# DISTRIBUTION OF TRANSPLANTED MARROW CELLS AND DEVELOPMENT OF FOCI OF DONOR HEMOPOIESIS IN IRRADIATED RECIPIENTS

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If bone marrow cells are injected intravenously into irradiated animals, multiple foci of donor hemopoiesis are formed in the hemopoietic organs of the true radiation marrow chimeras, and so far little attempt has been made to study the dynamics of their formation. According to reports in the literature [4-6], in irradiated animals the marrow cells and the blood cells are retained in the lungs for the first few minutes after injection, but later most of the cells recirculate and accumulate in the marrow, spleen, and liver. The subsequent fate of these cells has been studied for only 3 days, or in some cases not at all.

The object of the present investigation was to study the dynamics of development of foci of donor hemopoiesis in irradiated recipients. For this purpose the primary localization of the injected marrow cells, their subsequent recirculation, and their secondary deposition were examined.

## EXPERIMENTAL

Experiments were carried out on 72 male CC57W mice, receiving a single dose of whole-body irradiation on a type RUM-11 apparatus amounting to 700 R (voltage 180 kV, current 15 mA, filters 0.5 mm Cu, half-thickness layer 1 mm Cu, distance from tube anode 40 cm, dose rate 36 R/min). The donors of heterologous marrow were noninbred albino rats. Marrow from the recently sacrificed animals containing 80-90 million nucleated cells was injected intravenously 24 h after irradiation in a dose of 0.7-0.8 ml. The recipient mice were sacrificed 5 and 15 min, 1 and 2 h, and 1, 2, 3, 4, 6, and 9 days after transplantation; no fewer than 5 mice were investigated at each time. The cells of rat type were identified in the histological sections of the lungs, spleen and liver and in the peripheral blood films and the films prepared from marrow suspensions, by mean of the Gomori test for alkaline phosphatase. In the histological sections the cells with a positive reaction for alkaline phosphatase were counted in 20 fields of vision of the microscope (objective 40×, ocular 10×) and the result expressed per field of vision, while in the films (objective 20×, ocular 5×) the results were not converted in this way.* The values obtained were analyzed by statistical methods.

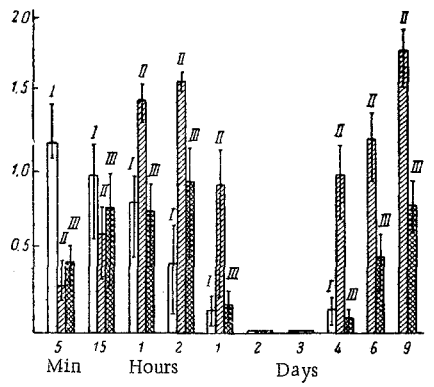


Fig. 1. Changes in the number of donor's granulocytes in the organs of irradiated recipients (confidence limits calculated for P=0.05). I) lung; II) spleen; III) liver. Abscissa) time after transplantation; ordinate) log of number of donor's granulocytes in one field of vision.

## EXPERIMENTAL RESULTS

Analysis of the results showed (Fig. 1) that 5 min after injection of the rat marrow cells granulocytes of donor type were found in the largest numbers relative to the liver and spleen (the difference is statistically significant) in the lungs, where they were located mainly in the capillaries of the interalveolar septa (Fig. 2a).

*The rat granulocytes in the blood and marrow films were counted by N. V. Butomo.

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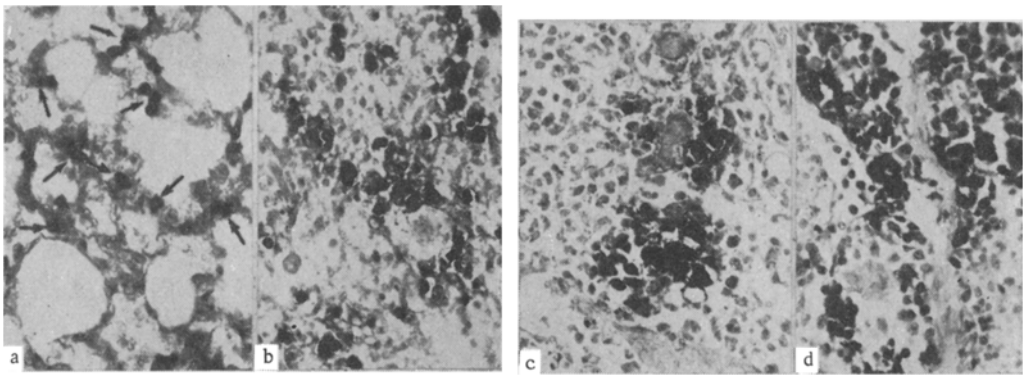


Fig. 2. Distribution of donor's granulocytes in the recipient's organs. a) Donor's granulocytes (indicated by arrows) in the lung capillaries 5 min after transplantation; b) diffuse arrangement of phosphatase-positive cells in the red pulp of the spleen 1 h after transplantation; c) appearance of foci of donor hemopoiesis in the red pulp of the spleen 4 days after transplantation; d) massive foci of phosphatase-positive cells in the spleen 9 days after transplantation. Gomori reaction. Magnification: objective 20×, ocular 20×.

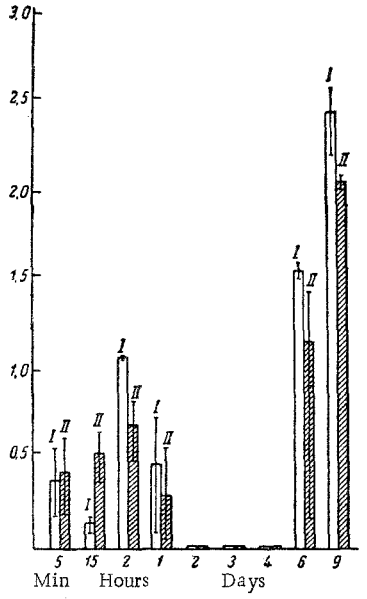


Fig. 3. Changes in the number of donor's granulocytes in films of blood and marrow of irradiated recipients (confidence limits calculated for P = 0.05). I) marrow; II) blood. Absciss) time after transplantation; ordinate) log of number of donor's granulocytes in 20 fields of vision.

The largest foci of these cells were discovered in the peripheral portions of the lungs, beneath the pleura, presumably because of the anatomo-physiological structural features of the capillaries, which in these parts are capable of storing the largest number of injected rat granulocytes. The phosphatase-positive cells in the capillaries were elongated in shape and lay in chains, often reproducing the outlines of the capillaries.

The donor's granulocytes were less numerous in the red pulp of the spleen, where they were often found near the lymphatic follicles but never in the Malpighian corpuscles. The phosphate-positive cells were slightly more numerous in the liver than in the spleen (the difference is not statistically significant), and they were located in the lumen of the intralobular capillaries. Donor's granulocytes were also present in the peripheral blood and marrow films, but they were much fewer than in the organs.

Fifteen minutes after transplantation, a tendency for the donor's cells in the recipient's body to undergo redistribution appeared. This phenomenon was seen most clearly 1-2 h after the injection, when the number of donor's granulocytes in the lungs had fallen and the number on the spleen had increased correspondingly (the figures are statistically significant, Figs. 1 and 2b). In the liver and marrow the number of donor's granulocytes had also increased, although less so than in the spleen. This redistribution of the injected marrow cells was accompanied by a small increase in the number of rat granulocytes in the recipient's peripheral blood (Fig. 3). This increase is not statistically significant.

By comparison with the indices 2 h after the injection, the number of donor's granulocytes in the recipients' organs and blood 24 h after injection showed a statistically significant decrease, and extracellular phosphatase-positive granules had begun to appear.

On the 2nd and 3rd days after transplantation no adult heterophilic granulocytes of donor type could be found in the irradiated recipients (Figs. 1 and 3).

Starting on the 4th day after injection of the rat marrow, a secondary increase in the number of granulocytes of donor type took place in all the organs studied histologically (Fig. 1). In contrast to the early periods of the investigation, at this time the character of the arrangement of these cells in the spleen and liver was totally different. In the spleen the phosphatase-positive cells were not diffusely scattered throughout the red pulp (Fig. 2b), but concentrated in small or larger foci located under the capsule and near the trabeculae (Fig. 2c). In the liver clusters of heterophilic granulocytes of donor type were seen around the septal veins. In the lungs, on the other hand, the character of the distribution of these cells was just as before, i.e., few were seen in the interalveolar capillaries (these cells were not counted). The development of foci of donor hemopoiesis on the 4th day after transplantation in the spleen and liver of the recipients was not accompanied by the appearance of donor's granulocytes in the peripheral blood (Fig. 3). Evidently, these cells were so few in number that they could not be detected in the blood in any significant number.

Not until the 6th day after transplantation did rat granulocytes appear in the recipients' blood, and their number increased progressively in the later periods of the investigation.

By the 6th-9th days after injection of the preparation, phosphatase-positive cells were found in the spleen in the form of large, confluent foci (Fig. 2d), and often the rat granulocytes were found also in the lymphoid follicles, apparently replacing them. The number of focal collections of phosphatase-positive cells in the liver and bone marrow was likewise increased.

The results of these experiments, in agreement with those of other workers [2, 5] who describe the deposition of a few heterologous marrow cells in the lung capillaries 5 min after their intravenous injection, may evidently help to explain the worsening of the animals' condition in the first 5-30 min after the injection, when severe dyspnea and adynamia were observed. These phenomena were evidently associated with the acute hypoxia developing as a result of the massive "obturation" of the lung capillaries by the marrow cells.

The phenomenon of primary retention of rat granulocytes by the recipient's lungs observed in these experiments does not confirm the observations of other investigators [1], who did not find it after the intravenous injection of homologous bone marrow into unirradiated rabbits. Possibly the luminescence method of identification of the donor's cells used by these workers is unsuited for experiments of this type, for the fluorochrome diffuses rapidly out of the cells into the surrounding medium and the cells lose their label.

Analysis of the distribution of phosphatase-positive cells in the organs and blood of the irradiated recipients showed that on the 2nd and 3rd days after transplantation no rat granulocytes could be detected. Evidently, most of the injected alkaline phosphatase-positive cells died quickly, and the retained young cells adapted themselves to the new conditions of existence, but without attaining the degree of differentiation at which they could be detected by the Gomori reaction. In these experimental conditions the adaptation period, with the subsequent proliferation and differentiation of the young heterologous hemopoietic marrow cells in the irradiated animals, ended on the 4th day after injection, when foci of donor hemopoiesis appeared in the hemopoietic organs.

When only the histochemical method of identification of the donor's cells in the recipient is used, only the distribution of the mature cells of the granulocyte series and the dynamics of development of only the leukoblastic foci of hemopoiesis can be determined. However, taking into consideration results obtained by investigators using other methods of identification of the donor cells, and who obtained approximately the same results of the primary retention and recirculation of the injected cells [2-6], it may be postulated that the distribution of the phosphatase-positive cells reflects the dynamics of circulation of younger forms of hemopoietic cells in the recipient's body, and the development of donor foci of the erythroid and magakaryocytic series takes place in the same way as the development of the granulocytic series of hemopoiesis.

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