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THE NATURE OF COMPENSATORY AND RESTORATIVE PROCESSES  
IN THE LIVERS OF ANIMALS IRRADIATED DURING HYPOKINESIA

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16. Abstract 150 nonlineal rats were divided into 4 groups and subjected to irradiation after varying periods of hypokinesia. Hepatocyte population counts, mitotic activity, binuclear cell content, and karyometric studies were done to ascertain the effects of combined hypokinesia and radiation. Hypokinesia was shown to change the nature and rate of postirradiation changes in the liver, the effect varying with the timing of irradiation relative to the length of hypokinesia.		13. Type of Report and Period Covered Translation	
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# THE NATURE OF COMPENSATORY AND RESTORATIVE PROCESSES IN THE LIVERS OF ANIMALS IRRADIATED DURING HYPOKINESIA

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Available information on the combined effects of hypokinesia and /42\* radiation on the body and certain of its functions is contradictory (V. M. Seraya and I. A. Abakumova; N. A. Ilyushko; R. A. Belitskaya and L. Ye. Yegorov; Keizer and Putten). This situation may be explained by the fact that the irradiation was done during varying periods of the hypokinetic state, which, according to current ideas (Ye. A. Kovalenko), consists of stages.

In view of this fact, we attempted with the aid of several quantitative tests to discover the nature of postirradiation repair in the livers of rats irradiated during periods of hypokinesia showing the greatest fluctuations in body resistance, as ascertained by survival rate studies, behavioral reactions, and body and organ weight figures.

## Materials and Methods

Experiments were done on 150 nonlineal male rats with initial weights of 150-170 g. A state of hypokinesia was achieved by keeping the animals in containers restricting movement in all directions. Total  $\gamma$ -radiation, in 800-rad doses with dose strengths of 65 rads per minute, was administered on a "Luch" installation. The experiment consisted of 4 series. Series I was irradiation of intact rats (control). In series II the animals were irradiated on the 3rd day of hypokinesia, when they had lost 12% of initial body weight and there was high functional activity of the hypothalamus-hypophysis-adrenal system (plasma hydroxycorticosteroid content was 14.83  $\mu\text{g}\%$ , versus 6.3  $\mu\text{g}\%$  in the intact rats). Series III of the experiments consisted of rats irradiated on the 20th day of hypokinesia, when weight figures and hypothalamus-hypophysis-adrenal system activity were at near-initial levels, and the survival rate of

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\*Numbers in the margin indicate pagination in the foreign text.

these animals by the 45th day significantly exceeded that of the controls ( $75 \pm 8.18\%$ , versus  $30 \pm 10.1\%$ ). Radiation sickness in series II and III rats was concomitant with the hypokinesia. In series IV the irradiation was done on the 3rd day of the recovery period following the 20-day hypokinesia, when the animals appeared excited by their release from the containers and their resistance to radiation remained elevated (survivability in this group amounted to  $58.33 \pm 10.1\%$  and significantly exceeded that of the controls;  $P < 0.02$ ). The animals in all series were decapitated 1, 3, 10, 30, and 45 days following irradiation. Liver sections were fixed in Carnoy's fluid and sealed in paraffin, stained with hematoxylin-eosin, and Feulgen stained for DNA. Hepatocyte population numbers were computed within the standard visual field (in an eyepiece grid square with a total magnification of 1350X), and the percentage of cells in the irradiated animals was calculated relative to that in the initial period prior to irradiation. The number of mitoses and binuclear cells per 1000 hepatocytes was counted in the liver preparations. The karyometry was done using an eyepiece micrometer. Large (L) and small (B) diameters were measured and the volumes were ascertained using the formula  $V = \frac{\pi}{6} LB^2$ . Ploidy was determined from nuclear volume, since it has been demonstrated (Mortreuil-Langlois; Z. A. Ryabinina and V. A. Benyush) that ploidy in the liver, as calculated from nuclear volumes, correlates with data obtained from DNA content measurements. Nuclei with volume logarithms of 2.05 ( $112 \mu\text{m}^3$ ) were categorized as diploid and those with volumes of  $224 \mu\text{m}^3$  and above were categorized as polyploid. In all series the counts and measurements were performed on 5 rats for each period, the results were subjected to variation statistics methods (I. A. Oyvin; Ya. Ye. Khesin), and the statistical significance was determined using Student's criteria. /43

## Results and Discussion

Irradiation of intact rats led to reduced numbers of hepatocytes by the end of the 1st day (table 1). As the variability in cell dimensions increased during the later stages of radiation sickness, population numbers differed insignificantly from initial data. Large hepatocytes initially predominated in the livers of rats irradiated on the 3rd and 20th days of hypokinesia and during the readaptation period. Subsequent

TABLE 1. HEPATOCYTE POPULATION NUMBERS FOR RATS IRRADIATED WITH 800 RAD DOSES UNDER VARIOUS EXPERIMENTAL CONDITIONS AND CALCULATED IN PERCENTS OF INITIAL VALUES SET AT 100 ( $M \pm m$ ).

Series #	Experimental Conditions	Back-ground	Observation period following irradiation, in days				
			1st	3rd	10th	30th	45th
I	Irradiation without additional influences	100±2,3	84±3,4	104±2,9	99±2,6	105±3,2	107±3,8
II	Irradiation on the 3rd day of hypokin. P	90±2,4 0,02	106±1,8 <0,01	137±0,9 <0,001	130±3,9 <0,001	134±4,6 <0,001	121±5,6 >0,05
III	Irradiation on the 20th day of hypokinesia P	75±0,8 0,001	143±5,2 <0,001	144±5,6 <0,001	163±6,9 <0,001	171±1,2 <0,001	162±6,6 <0,001
IV	Irradiation on the 3rd day of readaptation after hypokinesia P	92±1,1 0,02	118±3,9 <0,001	130±1,5 <0,001	130±4,2 <0,001	109±1,7 0,2	120±3,8 >0,05

Note: Here and in tables 2-4, P is the significance of discrepancies in series II, III, and IV relative to series I.

irradiation led to a steady increase in the population numbers because of increasing numbers of small hepatocytes. This process was more significantly shown in series III of the experiments.

The liver is known (D. S. Sarkisov) to be representative of a group of organs whose reparative regeneration under various pathogenetic influences is accomplished through a combination of intracellular hyperplastic processes in retained cells and intensified cell neoplasia consequent to increased mitotic activity. Varying levels of mitotic activity appeared in the initial (background) period when analyzing mitotic indices in the livers of the rats of series being compared (table 2). Irradiation on the 3rd day of hypokinesia was done upon a background of statistically significant increases in mitotic indices. Background mitotic activity figures in series III and IV did not differ from those in the controls. We should also note the similar trends of hepatocyte mitotic activities in series I and II (initial inhibition, then progressive increase) and contrasting shifts of mitotic activity in the group of rats irradiated on the 20th day of the experiment. Liver mitotic activity vacillated widely during the observation period when

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irradiation was performed during the readaptation period. I. B. Tokin considers that mitotic activity inhibition occurs during early stages following irradiation because most cells are repairing radiation damage. Blocking is reduced following the completion of repairs and the cells, under the influence of mitotic activity stimulators, rapidly return to the mitotic cycle. The lack of a mitotic inhibition phase in the livers of series III rats indicates either that the intracellular repair phase period is curtailed, or that the amount of damage to cell structures is reduced, or both.

The number of binuclear cells in the livers of rats irradiated without additional influences exceeded initial levels during the entire period of the experiments (table 3). Peak increase occurred on the 30th day of the experiment. By changing the binuclear cell content extant prior to irradiation, hypokinesia also affected the nature of postirradiation changes in this factor. Thus when irradiation was performed after 3 days of hypokinesia and during the readaptation period, the initial reaction consisted not of an increase, but of a reduction in the total number of binuclear cells. In the rats irradiated on the 20th day of hypokinesia, however, the number of binuclear cells rose more dramatically, after 3 days, than in the animals in the other series. The increase in the number of binuclear cells in our experiments basically either was consequent to or accompanied the elevation of mitotic activity. Other investigators have also found this regularity during acute radiation sickness (V. I. Bulgak). In the opinion of V. Ya. Brodskiy, only 46 20-25% of all binuclear cells in the liver may be formed as a result of mitosis. It is possible that the fraction of cells with incomplete mitosis rises following irradiation, when the frequency of chromosomal aberrations increases. The increased frequency of binuclear cells is viewed as a manifestation of adaptation to the effects of radiation (I. B. Tokin). Multinuclear cells are complete units with high mitotic activities and are considered a possible functional reserve for such organs as the livers of mammals (Z. A. Ryabina and V. A. Benyush).

Nuclear volume measurements indicated that there was a significant increase of this factor in the livers of series I irradiated rats on the 1st, 10th, and 30th days of the experiment (table 4). Here the number



of polyploid cells rose up to 85, 65, and 42%, respectively, versus 37% in the initial period. Average hepatocyte nuclear volumes in the rats irradiated on the 3rd day of hypokinesia exceeded those in the intact rats during the initial period, and the number of polyploid cells in them was 52% of the intact animals'. Enlarged nuclei were recorded only on the first day of radiation sickness in this series. In other periods of observation, a distinct tendency was noted for nuclear size and ploidy to diminish. Polyploid cells constituted 24% on the 10th day of the experiment and 20% on the 45th. Nuclear volumes increased before irradiation even more significantly in series III than in series II, while following irradiation the average value never once exceeded initial. The number of polyploid cells was also reduced in these rats, amounting to 51, 57, 52, 34, and 30%, respectively to the periods of the experiment, versus 75% in the initial period. A similar regularity was seen in series IV as well, where discrepancies in nuclear volume and number of polyploid cells from initial values were least.

Thus the results of our study indicate that hypokinesia alters the nature of postirradiation compensatory and restorative processes in the liver. With irradiation of intact rats, restoration of liver structures was provided at early stages of radiation sickness by cell hypertrophy consequent to delay of mitoses and increased hepatocyte ploidy; another form of reparative regeneration -- cell neoplasia -- gradually increased at later stages, and the fractional content of binuclear cells also rose. This conclusion agrees with the data of L. F. Romanova and V. N. Strel'tsova which showed the role of hepatocyte endomitosis and polyploidization to be a prominent type of post-irradiation restoration. A second type of restoration dominates during the entire observation period when irradiation occurs with hypokinesia. It is provided by the enlarged pool of dividing cells just before irradiation (series II) and by the lack of a mitotic division delay phase in the initial period of radiation sickness (series III and IV). We should note that the rate of cell proliferation in the initial phase of radiation sickness is higher in series III animals, but in groups I and II rats at later stages.

In our view, the changes in the nature of compensatory and restorative processes in the livers of rats irradiated during hypokinesia are caused by stress developing in the animals in response to isolation and restricted mobility. By changing neuroendocrine system activity, the latter stimulates cell and tissue repair mechanisms at a certain stage, which is essential in the body's reaction to subsequent irradiation. The intimate relationship of body radiosensitivity to cell and tissue repair system activity has been emphasized repeatedly (Köessler; I. G. Akoyev). It has been shown that forming products of lymphoid cell decomposition may serve as the signal for restorative process activation during stress (I. G. Akoyev).

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TABLE 2. MITOTIC INDEX (1:1000) IN THE LIVERS OF RATS IRRADIATED IN 800 RADDOSES UNDER DIFFERING EXPERIMENTAL CONDITIONS ( $M \pm m$ ).

Series	Experimental Conditions	Background	Periods of Observation Following Irradiation, Days				
			1	3	10	30	45
I	Irradiation alone						
II	Irradiation on 3rd Day Hypknsa.	$1.33 \pm 0.0390$	0.000	$0.66 \pm 0.0310$	$1.33 \pm 0.0323$	$2.76 \pm 0.0750$	$3.54 \pm 0.1910$
	p	$5.66 \pm 0.4810$	$1.66 \pm 0.1910$	$1.66 \pm 0.0820$	$2.66 \pm 0.0510$	$4.00 \pm 0.5100$	$5.00 \pm 0.2500$
		<0.001	<0.001	<0.001	<0.001	<0.05	<0.01
III	Irradiation on 20th day Hypkn.	$1.00 \pm 0.2130$	$2.70 \pm 0.2770$	$4.69 \pm 0.4410$	$2.06 \pm 0.1300$	$3.33 \pm 0.3650$	$0.33 \pm 0.0810$
	p	>0.2	<0.001	<0.001	<0.01	>0.1	<0.001
IV	Irradiation on 3rd Day of Readaptation Period	$1.66 \pm 0.2810$	$0.66 \pm 0.1010$	$1.66 \pm 0.0176$	$2.33 \pm 0.2130$	$0.66 \pm 0.0720$	$2.66 \pm 0.4740$
	p	>0.2	<0.01	<0.001	<0.01	<0.001	>0.1

TABLE 3. NUMBERS OF BINUCLEAR CELLS (1:1000) IN THE LIVERS OF RATS IRRADIATED IN 800 RAD DOSES UNDER DIFFERING EXPERIMENTAL CONDITIONS ( $M \pm m$ ).

Series	Experimental Conditions	Background	Periods of Observation Following Irradiation, Days				
			1	3	10	30	45
I	Irradiation alone						
II	Irradiation on 3rd Day Hypknsa	$27.7 \pm 2.96$	$33.0 \pm 1.2$	$40.0 \pm 2.02$	$34.33 \pm 1.22$	$62.0 \pm 2.55$	$40.3 \pm 1.50$
	p	$33.0 \pm 1.51$	$9.0 \pm 1.53$	$40.3 \pm 0.88$	$39.0 \pm 2.28$	$69.66 \pm 2.4$	$53.0 \pm 2.28$
		>0.1	<0.001	>0.2	>0.1	<0.1	<0.05
III	Irradiation on 20th Day Hypknsa	$13.66 \pm 1.76$	$22.7 \pm 1.58$	$47.66 \pm 1.87$	$50.33 \pm 1.39$	$76.33 \pm 3.24$	$58.66 \pm 2.31$
	p	<0.01	<0.001	<0.05	<0.001	<0.01	<0.01
IV	Irradiation on 3rd Day of Readaptation Period	$16.3 \pm 2.1$	$12.0 \pm 1.8$	$41.66 \pm 1.87$	$57.33 \pm 2.92$	$35.33 \pm 2.33$	$42.66 \pm 3.80$
	p	>0.02	<0.001	>0.5	<0.001	<0.001	>0.2

TABLE 4. POSTIRRADIATION FLUCTUATIONS IN NUCLEAR VOLUME ( $\text{mcm}^3$ ) OF HEPATOCYTES OF RATS IRRADIATED IN 800 RAD DOSES UNDER DIFFERING EXPERIMENTAL CONDITIONS ( $M \pm m$ ).

Series	Experimental Conditions	Background	Periods of Observation Following Irradiation, Days				
			1	3	10	30	45
I	Irradiation alone						
II	Irradiation on 3rd Day Hypknsa	$132.1 \pm 1.040$	$205.6 \pm 1.031$	$135.8 \pm 1.035$	$173.4 \pm 1.030$	$143.9 \pm 1.030$	$133.3 \pm 1.016$
	p	$161.8 \pm 1.035$	$175.4 \pm 1.033$	$145.5 \pm 1.012$	$126.3 \pm 1.010$	$134.0 \pm 1.030$	$116.1 \pm 1.035$
		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
III	Irradiation on 20th Day Hypknsa	$104.1 \pm 1.035$	$154.9 \pm 1.032$	$162.0 \pm 1.042$	$163.7 \pm 1.010$	$133.6 \pm 1.017$	$123.9 \pm 1.012$
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IV	Irradiation on 3rd Day of Readaptation Period	$141.3 \pm 1.042$	$119.9 \pm 1.035$	$147.9 \pm 1.042$	$132.7 \pm 1.010$	$153.5 \pm 1.039$	$116.1 \pm 1.035$
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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