EFFECT OF ABSENCE OF POLYPLOID HEPATOCYTES ON REGENERATION IN THE LIVER

T. K. Dubovaya, A. A. Kushch, and V. Ya. Brodskii

UDC 612.6.03:612.35

An autoradiographic study with [^{3}H]thymidine showed that the hepatocytes of young sexually mature guinea pigs commence the phase of DNA synthesis 25 h after partial hepatectomy. Peaks of the number of labeled nuclei were found 30, 45, and 60 h after the operation. Two waves of mitoses were found by counting mitotic figures in squash preparations: 40 and 55 h after hepatectomy. A cytophotometric study of the DNA content showed that practically all the mononuclear and binuclear hepatocytes contained diploid nuclei 3 and 5 days after the operation. By the end of the 7th day of regeneration there were 6% of mononuclear tetraploid cells. The number of binuclear cells fell during the period of regeneration studied from 16 to 8%. It is concluded that the principal cytological mechanism of liver regeneration in guinea pigs is normal mitosis terminating in separation of the cells.

KEY WORDS: liver; polyploidy; mitotic index; index of labeled nuclei; guinea pig.

Growth and regeneration of the mammalian liver are known to take place through increased proliferation and polyploidization of the hepatocytes.

Special attention is now being paid to the study of the cytological mechanisms of regeneration and the elucidation of the importance of polyploid cells in the development of these processes. Experimental studies in this direction as a rule have been undertaken on mice and rats, whose liver is characterized by the appearance of many polyploid hepatocytes during postnatal development [3, 4, 16].

The object of this investigation was to study the character of regeneration of the liver in animals in which physiological growth of the liver is not accompanied by polyploidization of the parenchymatous cells.

EXPERIMENTAL METHOD

The test object was the liver of young (weighing 220-250 g) and old (weighing 600-700 g) male guinea pigs.

The old animals were used to study the ratio between cells of different ploidy values at an age when the population of polyploid hepatocytes may be maximal. In the young guinea pigs partial hepatectomy was performed by the Higgins–Anderson method between 9 and 11 a.m. under ether anesthesia. The animals were killed between 15 and 80 h after the operation at intervals of 5 h; approximately 1 ml of a solution of [^{3}H]thymidine with specific activity of 5 Ci/mmole diluted with physiological saline was injected in a dose of 0.5 μCi/g body weight intraperitoneally 1 h before sacrifice. In the experiments to determine the DNA content, the investigation was carried out 5 and 7 days after the operation.

Three animals were used at each time of the investigation. The experiments were carried out in the spring and summer. Films of isolated cells were prepared from the liver and fixed in 96% ethanol for 20 min. The films were coated with type M (Photographic Chemical Research Institute) emulsion and exposed for 5 days. After development the films were stained with hematoxylin and the number of labeled nuclei counted

Department of Histology and Embryology, Second Moscow Medical Institute. Laboratory of Functional Morphology of the Chromosomes, Institute of Molecular Biology, Academy of Sciences of the USSR. Laboratory of Cytology, Institute of Developmental Biology, Academy of Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 221-224, August, 1977. Original article submitted March 16, 1977.

in 1000 cells. The number of binuclear hepatocytes was counted in films stained with azure II–eosin. From 3000 to 5000 hepatocytes were counted in each of three to five preparations from each animal.

For quantitative estimation of DNA in the nuclei the Feulgen reaction was carried out (hydrolysis in 5 N HCl at 20°C for 20 min followed by staining with Schiff's reagent for 1 h at room temperature). The DNA content in the nucleus was determined with the Vickers M-86 scanning cytophotometer. Lymphocytes from the peripheral blood or small lymphocytes from a lymph node were used as the standard diploid cells.

The number of mitotically dividing cells was counted in squash preparations. Pieces of liver were fixed in a mixture of 90% ethanol and glacial acetic acid (3:1) for 40 min, then transferred to 96% ethanol, after which the squash preparations were made and stained with carmine. To determine the number of mitoses 5000-6000 hepatocytes from each animal were examined and the number of mitoses per 1000 cells calculated (mitotic index).

Material was taken from the same animal for obtaining specimens for counting the number of mitoses and the number of labeled nuclei.

EXPERIMENTAL RESULTS

No data could be found in the literature on the parameters of the mitotic cycle and the duration of the prereplicative period of guinea pig hepatocytes during regeneration of the liver in response to partial hepatectomy. Such information is essential for choosing the times to study possible polyploidization of the parenchyma of the regenerating liver. It was also hoped that the experiments would show why the weight of the liver of young guinea pigs, unlike that of other mammals, is restored to only 68% of the original weight by the end of the first week after the operation [7].

The curve of the number of labeled nuclei during the first 80 h after hepatectomy is shown in Fig. 1. An increase in the number of labeled cells was observed 30 h (93 ‰), 45 h (170 ‰), and 60 h (82 ‰) after the operation. The reduction in the number of labeled cells after the first peak was not significant. The curve reflecting the changes in the number of mitotically dividing hepatocytes had two peaks, at 40 h (75 ‰) and 55 h (76 ‰); 80 h after the operation a tendency was again observed for mitotic activity to rise.

Mass commencement of the phase of DNA synthesis by hepatocytes during regeneration of the liver is known to occur 18-24 h in rats and 28-36 h in mice after hepatectomy; two waves of mitoses are found in the regenerating liver of these animals: in rats after 26-30 h and 45-48 h; in mice after 44-48 h and 68-72 h [1, 2, 6, 11, 13, 15]. Hence, the commencement of the S period and mitosis in guinea pigs occurs at about the same times as in mice.

The experiments showed (Fig. 1) that polyploid hepatocytes cannot be expected to appear sooner than 60 h after the operation, i.e., after the end of the main wave of mitotic divisions of the hepatocytes. Accordingly the cytophotometric study of the DNA content in guinea pig hepatocytes was carried out 5 and 7 days after hepatectomy.

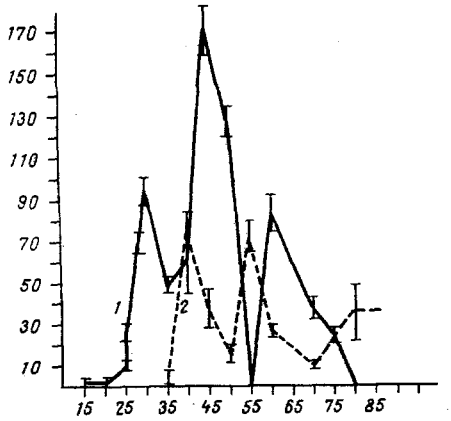

Fig. 1. Changes in number of labeled nuclei and mitotically dividing hepatocytes in regenerating guinea pig liver. Ordinate, number of labeled nuclei (1) and number of mitoses (2) (in ‰); abscissa, time after partial hepatectomy (in h).

TABLE 1. Body Weight and Weight of Liver of Normal Guinea Pigs (control group) and Guinea Pigs 7 Days after Partial Hepatectomy (experimental group)

Group of animals	Number of animals	Body weight, g	Weight of liver, g	Relative weight of liver, %	Weight of regenerating liver, % weight of control liver
Control	5	251±7,1	11,6±0,2	4,51	
Experimental	5	228±5,4	8,4±0,6	3,7	82

The results of the measurements showed that tetraploid mononuclear hepatocytes in the intact liver of young sexually mature guinea pigs account for 0.5%, and in old guinea pigs not more than 2%, of the total cell population. These figures are in agreement with information in the literature according to which mononuclear polyploid hepatocytes are absent in the guinea pig liver [12] or account for only 1-2% of its cells [7].

During the course of regeneration, on the 5th to 7th days after partial hepatectomy 6% of tetraploid mononuclear hepatocytes was found in the liver of the experimental animals.

The counting showed that about 16% of the cells in the liver of intact guinea pigs weighing 220-250 g are binuclear. The number of binuclear cells after hepatectomy initially increased up to 20% (after 25 h), but then (after 80 h) fell to 8%; consequently, it fell below its initial level.

Toward the end of the 7th day after hepatectomy the number of polyploid cells (mono- and binuclear) in the guinea pig liver thus remained virtually unchanged. Meanwhile, the number of polyploid cells in the regenerating rat liver is known to increase by 15-30% by the end of the first week after hepatectomy [8], whereas in mice nearly all the parenchymatous cells of the liver become polyploid [10]. In both species of animals cells of higher classes of ploidy (16N and 32N), not present in intact animals, begin to appear.

The maximal number of mitoses found in the regenerating guinea pig liver was 7.6%, rather more than the maximal mitotic index (5-6%) for the liver of mice and rats [5, 9, 14, 15]. Determination of the weight of the liver (Table 1) showed that by the 7th day after the operation the weight of the liver of the experimental animals (body weight 220-250 g) was restored up to about 82% of its initial value.

It can be concluded from these results that the principal cytological mechanism of regeneration of the liver in guinea pigs is normal mitosis, terminating in cell division. The small increase in the number of mononuclear tetraploid hepatocytes (0.5-6%) by the 7th day after the operation cannot significantly affect the course of regeneration. A similar conclusion was reached by investigators who studied regeneration in the liver of sexually immature guinea pigs [7].

It is difficult to understand at present why, during physiological growth and reparative regeneration, mitotic division of cells in the same organ but of different species of mammals should end either in division of the nucleus and cytoplasm or in union of the daughter chromosomes with the formation of a polyploid cell. Further experimental investigations are necessary in order to shed light on this fundamental problem concerning the cause of formation of polyploid somatic cells.

LITERATURE CITED

1. I. D. Belyaeva, Byull. Éksp. Biol. Med., No. 8, 99 (1969).
2. I. D. Belyaeva, Byull. Éksp. Biol. Med., No. 7, 75 (1971).
3. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
4. V. Ya. Brodskii, I. V. Uryvaeva, and V. M. Faktor, in: Age Patterns of Regeneration and Cell Division (Proceedings of a Symposium) [in Russian], Moscow (1974), p. 4.
5. L. D. Liozner, Z. A. Ryabinina, and V. F. Sidorova, Byull. Éksp. Biol. Med., No. 5, 96 (1959).
6. A. M. Polishchuk, Tsitologiya, No. 6, 652 (1967).
7. Z. A. Ryabinina and V. A. Benyush, in: Regeneration and Cell Division (Proceedings of the 5th Conference on Regeneration and Cell Division) [in Russian], Moscow (1968), p. 369.

8. Z. A. Ryabinina and V. A. Benyush, Polyploidy and Hypertrophy of Cells during Growth and Regeneration [in Russian], Moscow (1973).
9. V. F. Sidorova, Z. A. Ryabinina, and E. M. Leikina, Regeneration of the Liver in Mammals [in Russian], Leningrad (1966).
10. I. V. Uryvaeva and V. Ya. Brodskii, Tsitologiya, No. 10, 1219 (1972).
11. V. M. Faktor, Byull. Éksp. Biol. Med., No. 9, 97 (1971).
12. W. Donkhaus and D. Heimer, Gerontologia (Basel), 16, 1 (1970).
13. F. Gasparini, Arch. Ital. Anat. Embriol., 65, 284 (1960).
14. T. Kambara and J. Nohara, Arch. Pathol., 81, 525 (1966).
15. S. Lesner, A. N. Stroud, and A. M. Broes, Cancer Res., 20, 1341 (1960).
16. C. Nadal and F. Zajdela, Exp. Cell Res., 42, 117 (1966).

EFFECT OF REGENERATION OF THE SPLEEN UNDER DIFFERENT CONDITIONS ON ITS MEGAKARYOCYTES IN ALBINO RATS

A. A. Mezhlumyan

UDC 616.411-008.953.8-091-092.9

Megakaryocytes are found in the intact rat spleen in various forms: with a ring-shaped nucleus and as large multinuclear cells. The megakaryocytes divide by the formation of multipolar mitoses without subsequent cytotomy. During regeneration the number of megakaryocytes and their mitotic activity increased sharply. Gravitational overloading increased the number of megakaryocytes and their mitotic activity even more. Administration of splenic extract in addition to gravitational overloading reduced the number of megakaryocytes in the regenerating spleen but increased their mitotic activity. Under conditions of hypokinesia the number of megakaryocytes in the regenerating spleen fell sharply and no mitoses were observed in them.

KEY WORDS: megakaryocytes; regeneration of the spleen; gravitational overloading; hypokinesia.

There is only limited information in the literature on the morphological and functional characteristics of the megakaryocytes of the spleen. Some workers [2] state that in Werlhof's disease marked megakaryocytopoiesis is observed but thrombus formation is depressed. In another report [1] a case of megakaryocythemia is described in which the platelet count in the peripheral blood remained fairly high. Data has been obtained [8] on the role of the spleen, the organ controlling the content of thrombosthenin, which stimulates megakaryocytopoiesis. Information has also been published [5, 7] on the morphology of the megakaryocytes. No unequivocal solution has been found to the problem of the origin of the megakaryocytes.

Some workers consider that megakaryocytes arise as a result of fusion of several reticulum cells, and that lymphocytes and immature plasma cells are also involved in the process [6]. Other workers [9] have stated that the precursors of the megakaryocytes are large basophilic cells.

On the question of the method of division of the megakaryocytes it has hitherto been considered [3] that multipolar mitoses are characteristic of these cells and are more frequently found under experimental conditions. Information on the morphological characteristics of the splenic megakaryocytes under different experi-

Department of Histology and Pathological Anatomy, Erevan Zooveterinary Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 224-227, August, 1977. Original article submitted February 21, 1977.