SHORTENING OF THE PREREPLICATIVE PERIOD OF THE MITOTIC CYCLE OF HEPATOCYTES DURING REPEATED STIMULATION OF DIVISION

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Repeated stimulation of division at a short interval (2-3 days) after the first division causes shortening of the prereplicative period of the mitotic cycle of hepatocytes in the regenerating rat liver to 9-10 h. Cells dividing a second time after one stimulation passed through the G_1 period of the second mitotic cycle in the same length of time. It is suggested that cells with the minimal duration of the prereplicative period do not pass through a period of transformation. With an increase in the time between successive stimulations of division (to 4-5 days) the duration of stay in a resting state was increased for most hepatocytes and they lost their ability to maintain a shortened prereplicative period.

KEY WORDS: mitotic cycle; regenerating liver; prereplicative period.

In a number of papers published recently, describing in vivo investigations, the authors found shortening of the prereplicative period in cells of the salivary glands and kidneys during repeated stimulation of division [7, 9-11, 14, 15]. The prereplicative period was lengthened again if the interval between successive stimulations of division was increased [11]. The prereplicative period of cells and system has been studied in more detail in vitro. After stimulation of division in cell cultures spending different lengths of time in the stationary phase of growth it was shown that:

1) induction of proliferation induced structural and functional changes in the chromatin of the resting cell [5, 6, 8, 12]; the entry into the G_1 period of the mitotic cycle was preceded by a "residual G_0 phase" [6, 8] or by a "period of transformation" [5];
2) with an increase in the length of stay in the resting state the first prereplicative period of the cells when stimulated to divide was lengthened [6, 12];
3) the resting state itself is possibly "deepened" with time and may have different levels [6].

There is no information in the literature on the effect of repeated stimulations on the prereplicative period of cells of the regenerating rat liver. The results of experiments with fractionation of the dose of irradiation [4] suggest the existence of a period of transformation in hepatocytes when dividing for the first time after partial hepatectomy. The use of methods of kinetic analysis has shown that the G_1 period of the second mitotic cycle in repeatedly dividing hepatocytes must be shorter than the first prereplicative period [2]. Attempts to use the curve of labeled mitoses to determine the duration of the G_1 period in repeatedly dividing cells have not given clear results: some workers did not find shortening of the G_1 period in the second mitotic cycle [13], others found that the second wave of labeled mitoses was "discontinuous" and it was difficult to determine from it whether or not the G_1 period was changed [3].

The object of the present investigation was to study the effect of repeated stimulation on the duration of passage through the prereplicative period by regenerating rat liver cells and to determine the duration of the G_1 period of cells dividing repeatedly after a single resection of the liver.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160-180 g. The first partial hepatectomy (PHE 1), to the extent of 70%, was performed by the usual method. Cells stimulated to divide by

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TABLE 1. Changes in MI (in ‰) after PHE 2 and Mock Repeated Operations at Various Intervals after PHE 1 (M ± m) and in Control

Index	MI							
	interval between PHE 1 and PHE 2, days							
	2		3		4		5	
	time of sacrifice after PHE 2, h							
	20	24	20	24	20	24	20	24
PHE 2	37,6±13,7	29,1±18,0	14,3±7,0	10,3±4,5	1,4±1,2	20,0±16,6	3,3±3,8	12,9±7,9
Mock repeated operation	8,7±4,3	17,1±6,0	3,9±3,6	4,0±2,9	0,9±0,4	1,2±1,3	0,1±0,2	0,2±0,4
Difference in MI after PHE 2 and after mock repeated operation	28,9	12,0	10,4	6,3	0,5	18,9	3,1	12,7
PHE 1 (control)	20,8±14,5	21,2±4,0	7,7±4,4	4,6±1,1	1,0±1,0	1,5±1,3	1,1±	0,2±0,4

Legend. PHE 1 was performed simultaneously in all groups of rats and rats in group PHE 1 were sacrificed simultaneously with the rest.

PHE 1 will be described as primarily stimulated. At the second operation (PHE 2) the right lateral lobe, accounting for 47% of the weight of the liver remaining after PHE 1, was removed. PHE 2 was performed at an interval of 2, 3, 4, or 5 days after PHE 1. The animals were killed 20 h (8-20 rats at this time) and 24 h (6-14 rats at this time) after PHE 2 (at 8 a.m.). The second operation performed on animals of the second group at the same times was a mock operation (8-16 rats at each time), and these animals were killed simultaneously with those undergoing PHE 2. The rats of the third group were subjected to PHE 1 only, but were killed at different times after the operation (2-5 days + 20-24 h) simultaneously with those undergoing PHE 2. The level of mitotic activity (MI) was determined by counting 4000-6000 cells in each rat and was expressed in promille. To determine the duration of the mitotic cycle of cells dividing repeatedly after PHE 1, [^{3}H]thymidine was injected (0.15-0.2 μCi/g body weight) into the animals 20 h after the operation, and the animals were killed at 2-hourly intervals for 30 h (three rats at each time).

Histological sections of the liver were coated with type M liquid emulsion and exposed for 1.5 months. The proportion of labeled mitoses (in %) was determined by counting 50 mitoses in each rat.

EXPERIMENTAL RESULTS

The changes in MI 20 and 24 h after PHE 2 and the mock repeated operation, performed at different intervals after PHE 1, are given in Table 1.

As Table 1 shows, 20 h after repeated stimulation, applied 2 and 3 days after PHE 1, a high level of mitotic activity was observed; it was much higher than MI in animals undergoing the mock operation (in both cases $P < 0.001$) and also than MI after PHE 1.

PHE 2 was not only an additional stimulus to divide, but also acted as a stressor on the primarily stimulated cells, which were still sufficiently numerous in the regenerating rat liver 2-3 days after PHE 1. An additional experiment showed that PHE 2 and the mock repeated operation (3 days after PHE 1) inhibited cells in the G_1 period equally: 15 h after the procedure the mitotic activity in both cases was equally depressed: $0.4 \pm 0.3‰$ and $0.9 \pm 0.5‰$ respectively (MI in the control was $6.1 \pm 2.1‰$). After removal of the inhibitory action of stress, the equal number of cells held up as a result of it in the G_1 period must then have divided both in the case of PHE 2 and after the mock repeated operation. Consequently, the difference in MI 20 h after PHE 2 and after the mock repeated operation (Table 1) characterized division of the secondarily stimulated cells, which passed through the mitotic cycle in the minimal time (for rats weighing 160-180 g), i.e., in 20 h.

The total duration of ($S + G_2 + \frac{1}{2}M$) periods was determined for cells dividing 20 h after PHE 2, performed 3 days after PHE 1. For this purpose, [^{3}H]thymidine was injected into the animals 14, 12, 10, and 9 h before sacrifice. It was found that the secondarily stimulated cells passed through ($S + G_2 + \frac{1}{2}M$) in about 10 h. Consequently, the prereplicative period in these cells accounted for approximately 9-10 h.

In another experiment the mitotic cycle of cells dividing repeatedly after a single stimulation of division was determined. To plot the curve of labeled mitoses [^{3}H]thymidine was injected into the animals 20 h after PHE 1, and the animals were subsequently killed every 2 h for 30 h. The duration of the mitotic cycle was determined from the time intervals between the symmetrical points on the ascending limbs of the curve of labeled mitoses. As Fig. 1 shows, the mitotic cycle of the repeatedly dividing cells was about 19.5 h and they passed through the G_1 period in 9-10 h. Consequently, the prereplicative period of cells dividing repeatedly after PHE 1 had the same minimal duration as in cells stimulated to divide secondarily. After a 70% PHE, DNA synthesis in the cells of the regenerating liver of rats (weighing 160-180 g) is known not to begin until after at least 12-14 h. MI 20 h after a 50% or 70% PHE remained (in the present experiments) at a very low level: $0.7 \pm 0.6‰$ and $0.5 \pm 0.3‰$ respectively. As the results given show, 20 h after repeated stimulation, especially after an interval of 2 days, a high proportion of the secondarily stimulated cells had divided (Table 1). Consequently, shortening of the prereplicative period in these cells compared with the first prereplicative period after PHE 1 is a possibility. In cells dividing repeatedly after primary stimulation the duration of the G_1 period of the second mitotic cycle also was shorter than their first prereplicative period. Data in the literature [4] suggest that cells with the minimal duration of the G_1 period do not pass through a period of transformation on recommencing the mitotic cycle. These results agree with the observed effect of repeated stimulation of division on the prereplicative period in salivary gland and kidney cells [7, 9-11, 14, 15].

It can be concluded from the results in Table 1 that with an increase in the period between consecutive stimulations of division in the regenerating rat liver the absolute number of secondarily stimulated cells, which pass through the mitotic cycle in the minimal time, is reduced. This fact can evidently be explained as follows. By the end of the second day of regeneration, there are many cells in the rat liver which have just divided, and

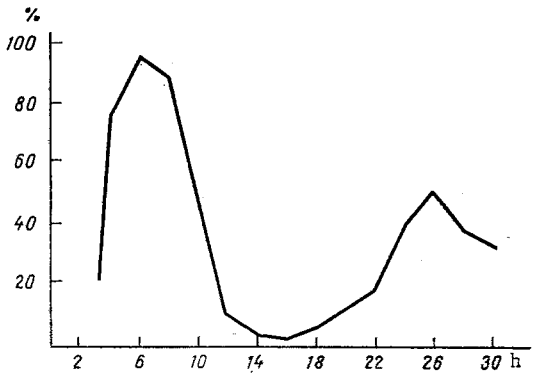

Fig. 1. Changes in percentage of labeled mitoses in regenerating liver of rats at different times after injection of [^{3}H] thymidine after 70% partial hepatectomy. Abscissa, time (in h) after injection of [^{3}H] thymidine; ordinate, percentage of labeled mitoses.

of this number some can divide a second time [1] whereas the rest pass into a resting state. Repeated (after 2 days) stimulation of division compels these cells to emerge from the resting state into the mitotic cycle, which they complete in the minimal time.

With an increase in the period of regeneration more and more cells which have passed into the resting state accumulate in the liver. The resting state for most hepatocytes is evidently so prolonged that if stimulation of division is repeated after 4-5 days only a very small proportion of them can pass through the mitotic cycle in the minimal time. It is also possible that the resting state in the regenerating liver is variable and changes (deepens) with time, as has been shown for other cells [6, 11]. After repeated stimulation of regenerating rat liver cells they evidently pass from the state of relatively prolonged rest through a period of transformation. If the period between the two consecutive stimulations of division is increased, the prereplicative period of the secondarily stimulated cells is therefore lengthened. A similar change in the duration of the prereplicative period has also been observed for salivary gland cells [11].

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