THE EFFECT OF SHORT TERM AND CHRONIC IRRADIATION ON LIVER REGENERATION IN WHITE MICE

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It was shown earlier [5, 7, 10] that, depending on the intensity of physiological regeneration present, different tissue systems are injured to a varying degree when the animals are exposed to radiation in varying dose outputs. In contrast to such cumulatively injured tissues as muscle and bone [2, 4, 9], in tissues with manifest physiological regeneration the extent of injury decreases sharply with low output irradiation [6, 8].

The purpose of this work was to compare the reaction of the mammalian liver to irradiation with various dose outputs in relation to the level of physiological regeneration in that organ.

Using the livers of partially hepatectomized white mice as the criterion of liver regeneration, we carried out a comparison of the degree of their injury after short term and prolonged irradiation with 60^{60} γ-rays.

According to data in the literature, the mammalian liver possesses a strongly developed capacity for regeneration. In the case of its subtotal removal, doubling of the liver mass occurs in 2-3 days [11]. Regeneration of the organ is accomplished by hypertrophy of the entire liver mass; in this case, a regenerate, as such, is not formed.

EXPERIMENTAL METHOD

The effect of ionizing radiation on the regeneration of the liver was studied in adult, male, white mice, weighing 23-25 grams. We investigated the course of regeneration in animals irradiated with 60^{60}γ-rays in a dose of 1000 r, administered quickly or over a prolonged period of time (dose output in the first case was 20 r/min, while in the second case – 0.0232 r/min; duration of the irradiation was 50 minutes and 30 days respectively).

The short term irradiation was carried out on the GUT-Co-400 apparatus, and the chronic exposure – on a constantly acting set up, switched off only during feeding of the animals. Dose outputs of 0.0232 r/min were attained by placing the container with the mice at a distance of 40 cm from the source of radiation. Dosimetric measurements were carried out by the Physico-Technological Laboratory of the Institute.

In the operation (under ether narcosis), we removed the entire left lateral lobe of the liver intact, which comprised an average of approximately 30% of the total weight of the organ. To determine the rate of liver regeneration, we weighed the removed lobe, and also the remainder of the organ at various intervals after the resection. The intensity of regeneration was judged from the increase in both natural and dry weight (the liver was desiccated at 100° until the weight became constant). In a preliminary step, we determined the weight of the liver in non-operated mice of the control group. Weighing of the liver in 10 animals showed that the fluctuation in weight (both wet and dry) was minimal. The mean natural weight of the liver, in milligrams, was equal to 1168 ± 24, and the mean dry weight – 362 ± 8.7.

EXPERIMENTAL RESULTS

In the first series of experiments, we studied the rate of liver regeneration in non-irradiated mice, following the resection. In different groups of experiments, we weighed the remainder of the organ immediately after the

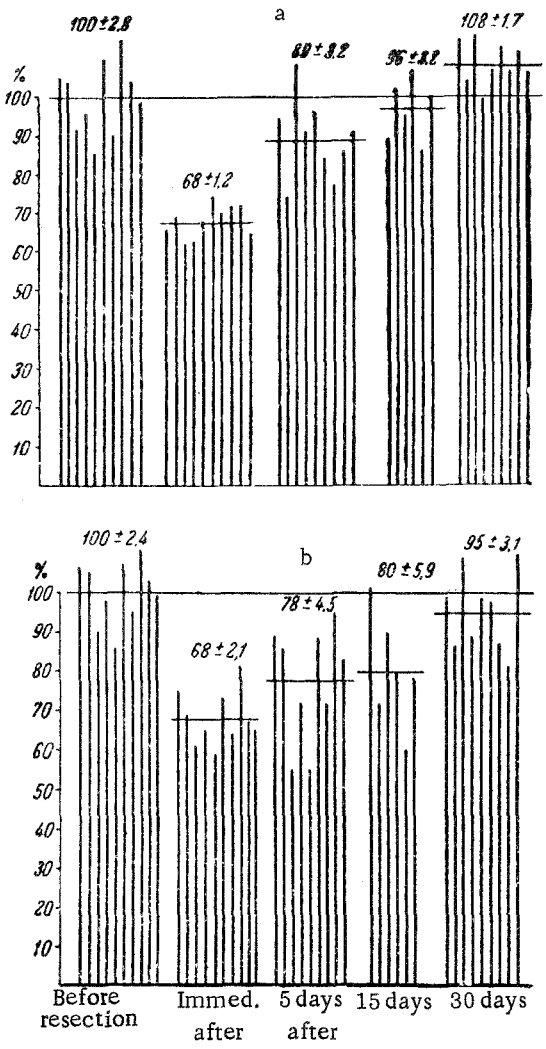

Fig. 1. Regeneration of the liver in non-irradiated mice following resection. a) moist weight of the liver; b) dry weight of the liver.

operation, and after 5, 15 and 30 days. The data obtained are presented graphically in Figs. 1a and b. The vertical columns reflect the weight of the regenerating liver of the individual animals as related to the mean weight of the liver in the non-operated animals, the latter taken as 100%; the unbroken horizontal lines represent the mean magnitude of the weight of the liver.

From the data presented, it follows that restoration of the weight of the liver after removal of 30% of the organ occurs rather quickly, and is essentially completed by the 30th day after operation. The weight of the liver undergoes its most intense increase during the first days after operation; five days after resection, the moist weight reaches an average of 80 ± 3.2% of the original, and the dry weight – 78 ± 4.5%.

It is important to emphasize that during regeneration both the moist and dry weight increase. Thus, these experiments show that regeneration of the liver does not occur through an increase in the weight of the organ due to water, although the somewhat greater increase in the moist weight of the liver than the dry weight does indicate that this process also takes place.

In the second series of experiments, we studied the course of liver regeneration after partial hepatectomy and subsequent short term irradiation of the mice, using a dose of 1000 r (dose output of 20 r/min, duration of the irradiation equal to 50 minutes). In this case, in calculating the weight of the regenerating liver, we took into account that, in irradiated mice, as a result of a general weight loss, there occurs a certain decrease in the weight of the liver. Thus, the weight of the regenerating organ of each mouse was figured as a percent of the liver weight in non-operated mice that had been irradiated under the same conditions and sacrificed at the same time as the operated subjects.

In Fig. 2 we present the data on the weight of the liver in the control and irradiated mice, 5 days after the operation (we were unable to make later observations, since by this time a large number of the mice had already died). The broken horizontal line in the graph shows the mean weight of the remainder of the organ following resection (68 ± 2.1%).

From the data presented, it follows that, under the conditions of irradiation that were applied, liver regeneration is markedly depressed. The increase in moist weight, 5 days after the operation, was approximately 50% of that in the control, and we were generally unable to record an increase in the dry mass of the liver at that period.

Thus, in contradiction to the opinion of certain authors, who did not observe marked morphological injury of liver tissue following irradiation, and concluded that it was insensitive [12, 13, 15, 16], in our experiments, where regeneration was used as the test of injury, it was proved that this organ is sensitive to irradiation.

In the third series of experiments, we studied the effect on liver regeneration of irradiation in the same dose as was used for the short term exposure (1000 r), but extended over a period of 1 month (dose output of 0.0232 r/min). It was shown that, in this case, in contrast to the single exposure, no slowing of the regeneration was observed, either from the moist or the dry weight (Fig. 3).

The opinion is widely held that the cellular elements in the liver of adult mammals do not undergo active replacement. Lately, however, data have appeared which contradict this opinion. Thus, in particular, in connection with studying the 24 hour periodicity of the mitotic activity in different tissues, it was recently shown that in the liver of mice, as well as in many other tissues, intense cell division occurs mainly at night and early morning hours [3]. This probably explains the long existing belief that liver cells cannot be replaced by mitotic division.

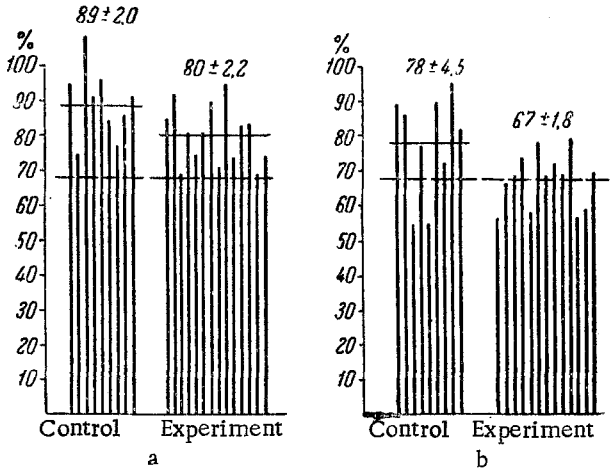

Fig. 2. Liver regeneration in mice irradiated with a dose of 1000 r, at a dose output of 20 r/min (5th day after resection). Symbols are the same as in Fig. 1.

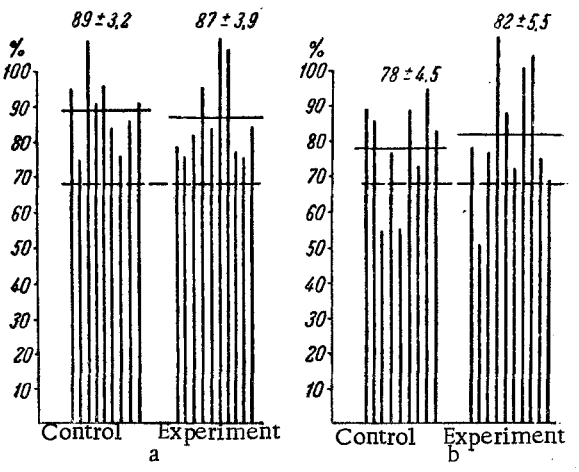

Fig. 3. Liver regeneration in mice irradiated with a dose of 1000 r, at a dose output of 0.0232 r/min. Symbols are the same as in Fig. 1.

Mitotic Activity in the Liver of Mice at Different Times of a 24 Hour Period

Time of day (in hours)	Number of mitoses per 100 visual fields
10	1.25±0.65
12	2.80±0.71
14	4.70±1.06
16	2.10±1.03
18	0.75±0.15
20	0.33±0.46
22	1.00±0.95
24	1.25±0.32
2	0.88±0.25
4	5.85±1.45
6	24.03±1.92
8	17.54±1.33

We repeated these experiments, in order to clarify the 24 hour rhythm of mitoses in the liver of the mice on which we performed our investigations.

Mice were sacrificed every 1-2 hours over a period of 24 hours. The livers were fixed in Zenker's solution, and after corresponding treatment, a series of sections, 8 micra in thickness, was prepared. The sections were strained with Karachchi's or Ehrlich's hematoxylin, and counter-stained with eosin. Mitoses were counted, using an ocular quadrate diaphragm (ocul. × 7, oil immersion obj. × 90), in 200 visual fields, and then their number was calculated per 100 visual fields (corresponding to an average of 8000 cells). We investigated the mitotic activity of the mouse livers in 3 series of experiments (96 animals), and found the results to be the same. The total data of these experiments are presented in the table.

The data obtained by us basically corresponds to the results of the cited work [3]. (It is interesting to note that the curve for the 24 hour periodicity of mitoses in the liver of mice coincided, in our experiments, with the curve for the mitotic activity of the cornea, studied at the same time as the liver).

On the basis of the data in the literature [1], as well as the data which we obtained, it may be concluded that physiological regeneration normally occurs in the liver of adult mice.

According to existing concepts, the duration of mitosis in the liver of mice is equal to approximately 1 hour[11]. Thus, replacement of the cell composition of the liver in mice, by mitotic division alone, can be accomplished, according to our data, in an average of 80 days, which approximately corresponds to the calculations carried out in the work of L. D. Liozner and V. F. Sidorova [3]. However, the possibility is not excluded that replacement of the cell composition of the liver may be accomplished more rapidly, since it occurs not only by means of mitotic division, but also through amitosis and endomitosis, which processes have been proved present in the liver by many authors.

Thus, both our own observations and the data in the literature show that physiological regeneration of the liver is amply manifest.

The experiments with Co^{60} γ-irradiation of mice showed that with a certain reduction of the dose output (extending the irradiation over 30 days), the effect of the radiation action is lost. It is still not clear whether or not this

depends on the fact that with prolonged irradiation the reparative processes succeeded in compensating for the radiation damage, and whether or not it is important that, with the single exposure, regeneration occurs against the setting of radiation sickness, while with prolonged exposure, there were no manifestations of radiation sickness and it could not thus influence the course of regeneration. The second concept is supported by data indicating that local irradiation depresses liver regeneration only at very high doses [14].

SUMMARY

The extent of injury of regenerating liver was compared on adult albino mice following brief (dose rate – 20 r/min) and prolonged (dose rate – 0.0232 r/min) irradiation with γ-rays (Co^{60}) in a dose of 1000 r. As demonstrated, hepatic regeneration was depressed after a single irradiation whereas after prolonged irradiation regeneration processes caused by trauma showed no significant depression. Decrease of irradiation efficacy with reduction of the dose rate may be associated either with the presence in the liver of physiological regeneration processes (which as shown on other test- objects decreased irradiation efficacy) or with the fact that radiation sickness was not marked in prolonged irradiation of animals.

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