experiments was the result of activation of enzymes of the microsomal fraction of the liver by the pesticides and, consequently, of the more rapid detoxication of the teratogens given, it will be evidence that potentiation of the action of the teratogens may be observed as a result of the inhibitory action of DDT and γ-BHC on these enzymes.

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KINETICS OF BONE MARROW CELLS DURING FRACTIONATED X-RAY IRRADIATION

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The harmful action of fractionated x-ray irradiation (12 rad daily, total dose 250, 500, 750, 1000, or 1500 rad) on hematopoiesis was studied in guinea pigs. The dynamics of the changes in erythro- and myelopoiesis after irradiation was phasic in character. In the first phase activation of proliferative processes was manifested as an increase in the mitotic index, shortening of the mitotic cycle of cells of the erythroid and myeloid series, and their more rapid differentiation, so that as a result a sufficient number of cells entered the blood stream and maintained a near-normal number of erythrocytes and leukocytes in peripheral blood. In the second phase weakening of proliferative processes was observed in the bone marrow, the mitotic index was reduced, the duration of the mitotic cycle was increased, and differentiation of cells of the erythroid and myeloid series was slowed, with the development of anemia and leukopenia in the peripheral blood.

KEY WORDS: *x-ray irradiation*; *blood*; *bone marrow*; *mitotic cycle*.

A single irradiation of animals with x rays in large [2, 4, 7, 9] and small doses [1, 3, 5, 6] causes considerable disturbance of the life cycle of the proliferating bone marrow cells. However, data in the literature are contradictory, evidently because of the use of different sources, conditions, and doses of irradiation, and also with the use of different methods of calculating the duration of the mitotic cycle and of its individual periods.

EXPERIMENTAL METHOD

Guinea pigs were subjected to fractionated x-ray irradiation (tube voltage 180 kV, current 10 mA, filters 0.5 mm copper and 1.0 mm aluminum, focal length 40 cm, daily dose of irradiation 12 rad, total doses 250, 500, 750, 1000, and 1500 rad, total duration of irradiation

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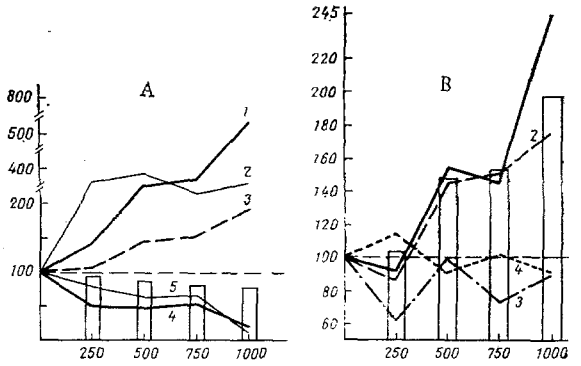

Fig. 1. Changes in cell composition of bone marrow during fractionated x-ray irradiation. A: 1) Reticular cells, 2) blast cells, 3) erythroid cells, 4) immature myeloid cells, 5) mature myeloid cells. Columns show number of myelokaryocytes; B: 1) proerythroblasts, 2) macroblasts, 3) basophilic normoblasts, 4) polychromatophilic normoblasts. Columns show total number of erythroid cells. Ordinate, change in number of myelokaryocytes (A) and erythroid cells (B) (in % of control); abscissa, total dose of irradiation (in rad).

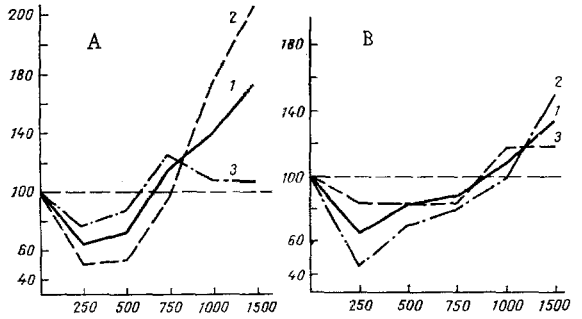

Fig. 2. Changes in duration of mitotic cycle of bone marrow cells and its individual periods during fractionated x-ray irradiation. A) Erythroid cells; B) myeloid cells. 1) Total duration of mitotic cycle; 2) duration of G_1 period; 3) duration of S period. Ordinate, change in duration of cycle and its period (in % of control); abscissa, as in Fig. 1.

1, 2, 3, 4, and 6 months respectively). At the end of each month a full blood count was carried out on the animals; the number of myelokaryocytes was determined in killed animals, films were prepared from the bone marrow for differential counting of myeloblasts and erythroblasts, the mitotic index was determined (separately for cells of the erythroid and myeloid series), and the duration of the mitotic cycle and of its various periods was measured by autoradiography with thymidine-^{3}H [8].

EXPERIMENTAL RESULTS

The experiments revealed considerable changes in the cell composition of the bone marrow in the course of radiation injury. A progressive decrease in the number of myelokaryocytes was accompanied by a quantitative redistribution on the various cells of the myelogram. The absolute numbers of erythroid, reticular, and blast cells increased whereas the number of immature and mature myeloid cells decreased (Fig. 1A).

If the total dose of irradiation was 250 rad the increase in the number of erythroid precursors was due mainly to an increase in the number of polychromatophilic normoblasts.

With large total doses of irradiation, there was a marked preponderance of basophilic normoblasts (500 and 750 rad) or proerythroblasts (1000 rad) (Fig. 1B).

The mitotic index of precursor erythrocytes increased, attaining a maximum value (130% initial) for a total radiation exposure dose of 500 rad, after which it was observed to decrease. The percentage of erythrocytes and hemoglobin in peripheral blood remained normal for a long time and not until the end (3-4 months) was a decrease of this value observed.

The study of the duration of the cell cycle of the erythroid series and of its individual periods revealed phasic changes. In the first phase, with total doses of 250 and 500 rad, the duration of the mitotic cycle was reduced by 26.7% compared with the control, mainly on account of shortening of the G_1 period (by 57%) and a small decrease in the length of the synthetic period. A further increase in the dose of irradiation led to an increase in the duration of the cell cycle on account of the G_1 period, which reached its maximal duration when the total dose of irradiation was 1500 rad (Fig. 2A).

Morphological and cytokinetic investigations of myelopoiesis revealed three phases of changes in the course of irradiation. In the first phase (250 rad) the number of cells in the bone marrow fell considerably on account of a decrease in the number of both immature and mature myeloid cells (Fig. 1A). A significant decrease in the leukocyte count to 83.9% of its initial level and the development of neutropenia and relative lymphocytosis were found in the peripheral blood. In the second phase (500 and 750 rad) the indices remained at the level thus attained during the second and third months of irradiation.

As essential role in the mechanism of development of the first and second phases was probably played by activation of poliferative processes of the cells of the myeloid series. For instance, there was an increase in the mitotic index and a decrease in the duration of the mitotic cycle on account of the G_1 period (Fig. 2B). This led to an increase in the number of cell divisions per unit time; the rate of maturation of the myeloid cells also was increased.

The third phase in the dynamics of chronic radiation injury to myelopoiesis developed toward the end of the fourth month of the experiment, when the total dose of irradiation reached 1000 rad. This phase was characterized by a marked decrease in the number of immature and mature myeloid cells in the bone marrow, to 17.1 and 16.2% respectively of its initial level (Fig. 1A), and also by marked leukopenia in the peripheral blood. Autoradiographic investigation showed considerable depression of proliferation of cells of the myeloid series, especially after a total dose of 1500 rad. The duration of the mitotic cycle was increased by 33.3% of its initial level and of the G_1 period by 50% (Fig. 2B). Meanwhile the number of pathological mitoses and the number of degeneratively changed cells increased considerably both in the bone marrow and in the peripheral blood.

It can be concluded from these results that in response to the harmful action of ionizing radiation proliferation of cells of the erythroid and myeloid series in the bone marrow is activated, especially after irradiation in total doses of 250 and 500 rad. The intensity of hematopoiesis reaches a maximum under these circumstances, and this is manifested by an increase in mitotic activity and a decrease in the duration of the mitotic cycle and of the period of cell differentiation, so that the development of anemia and of profound leukopenia in the peripheral blood is delayed. This phase can evidently be regarded as one of compensation.

With a further increase in the total doses (750, 1000, and 1500 rad) a phase of inadequate compensation of the disturbances caused by the harmful action of irradiation ensues. Activity of proliferative processes is reduced (the duration of the mitotic cycle is increased on account of the G_1 period, which is the most sensitive period to the action of ionizing radiation [10], and differentiation of the bone-marrow precursors of mature blood cells is delayed). This state of the cytokinetic processes during prolonged exposure to irradiation may evidently herald exhaustion of the reserve capacity of the bone marrow and the development of radiation pancytopenia.

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