OSTEOBLASTIC REACTION OF THE BONE MARROW OF IRRADIATED MICE AND GUINEA PIGS

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After whole-body x-ray irradiation of (CBA × C57BL) F_1 mouse hybrids and guinea pigs the number of osteoblasts was counted in the medullary cavity of the femoral diaphysis. A significant increase in the number of osteoblasts (by 2-5 times) was observed by the 10th day after irradiation in both species of animals, to be followed by a decrease in their number on the 20th day. Between the 20th and 30th days the number of osteoblasts reached the level of these cells in intact animals.

Close relationships exist between the bone and hematopoietic tissues as a result of their genetic, functional, and anatomical characteristics [1, 4]. Hematopoietic tissue is more radiosensitive than bone tissue [2, 6]: irradiation within the dose range producing a bone-marrow syndrome eliminates hematopoietic cells but causes less severe damage to bone. In this way the integrity of the system of hematopoietic and bone tissues is disturbed. For this reason it is important to know how the elements of osteogenic tissue behave under such situations: information in the literature on this subject is at present inadequate.

The object of the present investigation was to study the osteoblastic reaction of the bone marrow of irradiated mice and guinea pigs.

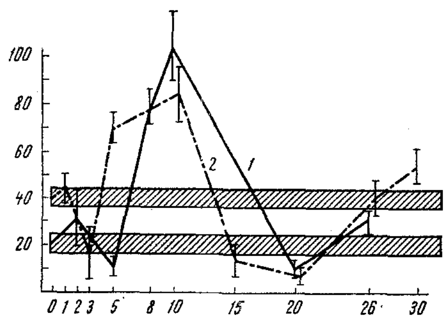

Fig. 1. Change in number of osteoblasts per standard area of femoral diaphysis of irradiated mice (1) and guinea pigs (2). Abscissa, days after irradiation; ordinate, mean number of osteoblasts.

EXPERIMENTAL METHOD

(CBA × C57BL) F_1 mouse hybrids and guinea pigs were used for the experiments. The mice were irradiated in a dose of 850-900 R on an EGO-2 apparatus (Co^{60}, dose rate 409-396 R/min). The animals were sacrificed after 2, 5, 8, 10, 20, and 26 days, and 7-15 animals were studied at each time. The guinea pigs were irradiated on the same apparatus in a dose of 300 R (dose rate 700 R/min). These animals were sacrificed after 1, 3, 5, 10, 15, 20, 26, and 30 days, and 5-7 guinea pigs were studied at each time. The femora were fixed in Bouin's fluid and decalcified in 5% nitric acid. The material was embedded in paraffin wax and longituidinal sections were cut to a thickness of 5-7 μ. The sections were stained with hematoxylin-eosin, and the number of osteoblasts in an area of diaphysis measuring 2.5 mm in length, starting from the boundary between the metaphysis and diaphysis, was counted under the microscope. The number of osteoblasts, which appeared as slightly elongated cells with a small quantity of basophilic cytoplasm and a hyperchromic nucleus, found at different times after irradiation was compared

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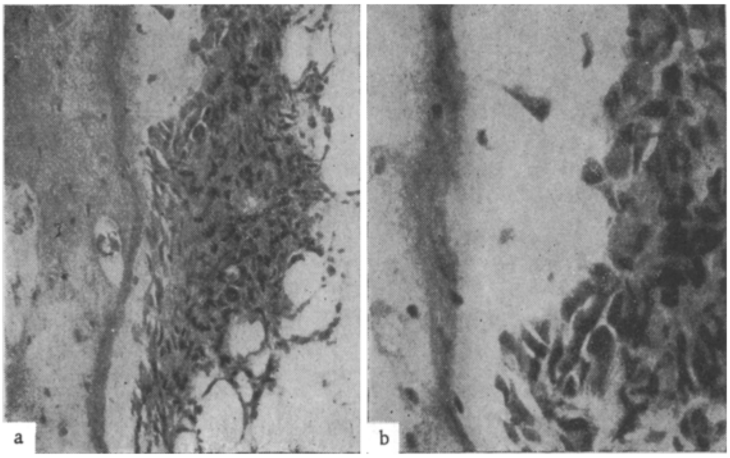

Fig. 2. Increase in number of osteoblasts in medullary cavity of the diaphysis of an irradiated mouse (10th day): accumulation of osteoblasts on surface of cortical bone of the diaphysis (left) and osteoid formation (right). Hematoxylin-eosin. Magnification: a) 200 ×; b) 400 ×.

with their number in the intact animals. The numerical results were subjected to statistical analysis [5]. Differences were regarded as significant when $P \leq 0.05$.

EXPERIMENTAL RESULTS

The character of the curve in Fig. 1 shows that after irradiation of the mice and guinea pigs the number of osteoblasts in the medullary cavity of the femoral diaphysis fluctuates. In intact animals of both species the number of osteoblasts located in the investigated area of bone in a single layer was 21 and 40, respectively. By the 10th day after irradiation the number of osteoblasts was sharply increased, reaching 104 in mice and 85 in guinea pigs ($P \leq 0.01$). The hematopoietic tissue in the mice at this time consisted of individual foci of erythroid and myeloid type, scattered among the hyperemic stroma. In the guinea pigs, on the 10th day the medullary cavity was uniformly filled with hypoplastic hematopoietic tissue. Osteoblasts, larger in size than in the control, were elongated in shape, with an extensive basophilic cytoplasm and a nucleus at the periphery. They formed one or two layers in the metaphysis and in the greater part of the diaphysis, or they lay as a group of cells producing osteoid (Fig. 2). By the 20th day the number of osteoblasts was reduced ($P \leq 0.05$): their number was 9 in mice and 8 in guinea pigs. At the same time, definite recovery of hematopoiesis was apparent in the animals of both species. However, the total number of hematopoietic cells in the bone marrow had not yet reached normal.

The maximum of the number of osteoblasts on the 10th day can be regarded as evidence that the precursor cells of the osteoblasts found in the bone marrow differentiate more intensively after irradiation, and this is accompanied by their appearance in large numbers in the diaphysis. The decrease in number of osteoblasts in the period between the 10th and 20th days is evidently due to the fact that pro-osteoblasts have ceased differentiating into osteoblasts, and are evidently engaged in maintaining the cell pool. The period between the 20th and 30th days, characterized by a gradual increase in the number of osteoblasts and hematopoietic cells, can be regarded as a later phase of the regenerative process of osteogenic and hematopoietic tissue.

These results demonstrate a phenomenon of transformation of the precursor cells of the osteoblasts under the influence of radiation into osteoblasts, with subsequent exhaustion of the precursors and restoration of the normal number of osteoblasts by the 30th day. In this connection it is interesting to note that investigation of the osteogenic properties of bone marrow, as revealed by heterotopic transplantation, showed that mouse bone marrow loses its osteogenic properties within a few days after irradiation in a dose of 825 R (although this dose does not possess a total inhibitory action on osteogenic cells immediately after irradiation) [3]. After the 26th day, the osteogenic properties of the radiochimeras are restored. If these results are compared with those of the present investigation, it can be postulated that loss of the osteogenic properties of bone-marrow tissue during the first few weeks after irradiation is dependent on exhaustion of the pool of osteoblast precursor cells as a result of their increased differentiation into osteoblasts.

The model of quantitative counting of osteoblasts used in this investigation is suitable for the study of the system of interactions between osteogenic and hematopoietic tissues when equilibrium between them is disturbed.

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