EFFECT OF DISTURBANCE OF RNA SYNTHESIS BY ACTIONMYCIN IN THE G_1 PERIOD ON THE MITOTIC CYCLE OF REGENERATING LIVER CELLS IN RATS

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Disturbance of RNA synthesis in regenerating liver cells of rats by actinomycin (0.1 μg/g) in the G_1 period retards entry of the cells into mitosis by 10-13 h. Evidently nearly all cells retarded at the beginning of the G_1 period complete their preparation for mitosis and divide. Delay of cells at the beginning of the G_1 period has no significant effect on the duration of the subsequent periods of interphase.

In the presynthetic (G_1) period of interphase, synthesis of RNA and protein takes place in cells in preparation for subsequent DNA replication (the S period) and mitosis. The need for synthesis of RNA and protein during the G_1 period for DNA replication has been demonstrated in various cells in tissue culture [4, 5, 7, 10, 11, 13] and regenerating rat liver cells [6, 8]. The relationship between RNA synthesis and DNA replication and their connection with mitosis in regenerating liver cells of mice and rats were described by the writer previously [1-3].

The object of this investigation was to study the mitotic cycle of regenerating liver cells of rats after disturbance of RNA synthesis by actinomycin at the beginning or in the middle of the G_1 period. The time of delay of the cells in the G_1 period and the effect of this delay on the duration of the subsequent periods of interphase were determined. No such information can be found in the known literature. The only relevant report is to the effect that disturbance of RNA synthesis by actinomycin in the G_1 period delays the progress of HeLa cells to DNA replication and increases the duration of replication [9].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180±20 g. Partial hepatectomy was performed by the usual method at 10 A.M. or 4 P.M. In the experiments of series I, immediately after the operation the animals were given actinomycin C in a dose of 0.1 μg/g. In series II, the same dose of actinomycin was given to rats 12 h after the operation. The animals were sacrificed in groups of 12-18 animals 26, 29, 33, 36, 39, 42, 45, 48, 54, 60, 66, and 72 h after the operation. Operations on the control and experimental animals and sacrifice of these animals were carried out simultaneously at all periods of the experiment (within 30-40 min). The number of mitoses was determined in histological sections (5000 cells were counted) and expressed in promille. In the experiments of series III, rats received actinomycin immediately after the operation, and in addition, at various times after the operation (22, 24, 27, 30, and 33 h) they received thymidine-H^3 in a dose of 0.3 μCi/g (specific activity about 300 mCi/mmole); the animals were sacrified (6-8 at each time) 1 h after injection of the isotope. Histological sections were coated with type "R" liquid photograph emulsion and exposed for 10-12 days at 4°. After development, the index of labeled nuclei was determined in sections stained with hematoxylin (by counting 1000 nuclei), and expressed in promille.

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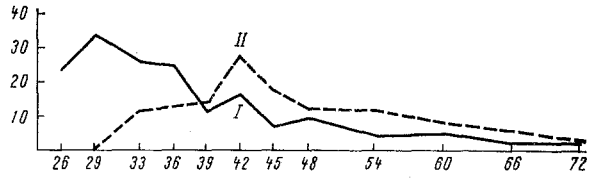

Fig. 1. Mitotic index of regenerating liver cells of rats following injection of actinomycin (0.1 μg/g) immediately after operation. I) Control; II) experiment; abscissa, time after operation (in h); ordinate, number of mitoses (in $^0/_{00}$).

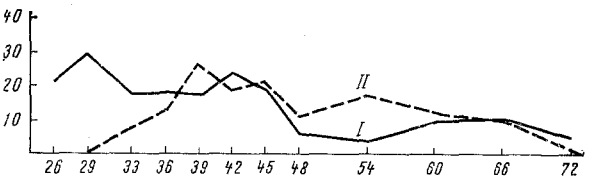

Fig. 2. Mitotic index of regenerating liver cells of rats following injection of actinomycin (0.1 μg/g) 12 h after operation. I) Control; II) experiment; abscissa, time after operation (in h); ordinate, number of mitoses (in $^0/_{00}$).

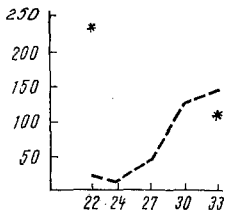

Fig. 3. Index of labeled nuclei in regenerating liver of rats receiving actinomycin (0.1 μg/g) immediately after operation. Asterisks denote control level of index of labeled nuclei 22 and 33 h after operation; abscissa, time after operation (in h); ordinate, number of labeled nuclei (in $^0/_{00}$).

EXPERIMENTAL RESULTS

In the experiments of series I (Fig. 1) the mitotic activity of the regenerating liver cells was studied in animals receiving actinomycin immediately after the operation. In this experiment (Fig. 1) mitotic activity reached a maximum 42 h after the operation, and in the control 29 h after the operation. Consequently, when RNA synthesis was disturbed by actinomycin at the beginning of the G_1 period the cells started division after a delay of 13 h. At all subsequent periods of observation the absolute number of dividing cells diminished gradually, the number of mitoses being relatively larger at all times in the experimental animals than in the controls. This may be due to the fact that all cells, including those dividing during the 3 days after the operation, were delayed as they passed through interphase. The level of mitotic activity in the experimental animals was thus higher than in the control, and was characteristic of the earlier periods of regeneration. The simple explanation of this fact may be that the time of delay differed for the different cells, and they entered into mitosis throughout the period of observation, so that a higher level of mitotic activity was apparent in the experimental animals.

The total number of cells which divided at all periods of observation was only slightly smaller in the experimental than in the control animals. Evidently most cells whose passage through interphase was delayed at the beginning of the G_1 period were able to continue their preparation for mitosis and to divide.

In the experiments of series II (Fig. 2), the mitotic activity of regenerating liver cells was studied in rats receiving actinomycin 12 h after the operation. At the time when the number of mitoses in the experimental animals reached its maximum (39 h after the operation), those cells which, at the time of injection of actinomycin, were the most advanced in their preparation for mitosis, i.e., were at the middle of the G_1 period, were evidently dividing. Consequently, when RNA synthesis was disturbed in the middle of the G_1 period, the cells were approximately 10 h late in starting mitosis. At subsequent periods of observation, cells which, at the time of injection of actinomycin, were in the earlier stages of the G_1 period, were evidently starting to divide. In this experiment, therefore, it was impossible to determine the fate of all the cells delayed at the middle of the G_1 period.

As was mentioned above, when RNA synthesis was disturbed at the middle of the G_1 period, a large proportion of the cell population began to divide after a delay of 10 h, i.e., the delay was about the same (or actually a little shorter) than when actinomycin was injected at the beginning of the G_1 period. It thus follows that disturbance of the continuity of RNA synthesis (at the middle of the G_1 period) has no more effect on the cell than its disturbance when the cell has just begun its preparation for division.

In the experiments of series III (Fig. 3) the effect of delay of the cells at the beginning of the G_1 period on the duration of the subsequent periods of interphase (S and G_2) was studied. A considerable increase in the number of nuclei incorporating thymidine-H^3 (synthesizing DNA) occurred 30-33 h after the operation (Fig. 3), i.e., 9-12 h before the observed maximum of mitotic activity. According to published data [12], the duration of the $S+G_2$ periods in regenerating liver cells of rats is about 10 h. This suggests that if cells are delayed at the beginning of the G_1 period, the duration of the subsequent periods of interphase remains almost unchanged.

It is evident that all the data described in this paper are characteristic of the mitotic cycle of regenerating liver cells of rats in which RNA synthesis was disturbed by administration of the above-mentioned dose of actinomycin C.

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