EFFECT OF REPEATED PARTIAL HEPATECTOMY ON CELL DIVISION IN THE REGENERATING RAT LIVER

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Repeated partial hepatectomy (PH), performed 24 h after a 70% PH, had the following effect on the mitotic cell cycle in the regenerating rat liver: it delayed (by about 2 h) the cells in the G_2 period, left the S period almost unchanged, and delayed the cells for 6-8 h in the G_1 period. A mock repeated operation had a similar effect. This indicates that the influence of the repeated PH on the mitotic cell cycle in the regenerating liver is due to operation stress. Additional stimulation of division by repeated PH affects the character of the regeneration process as a whole.

KEY WORDS: *repeated partial hepatectomy*; *stress*; *stimulation of division*; *periods of the mitotic cycle*.

Data in the literature on the effect of repeated partial hepatectomy (PH) or stress on cell division in the regenerating liver are few in number and contradictory in nature [2, 4]. The action of a second operation on cell division in the regenerating liver is due to several factors and, above all, evidently to operation stress and metabolic changes associated with the additional stimulation of division.

The object of this investigation was to determine the influence of repeated PH on the passage of regenerating rat liver cells through the mitotic cycle, to determine the role of operation stress in this effect, and to study the additional stimulating action of a repeated operation on the process of regeneration of the liver.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female rats weighing 160-180 g. The first PH (70%) was carried out by the usual method. The second operation consisted of removal of the right lateral lobe (about 45% of the liver tissue remaining after the first operation). The second PH was performed 24 h (23-27 h) after the first operation, when the maximal number of cells of the regenerating rat liver were in the mitotic cycle, and in only one experiment (with a double label) the interval between the operations was 36-39 h. A mock repeated PH caused operation stress (perhaps rather less in degree), but was not an additional stimulus for division. The mitotic index was determined by counting 4000-5000 cells. The duration of the individual periods of the mitotic cycle was established by an autoradiographic method after injection of [^{3}H]thymidine in a dose of 0.15-0.2 μCi/g. In the double-label experiment, [^{14}C]thymidine in a dose of 0.05 μCi/g was used as the second label. Sections were coated with type M emulsion and exposed for 3-6 weeks at 4°C. The percentage of labeled mitoses was determined by counting 50 mitoses, and the relative number of cells with one or other label by counting 1000 cells from each rat. The schemes of the experiments and the number of animals used are indicated in the description of the results.

EXPERIMENTAL RESULTS

Changes produced by the second operation in the passage of the regenerating liver cells through the mitotic cycle were determined by the duration of their preparation for division and, consequently, by the level of mitotic activity during the period of entry into mitosis. The mitotic activity of the regenerating liver cells at different times after the repeated

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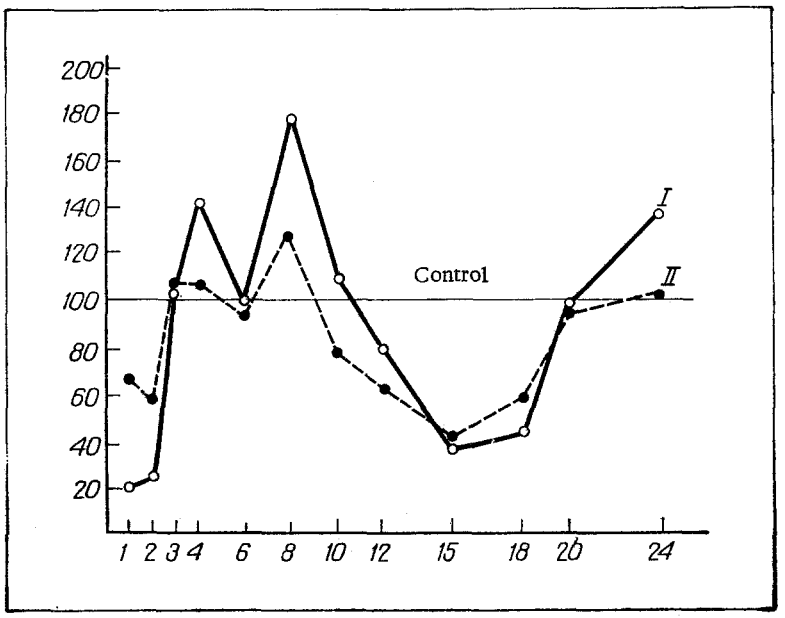

Fig. 1. Mitotic activity at different times after repeated (I) and mock repeated (II) PH. Abscissa, time (in h) after repeated or mock repeated PH; ordinate, mitotic activity (in % of control). Each point on curves is mean for 6-18 rats. Same number of animals as in experiment used in control.

and mock repeated PH, expressed as percentages of the control, which was determined for each experiment, is shown in Fig. 1. The control animals did not undergo a repeated operation and were sacrificed at the same times as the experimental animals, i.e., at equal intervals after the first PH.

After the repeated operation, cells which, at the moment of the operation, were at the end, in the middle, and at the beginning of the mitotic cycle, i.e., in the G_2, S, and G_1 periods respectively, entered into mitosis. From the results given in Fig. 1 the following conclusion can be drawn regarding the effect of the repeated PH on passage of the regenerating rat liver cells through the mitotic cycle. A relatively brief delay of many of the cells took place in the G_2 period (a low level of mitotic activity 1-2 h, and a return to normal 3 h after the repeated PH). The fact that the control level was exceeded 4 h after the repeated operation is probably evidence that not only was the total duration of the G_2 period increased, but cells also were delayed at some part of it, as a result of which their entry into mitosis was synchronized.

In the course of the entry into mitosis of cells which, at the time of the repeated PH, were in the S period the control level of mitotic activity was considerably exceeded 8 h after the second operation. The number of mitoses 2 h before and after this rise was close to normal. The control level was exceeded in this way in three experiments, in each of which 12 animals were used. It is impossible as yet to explain this fact.

A considerable decrease in mitotic activity 12-18 h after the repeated PH was evidence of delay of the cells in the G_1 period of the mitotic cycle; this delay was longer than in the G_2 period, namely 6-8 h. By 20 h after the repeated operation the level of mitotic activity had returned to normal, and by 24 h it was higher than normal. At that time cells held up in the G_1 period probably reached mitosis, and cells stimulated to divide by the repeated operation also entered into mitosis.

The mock repeated PH (Fig. 1, II) had mainly a similar effect on passage of the regenerating liver cells through the mitotic cycle.

This notion of the effect of repeated PH on the mitotic cycle of regenerating liver cells was also confirmed by the autoradiographic data. The duration of the G_2 period of the regenerating liver cells was determined in the early stages after the repeated operation from the increase in the percentage of labeled mitoses after injection of [^{3}H]thymidine (mean duration of the G_2 period minus interval between injection of the isotope and time of appearance of 50% of labeled mitoses). The duration of this period under these conditions was

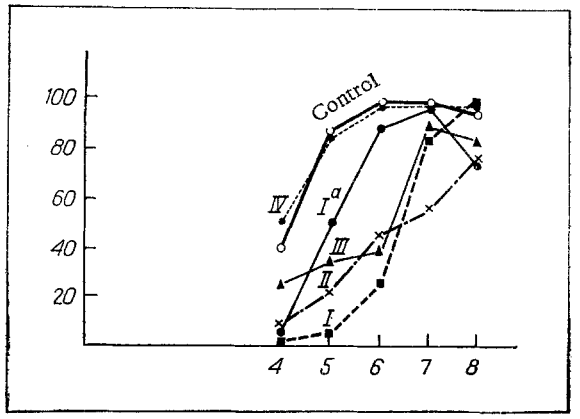

Fig. 2

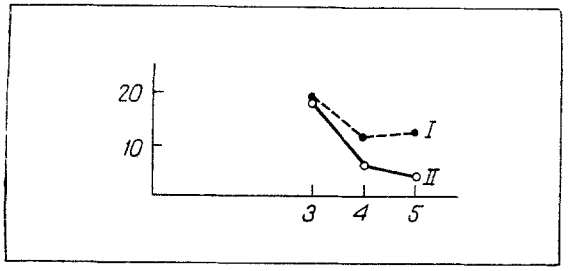

Fig. 3

Fig. 2. Number of labeled mitoses at different times after injection of [^{3}H]thymidine followed by repeated PH and in control. Abscissa, time (in h) after injection of [^{3}H]thymidine; ordinate, % of labeled mitoses; I, Ia) [^{3}H]thymidine injected 1 h before repeated or mock repeated PH; II, III, IV) [^{3}H]thymidine injected 2, 4, and 14 h after repeated PH. Each point on curves is mean for two to four rats.

Fig. 3. Mitotic index after one and two PH. Abscissa, time (in days) after first PH; ordinate, number of mitoses (in %); I) experiment; II) control. Each point on curves is mean for 6-12 rats.

increased by about 2 h (Fig. 2, I). In the case of the mock repeated operation (Fig. 2, Ia) this increase was much smaller. To discover whether delay of the cells in the G_2 period took place in the later stages after the repeated PH, [^{3}H]thymidine was injected 2, 4, and 14 h after the repeated operation, and the animals were sacrificed at the same time after injection of the isotope. The results of these experiments are given in Fig. 2. They show that if [^{3}H]thymidine was injected 2 and 4 h after the repeated operation the increase in duration of the G_2 period still continued (Fig. 2, II, III). This indicates that delay of the cells in this period also took place 6-12 h after the repeated operation, but the duration of the delay was the same, namely about 2 h. In the case of injection of [^{3}H]thymidine 14 h after the repeated operation (Fig. 2, IV) the duration of the G_2 period was the same as in the control animals, i.e., this time there was no delay of the cells.

Determination of the duration of the S period (from the curve of labeled mitoses) showed that after the repeated PH (7.0 h) it was almost the same as in the control (6.6 h). The absence of any significant increase in the duration of this period also makes it difficult to explain the rise in mitotic activity 8 h after the repeated operation, as was pointed out above.

To confirm the delay of the cells in the G_1 period a double-label experiment was carried out in which [^{14}C]thymidine was injected 1 h before the repeated PH and [^{3}H]thymidine was injected after an interval of 2, 4, and 6 h; the animals were killed 1 h later (the control rats received both labels at the same time as the experimental animals). The relative number of cells labeled with only ^{3}H or ^{14}C and the number with the mixed label were determined in sections. Under these experimental conditions the cells labeled with ^{3}H only were those which had passed into the S period during the time interval between injections of the isotope. The experimental results are given in Table 1 and they show that after the repeated and mock repeated PH the relative number of cells with only the ^{3}H label was smaller than in the control. This difference was most marked when the interval between injections of the isotope was 6 h and it demonstrates delay of the passage of the cells from the G_1 period into the S period of the mitotic cycle in the early stages after the repeated operation.

The stimulating effect of the repeated PH on cell division when performed a short time (24 h) after the first operation was revealed by an experiment in which the absolute and relative weights of the regenerating liver were determined 7 days after one PH (control) and after the same time interval during which a second (experimental) PH was performed. Both the absolute (7.25 ± 0.85 g for eight rats in the experiment and 7.22 ± 0.54 g for four rats in the control) and the relative (3.9 ± 0.5 and 3.8 ± 0.2%) weight of the regenerating liver

TABLE 1. Percentage of Cells with the ^3H Label Only in Double-Label Experiment (M ± m)

Interval between injections of thymidine labeled with ^{14}C and ^3H, h	Character of operation		Control
	repeated PH	mock repeated PH	
2	9,0±4,0	8,0±5,5	12,0±2,0
4	11,0±3,6	13,0±3,0	36,0±16,5
6	19,0±8,5	21,0±6,6	65,0±1,0

Legend. Three rats used at each point of experiment.

after one and after two resections were in fact identical. Since repetition of the operation involved the removal of a larger quantity of liver tissue, the identical result of the regeneration process could be achieved as a result of additional stimulation of division, mobilizing its reserve mechanisms (for example, a larger number of repeated cell divisions). That this second hypothesis may be correct follows from the results of an experiment in which the level of mitotic activity was determined at different times after one (control) and two (experiment) PH (Fig. 3): in the experimental animals the number of mitoses was greater than in the control animals 4 and 5 days after the first operation.

Repeated PH performed at short times after the first operation thus delays the cells of the regenerating rat liver in the G_2 period by approximately 2 h, it has hardly any effect on the duration of stay in the S period, and it delays by 6-8 h the cells in the G_1 period of the mitotic cycle. Since the mock repeated PH had mainly an analogous action of preparation of the cells for division, it can be concluded that the effect of the repeated PH on the mitotic cycle of the regenerating liver cells is due primarily to operation stress. The results are in agreement with statements in the literature that adrenal hormones act on the mitotic cycle of regenerating liver cells [1, 3]. The repeated PH is an additional stimulus to division for cells of the regenerating liver and it influences the character of the regeneration process as a whole.

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