BINUCLEAR RAT LIVER CELLS DURING REPARATIVE REGENERATION OF THE ORGAN

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UDC 612.6.03:612.35

In the early stages after partial hepatectomy (17 h after the operation) binuclear cells take part in proliferation (the number of binuclear cells in proportion to the total number of cells labeled 1 h after injection of thymidine-^{3}H was considerably smaller, whereas in the later stages (37 and 53 h after the operation) their relative fraction in the population was twice or three times greater. The formation of new binuclear cells from mononuclear cells (reflected in the number of labeled binuclear cells 20 h after injection of thymidine-^{3}H) took place most intensively in the early periods of regeneration (16-36 h after the operation) when about 20% of mitoses were acytokinetic and led to the formation of a binuclear cell. In the later periods only 8% of mitoses ended with the formation of binuclear cells.

KEY WORDS: binuclear cells; formation de novo; proliferation; reparative regeneration.

The fate of binuclear cells in the course of ontogeny and reparative regeneration is of evident interest, for polyploidization of the cells of the organ takes place with their participation. According to data in the literature, during the first 3 or 4 days of reparative regeneration there is a sharp reduction in the number of binuclear cells as a result of their mitotic division, leading to the formation of two mononuclear cells with nuclei of the next degree of ploidy [4, 5, 7, 8, 13, 15]. The object of this investigation was to discover whether binuclear cells can be formed in the course of regeneration from mononuclear cells and to determine the degree of their participation in proliferation at its various stages. Data in the literature on these matters are inadequate.

EXPERIMENTAL METHOD

Experiments were carried out on female noninbred albino rats weighing 170-190 g. Partial (70%) hepatectomy was performed in the usual way. At various times (16, 24, 36, and 52 h) after the operation the animals were given an injection of thymidine-^{3}H in a dose of 0.1 μCi/g body weight. To determine the degree of participation of binuclear cells in proliferation, one of the innominate lobes was removed under ether anesthesia from the rats 1 h later, and the animals themselves were killed 20 h after injection of the isotope. A cell suspension was obtained from the lobes of the liver (after perfusion with 1.2% sodium citrate), films were made, coated with type M nuclear emulsion, and exposed for 2-3 weeks at 4°C. The relative number of labeled and unlabeled mononuclear and binuclear cells was determined in the autoradiograph by counting 1000 cells from each animal.

EXPERIMENTAL RESULTS

Table 1 gives data on the total number of binuclear cells and their relative number among cells labeled at different times of regeneration of the liver. It follows from Table 1 that by 17 h after the operation (when the number of cells taking part in DNA synthesis was still small, so that the index of labeled nuclei in this group of animals was 49.0 ± 26.6%), binuclear cells took part in proliferation (the number of binuclear cells as a fraction of the total number of labeled cells 1 h after injection of thymidine-^{3}H) in much smaller numbers than their total fraction in the liver cell population (6.1 and 40.0% respectively). Throughout the subsequent periods of regeneration this ratio changed and became

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 347-349, April, 1979. Original article submitted May 24, 1978.

0007-4888/79/8704-0355$07.50

TABLE 1. Degree of Participation of Binuclear Liver Cells in Proliferation at Different Times after Partial Hepatectomy (M ± m)

	Time after operation, h			
	17	25	37	53
Total number of binuclear cells in population, %	40,0±11,2	26,0±8,8	10,3±2,8	4,4±1,6
No. of binuclear cells among total number of labeled cells (1 h after injection of thymidine-^{3}H), %	6,1±6,3	25,2±7,3	21,2±9,0	12,1±9,3

Note. Here and in Table 2, mean data for 5-12 animals given.

directly opposite: The number of binuclear cells as a fraction of the total number of labeled cells was about 2-3 times greater than their relative number in the population. These results are evidence that the lower level of participation of binuclear cells in proliferation in the early period observed previously (18 h after the operation) was evidently incorrectly attributed to the regeneration process as a whole [14].

Data on the formation of binuclear cells from mononuclear in the course of reparative regeneration are given in Table 2. All labeled binuclear cells 20 h after injection of thymidine-^{3}H were newly formed cells, for even in the case of surgical intervention (removal of the innominate lobe 1 h after injection of thymidine-^{3}H), this time was definitely longer than the total duration of the S and F_2 periods and mitosis [1], so that, consequently, all the labeled cells must have divided and become converted into mononuclear. It follows from Table 2 that the maximal number of binuclear cells was small in the early stage of regeneration of the liver (injection of thymidine-^{3}H 16 h, sacrificed 36 h after the operation) — only 11% of the total number of labeled cells were binuclear. Simple calculation indicates that in this case about 20% of mitoses were acytokinetic and led to the formation of a binuclear cell. At subsequent periods of regeneration the number of binuclear cells was a smaller proportion of the total labeled cells, namely about 4%, suggesting that at this time there were only 8% of acytokinetic mitoses. It will be clear that the formation of binuclear cells de novo was the result of acytokinetic mitosis of a mononuclear cell in the early stage of regeneration when the overwhelming majority of cells taking part in proliferation were mononuclear cells (in two of six rats 1 h after injection of label none of the labeled cells were binuclear, whereas 20 h after injection in the same animals, 9.0 and 9.3% of binuclear cells respectively were labeled). These results apply equally, evidently, to the later stages also, when binuclear cells also play an active part in proliferation, for the absence of morphological evidence (for example, of mitoses with two pairs of telophase plates) gives no grounds for assuming that the binuclear cells arise from another binuclear cell, as was the case for regeneration of the liver after injury by CCl_4 [9].

The results of the present experiments thus indicate that in the course of reparative regeneration of the liver in adult rats binuclear cells are formed from mononuclear cells, and the process is most marked in the earliest stage of regeneration. The change in the number of binuclear cells during the first 3 or 4 days of regeneration is the result of interaction between two processes — their reduction and their formation from mononuclear cells. The first of these processes is much more pronounced, so that the basic tendency is very marked toward a sharp decrease in the number of binuclear cells, as was observed previously.

TABLE 2. Formation of Binuclear Cells from Mononuclear at Different Times after Partial Hepatectomy

Time after operation, h		No. of binuclear cells among total no. of labeled cells (20 h after injection of thymidine-^{3}H), %
time of injection of thymidine-^{3}H	time of sacrifice	
16	36	10,7±3,0
24	44	4,0±2,9
36	56	3,6±2,4
52	72	4,8±3,1

formation of binuclear cells from mononuclear at this time, and this period of reparative regeneration could be contrasted with that of ontogenetic development as regards the fate of the binuclear cells [6, 10-12].

The fact that maximal formation of binuclear cells from mononuclear observed in this investigation in the earliest stages of regeneration can evidently be explained on the grounds that mononuclear diploid cells are among the first to take part in proliferation [2], and the transition to a binuclear cell is most evident for such cells from the point of view of the ontogenetic principles of development of the liver cells [3].

The formation of binuclear cells from mononuclear in the course of reparative regeneration is an additional reserve in the mechanism of polyploidization of the liver cells, and it evidently explains why cells of degrees of ploidy that are not found during ontogeny of the organ can appear during repeated partial hepatectomy [3].

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CHANGES IN THE LEVEL OF EPIDERMAL G_2 CHALONE AND MITOTIC ACTIVITY IN THE VAGINAL EPITHELIUM OF RATS

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UDC 611.671-018.825-092.9:612.621.31

The content of epidermal inhibitor of mitosis (G_2 chalone) at different stages of the estrous cycle in rats was determined by the radial immunodiffusion method. The chalone level correlates with the mitotic index of the vaginal mucous membrane and is minimal in proestrus and maximal in estrus. In aging (14-16 months) rats with regular cycles the content of G_2 chalone in the vaginal mucous membrane at all phases of the estrous cycle was significantly lower than in young (3-4 months) rats with a regular cycle. In castrated rats the mitotic index begins to rise 18 h after a single injection of estradiol benzoate (1 μg/100 g body weight). This increase is preceded by a significant decrease in the concentration of G_2 chalone 12 h after injection of the estrogen.

KEY WORDS: chalones; mitotic index; estrous cycle; estrogens.

In recent years great importance in the mechanisms of maintenance of tissue homeostasis has been attached to endogenous tissue inhibitors of proliferative activity (chalones). It

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Serebrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 349-351, April, 1979. Original article submitted June 13, 1978.