DISTRIBUTION OF BONE MARROW CELLS AFTER INJECTION INTO ANIMALS WITH EXPERIMENTAL HYPOLASTIC ANEMIA

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After intramedullary injection of bone marrow cells into rabbits with hypoplastic anemia due to benzene, the cells enter the blood stream and are deposited in the hematopoietic tissue of the bones and in the liver.

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The object of this investigation was to produce hypoplasia of the hematopoietic system in animals and to study the fate of donors' hematopoietic cells injected into them.

EXPERIMENTAL METHOD

Experiments were carried out on 10 rabbits weighing 3000-4000 g. Hypoplasia of the hematopoietic system was produced by benzene, injected subcutaneously into the abdominal wall mixed with an equal volume of peach oil in a dose of 0.5-1 ml pure benzene/kg body weight once or twice a week. Regular counts were made of the leukocytes, erythrocytes, reticulocytes, and platelets of the experimental animals and the leukocyte formula of the blood was determined. Healthy rabbits weighing 2000-2800 g acted as control. Their bone marrow was aspirated intravitally and labeled with fluorochrome–acridine orange (see [1]) for the technique) and injected by the intraosseous route into the distal epiphysis of the left femur of animals with hypoplasia of their hematopoietic system. To determine the character of distribution of the donors' cells in the recipient, blood samples, were taken from the auricular vein and bone marrow samples from the distal epiphysis of the right tibia every 1, 2, 3, 5, and 10 min after injection. The animals were sacrificed after 1 h and impressions of their lungs, liver, spleen, and bone marrow at the site of injection were studied. These specimens were examined in the luminescence microscope.

EXPERIMENTAL RESULTS

Hypoplastic anemia was produced in only 2 animals, for the other 8 died at various times (from 0.2 to 3 months). The observations on the rabbits in which hypoplasia of the hematopoietic system was successfully produced continued for 5.5 months (rabbit no. 1) and 1 year (rabbit no. 7). The first rabbit received 34 subcutaneous injections of benzene (55.5 ml) during this period, and the second received 62 injections (170.3 ml). The external manifestations of benzene poisoning were increasing lethargy of the animals, loss of appetite and weight, a blood-stained discharge from the eyes, nose, and mouth, and areas of necrosis of the skin.

The hematologic picture of chronic benzene poisoning was as follows: to begin with leukopenia developed, after 5-9 injections of benzene. In some cases (rabbits Nos. 4, 9, 5, 10), against the background of progressive leukopenia, sometimes transient rises in the number of leukocytes to normal values followed by a fall to 1000-500-150/mm^3 were observed in the terminal or preterminal period. In the two rabbits in which hypoplastic anemia was produced, a marked neutropenia, both relative and absolute, was observed. Morphological changes in the leukocytes consisted of the appearance of signs of degeneration (some neutro-

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TABLE 1

Blood cells	Rabbit No. 1	Rabbit No. 7
Hemoglobin	6.2 g%	6 g%
Erythrocytes/mm^3	2,600,000	2,590,000
Leukocytes/mm^3	1,700	550
Stab cells	–	1%
Polymorphs	15%	7%
Lymphocytes	82%	82%
Monocytes	3%	5%
Basophils	–	5%
Platelets/mm^3	18,000	Single cells in specimen
Reticulocytes	2.5%	4%
In 1 mm^3 bone marrow		
myelokaryocytes	26,000	10,000
megakaryocytes	3	None found

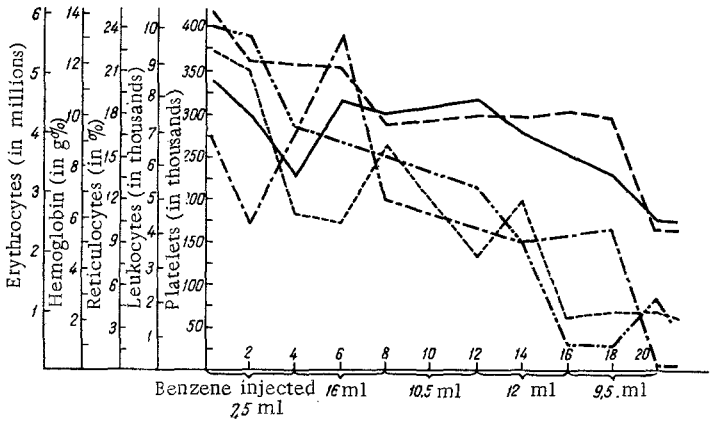

Fig. 1. Dynamics of numbers of blood cells and hemoglobin concentration in rabbit No. 1.

phils took up the stain poorly; vacuolation of the nucleus and cytoplasm, hypersegmentation and pycnosis of nuclei of the neutrophils, and the appearance of toxic granules in the cytoplasm were observed).

Thrombocytopenia developed most commonly after 5-9 injections of benzene and was progressive in character, although sometimes transient rises in the number of platelets to subnormal values took place. A sharp decrease in the number of platelets usually occurred in the terminal period and was accompanied by hemorrhagic manifestations.

Changes affecting erythropoiesis developed last of all. In the 8 rabbits which died either anemia was absent or there was only a small decrease in the number of erythrocytes for the hemoglobin concentration. As the anemia developed, the reticulocytosis observed initially was replaced by a decrease in the number of reticulocytes, sometimes with transient rises (to 71% in rabbit No. 7) in the preterminal periods. A tendency toward anemia usually appeared after 20 injections of benzene. The hemoglobin concentration and erythrocyte count fell in severe cases of poisoning to 6 g% and 2,590,000 respectively. The hematologic picture at the end of the experiment in the 2 rabbits in which hypoplastic anemia was produced is seen in Table 1.

These results clearly show that the pancytopenia in the circulating blood was accompanied by a sharp decrease in the number of myelokaryocytes and megakaryocytes in the bone marrow. The dynamics of changes in the hematologic indices of rabbit No. 1 are shown in Fig. 1.

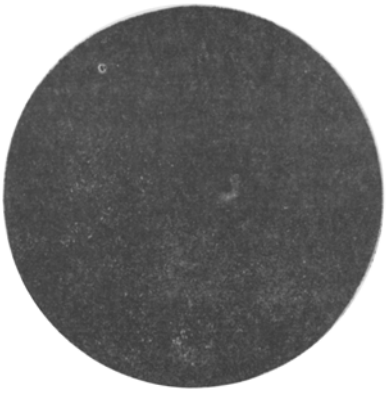

Fig. 2.

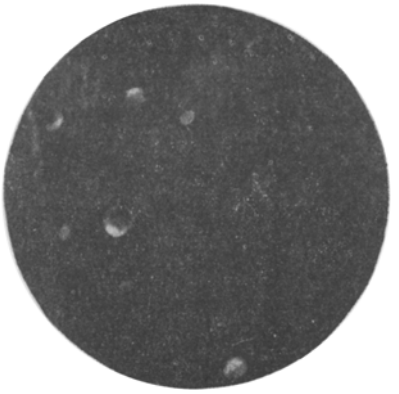

Fig. 3.

Fig. 2. Fluorescent bone marrow cells in the circulating blood of rabbit with hypoplastic anemia 1-2 min after intraosseous injection.

Fig. 3. Fluorescent donor's bone marrow cells in medulla of right tibia of a rabbit with hypoplastic anemia 5-10 min after intraosseous injection into left femur.

It must be stressed, when summing up these results, that hypoplasia of the hematopoietic system in rabbits can be produced only by the prolonged, repeated, and spread out (once or twice a week) subcutaneous injection of relatively small doses of benzene (0.5-1 ml/kg body weight) into large, adult animals. The frequent injection of benzene (three times a week) into rabbits causes the rapid development of leukopenia with the appearance of foci of necrosis and death of the animals. The transient rises in the number of leukocytes platelets and reticulocytes in the preterminal periods sometimes observed were evidently due to mobilization of all the compensatory powers of the animal.

Examination of films in the luminescence microscope revealed the following picture: hematopoietic cells appeared in the blood stream 1-2 min after intraosseous injection (Fig. 2). In subsequent tests the number of fluorescent cells in the circulating blood fell, and by 10 min they had disappeared completely. The opposite picture was observed in specimens obtained from the bone marrow. In this case solitary fluorescent cells were detected 1-2 min after injection of donor's bone marrow. The number of fluorescent cells reached a maximum 5-10 min after injection (Fig. 3). Most hematopoietic cells injected by the intraosseous route remained at the site of injection. No incorporation of fluorochrome-labeled cells into the fatty bone marrow was observed. No fluorescent cells were found in impressions of the internal organs (spleen, lungs) taken from animals with hypoplastic anemia and sacrificed 50-60 min after injection of labeled bone marrow. Only in the specimen from the liver were a few fluorescent cells found. This last fact is evidently explained by partial deposition of donor's hematopoietic cells in the liver of a rabbit with experimental benzene hypoplastic anemia, their phagocytosis by Kupffer cells, and by increased permeability of the blood-parenchyma barrier through toxic damage to the liver [2].

After intraosseous injection of the hematopoietic cells of a donor, most of them remain at the site of injection, but some are deposited in the epiphyses of other bones—in areas where hematopoiesis still continues. A few fluorescent cells are retained in the liver.

LITERATURE CITED

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