INDIVIDUAL VARIATIONS IN THE COURSE OF PROLIFERATION IN THE REGENERATING MOUSE LIVER

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Thymidine-H^3 was injected every 4 h into mice for 112 h starting from 34 h after removal of two-thirds of the liver. Individual variations in the number of labeled nuclei in the regenerating liver were less marked than usual but still remained significant. Hyperplasia and hypertrophy during regeneration of the liver evidently differ in intensity in individual mice. No correlation was found between the gain in weight of the regenerating liver and the index of labeled nuclei or the degree of hypertrophy of the hepatocytes in individual mice.

A previous investigation [3] showed that considerable individual variations in the level of DNA synthesis and mitotic activity occur in the regenerating mouse liver. The level of DNA synthesis was determined by injecting thymidine-H^3 into mice four times a day starting 18 h after the operation.

The problem arose whether the individual variations found in the indices of proliferation occurred because regeneration of the liver takes place in individual mice at different levels of proliferation or whether they are due to differences in the times of intensification of DNA synthesis after the operation in different individuals. To rule out this last possibility experiments were carried out in which thymidine-H^3 was injected later and for longer periods into mice.

EXPERIMENTAL METHOD

Two-thirds of the liver was removed from male (CBA × C57BL) F_1 hybrid mice weighing 15-20 g at 2-3 p.m. In series I injections of thymidine-H^3 into the mice began 34 h after the operation and continued at intervals of 6 h in a dose of 0.3 μCi/g. Altogether 12 injections were given over three days. In series II and III the isotope was injected only four times into the mice, the first time in series II 34 h after the operation and in series III 58 h after the operation, so that the time of injection of the isotope in these series corresponded to the first and second days of its administration in series I.

All the mice were sacrificed 12 h after the last injection at 6-7 a.m., which was 112, 64, and 88 h respectively after the operation. The liver was fixed by Carnoy's method. Sections 5μ in thickness were coated with type M emulsion, exposed for 20 days, and stained with Meyer's hematoxylin. On the day of the operation the mice were weighed and individually labeled. The lobes of the liver regenerating after resection were weighed in the fixed state, in order to verify the completeness of removal of the central and left lobes. The initial weight of the lobes remaining after the operation and the gain in weight as a result of regeneration were calculated on the basis of these results. In each case 50-80 fields of vision of the autoradiographs were examined and the number of labeled and unlabeled hepatocyte nuclei in the field of vision counted separately. The combined total was 1000. The index of labeled nuclei (ILN) was expressed in percent, and the mitotic index (MI) per 1000. In addition, in series I and II the diameter of 100 nuclei in each case was measured with an ocular micrometer. Pieces of liver removed at the operation were used as the control.

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TABLE 1. Change in Weight, ILN, and MI in the Regenerating Mouse Liver

Series No.	Number of mice	Time of injection of isotope (h after operation)	Time of sacrifice after operation (in h)	Relative weight of resected lobes of the liver	Relative weight of regenerating liver	Weight gain (in %)	ILN (in %)	MI (in ‰)
I	9	34—100	112	3,38±0,14	4,56±0,19	171,4±13,4	66,5±4,2	4,5±1,5
II	11	34—52	64	3,54±0,10	3,74±0,21	113,3±13,3	48,8±4,3	10,6±3,0
III	9	58—76	88	3,59±0,15	4,75±0,33	163,3±18,7	30,6±3,9	7,3±1,66

EXPERIMENTAL RESULTS

The main results are given in Table 1. In series I, in which the isotope was injected into the animals for three days, 70% of the nuclei were labeled. Most nuclei synthesized DNA from the 34th to the 52nd hour (see the results of series II), and rather fewer did so during the next day (see the results of series III). The number of cells entering the period of DNA synthesis thereafter fell sharply. This is shown by the fact that the combined total of labeled nuclei in series II and III was practically indistinguishable from their number in series I. If these results are compared with those of the previous investigation [3], it will be noted that with a change in the times of injection of the isotope to a later period (from 18-36 h to 34-52 h after the operation) not only was ILN increased (from 37.1 to 48.8%), but the individual variation also was considerably reduced. The coefficient of variation in the previous experiments was 64% but in the present experiments 29.1%. This means that individual variations in ILN in the regenerating liver were due partly to asynchronism of the beginning of activation of DNA synthesis in the different mice after hepatectomy.

The gradual equalization of the course of regeneration was also shown by changes in the relative weight of the liver. Although in the mice of series III sacrificed 88 h after the operation the relative weight of the liver was virtually indistinguishable from the relative weight of the liver in the mice of series I sacrificed 24 h later, the coefficient of variation of the relative weight by this time had fallen considerably (from 20.9 to 12.7). The variation in restoration of weight of the regenerating liver in individual animals was somewhat reduced in the course of regenerating of the organ. On the other hand, however, even when 12 injections of the isotope were given over a period of three days, the individual variations in ILN still remained, and in the mice of series I it varied from 33 to 87%. Individual variations in MI at all three consecutive periods (64, 88, and 112 h) were very great. The coefficient of variation was 94.4, 68.2, and 100% respectively.

The question arises whether there is any correlation between the level of proliferation and restoration of weight of the liver in individual mice. Whereas in the previous investigation [3], in which thymidine-H^3 was injected at the beginning of the period of activation of DNA synthesis, some degree of correlation, although only slight, was found between these indices (coefficient of correlation 50), in the present experiments no such correlation could be found in any of the series. The coefficient of correlation between ILN and the gain in weight 64 h after the operation was + 44, after 88 h it was + 0.08, but after 112 h it was − 0.03. The same pattern in principle was observed when ILN was compared with the absolute and relative weights of the regenerating liver. It is interesting to note that no correlation could be found between the size of the regenerating liver and indices of hypertrophy such as the increase in volume of the hepatocyte nuclei (in series I it increased by 81% over the intial control) and the number of cells in the field of vision (which was reduced by almost half in series I). No definite relationship could be found between the variation in these indices and the original weight of the mice. This result can evidently be attributed to variation in the manifestation of proliferation and polyploidy (which are known [1, 5, 6] to be responsible for regeneration of the liver in mice) in individual animals at different times of regeneration. Despite these definite patterns in the course of regeneration of the liver revealed by investigation of groups of animals, regeneration of the liver in each animal takes place very individually. As an example of the individual reaction of the liver to trauma, the distribution of zones of increased proliferation in the lobules of the regenerating liver can be cited. Most workers [7, 8] consider that activation of division of the liver cells begins from the periphery of the lobules. During the first 40 h after the operation most cells synthesizing DNA were found at the periphery of the lobules [7]. By 50 h the distribution was regular, and later most labeled nuclei were found in the center of the lobules. During repeated injection of thymidine-H^3 in the period from 18 to 36 h

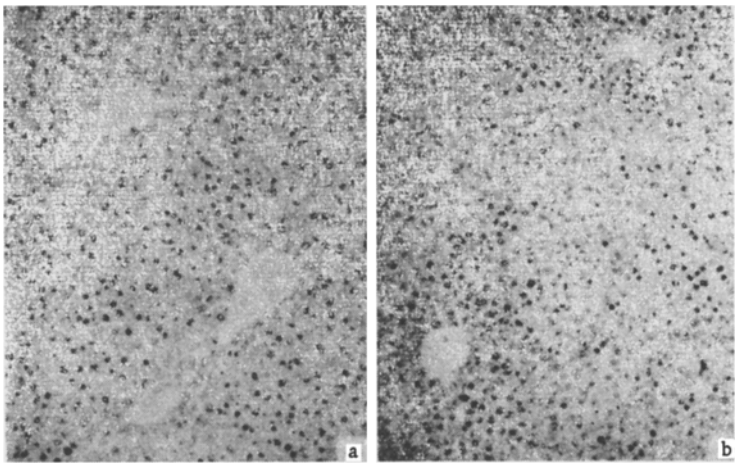

Fig. 1. Differences in localization of labeled nuclei in lobules of regenerating liver after injection of thymidine-H^3 by the same scheme (18, 24, 30, and 36 h after operation), ×100: a) pattern observed most frequently; b) pattern observed less frequently.

after the operation it was found that in most cases (eight of 15) such a pattern in fact was present, but in some mice labeled cells were arranged uniformly over the lobules or predominantly in the middle zone, while in three cases they were definitely predominant in the center of the lobules (Fig. 1).

Since the operation was carried out on young growing animals, an explanation of the differences in their reaction to trauma should possibly be sought in a difference of phase of the proliferative activity in the organ at the time of operation. Markelova [2], for instance, in an earlier investigation, obtained results showing that growth of the liver occurs rhythmically and not uniformly. Milyutina [4] also concluded that proliferation in the liver is pulsed in character. This, of course, is only one of the many possible explanations of individual variations in the response of the mouse liver to trauma.

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