THE DIURNAL RHYTHM OF MITOTIC ACTIVITY IN THE REGENERATING LIVER DURING ACUTE RADIATION SICKNESS

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At present we can consider the presence of a diurnal rhythm of mitoses during reparative regeneration of various tissues as established [1, 5, 6, 8, 10, 12]. The question arises whether it is retained in acute radiation sickness which occurs under conditions of a change in the activity of the regulating systems of an organism [2, 3, 9].

The results of the few investigations into this problem are contradictory. Jaffe, who studied mitotic periodicity in regenerating rat liver, detected a great difference between the average data obtained in animals killed during the morning and evening hours [12]. This difference persisted even after x-irradiation of the animals. G. P. Gruzdev investigated the physiological regeneration of the cells of corneal epithelium of mice totally irradiated with γ -rays of radioactive cobalt in a dose of 750 r [4]. He elicited by the 5th day of radiation sickness changes of the normal ratio of the number of mitoses in mice killed at 10:00 a.m. and 1:00 p.m. and on this basis concluded a disturbance of the the diurnal rhythm.

The present article is devoted to a study of the diurnal rhythm of mitoses in the cells of regenerating liver in acute radiation sickness.

EXPERIMENTAL METHOD

The investigation was carried out on white male rats weighing 200-250 g in which, under aseptic conditions

TABLE 1. Change in Mitotic Activity and Number of Binuclear Cells in Regenerating Liver of Unirradiated and Irradiated Rats at Different Hours of the Day

Time	Mitotic index (in %)		Number of binuclear cells (in %)	
	Control	Irradiation	Control	Irradiation
	$M \pm m$			
12	3,9±0,5	4,8±0,4	4,7±0,4	2,5±0,3
15	4,9±0,5	3,7±0,3	3,5±0,4	4,5±0,5
18	4,0±0,6	2,9±0,6	4,4±0,6	6,7±0,7
21	1,0±0,2	2,8±0,5	4,7±0,3	3,7±0,5
24	2,0±0,4	5,0±1,1	3,3±0,4	7,2±0,5
3	2,8±0,6	8,7±1,3	5,9±0,6	5,4±0,3
6	9,8±1,0	4,3±0,4	4,2±0,3	5,1±0,4
9	5,3±0,7	3,3±0,3	4,4±0,4	5,3±0,4
Average	4,2±0,6	4,4±0,7	4,4±0,4	5,0±0,4

TABLE 2. Percentage of Mitotic Phases in Regenerating Liver of Unirradiated and Irradiated Rats at Different Hours of the Day

Time	Prophase		Metaphase		Anaphase		Telophase		Coefficient of phases	
	Control	Irradiation	Control	Irradiation	Control	Irradiation	Control	Irradiation	Control	Irradiation
12	40,0	32,6	42,9	47,7	1,4	11,6	15,7	8,1	4,8	4,1
15	31,4	36,4	47,2	33,4	13,5	15,1	7,9	15,1	3,7	2,3
18	35,6	30,8	46,6	42,3	4,1	7,7	13,7	19,2	4,6	2,7
21	33,3	27,5	44,4	43,1	16,7	15,7	5,6	13,7	3,5	2,4
24	13,2	32,0	70,3	44,0	8,1	12,0	8,1	12,8	5,1	3,1
3	28,0	31,2	52,0	44,6	12,0	12,1	8,0	12,1	4,0	3,1
6	36,7	37,2	37,3	42,3	14,7	6,4	11,3	14,1	2,8	3,9
9	37,9	30,5	30,5	47,4	15,8	11,9	15,8	10,2	2,2	3,5
Average	34,1	32,4	43,1	43,4	11,2	11,1	11,6	13,1	3,3	3,1

with the use of ether, we removed the left lateral and central lobes of the liver by the method of Higgins and Anderson [11]. All operations were carried out at the same time of day (from 2 to 3:00 p.m.). Two series of experiments were set up: in the I, control, series of experiments the animals were not irradiated; in the II series of experiments partial hepatectomy was performed 3 days after single total-body x-irradiation in a dose of 600 r on the RUM-3 apparatus under standard conditions. This dose caused a drop in the number of leukocytes in the peripheral blood after 3 days on the average from 23,000 to 1700 in 1 cc. Of the 58 irradiated rats 10 died on the 1st day after the operation; not 1 of the unirradiated animals died after surgery. It is known that the maximal increase in the number of mitoses in the liver occurs 2 days after the operation [6, 11, 13]. Therefore, to study the mitotic activity the 3rd day after the operation was selected. For the irradiated rats it corresponded to the 5th-6th day of acute radiation sickness. In both series of experiments the rats were killed in groups of 3 animals at 3 h intervals. The material was fixed in Carnoy's fixing fluid. Paraffin sections 7 μ thick were stained with hematoxylin-eosin. For each animal we counted mitoses and the number of binuclear cells in the epithelium of regenerating liver per 6000 cells. The mitotic phases were considered in the count. On the basis of the data obtained we calculated the mitotic index and the percent of binuclear cells per 1000 cells. The numerical material was subjected to variance analysis.

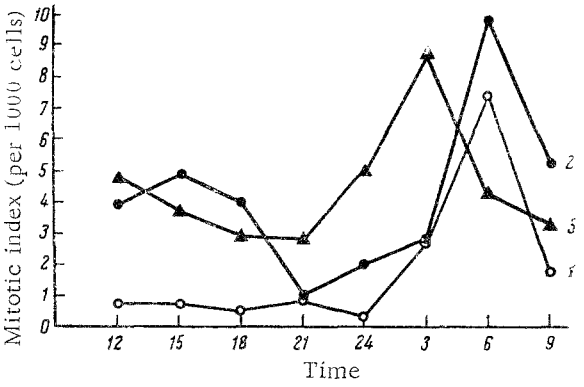
EXPERIMENTAL RESULTS

The results of the counts (for each period per 18,000 cells) are shown in Table 1. The mitotic index of unirradiated (control) animals reached a maximal value by 6:00 a.m. (9.8±1.0). A second negligible increase occurred at 3:00 p.m. (4.9±0.5). A statistical analysis of the data revealed that the difference in the number of mitoses at 6:00 a.m. and 3:00 p.m. with respect to the lowest index (1.0±0.2 at 9:00 p.m.) was significant ($P \ll 0.001$). The minimal number of dividing cells for the irradiated rats was also at 9:00 p.m. (2.8±0.5). Then an increase of mitotic activity began which reached a maximal value at 3:00 a.m. (8.7±1.3). A 2nd slight rise occurred at noon (4.8±0.4). A comparison of these data with the lowest index shows that here the difference is also significant ($P = 0.001$ and 0.006).

We must emphasize that our indexes of mitotic activity are appreciably higher than those cited by N. V. Krasil'nikova [5]. According to her data the mitotic index in reparative regeneration of the liver fluctuated from 3.0 to 0.04 (the range of fluctuations in our experiments was from 9.8 to 1.0). Undoubtedly this difference is because we removed not one but two lobes of the liver, as a consequence of which the process of regeneration occurred, on the whole, more intensely.

As is apparent from Table 2, at the same hours the percentage of individual mitotic phases of the unirradiated and irradiated rats varied within rather wide limits. However, the average daily indexes for each phase in both series of experiments were graphically the same. We note the unusually large number of early phases with respect to the late ones. The coefficient of phases (P + M/A + T) varied at different hours in the control from 2.2 to 5.1 (averaging 3.3), whereas with irradiation it varied from 2.3 to 4.1 (averaging 3.1). These indexes can hardly be considered random.

Wilson and cohorts who studied the cytological changes in regenerating mouse liver [13] also noted a low



Diurnal rhythm of mitotic activity in liver cells. 1) Physiological regeneration of mouse liver (according to the data of L. D. Liozner and V. F. Sidorova); 2) reparative regeneration of rat liver (our data); 3) reparative regeneration of rat liver in acute radiation sickness (our data).

percent of telophases. They explained this by the fact that nuclear division here goes to the end, however, in some cases it was not accompanied by division of the cytoplasm. In other words, the authors expressed a quite interesting thought concerning the origin of binuclear liver cells as result of mitosis.

We were not able to detect any patterns indicating the presence of diurnal fluctuations in the number of binuclear cells (see Table 1). Their average percent in both series of experiments was almost the same.

It is interesting to compare our data with the results of the investigation of L. D. Liozner and V. F. Sidorova [7], who studied the diurnal rhythm of mitoses during physiological regeneration in mice (see figure). The shape of the curves indicates that at the acme of acute radiation sickness the diurnal rhythm of mitoses persists in reparative regeneration of the liver. The maximum of mitotic activity, just as with reparative and physiological regeneration in unirradiated animals, occurs during the early morning hours. It is necessary to emphasize that in acute radiation sickness the average daily index of mitotic activity does not decrease in comparison with the control.

SUMMARY

In experiments on albino rats a study was made of the 24 h rhythm in the mitotic activity of hepatic cells and in the percentage of binuclear cells in the regenerating liver following a single total exposure to X-irradiation in a dose of 600 r. The finding was that at the peak of acute radiation sickness the 24 h mitotic rhythm was retained. As in the case with reparative and physiological regeneration in nonirradiated animals the peak of mitotic activity occured during the early morning hours. The mean 24 h coefficient of the cell mitotic activity (2-3 days following operation and correspondingly 5-6 days following irradiation) did not diminish as compared to control. No regularities were revealed that would suggest that during the 24 h period the number of binuclear cells in irradiated and nonirradiated rats was changed.

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