CHANGES IN PLOIDY OF HEPATOCYTE NUCLEI IN ANIMALS WITH TRANSPLANTABLE TUMORS DURING REGENERATION OF THE LIVER AND X-RAY IRRADIATION

A. I. Sizikov

UDC 616-006-092.9-06:616.36-001-07:
616.36-003.93-02:615.849.114

KEY WORDS: hepatocytes; regeneration of the liver; polyploidization; irradiation.

According to data in the literature, the leading role in the mechanism of post-traumatic regeneration of the liver in sexually mature rats is played by processes of cell proliferation, compared with hypertrophy of the hepatocytes and polyploidy of their nuclei [1, 2, 5]. Meanwhile, in the pathologically changed liver processes of polyploidy and hypertrophy are the main mechanism for restoration of liver function [3, 4, 8, 9]. In the liver of rats with primary tumors, a series of changes has been found, characterized by focal necrosis of the parenchyma, a sharp rise in the number of Kupffer cells, hypertrophy of the hepatocytes and enlargement of their nuclei — making their mark on the course of regenerative processes in the parenchyma after partial resection of the liver [7]. One of the features which distinguishes the course of regeneration in the liver in unirradiated and irradiated animals with tumors is the low level of cell proliferation together with marked enlargment of the hepatocyte nuclei [6]. It was accordingly decided to study changes in ploidy of hepatocyte nuclei in the regenerating liver in rats with transplantable tumors receiving x-ray irradiation, and the investigation described below was devoted to this task.

EXPERIMENTAL METHOD

Altogether 190 young sexually mature rats of both sexes weighing 110-120 g were used in 19 series of experiments. The following strains were used as primary tumors: sarcoma M1, sarcoma 45, and Guérin's carcinoma. Partial hepatectomy was performed by the method of Higgins and Anderson under general ether anesthesia 9 days after transplantation of tumors into the animals. Irradiation was given the day before the operation. The experimental conditions (for the various series) were as follows: 1) animals without tumors (intact); 2) animals with sarcoma M1 (30 days of growth of the tumor); 3) animals with sarcoma 45 (30 days of growth of the tumor); 4) animals without tumor plus partial hepatectomy (30 days after the operation); 5) animals with sarcoma M1 plus partial hepatectomy (16 days after the operation); 6) animals without tumor plus total irradiation in a dose of 360 R (30 days after irradiation); 7) animals with sarcoma 45 plus total irradiation in a dose of 360 R (30 days after irradiation); 8) animals without tumors plus total irradiation in a dose of 625 R (30 days after irradiation); 9) animals with sarcoma 45 plus total irradiation in a dose of 625 R (16 days after irradiation); 10) animals without tumors plus total irradiation in a dose of 360 R plus partial hepatectomy (30 days after operation); 11) animals with Guérin's carcinoma plus total irradiation in a dose of 360 R plus partial hepatectomy (30 days after the operation); 12) animals without tumors plus total irradiation in a dose of 540 R (24 days after irradiation); 13) animals with sarcoma 45 plus total irradiation in a dose of 540 R (24 days after irradiation); 14) animals without tumors plus total irradiation with a dose of 540 R plus partial hepatectomy (16 days after operation); 15) animals with sarcoma 45 plus total irradiation in a dose of 540 R plus partial hepatectomy (16 days after operation); 16) animals without tumors plus local irradiation in a dose of 540 R (30 days after irradiation); 17) animals with sarcoma 45 plus local irradiation in a dose of 540 R (30 days after irradiation); 18) animals without tumors plus local irradiation in a dose of 540 R plus partial hepatectomy (30 days after operation); 19) animals with sarcoma 45 plus local irradiation in a dose of 540 R plus partial hepatectomy (30 days after the operation).

Kirghiz Research Institute of Oncology and Radiology, Frunze. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 473-476, October, 1981. Original article submitted January 28, 1981.

0007-4888/81/9210-1415$07.50

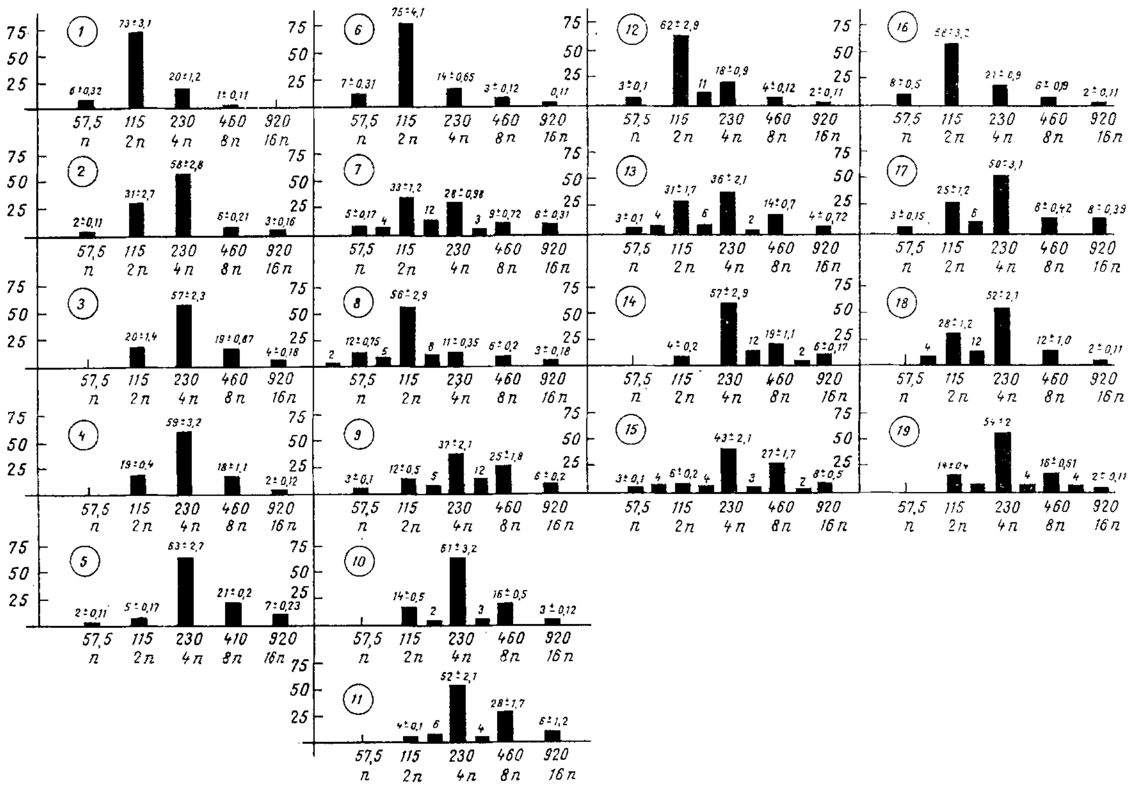

Fig. 1. Nuclear composition of hepatocytes in a series of experiments (explanation in text). Abscissa, ploidy of nuclei and volume (in μ^3); ordinate, number of nuclei (in %).

At the end of the experiment the animals were decapitated and pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. In histological sections 100 nuclei were measured with an ocular micrometer in each case (at least 1000 nuclei per series). The volume of the nuclei was calculated by the equation for the volume of a sphere. The numerical data thus obtained were divided into classes [1, 2] in which the volume of the hepatocyte nuclei, namely 57.5, 115, 230, 460, and 920 μ^3, corresponded, to the following ploidy classes: n, 2n, 4n, 8n, 16n, and so on. The Fisher–Student test was used for statistical analysis.

EXPERIMENTAL RESULTS

Four classes of hepatocyte nuclei were present in the liver of intact rats (Fig. 1:1). The modal class consisted of diploid nuclei, there were fewer tetraploid nuclei (20%), and very few octoploid (1%). Nuclei classed as haploid by their size counted for 6%. The modal classes in animals 30 days after transplantation of sarcoma M1 and sarcoma 45 consisted of tetraploid nuclei (Fig. 1: 2, 3). The increase in class of tetraploid and decrease in the class of diploid nuclei in animals with tumors compared with the control were statistically significant. Nuclei corresponding to 16 n appeared in the liver of these animals, whereas the 8n class in rats with sarcoma 45 accounted for 19%, i.e., the presence of a growing tumor in the rats led to an increase in the ploidy of the hepatocyte nuclei.

Resection of two thirds of the liver in rats without tumors caused an increase in ploidy of the hepatocyte nuclei; the histogram was almost identical with that of rats with tumors not undergoing the operation (Fig. 1:4). In rats with sarcoma M1 partial hepatectomy led to even more marked polyploidy of the nuclei (Fig. 1: 5): The class of diploid nuclei fell to 6% whereas tetraploid and octoploid nuclei rose to 63 and 21%, respectively (difference statistically significant for 8n).

Total x-ray irradiation of rats without tumors in a dose of 360 R caused hardly any change in ploidy of the hepatocyte nuclei (Fig. 1: 6). Meanwhile, similar irradiation of rats with sarcoma 45 led to certain changes in the nuclear composition of the hepatocytes (Fig. 1: 7): The diploid and tetraploid classes became almost equal in value, the 16 n class was increased, and intermediate classes appeared, one of which, occupying an intermediate position between diploid and tetraploid, accounted for 12% of all nuclei. On the whole, the histogram showed a very small shift toward an increase in ploidy of the nuclei.

Total x-ray irradiation of rats without tumors in a dose of 625 R led to more marked changes than were found after irradiation in a dose of 360 R (Fig. 1: 8). This was shown by the appearance of nearly all the intermediate classes and of very small nuclei, less than haploid in volume, but the modal class still remained diploid. After similar irradiation of rats with sarcoma 45 there was an increase in ploidy of the hepatocyte nuclei on account of an increase in the class of octoploid nuclei to 14% (Fig. 1: 9). The difference from the group of rats irradiated in a dose of 360 R was statistically significant for the 2n, 4n, and 8n classes.

Total x-ray irradiation in a dose of 360 R had almost no effect on the nuclear composition of the regenerating liver in rats without tumors (Fig. 1: 10), whereas in rats with Guérin's carcinoma it led to an increase in the class of octoploid nuclei to 28% and to the almost complete disappearance of the class of diploid nuclei (Fig. 1: 11); the intermediate classes also were increased (the difference is statistically significant for the 4n, 8n, and 16 n classes).

After total irradiation in a dose of 540 R, fairly large intermediate nuclear classes also appeared in the liver of rats without tumors (Fig. 1: 12), but in animals with sarcoma 45 the modal class became tetraploid, and the class of octoploid nuclei increased to 25% (Fig. 1: 13), i.e., there was a shift toward an increase in ploidy of the hepatocyte nuclei (the difference compared with the group of rats irradiated in a dose of 360 R was statistically significant for the 4n and 8n classes). In the regenerating liver of rats without tumors, under these same conditions, there was a decrease in the class of haploid nuclei and an increase in the intermediate 8n and 16n classes compared with the group of rats irradiated in a dose of 360 R (Fig. 1: 14). In rats with sarcoma 45 under these same conditions changes in the regenerating liver were approximately the same as after irradiation in a dose of 360 R, but all the intermediate nuclear classes appeared (Fig. 1: 15).

Local irradiation of the liver in rats without tumors and with sarcoma 45 caused a very slight change in the nuclear composition of the hepatocytes (Fig. 1: 16, 17). In the regenerating liver of rats without tumors and with sarcoma 45, irradiation in this dose led to the appearance of intermediate nuclear classes of hepatocytes with preservation of the same modal classes as in animals without irradiation (Fig. 1: 18, 19).

The presence of a growing primary tumor in animals thus leads to changes in the nuclear composition of the hepatocytes, namely an increase in their ploidy. X-ray irradiation induces an increase in ploidy of the nuclear composition of the hepatocytes, and the higher the dose the greater the increase. In rats with tumors under these conditions polyploidy of the hepatocyte nuclei was more marked than in animals without tumors. A sharp increase in ploidy of hepatocyte nuclei takes place in the regenerating liver in animals with tumors, whether irradiated (especially so in this case) or not.

LITERATURE CITED

1. Z. A. Ryabinina, Tr. Mosk. Ova. Ispyt. Pire., 11, 56 (1964).
2. Z. A. Ryabinina, Byull. Éksp. Biol. Med., No. 3, 102 (1964).
3. D. S. Sarkisov, Regeneration and Its Clinical Importance [in Russian], Moscow (1970).
4. D. S. Sarkisov, L. D. Krymskii, and K. V. Botsmanov, Byull. Éksp. Biol. Med., No. 3, 103 (1969).
5. V. F. Sidorova, Z. A. Ryabinina, and E. M. Leikina, Regeneration of the Liver in Mammals [in Russian], Leningrad (1966).
6. A. I. Sizikov, Byull. Éksp. Biol. Med., No. 4, 90 (1971).
7. A. I. Sizikov, Vopr. Onkol., No. 2, 47 (1979).
8. B. P. Solopaev, "Reparative regeneration of the normal and pathologically changed mammalian liver," Author's Abstract of Doctoral Dissertation, Moscow (1962).
9. B. P. Solopaev, I. A. Kovalenko, V. V. Sadovnikova, et al., in: Mechanisms of Regeneration and Cell Division [in Russian], Moscow (1971), p. 163.