POLYPLOIDIZATION OF MOUSE HEPATOCYTES AFTER REPEATED EXPOSURE TO CARBON TETRACHLORIDE

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UDC 612.35.014.24:612.6.052].014.46:547.412.133

KEY WORDS: carbon tetrachloride; regeneration of the liver; polyploidization of hepatocytes.

According to one opinion, liver cells have unlimited capacity for self-support, and this is manifested in particular during repeated surgical operations on chemical injury to the liver [13]. The special course of cell division in the liver is also known to be determined by polyploidizing mitoses, as a result of which the ploidy of the cell increases [3, 14]. Under these circumstances the rates of polyploidization are so great that after repeated stimulation of growth, it would evidently be possible to obtain a liver consisting practically entirely of polyploid cells.

In the investigation described below repeated (up to 8 times) stimulation of reparative growth of the liver by CCl_4 was used in order to assess the degree of development of polyploidy attainable under those circumstances and the powers of regeneration of the polyploid organs. CCl_4 was injected into the animals at intervals of one month, to allow complete recovery of the liver by the time of each consecutive regeneration [11]. The more frequent administration of the poison is known to induce pathological changes in the organ, leading to the development of cirrhosis and tumors [2, 9].

EXPERIMENTAL METHOD

Male CBA × C57BL/6 mice aged 2 months at the beginning of the experiment were used. The duration of the experiment was 7 months. Regeneration of the liver was induced by poisoning with CCl_4 vapor from one to eight times in succession, with an interval between poisonings of one month. The mice were placed for 15 min, 6 to 8 animals at a time, in a 3-liter exsiccator, into which 0.1 ml CCl_4 was introduced. The result of the action of CCl_4 was assessed by the development of foci of necrosis in the liver 24 h after each consecutive poisoning, for by that time the histological picture of injury to the parenchyma was formed [11]. The animals were decapitated in groups of four, pieces of liver were fixed in 10% neutral formalin, and sections, cut to a thickness of 5 μ, were stained with hematoxylin–eosin.

Changes in the cell composition of the liver in the course of consecutive regeneration were recorded 8–10 days after each dose of CCl_4, when processes of cell proliferation were largely complete and the structure of the liver was restored [11]. The animals (from 2 to 4, less frequently 4 animals at a time) were killed under superficial ether anesthesia. Isolated cell preparations were obtained from the liver by the method described previously [7]. Films were stained by Feulgen's method (hydrolysis in 5 N HCl for 15 min at 37°C, staining in Schiff's reagent for 1 h at room temperature). Mononuclear and binuclear hepatocytes were marked on a scheme of the preparation during observations in phase contrast. The content of DNA-fuchsin in the identified nuclei was measured on a Vickers M-86 scanning microdensitometer in the 540 nm region. For each animal 100 to 150 cells were measured.

At the beginning and end of the experiment four normal mice were taken and isolated cell preparations were also obtained from their liver.

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 614-616, November, 1980. Original article submitted February 12, 1980.

0007-4888/80/9011-1605$07.50

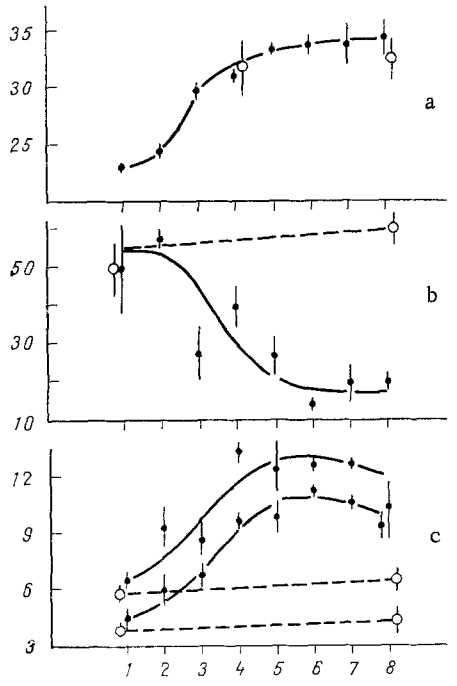

Fig. 1

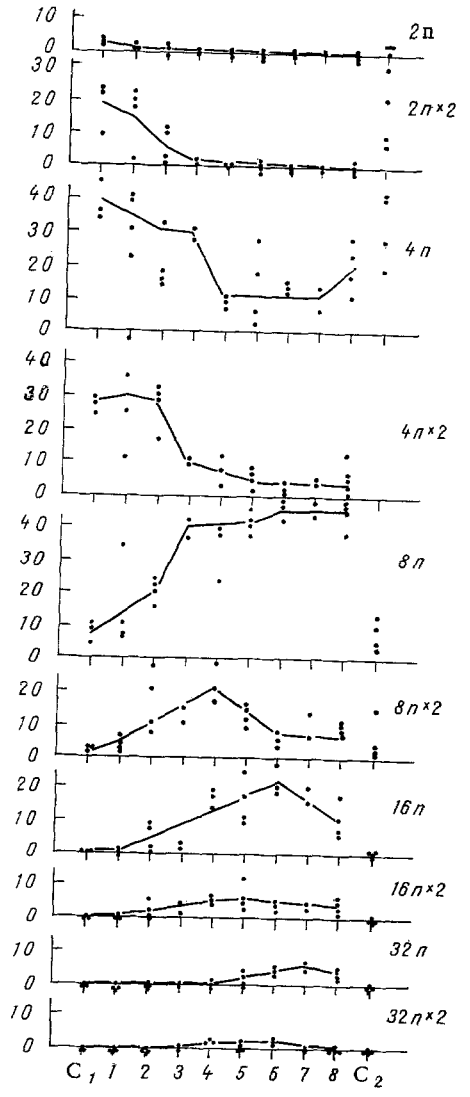

Fig. 2

Fig. 1. Changes in weight of liver (a), number of binuclear cells (b), and coefficients of average ploidy (c) of nucleus (below) and cell (above), depending on number of administrations of CCl_4. Filled circles and continuous lines represent experimental animals; empty circles and broken lines, control animals; vertical lines show error of arithmetic mean. Abscissa, number of administrations of CCl_4; ordinate: a) body weight of mouse (in g), b) relative number of binuclear cells (in percent), c) coefficient of average ploidy of nucleus and cell (in ploidy units).

Fig. 2. Changes in cell composition of mouse liver during consecutive regenerations following repeated poisoning by CCl_4. C_1 and C_2) same age control at beginning and end of experiment. Each point represents one animal. Abscissa, number of doses of CCl_4; ordinate, relative number of cells (in percent).

EXPERIMENTAL RESULTS

Each of the eight consecutive poisonings of the animals with CCl_4, at intervals of one month, led to the development of centrilobular necrosis in the liver. The area of damaged liver tissue was independent of the number of pathogenic exposures and varied within the same limits as after administration of a single dose of CCl_4. It was shown previously that this area averages about 40% [4]. After 8-10 days the foci of necrosis were eliminated and the structure of the lobules restored. In all cases the liver had a normal structure with no signs of cirrhosis. Repeated administration of the hepatropic poison did not affect the growth of the animals. The weight of the experimental and control mice of the same age

did not differ. Since the weight of the liver correlates during normal development with body weight [11], these data are evidence not only of the completeness of regeneration, but also of continuation of the ontogenetic growth of the liver (Fig. 1a).

Each time, injury to the liver with CCl_4 induced a process of proliferation leading to restoration of the weight of the liver. However, the composition of the newly formed cells differed sharply from that of the original population as regards the ploidy of their nuclei. With an increase in the number of stimulations of reparative growth, cells with diploid nuclei (2n and 2n × 2) disappeared (Fig. 2). The number of cells with tetraploid nuclei (4n and 4n × 2) was considerably reduced. Octaploid cells became the dominant form, and after the 5th dose of CCl_4 they accounted for about 50% of all cells of the parenchyma. The other half consisted mainly of cells with nuclei of such high levels of ploidy as 8n × 2, 16n, 16n × 2, 32n, and 32n × 2.

During the first two regenerations of the liver, virtually total polyploidization of the hepatocytes took place, and the whole subsequent ontogenetic and reparative growth of the organs was due to proliferation of polyploid cells. The ability of polyploid cells to proliferate was demonstrated by the writers previously using labeled precursors for DNA synthesis and for chromosomal analysis of dividing cells [6, 8]. During regeneration of the liver after administration of CCl_4, causing death of about 40% by weight of the parenchyma, all cells which remained intact entered the proliferative pool and commenced the mitotic cycle at least once [14]. Consequently, during eightfold induction of proliferation by CCl_4 the cells must have completed at least eight additional cycles of reproduction, besides those maintaining ontogenetic growth of the liver. The ability of the liver to undergo repeated regeneration must evidently suggest that the main mass of the hepatocytes possess unlimited powers of proliferation.

Curves reflecting the character of the change in the number of cells of the different ploidy values, depending on the number of replication cycles completed (Fig. 2) are in harmony with the view that mononuclear and binuclear states alternate in the course of an increase in cell ploidy [8]. Accumulation of binuclear cells always precedes an increase in the number of mononuclear cells at the next level of ploidy. The total number of binuclear cells falls rapidly after each cycle of stimulation of reparative growth (Fig. 1b).

The rate of increase in the level of polyploidy in the liver reflects indices of mean ploidy of nucleus and cell well (Fig. 1c). Maximal mean ploidy is observed after the 6th injection of CCl_4, with a mean value of 11.1n for the nucleus and 12.5n for the cell. These figures are 2.8 and 2.2 times respectively higher than the initial levels of mean ploidy, and they indicate extremely intensive polyploidization of the hepatocytes. Induction of proliferation in the liver by triple resections of part of the organ led to an increase in the mean ploidy of the nucleus by only 1.86 times, compared with 2.8 times in the present experiments [7]. However, despite the sharp increase in the degree of development of polyploidy in the population as a whole, the highest level of polyploidy achieved showed virtually no change compared with the normal liver of old animals [7]. In the experimental group it was 64n. The frequency of discovery of such cells did not exceed 1% in individual animals. Estimation of the upper limit of polyploidization of the hepatocytes in the experimental series must take into account the fact that each of the 8 consecutive injuries to the liver by CCl_4 was accompanied by death of the cells in the central parts of the lobules, i.e., those with highest levels of ploidy [12, 15]. This could be the reason for failure of giant hepatocytes with a nuclear ploidy of 64n or higher to accumulate in the tissue. At the same time the possibility cannot be ruled out that the unique features of structure and function of the liver could be responsible for the existence of an upper limit of polyploidization and, consequently, of enlargement of the cell. A ploidy of 64n in that case reflects the actual limits of polyploidization of the hepatocyte nucleus. In animals of other strains, cells with higher ploidy exist. For instance, Gerhard et al. [10] observed cells with nuclear ploidy of 128n in the liver of normal 5-month-old NMRI mice, but in that case also the number of these cells was extremely small, only 0.001%.

After eight poisonings with CCl_4 a tendency was observed for the coefficients of average ploidy to fall somewhat (Fig. 1c). The decrease in the relative number of high-ploidy cells of the 8n + 2, 16n, and so on, type may be connected with stimulation of division of low-ploidy cells, tetraploid for example, at this stage of induced growth of the liver (Fig. 2). During reparative growth of the liver, besides polyploidizing mitoses, ordinary division of mononuclear hepatocytes may also take place [14].

The fate of the population of diploid hepatocytes found in individual animals in vanishingly small numbers even after eight stimulations of growth also is interesting. There are two alternative explanations of this phenomenon: replenishment of diploid cells on account of self-support [14] and long preservation of these cells in the tissue in the undividing state [5]. The available results do not enable any decisions to be made regarding the mechanisms which lie at the basis of preservation of extremely small numbers of diploid cells during intensive reparative growth of the liver.

LITERATURE CITED

1. V. A. Benyush, Tsitologiya, 12, 1497 (1970).
2. D. S. Sarkisov and L. S. Rubetskoi, Ways of Repair of the Cirrhotically Changed Liver [in Russian], Moscow (1965).
3. I. V. Uryvaeva and V. Ya. Brodskii, Tsitologiya, 14, 1219 (1972).
4. I. V. Uryvaeva and V. M. Faktor, Tsitologiya, 18, 1354 (1976).
5. I. V. Uryvaeva and V. M. Faktor, Dokl. Akad. Nauk SSSR, 249, 1225 (1979).
6. V. M. Faktor and I. V. Uryvaeva, Tsitologiya, 14, 868 (1972).
7. V. M. Faktor and I. V. Uryvaeva, Tsitologiya, 17, 909 (1975).
8. V. Ya. Brodskii (W. Y. Brodsky) and I. V. Uryvaeva, Int. Rev. Cytol., 50, 275 (1977).
9. J. E. Edwards and I. Albert, J. Natl. Cancer Inst., 3, 1941 (1942).
10. H. Gerhard, B. Schultze, and W. Maurer, Exp. Cell Res., 69, 223 (1971).
11. H. Gerhard, B. Schultze, and W. Maurer, Arch. Path. Anat. Abt. B. Zellpath., 10, 104 (1972).
12. H. Gerhard, B. Schultze, and W. Maurer, Arch. Pathol. Anat. Abt. B. Zellpath., 14, 345 (1973).
13. L. G. Lajtha, Nouv. Rev. Fr. Hématol., 21, 59 (1979).
14. B. Schultze, H. Gerhard, E. Schump, et al., Arch. Pathol. Anat. Abt. B. Zellpath., 14, 329 (1973).
15. E. Stöcker, B. Schultze, W.-D. Heine et al., Z. Zellforsch., 125, 306 (1972).

FUNCTIONAL MORPHOLOGY OF THE MYOCARDIAL ULTRASTRUCTURE DURING LONG-TERM ADAPTATION TO PRESSURE CHAMBER HYPOXIA

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UDC 612.172.6-06:612.273.2

KEY WORDS: myocardium; ultrastructure; pressure chamber hypoxia.

During adaptation of man and animals to high-altitude hypoxia a combination of compensatory and adaptive processes develops in the heart, in the form of hypertrophy of the muscle, an increase in the intensity of functioning of the intracellular structures, and an increase in the capacity of the coronary circulation [2]. However, insufficient attention has been paid in the literature to the morphofunctional aspects of myocardial adaptation to the prolonged, intermittent action of pressure chamber hypoxia. It was therefore decided to undertake the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 108 adult noninbred male albino rats weighing 200-250 g. Daily for 6 h the animals were "raised" in a pressure chamber successively to altitudes of 2000, 3000, 4000, and 5000 m, after which for 2.5 months they were raised to an altitude of 6000 m. The period of the experiments was calculated from the number of days which the

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(Presented by Academician of the Academy of Medical Sciences of the USSR M. I. Kuzin.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 616-619, November, 1980. Original article submitted May 26, 1980.

0007-4888/80/9011-1608$07.50