MITOTIC CYCLE OF HEPATOCYTES IN THE REGENERATING LIVER

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An autoradiographic study using thymidine-H^3 has shown that after removal of two-thirds of the liver in CBA mice three waves of labeled mitoses appear. This is evidence of repeated division of the hepatocytes.

Workers who have studied the mitotic cycle in the regenerating liver [1, 4, 7, 8] did not obtain a second wave of labeled mitoses and they could not therefore determine the duration of the whole cycle. However, in these investigations the duration of the experiment (the time after administration of thymidine-H^3) did not exceed 38 h.

In a previous investigation in mice [3], after resection of two-thirds of the liver labeled thymidine was injected 4 times in the course of 24 h. Between 70 and 100% of labeled mitoses was found 33 h after the last injection. Since the time interval between the waves of DNA synthesis and of mitoses in the regenerating liver was 9-10 h, this fact could be explained either by repeated division of the cells labeled during the period of injections or by reutilization of the thymidine-H^3 label by cells synthesizing DNA in the subsequent period [5, 6].

To solve this problem and to determine the duration of the mitotic cycle in regenerating liver cells, 2 series of experiments were carried out.

EXPERIMENTAL METHOD

In series I, two-thirds of the liver was resected from 48 hybrid (CBA × C57B1) male mice weighing 14-20 g between 11 a.m. and 12:30 p.m. Altogether 41 mice survived. Next day between 11:30 p.m. and midnight the mice received an intraperitoneal injection of thymidine-H^3 of Soviet manufacture with a relative activity of 1.4 Ci/mmole in a dose of 0.6 μCi/g. The mice were sacrificed in groups of two or three 2, 3, 4, 5, 6, 8, 10, 11, 12, 14, 15, 17, 20, 24, 28, 30, 32, 34, and 36 h after the injection.

The experiments of series II were carried out on male CBA mice with a mean weight of 18 g. In CBA mice [6], just as in CBA × C57B1 hybrids [3], mass entry of hepatocytes into the period of DNA synthesis is observed 36-38 h after removal of two-thirds of the liver; the operations on 55 mice were performed between 10:30 a.m. and 12:30 p.m. Altogether 38 mice survived, and next day between 11:30 p.m. and midnight they were injected with thymidine-H^3 in a dose of 0.7 μCi/g. These mice were sacrificed 9, 31, 36, 41, 46, 50, 54, 58, 62, 66, 70, 74, 78, and 82 h after injection of thymidine-H^3.

So that the second wave of labeled mitoses should not be missed, all the mice of series II except those sacrificed at the first two times received an intraperitoneal injection of colchamine (the Soviet preparation identical with democolcine) solution in a dose of 5 mg/kg 4-5 h before sacrifice. The total duration of the experiment in series I was 72 h and in series II 118 h.

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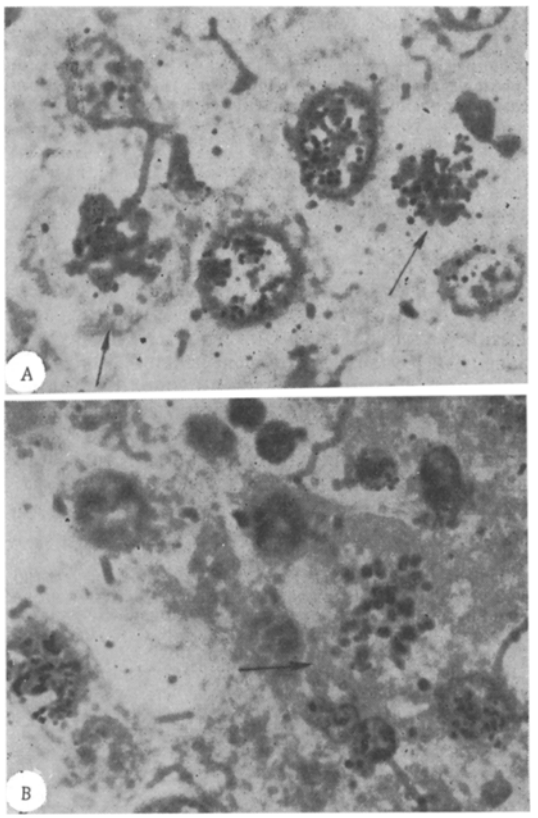

Fig. 1. Labeled and unlabeled colchicine mitoses (indicated by arrows) in regenerating liver 41 h after treatment with thymidine-H^3 (second wave of labeled mitoses): A) labeled mitoses; B) unlabeled mitosis.

The liver was fixed in Carnoy's fluid. Paraffin sections, 5 μ in thickness, were coated with type M (NIIKhIMFOTO) emulsion. The duration of exposure was 9 days in series I and 23 days in series II. The sections were stained with Mayer's hematoxylin. Depending on the frequency with which mitoses were found under a magnification of 1350 times, from 2300 to 12,000 hepatocyte nuclei were examined in series I and from 7,000 to 34,000 nuclei in series II.

The total mitotic index, the index of labeled mitoses, the index of labeled nuclei, and the number of labeled mitoses as a percentage of the total number of mitoses were determined. The mean number of grains of silver above the labeled mitoses also was determined: in series I mitoses above which there were at least three grains of silver were counted as labeled, but in series II only those with at least seven grains of silver were so counted because the colchicine mitoses occupy a larger area than normal mitoses and interphase nuclei (Fig. 1). In this case, however, the percentage of mitoses with 1 or 2, 3, or 4, and 5 or 6 grains of silver was estimated separately.

Cases in which at least 20 mitoses were found were used to plot the curve of labeled mitoses. On the average there were 60 mitoses per case in series I and 53 in series II.

EXPERIMENTAL RESULTS

The curve of the percentage of labeled mitoses plotted from the results of series I is shown in Fig. 2A (1). According to this curve, the total duration of the G_2-period + 1/2 mitosis in the regenerating mouse liver was 5 h, and that of the S-period 7 h. The curve did not reach the 100% level, evidently because of the insufficient exposure in this series. However, the results mainly agreed with those in the literature [1, 4, 7, 8]. Only in an investigation by Li [2], who studied the mitotic cycle in the regenerating liver at later periods of regeneration (starting with 70 h), was the S-period longer in duration (8.8 h).

No second wave of labeled mitoses was obtained in series I. The very slight rise after 28 h took place because of mitoses with a very small mean number (3.2) of silver grains. During the first wave, the mean number of grains above the mitosis was 17.

In series II, 9 h after injection of thymidine-H^3, 95% of mitoses were labeled. The mean number of grains of silver above a mitosis was 42. The next two periods (31 and 36 h) were characterized by a low percentage (5 and 8) of labeled mitoses. The investigations at these 3 times, repeating the corresponding times of series I, thus confirmed its results. Further, as is clear from Fig. 2A (2), the curve of labeled mitoses gave two waves (a second and third) with maxima at 46 h and 68 h, reaching the 50% level. The mean number of grains above the labeled mitoses during the second and third waves was almost identical (26 and 27). The maximum of the second wave was separated from the maximum of the first wave of labeled mitoses by 37 h, and the maximum of the third wave from that of the second by 20 h.*

Changes in the total mitotic index and index of labeled mitoses (the number of labeled mitoses per 1000 cells) in the course of the experiment are shown in Fig. 2B. The morning wave of mitoses on the

*Inclusion of mitoses with 5-8 and 3-4 grains of silver above them among the labeled group increases the level of the curve of labeled mitoses in the first case by about 5%, and in the second by 15-30%, but the curve still remains similar in character.

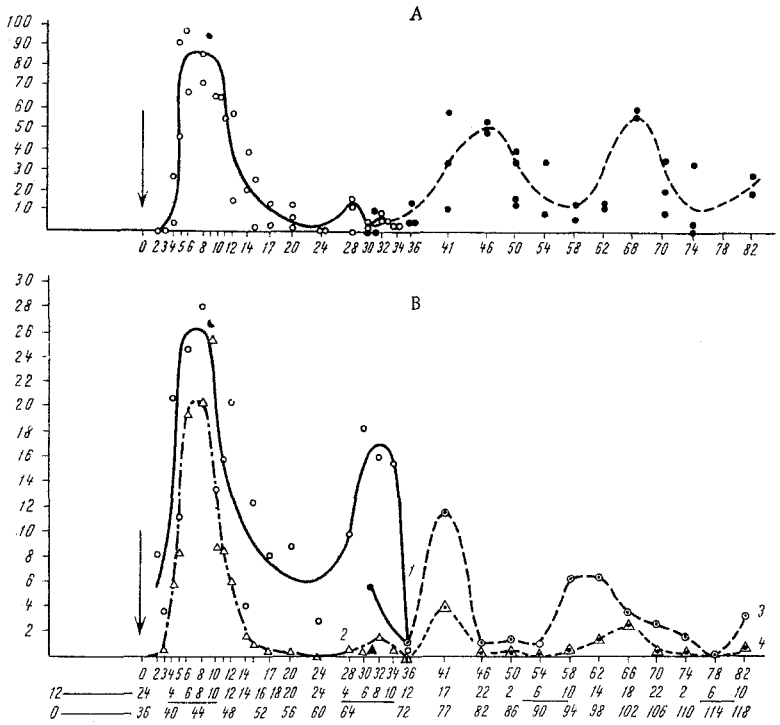

Fig. 2. Change in percentage of labeled mitoses in regenerating mouse liver of series I (continuous line) and II (broken line) during experiment (A); change in total mitotic index (1, 3) and index of of labeled mitoses (2, 4) in regenerating mouse liver (B) (starting from 36 h after injection of thymidine-H^3, mitotic indices were obtained for mice receiving demecolcine 4-5 h before sacrifice). Ordinate: A) percentage of labeled mitoses, B) mitotic index (in $^0/_{00}$), abscissa: A) time after injection of thymidine-H^3 (in h); B) (numbers from top to bottom respectively) time after injection of thymidine-H^3 (in h); time of day; time after operation (in h).

first day of increased proliferation in the regenerating liver was due mainly to cells synthesizing DNA at midnight (at the time of injection of thymidine-H^3). Because of the more regular exposure in series II, a smaller gap could be obtained between the total index of mitoses and the index of labeled mitoses (26-25$^0/_{00}$). The fraction of cells of the first wave of mitoses virtually did not participate in the increase in mitotic activity during the morning of the following day. Conversely, in the evening and at night 30-50% of all mitoses were cells of the first wave dividing a second time. The absolute size of the fraction of repeatedly dividing cells was small, as is clear from Fig. 2B (4), especially when it is remembered that the mitotic index (curves 3 and 4) was the result of accumulation of mitoses for 4-5 h under the influence of demecolcine.

The morning increase in the mitotic index on the 5th day of the experiment (94 h after the operation) was much lower than the preceding rise, but also took place principally on account of unlabeled mitoses. The third wave of labeled mitoses again occurred in the evening. The total number of mitoses forming the second wave of labeled mitoses was slightly more than 4% of their total in the first wave of labeled mitoses. If it is accepted, as a rough guide, that the number of cells in the liver was doubled during the period between the two waves, it can be assumed that 9-10% of the cells participating in the first increase in the number of mitoses divided a second time within 36 h.

The considerable fluctuations in mitotic activity in the course of the experiment considerably added to the difficulty of determining the duration of the mitotic cycle from the curve of percentage of labeled mitoses. For instance, comparison of the curves in Figs. 2A and 2B shows that the high percentage of labeled mitoses 46 h after injection of thymidine-H^3 can largely be attributed to a decrease in the number of unlabeled mitoses. A true increase in the number of labeled mitoses occurred at 41 h. It was therefore

considered that a more correct estimate of the time T would be 33 h. The time G_1 in this case would be about 21 h. The corresponding values determined from the curve of percentage of labeled mitoses were 37 and 25 h.

To ascertain the causes of appearance of the third wave of labeled mitoses obtained in these experiments, several possible interpretations must be considered.

1. Cells labeled at the beginning of the experiment divided 3 times (passed through two mitotic cycles).

2. Cells dividing in the period of the first wave of mitoses started a new cycle at different times and gave two maxima of labeled mitoses in the evenings of two consecutive days. Most of them either did not divide a second time in the period of regeneration or divided later still.

3. The third wave of labeled mitoses was the result of reutilization of the labeled DNA of dying lymphoid cells by cells preparing for mitosis.

An argument against the first suggestion is the fact that the second and third waves were too close together, and the number of grains of silver above the labeled mitoses during the third wave was too high. This last point also is an argument against the hypothesis of reutilization of the label. During reutilization of labeled DNA, a weaker but uniform intensity of labeling above the mitoses would be expected.

The second hypothesis seems most probable, but a further question arises: why did the cells which participated in the first wave of proliferation divide a second time in the evening but did not take part in the more natural morning maxima of mitotic activity?

No regular pattern of change in the number of grains of silver above the labeled mitoses in the course of the experiment could be detected, such as is usually used as an additional criterion when determining the duration of the mitotic cycle. The index of labeled nuclei gave very sharp fluctuations (for example, from 1 to 49%) among individual mice both at the beginning and at the end of the experiment, but on the whole it remained at approximately the same level.

The mean number of silver grains above the labeled mitoses, which also fluctuated, fell during the 58 h after injection of thymidine-H^3, but then returned to its original level, evidently because of reutilization of labeled DNA.

LITERATURE CITED

1. I. D. Belyaeva, Byull. Éksperim. Biol. i Med., No. 8, 99 (1969).
2. S. E. Li, Effect of Glycosides of Eleuterococcus on Regeneration of the Mouse Liver after Partial Hepatectomy. Author's abstract of candidate's dissertation, Moscow (1970).
3. I. V. Markelova and L. D. Liozner, Byull. Éksperim. Biol. i Med., No. 12, 72 (1969).
4. A. M. Polishchuk, The Action of Radiation on DNA Synthesis and on the Kinetics of the Cell Population in Regenerating Rat Liver. Author's abstract of candidate's dissertation, Novosibirsk (1969).
5. B. J. Bryant, Exp. Cell Res., 27, 70 (1962).
6. B. J. Bryant, J. Cell Biol., 18, 515 (1963).
7. J. I. Fabrikant, J. Cell Biol., 36, 551 (1968).
8. E. Stöcker and U. Pfeifer, Z. Zellforsch., 79, 374 (1967).