MITOTIC CYCLES OF "EARLY" AND "LATE" DIVIDING CELLS OF THE REGENERATING RAT LIVER

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In "early" (22 h after the operation) dividing cells of the regenerating rat liver the mitotic cycle was shorter than in "late" (48 h after operation) dividing cells. In addition, a more synchronized passage of individual cells through all stages of the mitotic cycle was observed in the case of the "early" dividing cells.

The first mitoses appear in the regenerating liver of adult rats (weighing 150-200 g) between 20 and 22 h after partial hepatectomy. They increase rapidly in number to reach a maximum (30-35 $^0/_{00}$) by 26-30 h after the operation. The mitotic activity then gradually declines, and by 72 h after the operation it is at a low level (1-3$^0/_{00}$) [1, 2, 4].

According to data in the literature [3], each parenchymatous cell in the regenerating liver divides, as a rule, once (only a very few divide more than once). Consequently, the cells which divide during the first 3 days of regeneration began mitosis at different times after partial hepatectomy – some 20-22 h after the operation, many of them 26-30 h after, and some much later still, 48-72 h after the operation. This asynchronous commencement of mitosis of the parenchymatous cells of the regenerating liver is observed if the stimulation of division (by which is implied the time of the operation) takes place at the same time for all cells.

The objective of the present investigation was to compare the mitotic cycle of the parenchymatous cells of the regenerating rat liver starting mitosis 22 h after the operation (some of the first group) and of cells starting mitosis much later, viz. 48 h after the operation. In other words, it was hoped to discover whether the relative delay between the time of stimulation of division and the beginning of the mitotic cycle has any effect on the character of the cycle. So far as the author is aware, this problem has not previously been discussed in the literature with respect to cells of the regenerating liver.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats, males and females weighing 170-200 g. In the experiments of series I the animals were sacrificed 22 h after partial hepatectomy, performed by the usual method, while in series II they were killed 48 h after the operation. In both series of experiments the animals received an injection of thymidine-H^3 in a dose of 0.1 μCi/g (specific activity about 800 mCi/mmole) 14,12, 11, 10, 9, 8, 6, 5, 4, 3, and 2 h before sacrifice (3-6 animals for each time). Sections of the liver were coated with type "R" liquid nuclear emulsion and exposed at 4°C for 3-4 weeks. After development, the sections were stained with Mayer's hematoxylin, and the percentage of labeled metaphases was determined. In cases in which the total number of mitoses was higher than 5$^0/_{00}$ (this was the overwhelming majority), the percentage of labeled metaphases was determined by counting 100 metaphases in each rat, but if, on the other hand, the total number of mitoses was less than 5$^0/_{00}$, 50 metaphases were counted in each

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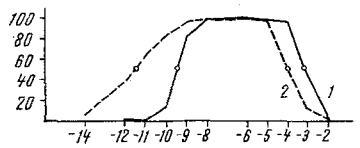

Fig. 1. Changes in percentage of labeled metaphases in regenerating rat liver after injection of thymidine-H^3 at various times before sacrifice: 1) "early" dividing cells (22 h after operation); 2) "late" (48 h after operation) dividing cells. Abscissa, time (in h) before sacrifice (mitosis); ordinate, number of labeled metaphases (in %).

animal. By determining the percentage of labeled metaphases after injection of thymidine-H^3 at different times before sacrifice (mitosis), it was possible to establish the limits of the individual periods of the mitotic cycle for the cells concerned.

EXPERIMENTAL RESULTS

The results of the experiments of series I and II are illustrated in Fig. 1. They show that the first labeled metaphases in the "early" dividing cells* are found when thymidine-H^3 was given 10 h before sacrifice (before mitosis). This indicates that at this time the cells were starting to synthesize DNA. The maximum number of labeled metaphases (94-98%) was found when the isotope was given 8-4 h before sacrifice. The number of labeled metaphases subsequently fell rapidly: when thymidine-H^3 was given 2 h before sacrifice only 2% of labeled metaphases were found, i.e., in the time interval between 4 and 2 h before mitosis the synthetic (S) period of interphase was completed.

Meanwhile, the first labeled metaphases in the "late" dividing cells were observed when the isotope was injected 14 h before sacrifice (these cells started DNA synthesis somewhat earlier than the "early" dividing cells). The number of labeled metaphases reached a maximum (94-97%) if the isotope was injected 9-5 h before mitosis. All the cells ceased to incorporate labeled thymidine 2 h before mitosis, i.e., they had concluded the synthetic period of the interphase.

Comparison of the curves of labeled metaphases for the "early" and "late" dividing cells leads to the following conclusions regarding the character of their mitotic cycles.

First, all the periods of the mitotic cycle are shorter in the "early" dividing cells than in "late" dividing cells. The minimal duration of the G_1-period of the "early" dividing cells is evidently accompanied by a relatively shorter duration of their S- and G_2-periods. The duration of the S-period of the "early" dividing cells is about 1.2 h shorter than the duration of the S-period of the "late" dividing cells (it was determined as the time between the 50% level of labeled metaphases on the ascending and descending segments of the curves and is shown in Fig. 1 by the circles). The G_2-period of the "early" dividing cells was also shorter. It was impossible to answer the question of to what extent the G_1-period was lengthened in the "late" dividing cells because it is not yet known whether prolongation of the G_1-period is all that takes place or whether, in addition, there is marked delay in the movement of cells from the G_0- into the G_1-period.

The mitotic cycles of the "early" and "late" dividing cells evidently also differ, in that less variability is observed in the population of "early" dividing cells in the duration of the individual periods of the mitotic cycle. This is reflected graphically by the steeper rise and fall of the curve of labeled metaphases of these cells.

Hence, the "early" dividing cells of the regenerating rat liver not only have a shorter mitotic cycle, but they also have a more synchronous or "collective" passage of the individual cells through all the periods of the mitotic cycle. In the case of the "late" dividing cells, an increase in the total duration of the mitotic cycle is accompanied by a significantly lower degree of synchronization of its course.

Probably the differences observed between the character of the mitotic cycle of these two groups of cells can be attributed to the relative length of the interval between mitosis and the time of stimulation of division. Cells dividing at the shortest period after stimulation pass through the mitotic cycle more rapidly and also more synchronously. A longer interval after stimulation of division is associated not only with a longer duration of the mitotic cycle, but also with much greater asynchronism in the passage of individual cells through its period.

*Cells dividing 22 h after the operation (experiments of series I) will in future be called "early" dividing, while those starting mitosis 48 h after the operation (experiments of series II) will be called "late" dividing cells.

Similar data concerning the character of the mitotic cycle of transplantable tumor cells have been published. In most papers on this problem [5-8] the duration of the mitotic cycle of the cells is considered to be directly dependent upon the time elapsing from transplantation (this procedure is known to stimulate the mitotic division of the transplanted cells).

The distinguishing features of the mitotic cycle of "early" and "late" dividing cells of the regenerating rat liver examined above evidently reflect general principles governing the changes in the character of the mitotic cycle of cells, depending on the relative duration of the interval between mitosis and the stimulation of cell division.

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