate hepatic cells and their cytoplasm in normal and regenerating rat livers at various times. 1) Area of control cells; 2) area of cells in regenerating liver; 3) area of cytoplasm in the control; 4) area of cytoplasm in cells in the regenerating liver.

From the 11th month onwards, there was a small reduction of the nucleus/cytoplasm ratio in the cells of the control rats, and the change was more marked after 17 months. The effect was brought about by the more rapid decrease of the nucleus than of the cytoplasm (see Table 2).

In the regenerating liver, the hypertrophy of the cell and nucleus which occurred during the early stages of regeneration (usually on the 1-2nd day after the operation) was maintained for five months; the area of the cell is given by the figure 132.7%, that of the nucleus by 133.3%. Subsequently the dimensions of the cytoplasm and of the nucleus began to fall, and this effect could be observed for 17 months when the cell and nuclear areas fell to 76 and 69.6% respectively. Here the process of reduction of the area of nucleus and cytoplasm of the regenerating liver was more rapid than in the intact liver (see Figs. 1, 2, and Table 2).

The nucleus/cytoplasm ratio of the regenerating liver remained constant for a long time, until the 11th month, and did not differ from the value of the hepatic cells of the two-month-old control rats, and it was not until 17 months after the operation that there was some reduction (see Table 2).

The undamaged liver was rich in glycogen. It filled the whole of the cytoplasm of the hepatic cell in the form of large clumps, and was distributed unevenly between the different liver cells; cells lying at the periphery of a lobe, and especially those surrounding the central vein were very rich in glycogen; hepatic cells lying centrally in a lobe contained a moderate number of clumps.

As the animal aged, the amount of glycogen in the intact liver fell; isolated hepatic cells or groups of them with no glycogen in the cytoplasm appeared, though the glycogen content remained high in the cells surrounding the central vein.

TABLE 2. Area of Uninucleate Liver Cells, of their Nuclei and Cytoplasm, and the Nucleus/Cytoplasm Ratio in Rat Liver at Various Times

Duration of the experiment (in months)	Group of animals	Area						Ratio nucleus / cytoplasm	Significance (P) of the difference between expt. and control animals	
		of the cell	Of the cytoplasm	Of the nucleus	Of the cell	Of the cytoplasm	Of the nucleus		For the area of the cell	For the area of the nucleus
		in microns			in % of control					
2	Control	287	254	33	100	100	100	1:8		
	Experiment	392	346	46	136,5	136,2	139,3	1:8	0,001	0,C00
5	Control	354	310	44	123,3	122,0	133,3	1:7		
	Experiment	381	338	43	132,7	133,0	133,3	1:8	0,138	1,000
11	Control	338	303	35	117,9	115,3	106,0	1:9		
	Experiment	278	246	32	96,9	96,9	96,9	1:8	0,117	0,08
17	Control	325	296	29	113,2	116,5	87,9	1:10		
	Experiment	218	195	23	76,0	76,7	69,6	1:9	0,000	0,C2

A study of the distribution of glycogen in the regenerating rat liver two months after operation revealed no essential deviations from normal. After five months, in the cells of the regenerating liver the amount of glycogen remained high, but its distribution in the cytoplasm had changed. It took the form of larger clumps than were observed in the cytoplasm of the hepatic cells of non-operated rats of the same age. From 11 months onwards its amount became reduced, in all the lobes large groups of cells almost free from glycogen could be found in the center of the lobe, although quite close to them there were cells whose cytoplasm contained a large amount of strongly stained glycogen clumps. Cells lying in the periphery of the lobe were rich and those round the central vein richer in glycogen, although here too there were some cells which contained none.

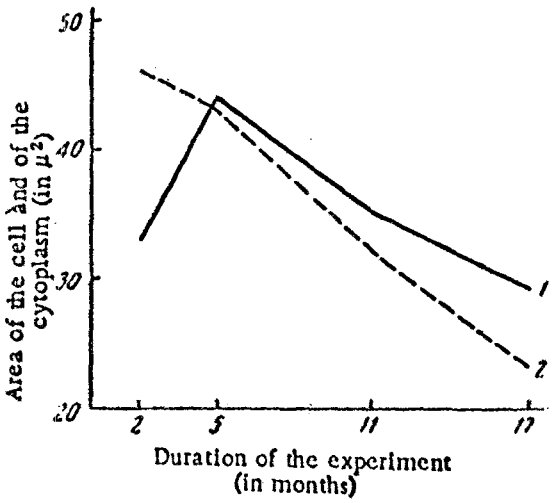

Fig. 2. Change in the area of the nuclei and of the uninucleate hepatic cells in (1) normal and (2) regenerating rat liver, at various times.

The most definite picture of the sharp fall in the amount of glycogen in the hepatic cells was observed in the regenerating liver of old rats, i.e., 17 months after removal of 70% of the hepatic tissue.

At the present time it is difficult to say whether the loss of glycogen from the isolated or grouped hepatic cells is related to their functional activity, or to some other cause.

Histological study of the regenerating liver revealed a number of morphological changes. In addition to the unchanged hepatic cells there were quite a large number of cells with a vacuolized cytoplasm, and in some cases the nuclei were disrupted or pyknotic.

Staining for reticular fibers showed that in some parts of the liver they had increased and had become noticeably thickened. In the control livers, by the 17th month there were also a number of age changes, and a more profuse development of the reticular framework; however, the outgrowth of the reticular fibers in the liver of operated rats was always much greater [4].

Therefore, during the aging process of the control rats, the following age changes were present: the area of the cytoplasm and nucleus of the uninucleate hepatic cells was reduced, as was also the amount of glycogen and the outgrowth of reticular fibers.

The morphological and cytochemical changes in the regenerating rat liver which occurred at the late stages, after partial liver resection, are of the same nature but more marked than in the liver of control animals of the same age.

The appearance of these changes in the operated liver is probably due to the development in it of dystrophic processes. The latter develop very slowly, and appear only in old rats.

Further investigations will be made to determine what factors influence the condition of the regenerating liver and how the dystrophy may be prevented.

SUMMARY

Measurements of uninucleate hepatic cells and of their nuclei were made in intact (control) female rats and in the regenerating liver 2 and 5 months after the excision of 70% of hepatic tissue. The initial weight of these animals ranged from 130 to 150 g. There was a considerable hypertrophy of the uninucleate cells and of their nuclei; these elements were also enlarged in the liver of the control rats, though to a lesser degree.

At 11 and 17 months, age-induced changes in the non-operated livers of control rats occurred: in the uninucleate hepatic cells there was a reduction of the area of the cytoplasm and of the nucleus, and a reduction also in the amount of glycogen while there was less proliferation of reticular fibers.

In the operated livers of old rats, on account of dystrophic processes the changes were of the same nature but more pronounced.

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