INFLUENCE OF GAMMA-IRRADIATION ON THE MITOTIC CYCLE OF BONE MARROW CELLS

(UDC 612.419.014.482.084)

A. I. Zosimovskaya

Laboratory of Experimental Cytology and Cytochemistry, Institute of Radiation and Physicochemical Biology (Director, Academician B. A. Éngel'gardt), AN SSSR Moscow
(Presented by Active Member AMN SSSR L. M. Shabad)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 57, No. 6, pp. 91-95, June, 1964
Original article submitted April 16, 1963

The best known cellular reaction to irradiation is the loss of ability to divide. Recently it has been shown by radioautography that with various kinds of radiation different periods of the mitotic cycle may be blocked, so that the cell are unable to perform a normal mitotic cycle and to undergo mitosis [1,4,11,12]. In such a case the cells are able to respond to this kind of block produced not only by irradiation but by other unfavorable circumstances [2].

We have set out to determine how irradiation affects the mitotic cycle of bone marrow cells in the case of both myelo- and erythropoiesis.

METHOD

The experiments were carried out on mice. The effect was found of total irradiation with the gamma-instrument of the Institute of Biophysics, AN SSSR; a dose of 400R at a rate of 278R/minute was used. The nature of the disturbance and the recovery of the mitotic index in the bone marrow was investigated on white mice of a mixed strain. For this purpose we took samples for 60 minutes at 5 or 15 minute intervals for 3, 6,9,18,24,48,72, and 120 hours after irradiation. We counted the number of mitoses in 3,000 cells of the myeloid series and in 3,000 cells of the erythroid series. The numbers were expressed as a percentage of the control value.

To study the action of irradiation on the different periods of the mitotic cycle we use the method of radioautography by means of H^3-thymidine. These experiments were carried out on mice of the SVA line. H^3-thymidine with a specific activity of 6.6 Ci/mM (produced by the Radiochemical Center in Amersham, Great Britain) was injected intravenously in a dose of 20μ Ci/mM per mouse 1,2,4,8,18,48,72, and 120 hours after irradiation; the animals were then killed in every case seven hours after injection of the isotope. The method of obtaining the autographs has been described in an earlier work [3]. In the autographs we counted the number of labelled cells and the labelled mitoses in 3,000 myeloid and 3,000 erythroid cells. The nuclei were counted as marked if there were not less than three grains of silver above them.

RESULTS

First of all we followed the action of radiation on the mitotic cycle in the bone marrow. As shown in Fig. 1, A, after irradiation the number of mitoses falls to zero for one hour, and for some time there were no mitoses in either the white or the red cell series. The ionizing radiation inhibits the entry of cells into mitoses and the prophase stage as shown by the rapid reduction in the number at this stage to zero after the brief period of 35 minutes (see table). Cells caught by the radiation at other stages of division terminated the division. These results indicate the occurrence of a block between the premitotic period and mitosis.

A small percentage of cells of the myeloid series, and somewhat more cells of the erythroid series appeared three hours after irradiation. This result indicates a partial removal of the block and the renewed entry of the cells from the premitotic stage into mitosis. However, complete return to normal of cellular division at the times investigated never occurred (Fig. 1, B, 1,2).

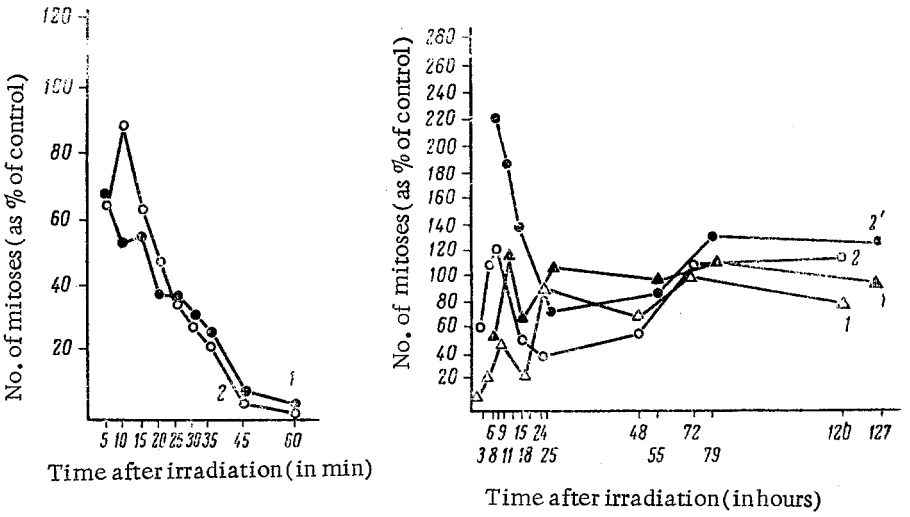

Fig. 1. A) Change in the number of mitoses of mouse bone marrow cells at various times after irradiation. 1) Myeloid series; 2) erythroid series. B) change in the number of mitoses in mouse bone marrow cells after irradiation, with and without the injection of H^3-thymidine. 1) Myeloid series without H^3-thymidine; 1') myeloid series with H^3-thymidine ; 2) erythroid series; 2') erythroid series with H^3-thymidine .

Relationship of the Mitotic Phases in Mouse Bone Marrow Cells after Irradiation

Tissue investigated	Mitotic phase	Control	After irradiation			
			After 10 min	After 15 min	After 20 min	After 35 min
Myeloid series	Prophase	7.3	2.0	1.1	0.3	0.1
Erythroid "		10.8	6.6	3.3	1.5	0.3
Myeloid "	Metaphase	8.1	5.6	5.6	1.8	0.3
Erythroid "		10.6	10.0	7.6	5.1	0.5
Myeloid "	Anaphase	2.8	1.6	1.6	2.8	0.8
Erythroid "		5.0	4.0	2.6	2.5	1.1
Myeloid "	Telophase, reconstruction	2.1	2.6	2.1	2.6	3.0
Erythroid "		2.9	5.0	4.0	4.0	4.8

To determine what processes in the mitotic cycle are damaged by irradiation we used the method of radioautographic analysis. The nature of the curves reflecting the change in the percentage of dividing cells in the myeloid and erythroid series at various times after irradiation were not changed by injection of the mice with isotope (Fig. 1, B, 1', 2'), although the level of mitotic activity in the experiment with H^3-thymidine was higher than under normal conditions, particularly in the red series. This effect was possibly related to some stimulating action of the isotope on the rate at which cells pass through the stage of synthesis of DNA [6].

In the erythroid and myeloid bone marrow cells, one hour after irradiation a reduction in the mean number of silver grains per cell was observed, indicating a 50 - 60% reduction in the rate of DNA synthesis (Fig. 2, A and B, 1). At the same time there was also a 65% and 90% reduction in the myeloid and erythroid series respectively, indicating not only a suppression of synthesis itself, but also a delay in the transition of the cells from the presynthetic period to the period of DNA synthesis. This result indicates the development of a block between these periods, a block which is more marked in cells undergoing erythropoiesis (Fig. 2, A and B, 2).

The first labelled mitoses appeared eight hours after irradiation, and amounted to 10-15% of the control indices (Fig. 2, A and B, 3). Cells which performed in this manner were those which were caught by the irradiation at the end of the period of DNA synthesis. By the eighteenth hour after the action of the ionizing radiation there was some increase in the mean number of silver grains per labelled cell (see Fig. 2, A and B, 1), where the percentage of labelled cells was still very low (2 in the red series and 14 in the white series; see Fig. 2, A and B, 2). Consequently the restoration of the mean number of silver grains per cell preceded the increased percentage of labelled cells. By

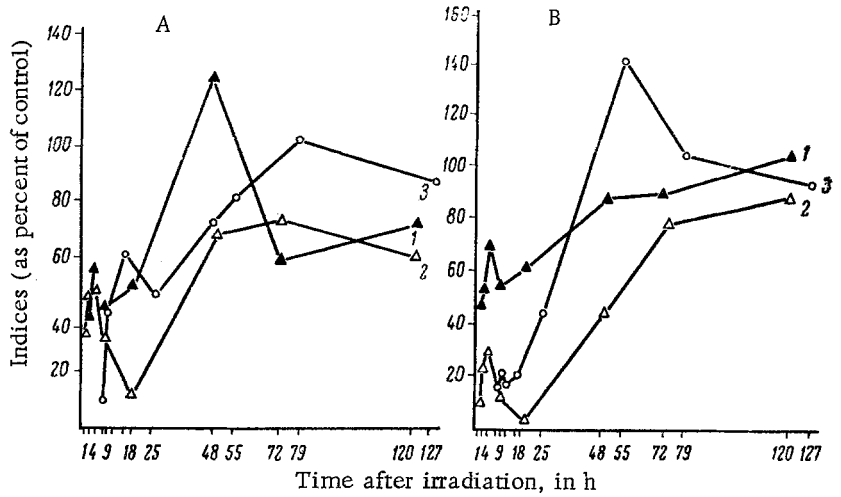

Fig. 2. Action of irradiation on the periods of the mitotic cycle as shown by cells of (A) the myeloid and (B) the erythroid series in mouse bone marrow. 1) Mean number of silver grains per labelled cells; 3) labelled mitoses

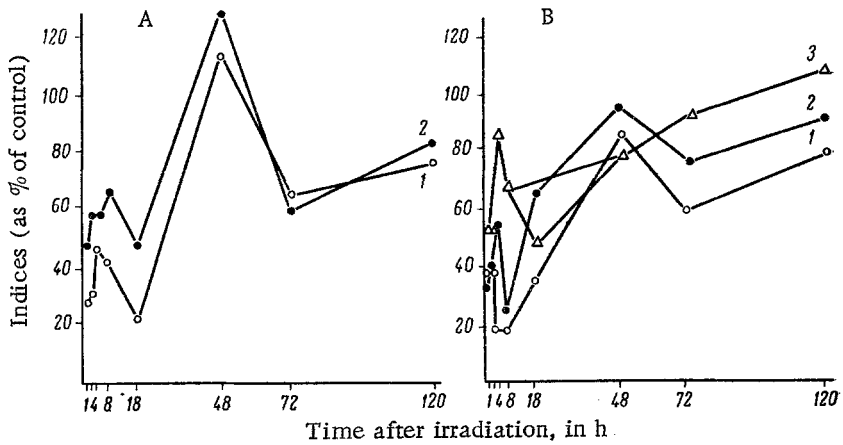

Fig. 3. Reduction in the number of silver grains per labelled cell of (A) the myeloid and (B) the erythroid series of cells in mouse bone marrow at various times after irradiation. A: 1) Young elements (hemocytoblasts, myeloblasts, promyelocyte); 2) myelocytes; B: 1) erythroblasts, pronormoblasts; 2) basophile normoblasts; 3) polychromatophile normoblasts.

the forty-eighth hour there was an increase in the percentage of labelled cells (up to 70% in the myeloid and 45% in the erythroid series), a result which indicates a renewed transition of cells from the presynthetic period into the period of DNA synthesis, i.e. a removal of the block between these periods (see Fig. 2, A and B, 2).

By this time the rate of DNA synthesis in the cells of the myeloid series was greater than normal (120%), whereas for the erythroid series the rate was still at 80% of normal. Thus the onset of recovery of DNA synthesis in the S period and the renewed transition of cells from the presynthetic to the DNA synthesis period in cells of the myeloid series occurred earlier in the myeloid than in the erythroid series; outside this period of investigation made 120 hours after irradiation the recovery indices for cells undergoing erythropoiesis was closer to the control value. This effect may probably be attributed to the shorter mitotic cycle of the erythroid cells. The time of generation of the myeloid cells was 20.8 to 21.5 hours, and for cells of the erythroid series it is 14.3 - 14.5 hours [3].

Knowing the different radio sensitivities of bone marrow cells we then investigated the change in the rate of DNA synthesis in the different categories of myelo-and erythropoiesis after irradiation. In the young cells undergoing myelopoiesis (hemocytoblast, myeloblast, promyelocyte), the mean number of silver grains per labelled cell

was reduced to a greater extent than it was in the more mature cells – the myelocytes (Fig. 3, A, 1,2). In erythropoiesis the same effect was observed (Fig. 3, B, 1,2). The reduction in the rate of DNA synthesis was manifested earliest of all (2 hours after irradiation) in the young cells (erythroblasts, pronormoblasts), next it was shown in the basophile normoblasts (after 8 hours), and finally in the polychromatophile normoblasts (not until after 18 hours). The increased rate of DNA synthesis observed after 48 hours in the cells of the myeloid and erythroid series is to be attributed both to the recovery of DNA synthesis as well as to the presence of binucleate cells which disappeared after this time. Cells which had lost the ability to divide properly once more passed through a period of DNA synthesis, consequently the number of grains of silver per nucleus is increased.

Thus the effect of gamma-irradiation on bone marrow cells is to reduce the mitotic index and the number of silver grains per labelled cell; there is also a drop in the percentage of labelled cells, i.e. there is a reduction in the rate of synthesis of DNA, as well as a blockage of the different stages of the mitotic cycle. Essentially all periods of the mitotic cycle are affected. From published reports it is known that the nature of the changes observed in the mitotic cycle does not depend upon the kind of radiation. Thus studies on the action of the ultraviolet on L-fibroblasts of the mouse [11] has shown that DNA synthesis is inhibited in the S period. These results agree with observations on the uptake of P^{32} in the thymus when rats were totally irradiated with 200-400R of x-rays [10], which caused a 40-50% reduction in the labelling, whereas a dose of 1,000-500 R caused an increase of labelling of up to 70%. The results of these investigations may be compared with those obtained on irradiation of the mouse spleen in vivo [7]. The three steps in the curve of the uptake of H^3-thymidine in lymphocytes are attributed by the authors to a three-phase interference with the DNA synthesis, the degree of impairment being related to the dose. The most radio-sensitive factor, indicated as S_0, was revealed only in lymphocytes in vivo; it was responsible for the first 35% of the damage when the dose was 200R, and was related to the generation in the nucleus of high-energy phosphates [8]. The next two factors, S_1 and S_2, were identified in early experiments in vitro [5,8]. Factor S_1 was less radiosensitive and caused a subsequent 35% damage after irradiation with a dose of 200-2,000 R. This component is associated with the process of phosphorylation of nucleosides and with the intracellular formation of triphosphates; it is responsible for the normal rate of the process in the presynthetic period (G_1), and also for the normal rate of DNA synthesis in the period of synthesis.

Factor S_2 is relatively insensitive to the action of radiation, it is responsible for 10-20% of the damage which may be the result of the direct gradual distraction of the DNA matrix by increasing the doses of radiation (above 2;000 R).

In our work with gamma-irradiation on mice with a dose of 400 R, in the bone marrow cells 1 h after irradiation, both DNA synthesis (up to 50-60%), and the transition of the cells from the presynthetic into the synthetic period were suppressed. Guided by the results reported in [8] we may suppose that here there is a reduction of S_0 and S_1-factors, so that a transition of the cells from the presynthetic period G_1 into the period of synthesis S is blocked, and that there is also interference with the process of DNA synthesis itself. The results indicate that during irradiation there is a change in the production of bone marrow cells in all periods of the mitotic cycle. As a result of the development of the first block (between the premitotic period and mitosis) there is a brief restraint of the cells in the premitotic period, which lasts about 2-3 h, and which Yamada and Puck [12] interpret as a consequence of disturbance of the condensation of the chromosomes. The development of a second block (between the presynthetic period and the period of DNA synthesis) brings about a prolonged delay of these cells in the presynthetic period which lasts about 18-20 h. A similar alteration to the passage of cells through the phases of the mitotic cycle was encountered also with x-irradiation of regenerating liver [10] and in the action of ultraviolet light on L-fibroblasts [11].

The radioautographic investigation in conjunction with published reports confirms the opinion of Mazia [9] concerning the existence in the mitotic cycle of the so-called irreversible points, which separate the different periods of the cycle, and which are variously sensitive to controlling factors. Thus the establishment in the mitotic cycle of blocks may be considered a general feature which is characteristic of the regulatory power of the cells.

LITERATURE CITED

1. M. N. Veselkina. Tsitologiya, No. 5, (1962), p. 571.
2. O. I. Epifanova and I. N. Smolenskaya. In book: Contributions to the Third Conference on Problems of Regeneration and Cellular Multiplication. [in Russian], Moscow, (1962), p. 55.
3. A. I. Zosimovskaya. In book: Contributions to the Third Conference on Problems of Regeneration and Cellular Multiplication. [in Russian], Moscow, (1962), p. 69.

4. M. G. Chumak. In book: Contributions to the Third Conference on Problems of Regeneration and Cellular Multiplication. [in Russian], Moscow, 1962, p. 190.
5. R. J. Berry, E. Hell, and L. G. Lajtha. Int. J. Radiat. Biol., 4, (1961), p. 61.
6. R. C. Creulich, I. L. Cameron, and J. D. Thrasher. Proc. nat. Acad. Sci. (Wash.), 47, (1961), p. 743.
7. G. H. Huntley and L. G. Lajtha. Int. J. Radiat. Biol., 5, (1962), p. 447.
8. L. G. Lajtha, R. Oliver, and T. Kumatori. et al., Radiat. Res., 8, (1958), p. 1.
9. D. Mazia. In book: The Cell. New York, 3, (1961), p. 108.
10. M. Ord and L. Stocken. Nature, 182, (1958), p. 1787.
11. W. F. Powell. Biochim. biophys. Acta, 55, (1962), p. 969.
12. M. Yamada and T. Puck. Proc. nat. Acad. Sci. (Wash.), 47, (1961), p. 1181.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.