BINUCLEAR CELLS AND PROLIFERATIVE PROCESSES IN THE INTACT AND REGENERATIVE LIVER OF RATS

Z. A. Ryabinina

Laboratory of Growth and Development (Head, Doctor of Biological Sciences L. D. Liozner), Institute of Experimental Biology (Director, Professor I. N. Maiskii) of the AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 55, No. 3, pp. 105-109, March, 1963
Original article submitted February 20, 1962

A fair degree of unanimity has been reached over the cytochemical changes and intensity of proliferation of cells after partial hepatectomy [1, 3, 5, 6, 7, 12, 13], yet conflicting estimates have been made of the number, situation, and formation of binuclear cells in the normal and regenerating liver. Some writers [4] consider that the percentage of binuclear cells is greatest in the liver of young (45 g) rats, and that with age their number falls to a certain level, which is then maintained throughout life. Other writers [9,10] suggest that with age the number of binuclear cells in the animal liver increases. The number of these cells in the regenerating liver decreases during intensive proliferation [4, 6, 14].

Differences of opinion also exist concerning the origin of the binuclear cells in the liver. It has been suggested that they are formed as a result of amitosis [8, 9], or mitosis not terminating in division of the cell body [6, 15].

There is little information concerning the late results of partial hepatectomy. In our earlier reports we described changes taking place in mononuclear liver cells, reticulin fibers, and the glycogen of the regenerating liver of rats 17 months after partial hepatectomy [2, 3]. In the present research we determined the number and size of the binuclear cells and counted the number of dividing cells in normal and regenerating liver from 2 to 17 months after partial hepatectomy.

EXPERIMENTAL METHOD

Experiments were carried out on 39 female rats initially weighing 135-150 g. In 21 animals two-thirds of the liver was resected (central and left lateral lobes) under ether anesthesia by the method of Higgins and Anderson [7], a ligature first having been applied to the base of the resected lobes. The remaining 18 rats, not undergoing the operation, served as controls. After the operation, all the animals received white bread and water ad lib. for a period of 1-3 days, after which they were transferred to a pellet diet. The animals were sacrificed 2, 5, and 17 months after the beginning of the experiment. Pieces of the liver were fixed in Carnoy's fluid. The material was embedded in paraffin wax in the ordinary way. Sections were cut to a thickness of 9 μ. In sections stained by Feulgen's method and with light green, from both the intact and regenerating liver, the numbers of liver cells with nuclei dividing by mitosis, amitosis, and fragmentation were counted, and the number of binuclear cells in a total of 4500-6500 cells was determined. We assumed, by amitosis, a form of cell division in which the nucleus was shaped like a dumbbell. A separate group included dividing liver cells with nuclei in various stages of fragmentation, according to the classification suggested by Nagata [8]. Counting was carried out under an oil immersion objective ($\times$60) and ocular ($\times$10) of a binocular microscope. The field of vision of the microscope was bounded by a diaphragm, the aperture size of which was 0.7 $\times$ 0.7 mm. The results of the counting of the binuclear cells were manipulated by Pfuhl's formula [11].

The dimensions of the binuclear cells and of their nuclei were obtained, measuring the large and small diameters of the cell and the diameter of one of the nuclei. In the liver of each rat 100 cells and 100 nuclei were measured in this way. The area of the nucleus was calculated from the formula for the area of a circle (πr^2), and the area of the cell was calculated as the product of its two diameters. The numerical results thus obtained were analyzed statistically by the Fisher-Student formula.

EXPERIMENTAL RESULTS

The results of the counting of the dividing and binuclear cells in the intact and regenerating liver are shown in Table 1. Mitoses and amitoses were found extremely rarely in the intact liver of the control rats 2 and 5 months after the beginning of the experiment: 1 mitosis and 0.2-4 mitoses per 10,000 cells. Seventeen months after the beginning of the experiment, examination of 4500 cells failed to reveal a single liver cell the nucleus of which was dividing by mitosis or amitosis.

In the liver of the intact rats the number of fragmenting cells increased gradually with age from 10‰ 2 months after the beginning of the experiment to 31 ‰ after 17 months.

In the regenerating liver of the rats 2 and 17 months after partial hepatectomy, mitoses and amitoses were extremely rare, as in the intact organ: 1 mitosis and 1-2 amitoses per 10,000 cells. Mitoses and amitoses were more common in the regenerating liver 5 months after the operation: 7 mitoses and 11 amitoses per 10,000 cells. Throughout the period of the investigation from 2 to 17 months, the number of fragmenting liver cells underwent little change: 21-34 per 10,000 cells.

The distribution of binuclear cells within the lobules was irregular; they were commoner around the central vein, and their nuclei were smaller here. As the animals grew older (2-17 months), the number of binuclear cells in the intact liver rose gradually from 5.9 to 14.1% ($P < 0.001$). In the later stages of the investigation, however, there were considerable individual variations in the number of binuclear liver cells. For this reason the counts of these cells after 17 months were not always in agreement. For instance, in 4 of the 6 intact animals, the number of binuclear liver cells was unchanged by comparison with the earlier periods of the investigation (5 months), and amounted on the average to 11%, whereas in the other two rats their number rose sharply (up to 23.9%).

TABLE 1. Average Number of Dividing, Binuclear, and Mononuclear Liver Cells

Duration of experiment (in months)	No. of rats	Number of		Number of cells						Total no. of cells counted
		mitoses	amitoses	fragmenting	binuclear	with 3 nuclei	with 4 nuclei	binuclear	binuclear, by Pfuhl's formula	
Regenerating liver										
2	7	0.43	–	14.7	264	2.0	–	6.1	12.9	4284
5	7	3.10	5.10	15.4	629	6.4	0.57	12.9	20.7	4794
17	7	0.60	1.00	11.2	355	1.9	–	6.9	9.6	5189
Intact liver										
2	6	0.50	0.17	10.5	235	2.5	–	3.6	5.9	6584
5	6	0.70	2.10	29.9	317	0.8	0.16	6.6	11.3	4751
17	6	–	–	31.5	428	3.7	0.80	9.3	14.1	4564

TABLE 2. Dimensions of Binuclear Liver Cells and of their Nuclei, and Ratio between Area of Nucleus and Area of Cytoplasm

Duration of experiment (in months)	No. of rats	Diameter (in μ)			Area (in μ^2)			Ratio between area of nucleus and area of cytoplasm
		of cell	of cell body	of nucleus	of cell	of cytoplasm	of nucleus	
Regenerating liver								
2	7	27.6	20.8	8.8	574.1	513.3	60.8	1:9
5	7	29.8	20.8	7.8	619.8	573.2	46.6	1:13
17	7	22.0	17.2	6.1	378.4	349.2	29.2	1:13
Intact liver								
2	6	24.1	16.4	7.5	395.2	351.0	44.2	1:9
5	6	25.8	18.6	7.5	479.9	435.7	44.2	1:11
17	6	26.4	19.4	7.4	512.2	469.2	43.0	1:12

The number of binuclear cells in the regenerating liver 2 and 5 months after partial hepatectomy was twice their number in the intact organ ($P < 0.001$), and it was not until 17 months after the operation that they became fewer in number than in the liver of the control animals of the same age: 9.6% in the regenerating liver, 14.1% in the control rats ($P = 0.07$).

At all periods of the investigation the number of multinuclear cells in the intact and regenerating liver of the animals was small, ranging from 0 to 8 cells per 10,000 with 3 and 4 nuclei. This figure was slightly exceeded only in the case of the regenerating liver of rats sacrificed 5 months after operation (13 per 10,000 cells).

Throughout the period of the investigation (2, 5, and 17 months after operation) the size of the nuclei of the intact liver cells of the control animals showed hardly any change (see Table 2), and their average area was 43.0-44.2 μ^2. On the other hand, the area occupied by the cytoplasm gradually increased with age, so that the ratio between area of nucleus and area of cytoplasm fell.

From 2 to 5 months after hepatectomy, hypertrophy of the nucleus and cytoplasm of the binuclear cells of the regenerating liver took place by comparison with the liver of control rats of the same age, and hypertrophy of the nucleus was more marked 2 months after operation, while hypertrophy of the cytoplasm was more marked after 5 months ($P = 0.009$). After 17 months the areas of the nucleus and cytoplasm of the binuclear cells fell to less than two thirds of the areas of the nucleus and cytoplasm of the binuclear cells in the liver of the control animals ($P = 0.018$ for the cell and $P = 0.008$ for the nucleus).

If the changes in the area of the nucleus and cytoplasm of the binuclear liver cells in the regenerating liver of the rats after 2, 5, and 17 months are compared, it will be seen that the nuclei of these cells gradually became smaller: from 60.8 μ^2 2 months after operation to 29.2 μ^2 after 17 months ($P = 0.014$), while the area occupied by the cytoplasm was not reduced until 5 months after hepatectomy ($P = 0.001$).

The ratio between nucleus and cytoplasm in the regenerating liver of the rats fell during the period from 2 to 5 months, and then became stabilized; it remained slightly lower than in the liver of the control rats of the same age.

Hence, the changes in the number of binuclear liver cells and in the dimensions of these cells and of their nuclei in the intact and regenerating liver of the rats in the course of aging were different in character. The number of binuclear cells in the liver of the control rats increased with age, i.e., as the animal grew older their importance increased, while in the regenerating liver this process took place only between the 2nd and 5th months.

The changes in the size of the nuclei and cytoplasm of the binuclear cells also differed in the intact and regenerating liver. In the intact liver the size of the binuclear cells gradually increased with age (2-17 months), whereas the size of the nucleus was unchanged. An increase in the size of the binuclear cells in the regenerating liver was observed only until the 5th month after hepatectomy, after which the cytoplasm of the cells shrank; the nucleus gradually decreased in size with effect from 2 months after the operation.

The changes developing in the regenerating liver of rats 17 months after operation [2, 3] also affected the binuclear cells, causing some of them to atrophy, as shown by the considerable decrease in the size of these cells and their nuclei.

SUMMARY

In the nonoperated liver of control female rats 2 to 17 months after the beginning of the experiment there was a gradual increase of the number of binuclear cells and their sizes; the size of the nucleus remained unchanged. 2-5 months after the removal of two thirds of the liver the percentage of binuclear cells was higher than in the liver of the control animals of the same age; moreover hypertrophy of binuclear cells and their nuclei was observed at these periods. At the late postoperative dates (in 17 months) the percentage of binuclear hepatic cells decreased; their size and the size of their nuclei also became smaller.

LITERATURE CITED

1. Z. A. Ryabinina. Byull. éksper. biol., 3, 105 (1960).
2. Z. A. Ryabinina. Byull. éksper. biol., 4, 114 (1961).
3. Z. A. Ryabinina. Byull. éksper. biol., 2, 101 (1962).
4. A. M. Brues and B. B. Marble, J. exp. Med., 1937, v. 65, p. 15.
5. N. L. R. Bucher and A. D. Glinos, Cancer Res., 1950, v. 10, p. 324.
6. E. Grundmann and G. Bach, Beitr. path. Anat., 1960, Bd. 123, S. 144.

7. G. M. Higgins and R. M. Anderson, Arch. Path., 1931, v. 12, p. 186.
8. T. Nagata, Med. J. Shinshu Univ., 1959, v. 4, p. 179.
9. T. Nagata and S. Momozé, Acta anat. nippon., 1959, v. 34, p. 187.
10. T. Nagata, K. Shimamura, T. Kondo et al., Med. J. Shinshu Univ., 1960, v. 5, p. 153.
11. W. Pfuhl, Z. mikr.-anat. Forsch., 1930, Bd. 22, S. 557.
12. J. M. Price and A. K. Laird, Cancer Res., 1950, v. 10, p. 650.
13. R. E. Stowell, Arch. Path., 1948, v. 46, p. 164.
14. N. M. Sulkin, Am. J. Anat., 1943, v. 73, p. 107.
15. J. W. Wilson and E. H. Leduc, Am. J. Anat., 1948, v. 82, p. 353.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
