

# REGENERATION AND GROWTH OF THE LIVER IN 3-WEEK-OLD MICE OF DIFFERENT STRAINS

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At the age of 3 weeks, C57BL mice have comparatively low proliferative activity of their hepatocytes when the liver grows normally, but also during regeneration of the liver 44 h after its extensive resection (mitotic index 16‰). Animals of the same age but of other strains (noninbred, CBA, and CC57BR), however, in most cases have a higher mitotic index of their hepatocytes both under normal conditions and during regeneration of the liver (42, 70, and 60‰). This pattern of interlinear differences in mitotic activity of the hepatocytes during growth and regeneration of the liver was still found 7 days after the beginning of the experiment. The results indicate genetic determination of the level of proliferative activity of cells.

KEY WORDS: mitotic index; growth and regeneration of the liver; interlinear differences.

Recent investigations have clearly shown that the cytological features of the response of the liver in adult mice of different strains to injury are determined by differences of genotype [1, 3, 6, 7]. For a final solution to the problem of whether the observed differences are connected with genetically determined properties of a particular strain of animals, it is important to know the character of the response of the liver to injury not only in adult forms, but also in animals in the earlier stages of individual development.

Establishment of the periods when genetically determined properties are expressed is also important in connection with the study of problems arising during research when animals of different strains and different ages are used in the same experiment [2].

It was accordingly decided to study the character of regeneration of the liver in mice of different strains after partial hepatectomy in the early postnatal period.

## EXPERIMENTAL METHOD

Mice of both sexes, aged 3 weeks, noninbred or of strains CBA, CC57BR, and C57BL, were used. Earlier than this age mice (especially C57BL) tolerate extensive resection of the liver badly; a high proportion of the animals die, especially 1-2 days after the operation. Even with mice aged 3 weeks it was often necessary to repeat the operation on several occasions in order to be able to choose a sufficient number of animals (especially C57BL mice). Each of the four groups of mice used in the investigation therefore consisted of at least 25 experimental and 15 control animals. Partial hepatectomy was performed by the method of Higgins and Anderson (removal of 68-70% of the liver) on the experimental animals. The liver of the experimental and intact animals was fixed at 9 a.m. in Carnoy's fluid 44 h and 7 days after the operation, i.e., at the time of maximal expression of proliferative activity of mouse hepatocytes [1, 2, 4] and in a period of almost complete restoration of the weight of the regenerating organ [1, 4]. The degree of increase in weight of the liver and also the level of proliferative activity of its cells were used as criteria of regeneration and growth of the organ.

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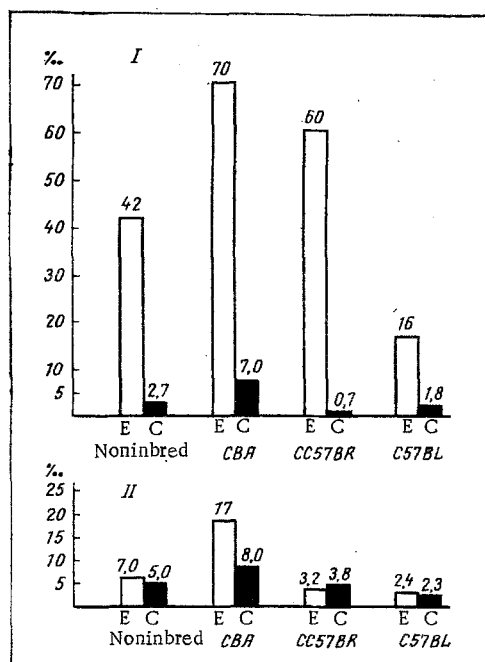


Fig. 1. Proliferative activity of hepatocytes in liver of 3-week-old mice of different strains under normal conditions and at various times after partial hepatectomy: I) 44 h, II) 7 days after, E) Experiment, C) control. Ordinate, mitotic index of hepatocytes (in  $\%$ ).

The liver of 116 experimental and 72 control animals was examined histologically. Paraffin sections 6-7  $\mu$  thick were stained with hematoxylin-eosin. The number of mitoses was counted in 3000-4000 hepatocytes and the values obtained (mitotic index) were expressed in promille.

The significance of differences between individual indices was determined by the Fisher-Student criterion.

## EXPERIMENTAL RESULTS

In a previous investigation [1] of regeneration of the liver in adult mice of different strains they were found to respond differently to the operation. Similar results were obtained in the present experiments. Noninbred mice and CBA mice were found to tolerate partial hepatectomy best (their survival rate was 60-70%); C57BL mice tolerated the operation worst (survival rate about 20%). In the early stages of postnatal development, at the age of 3 weeks, C57BL mice thus behaved differently from the animals of the other strains.

Interlinear differences also affected normal growth and regeneration of the liver in mice aged 3 weeks. Differences were found both in the rate of increase of mass of the liver during regeneration and growth and in the level of proliferative activity of the hepatocytes in mice of the different strains. For instance, the index of restoration of weight of the liver (the relative weight of the liver in the experimental series as a fraction of the relative weight of the liver in the control series, expressed in per cent) differed in the mice of different strains both 44 h and 7 days after the operation. The index of restoration of weight of the liver 44 h after partial hepatectomy was 70% in noninbred mice aged 3 weeks, 71% in CBA mice (I), 75% in CC57BR mice (II), and 60% in C57BL mice (III) ( $P_{I-III}=0.84$ ,  $P_{II-III}=0.0001$ ). Seven days later this index in the noninbred mice and the mice of the three strains used was 93, 90, 96 (III) and 80% (IV), respectively ( $P_{III-IV}=0.0001$ ).

The mitotic index of the hepatocytes of 3-week-old noninbred mice and of the CBA, CC57BR, and C57BL mice 44 h after partial hepatectomy was 42, 70, 60, and 16  $\%$  respectively, and 7 days after the operation it was 7.17 (I), 3.2 (III), and 2.4  $\%$  (III), respectively ( $P_{II-III}=0.99$ ). It follows from these

values of mitotic activity of the hepatocytes in the regenerating liver of the young mice that the liver of C57BL mice possessed the lowest level of proliferation.

Another very important fact deserves attention. It was found that the liver of 3-week-old C57BL mice during the period of normal growth also had a lower level of proliferation than the growing liver of 3-week-old CBA or noninbred mice (Fig. 1). This fact suggests that the degree of proliferative activity of the hepatocytes in the growing liver under normal conditions (in mice of the strains used) determines the level of cell proliferation during regeneration of that organ. The validity of this hypothesis becomes particularly evident when diagrams showing the level of proliferative activity of the hepatocytes in the growing and regenerating liver 7 days after operation are analyzed (Fig. 1).

On the whole the following conclusions can be drawn from the results. First, differences in the degree of response of mice of the different strains to injury to the liver are unquestionably a genetically determined feature, which evidently is manifested very early in ontogeny of mice. Second, the intensity of growth of the liver in the early stages of postnatal ontogeny and the degree of proliferation of its cells largely determine both the rate of its regeneration and the level of mitotic activity of its cells after resection. At the same time, although the cellular mechanisms or pathways used for growth and regeneration of organs are similar in several respects [5], the degree to which they are used is largely determined by hereditary factors.

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