INDIVIDUAL VARIATIONS IN DNA SYNTHESIS IN THE REGENERATING MOUSE LIVER

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The number of labeled nuclei and mitoses and the increase in weight of the liver were determined in (CBA × C57Bl) mice 45 and 65 h after removal of two-thirds of the liver. After the operation the mice received 4 injections of thymidine-H^3 (after 18, 24, 30, and 36 h). Marked variation in the number of labeled nuclei and mitoses and also in the increase in weight of the liver was observed among individual mice. A correlation was found between these values, indicating differences in the rate of proliferation in individual animals persisting for longer than 24 h.

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Attention was drawn long ago to marked variations of the mitotic index (MI) in the regenerating mouse liver [1]. Later one of the writers [2] postulated that variations of this type can be attributed to the existence of periodic changes in the level of proliferative activity in certain organs: changes occurring over a longer period of time than diurnal fluctuations. Their nature has not yet been explained.

It was therefore necessary to investigate variations in the level of DNA synthesis preceding mitosis, the value of MI, and the increase in weight of the regenerating liver in individual mice, for a comparison of these three indices of regeneration can provide a more complete picture of the variation in its manifestations in individual animals.

EXPERIMENTAL METHOD

Two-thirds of the liver was removed from F_1 hybrid mice (CBA × C57Bl) weighing 18-20 g by the method of Higgins and Anderson originally suggested for rats. The operation was carried out between 11 a.m. and 12 noon (Table 1). The following day, 18, 24, 30, and 36 h after the operation, i.e., at 6 a.m., noon, 6 p.m. and midnight, the mice received an intraperitoneal injection of thymidine-H^3 in a dose of 0.7 μCi/g body weight. The object of these repeated injections of thymidine was to detect as many nuclei as possible synthesizing DNA in the course of the 24-h period, and thus to discover if differences between individual animals tend to disappear under these circumstances. The mice were sacrificed 45 and 69 h after the operation, at 9 a.m., when an increase in mitotic activity is usually observed. The liver was fixed in Carnoy's fluid. Type R (NIKFI manufacture) nuclear emulsion was applied to sections 5 μ in thickness. Exposure lasted 19 days. The number of labeled nuclei in 1500-2000 cells and the number of mitoses in 5000-7000 cells were counted in sections stained with Mayer's hematoxylin. The removed lobes of the liver were weighed at the time of operation, and the regenerating liver was weighed at autopsy. Since the same weight of liver (67%) was always removed, it was easy to calculate the approximate weight of liver remaining after the operation and its increase in weight during the experiment.

EXPERIMENTAL RESULTS

Six mice were sacrificed before the last injection of thymidine-H^3 (at midnight) in order to determine what incorporation of thymidine-H^3 had taken place into the liver cells before this time. The mean number

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TABLE 1. Mean Indices of Proliferation in Regenerating Mouse Liver

Days of experiment		1	2	3	4	5
Character of procedure		Operation	Injection of thymidine-H^3	—	—	Control
Time of procedure	Time of day	11—12	6 12 18 24			
	Hours after operation	0	18 24 30 36			
Time of sacrifice of mice	Time of day		23	9	9	9
	Hours after operation		35	45	69	—
Index of labeled nuclei (in %)			2,01 ±2,3	37,1 ±6,2	47,0 ±5,7	0,06 ±0,03
Mitotic index (in %)			0,17 ±0,01	35,3 ±10,6	15,7 ±4,9	0,04 ±0,02
Number of silver grains	above nucleus			37,1	14,3	16,9
	above mitosis			41,0	7,3	

of labeled nuclei in these mice was 2.01%, i.e., DNA synthesis preceding the morning rise in mitotic activity in the regenerating liver was still slight in degree. In mice sacrificed at the next period, 45 h after the operation, 37.1% of labeled nuclei could be counted (Table 1), i.e., the last injection of isotope coincided with a sharp increase in DNA synthesis in the liver cells. The mitotic index calculated 45 h after the operation was 35.3%. However, it must be remembered that the number of mitoses reflects their actual number at a given time, while the number of labeled nuclei relates to the period of injection of thymidine (during the 9 h before sacrifice).

Sharp variations were found in the number of labeled nuclei in individual mice (Table 2)—from 0.8 to 76.3%. In other words, the level of DNA synthesis differed in different mice, and the differences were very considerable: from the almost total absence of synthesis to participation of most nuclei in synthesis. The number of mitoses also varied sharply from one mouse to another—from 0 to 167%.

It is interesting to note that, although no direct relationship exists between the level of DNA synthesis in individual mice and the number of mitoses found, there is nevertheless a definite tendency toward a higher index of labeling in mice with a higher number of mitoses. The coefficient of correlation between the number of mitoses in individual mice and the number of labeled nuclei was 0.63 (P = 0.99). A less close correlation was observed between the index of labeling and the increase in weight of the liver in individual mice, the coefficient of correlation in this case being 0.50 (P = 0.93).

All mitoses in the liver of mice sacrificed at this period were labeled, which is not surprising because thymidine-H^3 was injected 9 h before sacrifice, when the liver cells were in the S period.

The number of labeled nuclei in mice sacrificed at the next period, i.e., 69 h after the operation (Table 1), was 47%. A larger increase in the number of labeled nuclei over that at the preceding period might have been expected on the grounds that all labeled cells had divided. However, the possibility must also be remembered that some cells were comparatively weakly labeled even after division, when the number of grains in them was reduced by half, and they could have been taken as unlabeled. The number of mitoses 69 h after the operation was 15.7%, i.e., less than at the previous period.

Individual variations in the number of labeled nuclei and mitoses in mice sacrificed 69 h after the operation were no less marked than in the preceding group of mice (Table 3). The labeling index varied from 7.0 to 86.9% and MI from 0.0 to 75.3%. No correlation was found at this period of investigation between the index of labeling and the mitotic index. The coefficient of correlation was 0.28, i.e, no correlation was present. At the same time, correlation existed between the labeling index and the increase in weight of the liver in individual mice during regeneration, the coefficient of correlation being 0.57 (P = 0.95). The liver of intact control mice contained 0.06% of labeled nuclei and 0.04% of mitoses.

TABLE 2. Indices of Proliferation in Regenerating Liver of Individual Mice 45 h after Operation

№	Index of labeled nuclei (in %)	MI (in ‰)	Increase in weight of liver (in %)
1	0,8	0,0	6
2	7,9	12,2	40
3	12,3	0,7	—12
4	15,7	16,1	27
5	26,2	4,8	20
6	27,8	39,4	33
7	30,1	30,6	42
8	30,3	34,3	23
9	37,8	18,1	90
10	42;1	10,4	—
11	51,7	15,9	87
12	63,0	93,3	57
13	66,5	167,2	51
14	68,4	49,3	137
15	76,3	37,0	31
Mean	37,1	35,3	45

TABLE 3. Indices of Proliferation in Regenerating Liver of Individual Mice 65 h after Operation

№	Index of labeled nuclei (in %)	MI (in ‰)	Increase in weight of liver (in %)
1	7,0	15,9	75
2	16,3	6,0	32
3	22,4	75,3	20
4	36,0	15,9	47
5	37,7	39,4	85
6	39,8	2,0	87
7	48,6	0,4	—
8	49,5	13,5	80
9	50,5	2,7	—
10	52,4	11,5	140
11	55,2	0,0	35
12	56,8	6,9	133
13	61,9	18,4	84
14	83,9	18,4	90
15	86,9	8,8	123
Mean	47,0	15,7	79

Most mitoses in mice sacrificed 69 h after the operation were labeled (from 70 to 100% in individual mice), although a long time had elapsed after injection of the isotope. This phenomenon can be explained either by reutilization of thymidine-H^3 by the dividing liver cells or by repeated division of labeled cells Without additional investigation it is impossible to decide which suggestion is correct.

Correlation between the number of labeled nuclei and MI for individual mice 45 h after the operation was naturally present, indicating that division of all liver cells is preceded by DNA synthesis, taking place some 6-9 h earlier. Results obtained by Milyutina [3], who considers that the regenerating mouse liver contains a population of cells at the G_2 stage, synthesizing DNA a long time before the operation, are unconvincing because, in her experiments, she failed to take into account the fact that DNA synthesis and MI reach their maximum at different times of day.

The results obtained clearly show that sharp individual variations exist in the course of proliferative processes during regeneration of the liver in mice. It can be postulated that proliferative processes in some animals take place at a low level, and in others at a high level, and that these differences are stable in character. In other words, individual mice differ in their capacity for regeneration. On the other hand, it can be postulated that the differences demonstrated in these experiments are due to asynchronization of the onset of DNA synthesis after partial hepatectomy in individual mice, although this process ultimately follows the same course in all of them. It was impossible to solve this problem because administration of thymidine-H^3 to the mice was stopped too early. Had it been continued, it could have been shown whether increased DNA synthesis occurs in all mice in the course of time or in some of them, and also if it remains resistant. The problem can thus be solved only by further investigations.

Nevertheless, it must be remembered that in those mice in which a low level of DNA synthesis was found, the increase in weight of the liver was slight, i.e., a correlation was present between the labeling index and the increase in weight of the liver. Since this phenomenon was found 69 h (i.e., almost 3 days) after the operation, it indicates that differences between the level of proliferation in individual mice are of long duration.

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