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ANALYSIS OF THE RESULTS OF CYTOGENETIC EXAMINATION OF BONE MARROW UNDER EXPERIMENTAL IRRADIATION

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Depending on the radiation dose and its distribution throughout the human or animal body, the timing and causes of their death vary. The most common is the bone marrow form of acute radiation sickness, and depending on the type of mammal, death occurs 7-30 days after exposure, and the causes of death are most often hemorrhagic syndrome or infectious complications [3, 10, 11].

Ionizing types of radiation include electromagnetic vibrations with a short wavelength, X-rays and gamma radiation, streams of α- and β-particles (electrons), protons, positrons, neutrons and other charged particles, α-radiation and X-ray radiation have a high penetrating power, β-radiation has a lower penetrating power [5]. Radioactive substances can enter the body through intact skin, gastrointestinal tract, and respiratory organs. After that, they are transported by blood and lymph flow to organs and tissues [3, 9, 12].

It has been proven that the hematopoiesis system of the body is most susceptible to the effects of radiation, especially for bone marrow cells. Under the influence of radiation, aplasia of the bone marrow develops, inhibition of mitotic processes in the organs of hematopoiesis, and total death of low-grade bone marrow cells. A decrease in hematopoiesis is accompanied by the appearance of hemorrhagic syndrome [2, 5, 8].

Chronic radiation sickness is a complex clinical syndrome that develops in the case of prolonged exposure to ionizing radiation in doses that exceed the permissible. Characteristic manifestations: duration and undulation of the course; the presence in the clinical symptoms of both signs of damage to the body from the effects of radiation, as well as manifestations of restorative and adaptive reactions. Periods of development of chronic radiation sickness: the period of formation, or actually chronic radiation sickness; the recovery period; the period of consequences of radiation sickness [4, 7].

The aim of the study was to study and evaluate cytogenetic changes in the bone marrow cells of white outbred rats under chronic and acute experimental radiation in a comparative aspect.

Materials and methods of research. To carry out the planned studies, 30 white mongrel rats weighing 150-180 g of male were used, kept in standard vivarium conditions (room temperature 21-220 ° C, relative humidity 50-60%, light mode - 12 hours of darkness and light each). The maintenance of laboratory animals, feeding and caring for them, selection of animals, cleaning and disinfection of the vivarium premises were carried out according to Nuraliev N.A. et al. [6].

All laboratory animals (white mongrel rats) were obtained from the same nursery and of the same age. Before the start of the experimental studies, all laboratory animals were kept in quarantine for

21 days. When working with experimental animals, all ethical principles of working with laboratory animals and rules of biological safety were strictly observed [1, 6].

All laboratory animals were divided into the following groups: The first group consisted of white outbred rats (n=12) who received acute radiation once at a dose of 5 Gy; The second group consisted of white outbred rats (n=12) who received chronic radiation for 20 days at 0.2 Gray daily; The third group consisted of intact white outbred rats (n=6) who did not receive acute and chronic radiation. During cytogenetic studies, all work with growth media and preparations was carried out under sterile conditions using a laminar flow box. The buffers were prepared in bidistilled water, filtered through membrane filters (0.22 microns "Millipor", Germany) and autoclaved at 1.2 atm. 30 minutes. The glassware is pre-sterilized at 1600C for 120 minutes before use. Equipment, fixtures, and tableware made of polymer materials were exposed to ultraviolet light for 30 minutes. For experimental studies, bone marrow was selected from the femur of white outbred rats during the autopsy of the animal.

Cytogenetic changes in rat bone marrow cells were studied using the direct method. The method included the following steps: bone marrow was washed out of the femur of white outbred rats involved in the experiment of all three study groups with RPMI 1640 nutrient medium with 0.04% colchicine (which destroys the spindle of division and chromosomes do not diverge to the poles during mitosis, forming a polyploid organism) into a centrifuge tube and incubated for 2-2.5 hours in thermostat at 370C; incubated with hypotonic solution of CSl for 40 minutes in a thermostat at 370C; after hypotonization, the fixative was treated three times in the proportion of one part glacial acetic acid and three parts 96-1000 ethyl alcohol; the resulting precipitate was applied to a pre-cleaned degreased slide and stained with Giems dye; metaphase search was performed under a Leica microscope (Germany) at 200 magnification, metaphase plates were analyzed at 1000 magnification, in each sample From 15 to 25 cells with metaphase plates were analyzed. Statistical processing was carried out using generally accepted methods of variation statistics using programs for statistical analysis of biomedical research. The significance level of the indicator of the reliability of differences was considered to be $P<0.05$. When organizing and conducting research, the principles of evidence-based medicine were observed.

Research results and their discussion. For the analysis, we used bone marrow cells from laboratory animals that received and did not receive different types of radiation, in which elements of the mitotic apparatus were detected (Table).

Table

Results of cytogenetic analysis of bone marrow cells of white outbred rats exposed to acute and chronic radiation

Group	Number of investigated			Polyploidy	Premature condensation of chromosomes
	Dividing cells	Metaphase	Prophase		
First group, n=12	123	89 / 72,36	15 / 12,19	7 / 5,69	12 / 9,76
Second group, n=12	125	60 / 48,0	11 / 8,80	3 / 2,40	51 / 40,80
Third group,	75	75 / 100,0	0	0	0

n=6					

Note: the numerator is absolute; the denominator is relative (%)

In the first group (acute irradiation) of 123 bone marrow cells studied in laboratory animals, 72.36% (n=89) of the cells showed normal metaphase plates, 12.19% (n=15) of the cells were at the prophase stage. It should be emphasized that 5.69% (n=7) of the cells had polyploid cells (polyploidy), 9.76% (n=12) of the cells had premature condensation of chromosomes. Metaphase plates are an accumulation of chromosomes in a plane perpendicular to the axis of division (the equatorial plane), in which the chromosomes are equatorially located in the metaphase of mitosis (the second phase of somatic cell division). The number of chromosomes in rats is normally 42 (diploid set).

Thus, the low content of cells (9.76%) with premature condensation of chromosomes and the absence of cells with pulverization and scattering of chromosomes indicates minor changes in the mitotic division of bone marrow cells in laboratory animals of this study group. The absence of animals in this group with low cellular density and low blast transformation (8.3%, n=1) indicates the normal mitotic activity of bone marrow cells in all (n=12) laboratory animals. There is no pathology of mitosis in their bone marrow cells. Apparently, this fact is explained by the short observation period (5 days) of animals after a single acute exposure, since, depending on the type of mammal, death occurs on 7-30 days from the moment of exposure [2, 5].

The conducted studies have proved that after a single acute irradiation (5 Gray) during the first 5 days, there are practically no changes in the mitotic division of bone marrow cells, chromosomal aberrations do not appear, and mitotic activity does not decrease.

Further, the same studies were conducted with white outbred rats that received chronic radiation (the second group).

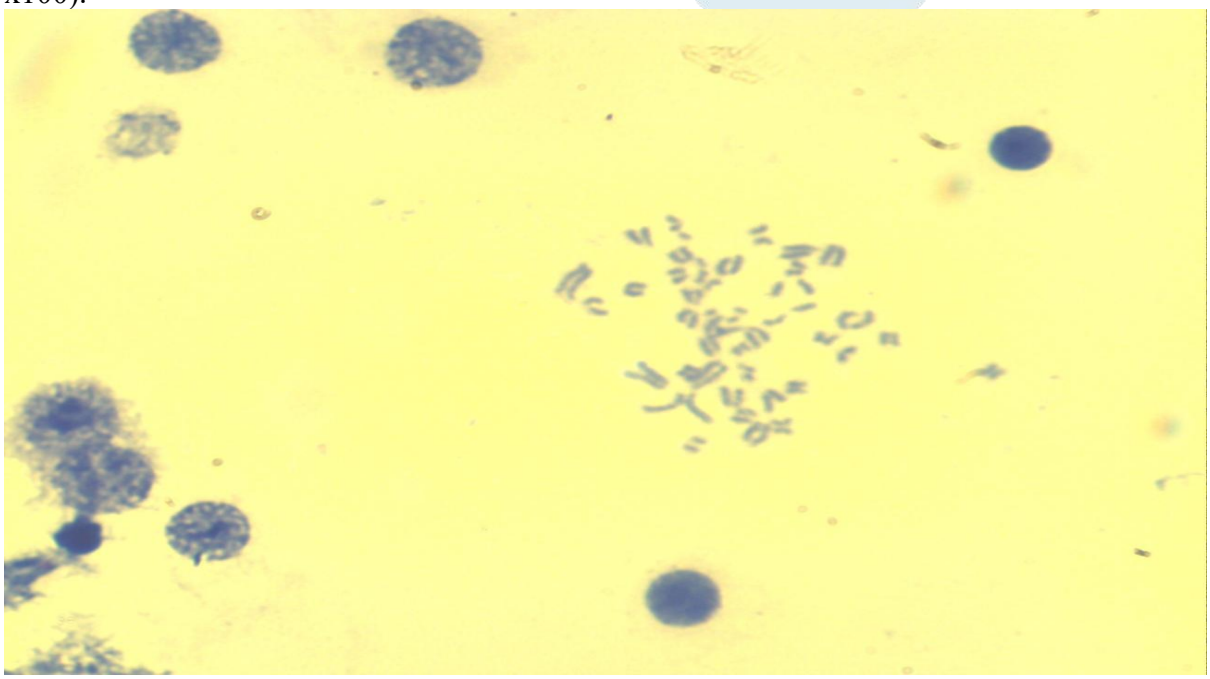
Of the 125 bone marrow cells studied in laboratory animals of the second group, normal metaphase plates were found in 48.0% (n=60) of the cells, prophase stage was observed in 8.80% (n=11) of the cells, polyploid cells were found in 2.40% (n=3) of the cases, and 40.80% (n=51) of the cells Cells with premature condensation of chromosomes were observed.

Of the 12 animals of the second group, 1 rat (8.33%) did not have mitotically dividing cells on the preparations, low cell count, low blast transformation and inhibition of mitosis were observed. The presence of cells with pulverized chromosomes indicates a pathology of mitosis. The presence of a high concentration of cells (40.80%) with premature condensation of chromosomes in the bone marrow cells of rats of the second group indicates an inhibition of the normal mitotic cycle, which affects the proliferative activity of this tissue and the presence of cell clones with genetic pathology.

The following figures 1 (metaphase plate with normal karyotype) and 2 (normal early metaphase plate) confirm the absence of changes in the microscopic picture of bone marrow cells in laboratory animals that received a single acute radiation dose of 5 Gy on the 5th day after irradiation.

Fig. 1. Bone marrow cells. A metaphase plate with a normal karyotype (the first group is acute irradiation, Approx. x10, Vol.

x100).



In contrast to the laboratory animals of the first group, which were observed on the 5th day after acute radiation, a different picture was observed in the second group of laboratory animals, which were examined after 20 days of chronic radiation with a daily dose of 0.2 Gray. Pathology was noted during bone marrow cell division.

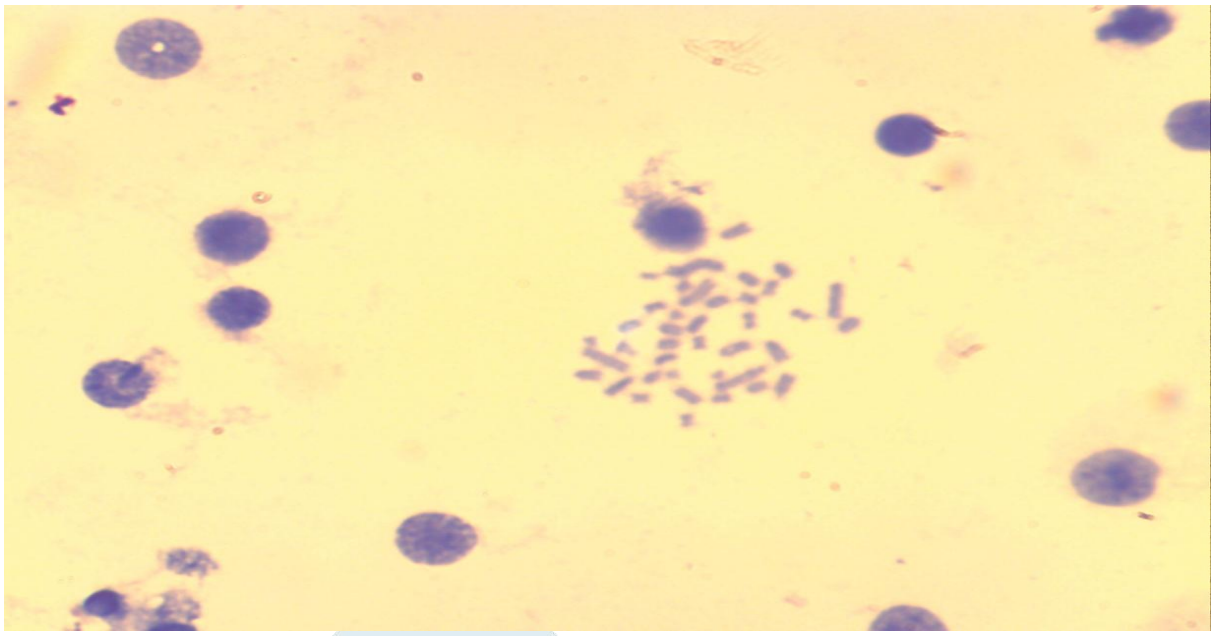


Fig. 2. Bone marrow cells. Normal early metaphase plate (the first group is acute radiation, Approx. x10, Vol. x100).

Figure 3 shows that the nucleus of an animal bone marrow cell belonging to the second group contains an early phase with premature condensation of chromosomes. To the right and bottom of the cell, which is visible in Fig. 3, there are interphase nuclei.

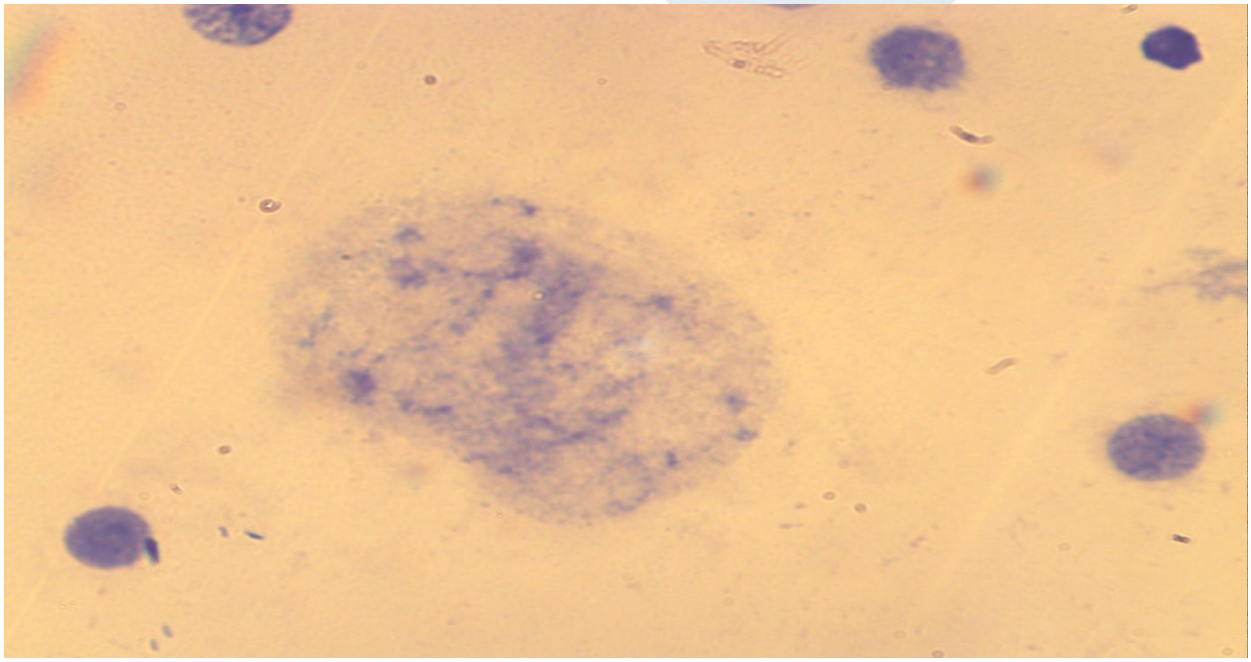
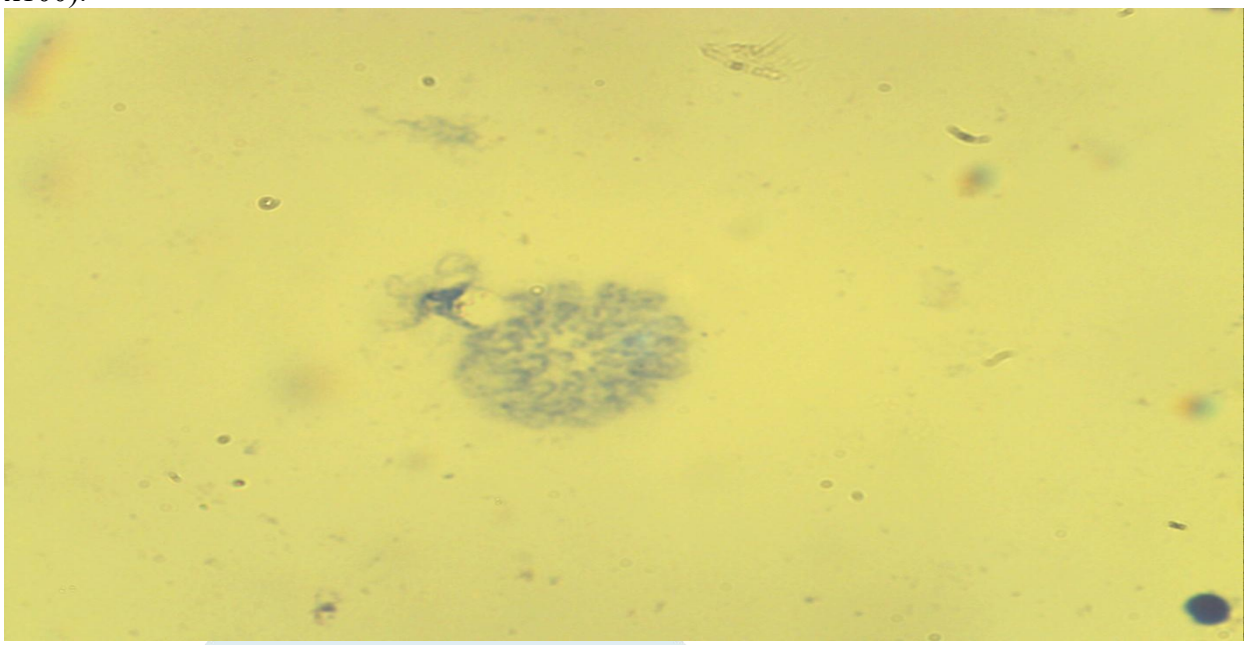


Fig. 3. Bone marrow cell. Premature condensation of chromosomes (the second group was chronic irradiation, Approx. x10, Vol. x100). In addition, a late phase of premature chromosome condensation was also observed in the nucleus of bone marrow cells in laboratory animals (Fig. 4). In another figure (Fig. 5), a nucleus with premature condensation is observed in the center of an animal bone marrow cell after chronic irradiation. condensation of chromosomes, and interphase nuclei are visible around it.

Fig. 4. Bone marrow cell. The nucleus has a late phase of premature chromosome condensation (the second group is chronic radiation, Approx. x10, Vol. x100).



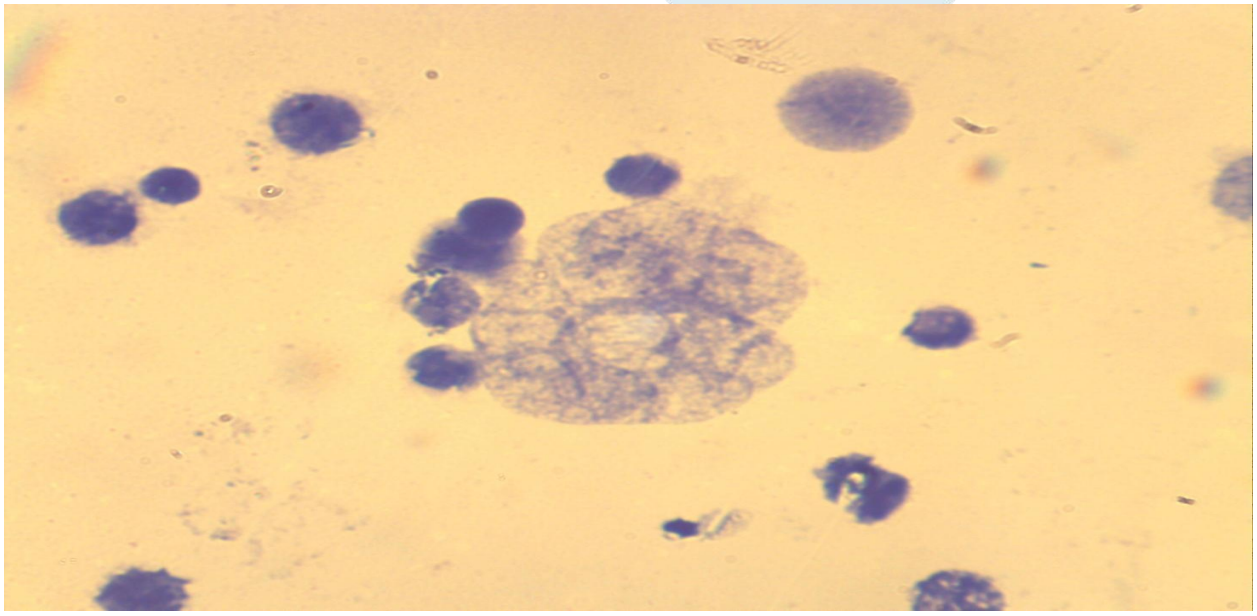
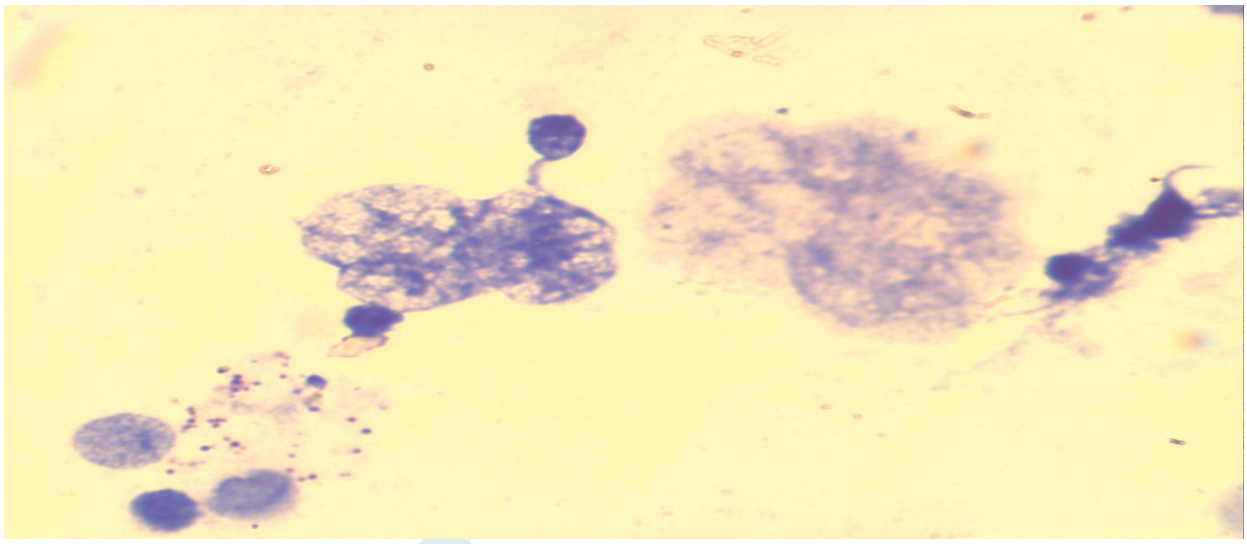


Fig. 5. Bone marrow cells. In the center is a nucleus with premature condensation of chromosomes. There are interphase nuclei around (the second group is chronic radiation, Approx. x10, Vol. x100).

The pathology of mitosis can also be observed in Fig. 6, where the nuclei with premature condensation of chromosomes are visible in the center, and the nucleus with pulverization of chromosomes is observed on the left (the second group is chronic radiation).



6. Bone marrow cells. In the center is a nucleus with premature condensation of chromosomes, on the left is a nucleus with pulverized chromosomes (the second group is chronic radiation, Approx. x10, Vol. x100).

Unlike laboratory animals of the first and second groups, which underwent acute and chronic irradiation, no changes in bone marrow cells and the course of cell division were observed in the bone marrow cells of white outbred rats of the third group (intact). In all cases, a normal karyotype was found - late (Fig. 7) and early (Fig. 8) metaphase

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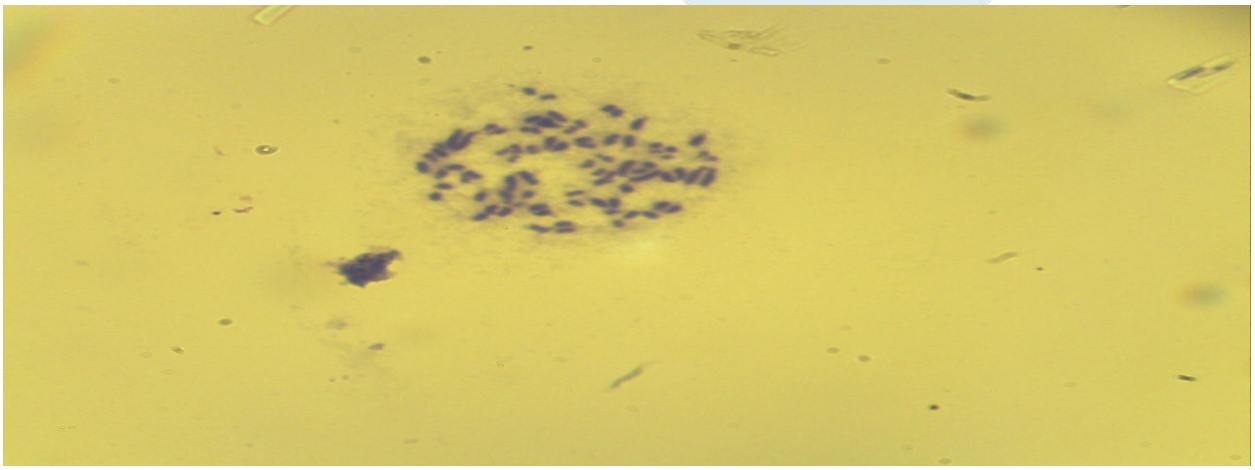
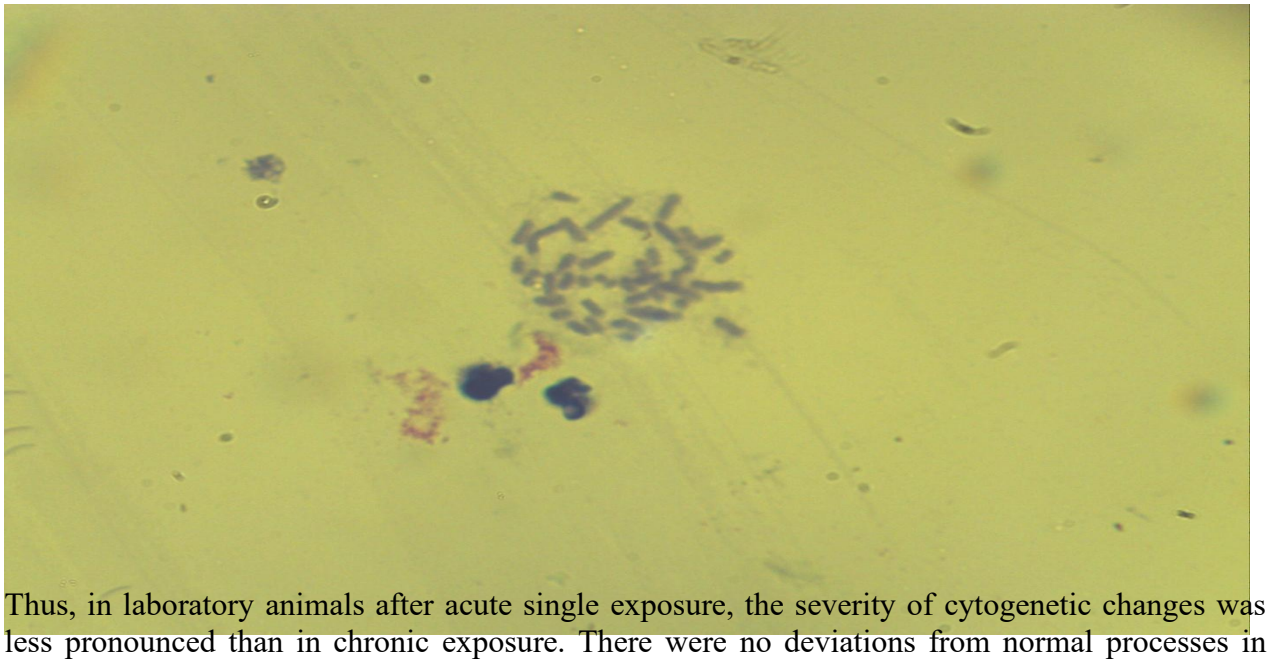


7. Bone marrow cells. Normal karyotype, late metaphase (the third group - without acute and chronic radiation, Approx. x10, Vol. x100).

8. Bone marrow cells. Normal karyotype, early metaphase (the third group - without acute and chronic radiation, Approx. x10, Vol. x100).



Thus, in laboratory animals after acute single exposure, the severity of cytogenetic changes was less pronounced than in chronic exposure. There were no deviations from normal processes in intact animals. Based on the conducted studies, cytogenetic changes in bone marrow cells of laboratory animals exposed to acute and chronic radiation were studied and evaluated. The data obtained make it possible to use the proposed recommendations to improve the effectiveness of the methodology for studying and evaluating cytogenetic changes in bone marrow cells of laboratory animals in experimental studies to determine the effect of different doses of radiation on the body.

Conclusions.

1. In the first group (acute irradiation) of 123 bone marrow cells studied in laboratory animals, normal metaphase plates were detected in 72.36% of the cells, 12.19% of the cells were at the

prophase stage. It should be emphasized that 5.69% of cells were polyploid (polyploidy), 9.76% of cells had premature condensation of chromosomes.

2. The low content of cells (9.76%) with premature condensation of chromosomes and the absence of cells with pulverization and scattering of chromosomes indicates minor changes in the mitotic division of bone marrow cells in laboratory animals of the first study group. The absence of animals with low cellular density and low blast transformation (8.3%) indicates the normal mitotic activity of bone marrow cells in all laboratory animals. Apparently, this is due to the short observation period (5 days) of animals after acute irradiation, since it is assumed that, depending on the type of mammal, death occurs on 7-30 days from the moment of irradiation.

3. Studies have proven that after a single acute irradiation (at a dose of 5 Gy) during the first 5 days, there is practically no pathology of mitosis (changes in the mitotic division of bone marrow cells), chromosomal aberrations do not appear, and mitotic activity does not decrease. In this regard, we believe that at this time it is possible to carry out therapeutic and preventive measures to maintain the proliferation and differentiation of bone marrow cells, increase the activity of the immune system.

4. Of the 125 bone marrow cells studied in white outbred rats of the second group (chronic irradiation), normal metaphase plates were found in 48.0% of the cells, the prophase stage was observed in 8.80% of the cells, polyploid cells were found in 2.40% of the cases, and cells with premature chromosome condensation were observed in 40.80% of the cells. Of the 12 animals of the second group, 1 rat (8.33%) did not have mitotically dividing cells on the preparations, low cell count, low blast transformation and inhibition of mitosis were observed. The presence of cells with pulverized chromosomes indicates a pathology of mitosis.

5. The presence of a high concentration of cells (40.80%) with premature condensation of chromosomes in the bone marrow cells of rats of the second group indicates an inhibition of the normal mitotic cycle, which affects the proliferative activity of this tissue and the presence of cell clones with genetic pathology. 6. In laboratory animals, after acute single exposure, the severity of cytogenetic changes was less pronounced than in chronic exposure. There were no deviations from normal processes in intact animals.

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