THE MITOTIC CYCLE OF HEPATOCYTES OF THE REGENERATING MOUSE LIVER DIVIDING AT THE BEGINNING AND END OF THE PROLIFERATION PERIOD

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The mitotic cycle of mouse liver cells dividing 36 and 72 h after partial hepatectomy was determined by the labeled mitosis curve method. Differences were found in the shape of the compared labeled mitosis curves, evidently due to prolongation of the G_2- and S-periods and to repeated divisions of some cells at the end of the period of active proliferation.

Using the regenerating liver, in which there is mass proliferation of hepatocytes, as the model, many investigations have been undertaken to study the kinetics of cell reproduction. The study of the character of changes in the duration of the mitotic cycle in the course of regeneration is of particular interest. Some results have already been obtained in this direction [1, 10].

The object of the present investigation was to determine the parameters of the mitotic cycle of regenerating mouse liver cells dividing at the beginning and at the end of the period of proliferative activity.

EXPERIMENTAL METHOD

F_1 hybrid (CBA×C57BL6) male mice weighing 26-29 g and aged 3 months were used. Curves of labeled mitoses were compared from animals taken 36 h (beginning of increase in mitotic activity) and 72 h (end of the period of increased mitotic activity) after removal of two-thirds of the liver by the method of Higgins and Anderson. The animals of group 1 (27 mice), taken 36 h after the operation, received an intraperitoneal injection of thymidine-H^3 with specific activity 1.04 μCi/mg in a dose of 0.5 μCi 1.5-24 h before sacrifice. The animals of group 2 (20 mice), taken 72 h after the operation, received thymidine-H^3 with a specific activity of 18.8 μCi/mg in a dose of 0.5 μCi 1.5-16 h before sacrifice. The animals were decapitated, and pieces of the caudate lobe of the liver were fixed in Carnoy's fluid and embedded in paraffin wax. Liver sections, 5 μ in thickness, were coated with type M (NIIKHIMFOTO) emulsion and exposed for 20 days. After development the sections were stained with Mayer's hematoxylin and the percentage of labeled mitoses was calculated after examination of 150-250 mitoses for the animals of group 1 and 50 mitoses for the animals of group 2.

EXPERIMENTAL RESULTS

Hepatocytes start mitosis on a large scale in mice 36 h after the operation. Later, 72 h after the operation, cells which for some reason or other have begun to divide later than the others, and also hepatocytes passing through the second mitotic cycle, are dividing. The proportion of the latter must be small, and in some animals no such cells in general have been observed [3, 4].

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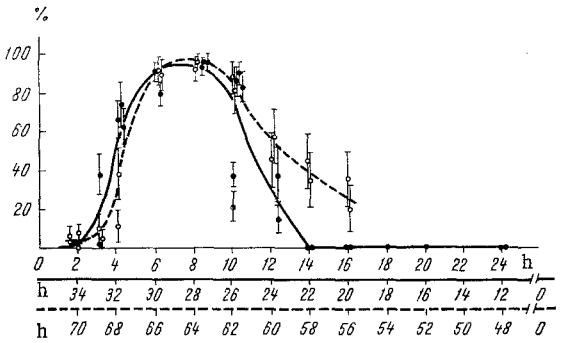

Fig. 1. Changes in percentage of labeled mitoses at various times after injection of thymidine-H^3. Abscissa, time interval (in h) between injection of thymidine-H^3 and sacrifice; time between operation and injection of thymidine-H^3 is shown in the opposite direction. Ordinate, percentage of labeled mitoses. Filled circles and continuous line – 36 h, empty circles and broken line – 72 h after partial hepatectomy. 95% confidence limits are shown. Standard error calculated from binomial distribution $\pm\sqrt{\frac{p(1-p)}{n}}$ [12], in which p is the frequency of finding labeled mitoses (in %) and n the total number of mitoses examined.

The mean mitotic index for the mice of group 1 was 26.3 ‰ (from 3 to 97 ‰ in individual animals), and for the mice of group 2 it was 3.6 ‰ (from 0.1 to 15 ‰). As Fig. 1 shows, the curve of labeled mitoses for cells dividing 36 h after hepatectomy had the typical symmetrical shape characteristic of a homogeneous proliferating population. In relation to the method of calculating the duration of individual parameters of the mitotic cycle generally used [7], it can be taken that the minimal duration of the G_2-period is 1 h 40 min, and that the duration of the S-period is 7 h, in good agreement with data in the literature [2-6]. Analysis of the curves in Fig. 1 showed that they differ slightly in shape, especially due to the fact that hepatocytes dividing 72 h after partial hepatectomy constitute a heterogeneous cell population. The heterogeneity observed may be based, first, on an increase in variability of the G_2- and S-periods at the end of proliferative activity, while second, the possibility of repeated division of some of the cells cannot be ruled out. The possibility of finding such repeatedly dividing cells is due to the experimental conditions, for thymidine-H^3 was injected at different times after the operation. If the isotope was injected 14-16 h before sacrifice, i.e., 56-58 h after hepatectomy, some of the cells which were labeled at the time of injection and circulation of the thymidine-H^3 could evidently have passed through two successive cycles of division. This possibility is ruled out in the case of animals taken 36 h after partial hepatectomy, for virtually no mitoses were found in the liver before this time.

The difference between the compared curves can also be attributed to the fact that preparation of some cells for mitoses takes longer on account of the gradual transition of the cells from proliferation to differentiation.

During regeneration there is a marked decrease in length of the mitotic cycle [2-6, 8, 9, 11]. This evidently takes place as the result of the predominance of syntheses promoting division in the cells, at the expense of processes providing for the specific functions of the liver cell. At the end of the period of active proliferation, the opposite process – intensification of specific syntheses – can be considered to have commenced in some of the cells, leading to prolongation of the period of preparation for division.

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