KINETICS OF CELL PROLIFERATION IN THE REGENERATING RAT LIVER AFTER SUBTOTAL RESECTION

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Between 78 and 82% of the weight of the liver was resected in noninbred male rats. Maximal mitotic activity of the hepatocytes was observed 38-48 h after the operation. Pulse labeling with [^{3}H]-thymidine showed that the G_2 period is 6 h and the minimal duration of the S period is also 6 h. During regeneration of the liver after subtotal resection asynchronization of the entry of the hepatocytes into the S period and mitosis and delay of the cells in the G_1 and G_2 periods were observed. The above-mentioned changes in proliferation of the hepatocytes are the result of a disturbance of intracellular metabolism in response to an extremal functional demand.

KEY WORDS: regeneration; cell cycle; mitotic regime; subtotal resection.

The level of proliferative activity of the hepatocytes is known to depend to some degree on the extent of resection: The larger the part of the liver removed at operation, the higher the proliferative pool of cells of the regenerating organ [7, 10]. In the course of regeneration of the liver in rats 17-20 h after removal of two-thirds of the organ by the method of Higgins and Anderson [9] 30% of the hepatocytes enter the S period synchronously and start to synthesize DNA [13]. However, after subtotal hepatectomy (removal of 78-82% of the weight of the liver) desynchronization of hepatocyte proliferation is observed, and after removal of more than 90% of the weight of the organ most of the hepatocytes of the residual fragment are no longer capable of synthesizing DNA and of dividing by mitosis [6, 14, 15]. The reasons for this change in the proliferative activity of the hepatocytes after subtotal hepatectomy are unknown.

The object of this investigation was to study the dynamics of proliferation of hepatocytes after subtotal resection and to determine how metabolic overloads arising as a result of this type of operation affect the cell cycle of the hepatocytes.

EXPERIMENTAL METHOD

In the course of a one-stage operation on noninbred male albino rats weighing 250-350 g, between 10 a.m. and 12.30 p.m., the central, left, and right lateral lobes of the liver, amounting on average to 78-82% of the total mass of the organ, were removed. [^{3}H]-thymidine was injected intraperitoneally as a single dose of 0.5 μCi/g body weight into all the experimental animals 23-24 h after the operation. The rats were killed in groups of 2 or 3 every 2 h during the 24 h after injection of the isotope. The liver was fixed in Carnoy's fluid, embedded in paraffin wax, and sections cut to a thickness of 5-7 μ were coated with type R emulsion (Photographic Chemical Research Institute) and exposed for 3 weeks. After development the sections were stained with hematoxylin-eosin. The mitotic index (MI) was determined by counting the number of mitoses in 2000-6000 hepatocytes. The percentage of labeled mitoses was determined by examination of 50-140 mitoses in each animal. The duration of the cell cycle was determined by the usual method [3].

EXPERIMENTAL RESULTS

Comparatively few animals were found to survive after subtotal resection of the liver. During the first 48 h after the operation 40 of the 77 hepatectomized rats died. Examination of the operative field at autopsy revealed no errors of operative technique: The ligatures tied around the roots of the lobes were in place and no bleeding was found. The animals perhaps died after subtotal hepatectomy as a result of the development of acute hepatic failure. After removal of 66-68% of the weight of the liver, as a rule, all the animals survived [9, 13], and their state in the late periods after the operation was satisfactory. With the results of the present

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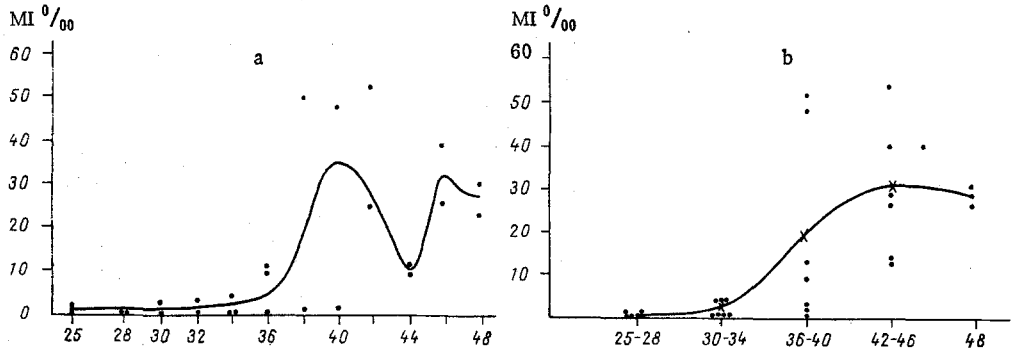

Fig. 1. Changes in MI in regenerating liver at various times after subtotal resection: a) values of MI in liver of animals killed at 2-h intervals after operation; b) pooled values of MI in liver of same animals at intervals of 4-6 h after operation. Abscissa, time after operation (in h); ordinate, values of MI (in ‰).

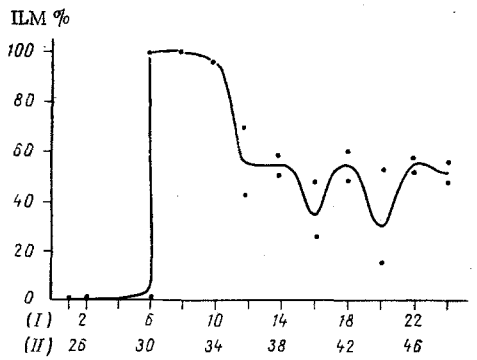

Fig. 2. Changes in percentage of labeled mitoses (ILM) in hepatocytes at various times after injection of [^{3}H]-thymidine. Abscissa, time (in h) after injection of [^{3}H]-thymidine (I) and after operation (II); ordinate, number of labeled mitoses, in %.

experiments in mind it can be concluded that the allowable limit for resection of the liver in mammals (using survival as the criterion, is removal of 80% of the weight of the organ.

Subtotal resection of the liver did not prevent its regeneration in the surviving animals. However, the dynamics of the increase in the number of mitoses in the regenerating liver in this case differed appreciably from the dynamics of proliferation of hepatocytes in the liver after removal of two-thirds of the organ. The complete absence of mitoses in the liver of many of the animals even 30-36 h after subtotal resection was noted (Fig. 1). The highest values of MI in most of the experimental rats were observed 36-48 h after the operation (Fig. 1b). After removal of 66-68% of the weight of the liver in sexually mature rats of the same body weight the highest mitotic activity of the hepatocytes occurred 28-30 h after the operation [5, 10, 13]. Delay of entry of the hepatocytes into the S period by 10 h or more, followed by delayed entry into mitosis after subtotal resection of the liver was observed in rats by Weinbren [14, 15]. Consequently, lengthening of the period between the operation and the time of maximal mitotic activity of the hepatocytes can be considered to be a regular phenomenon after subtotal resection.

The second distinguishing feature of the regenerative response of the liver after subtotal resection was the presence of considerable individual variations in the time of entry of the cells both into the S period and into mitosis. The large fluctuations of MI (from 0 to 60‰) in the different animals point to desynchronization of the entry of the hepatocytes into mitosis. A parallel analysis of the dynamics of mitotic activity and of the duration of individual periods of the cell cycle showed that accumulation of cells in the G_2 period capable of subsequently entering into mitosis synchronously did not take place; in none of the animals was the level of proliferation of the hepatocytes above 50-60‰. A similar response of the hepatocytes was observed during regeneration of the liver in mice [8], when under the influence of further resection the maximal mitotic activity did not increase, but remained the same (30‰) as after removal of two-thirds of the liver. The maximal num-

ber of hepatocytes dividing simultaneously by mitosis is thus a fairly constant parameter for the regenerating liver, and hyperfunction may play the role of desynchronizer of the entry of the hepatocytes into mitosis.

The individual periods of the cell cycle could be determined by analysis of the curve of labeled mitoses (Fig. 2). The minimal duration of the S period was 6 h and the duration of the G_2 period was increased to 6 h. A special feature of the curve of labeled mitoses of the hepatocytes of the regenerating liver after subtotal resection was the absence of any decrease in the percentage of labeled mitoses in the late periods after injection of [^{3}H]-thymidine (Fig. 2). Starting from 12 h after injection of the isotope (36 h after the operation) and until the end of the experiments (48 h after the operation) the number of labeled mitoses remained at approximately the same level (50-60%).

The duration of the G_2 and S periods is known to be fairly constant: For most mammalian cells the S period is 6-8 h, and the G_2 period 3-5 h [11, 12]. For the hepatocytes of the regenerating liver after removal of 66-68% of the weight of the organ the G_2 period is 3 h [13]. At the same time, it has been shown that there are two critical periods in the life cycle of the cell: the moment of transition from the G_1 to the S period, when duplication of the chromosomes begins, and from the G_2 period to mitosis. The signal determining the onset of mitosis is unknown. However, several factors (irradiation, hormones) which can block cells in the G_2 period and so prevent their entry into mitosis have been discovered [2, 4, 5]. The development of a block in the G_2 period may be connected not only with exogenous factors, but also with a change in the intracellular synthesis of protein and RNA [1] or a change in the concentration of substances of the chalone type.

Changes in the dynamics of proliferative activity of the hepatocytes after subtotal resection of the liver, delay of entry of the cells into the S period in some animals, and lengthening of the G_2 period were probably due to disturbances of intracellular metabolism after this type of operation. It can tentatively be suggested that these disturbances are based both on the inhibition of RNA transcription at individual stages of the cell cycle and on a reduction in the intracellular reserves of proteins functionally connected with mitosis. It can be postulated that during metabolic overloads such as those which follow subtotal resection of the liver it is likely that a block will develop in the G_2 period, which the writers have called the metabolic period.

To sum up the results obtained the following conclusions can be drawn: 1) An excessive functional load on the liver after subtotal resection is not a stimulus for increased proliferation of hepatocytes; 2) hyperfunction after reparative regeneration of the liver leads to desynchronization of the entry of the hepatocytes into the S period and mitosis; 3) hyperfunction promotes lengthening of the G_2 period.

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