

CHANGES IN BONE MARROW HEMOPOIESIS IN DONOR RABBITS

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Because of the use of bone marrow in the treatment of many diseases of the blood system (leukemias, aplastic and hypoplastic anemias) and, in particular, of acute radiation sickness, the study of bone marrow is very important. Furthermore, it has been shown [5, 6] that transplantation of bone marrow may be used to prevent the development of anemia and leukopenia during the treatment of various neoplastic diseases with chemical cytostatic preparations. Accordingly, the problem of methods of obtaining bone marrow in the necessary amounts, and of the effect of aspiration of bone marrow on the donor, is very important. Insufficient attention has been given to it in the literature. Only a few papers have been found [1-4, 7] describing observations on marrow donors; in these cases a single removal of bone marrow did not lead to pathological changes in the donor.

The object of the present investigation was to examine how 2, 3, or more removals of bone marrow affects the donor and to study the after-effects of such procedures, depending on the removal of different volumes of marrow.

EXPERIMENTAL METHOD

Male and female rabbits weighing 2200-3500 g were used as marrow donors. The animals were kept in ordinary conditions and on a normal diet.

Bone marrow was extracted from the living rabbits with a modified Kassirskii's needle, by aspiration from the cancellous bone of the femur, tibia, fibula, and ilium.

The state of hemopoiesis in the donor rabbits was determined from the blood and marrow pictures. Blood was taken from the auricular vein, the hemoglobin concentration and the number of erythrocytes, leukocytes, and reticulocytes was determined, and the blood formula calculated. The number of myelokaryocytes in the myelogram was also counted. All these indices were obtained for each animal before the experiment, on the 3rd day after removal of the bone marrow, one week later, and thereafter daily for 1 month and then monthly for the duration of the experiment.

The experimental animals (17 rabbits) were divided into several groups.

Group 1 (5 rabbits) consisted of animals from which marrow was extracted once only, in a volume of 10-20 ml. Observations on these animals were made for 6 months.

Group 2 also consisted of 5 rabbits. Marrow from these animals was extracted in a volume of 10-20 ml (i.e., 5 ml/kg body weight) twice or three times at intervals of 4-5 months in the course of 1 year. The number of myelokaryocytes in the extracted material varied within wide limits. The first samples of marrow usually contained many myelokaryocytes—from 98,000 to 182,000 per mm^3 , while later samples were mixed with considerable amounts of blood so that the number of myelokaryocytes varied from 4000-6000 to 10,000-17,000 per mm^3 of material extracted.

Group 3 consisted of 4 rabbits from which marrow was extracted in a volume of 25-50 ml 3 or 4 times at intervals of 4-5 months. These animals remained under observation for 16-24 months.

Group 4 consisted of 3 rabbits from which bone marrow was extracted in a volume of 10-25 ml 7 or 8 times at intervals of 1.0-1.5 months. Observations on these animals continued for 1 year.

EXPERIMENTAL RESULTS

The state of all the rabbits except those of group 3 remained good throughout the experiment, and they neither lost their appetite nor failed to gain in weight.

So far as the results of the hematological investigations were concerned, the animals of groups 1 and 2 could be considered together. In these animals, on the 3rd day after removal of bone marrow, a very slight fall in the hemoglobin and in the erythrocyte count was observed, accompanied by a constant increase in the number of reticulocytes. As a rule, the leukocyte count showed a marked fall. However, these changes were not long lasting, and by the beginning of the 2nd week after removal of the marrow a tendency towards the normalization of these indices was observed. One month after removal of marrow the hemoglobin concentration and the erythrocyte and leukocyte counts had usually returned to normal, or were even above normal. The relative reticulocyte count at this period, as a rule, had not yet returned to the initial level. Blood films taken during the first 2 weeks after the removal of marrow revealed anisocytosis, poikilocytosis, and polychromatophilia, together with the presence of normoblasts and erythroblasts. In some animals the number of monocytes in the blood had increased to 12-15%. In the subsequent months all the blood indices investigated varied within normal limits. During the first month after the removal of marrow the total number of myelokaryocytes fell significantly. Their initial level was regained only after 2-3 months. The myelogram also showed marked disturbances: the relative numbers of immature leukoblastic and erythroblastic cells were modified with an increase in the number of primitive white cells. These fluctuations corresponded to the changes in the myelokaryocytes. Restoration of the original picture of the myelogram likewise was not observed until 2-3 months after removal of the marrow.

The changes observed after repeated removal of bone marrow were roughly the same.

Different results were obtained with the animals of group 3 from which marrow was extracted 3-4 times in the course of 1 or 1½ years. Each time a total volume of 25-50 ml of bone marrow could be obtained from 6 punctures. In the course of the whole experiment, between 100 and 130 ml of blood was taken from each animal. In these conditions, besides true bone marrow cells, a considerable volume of blood was naturally extracted. Whereas the first samples of material were very rich in myelokaryocytes (their mean count was 118,000 per mm³), in later samples their number was hardly greater than the number of leukocytes in the peripheral blood (mean count 7500 per mm³ of material extracted).

In this group of rabbits the changes in the blood count after the first removal of marrow were in general similar to those described above. The hematological reaction to the removal of marrow was expressed as a very slight fall in the hemoglobin concentration and erythrocyte count. The total leukocyte count showed a rather greater fall (in 1 rabbit, for example, it fell from 10,450 to 7,050, and in another from 9,000 to 6,650 per mm³ of blood). All these changes were essentially the same as those observed in the two preceding series of experiments. The disturbances affecting the reticulocytes were more marked: their number rose considerably, almost certainly as the result of the removal of large volumes of bone marrow.

In the marrow itself the number of myelokaryocytes fell considerably. In the myelogram, the relative percentages of primitive white and red cells were changed, although this time in the direction of an increase in the number of red cells on account of the appearance of large numbers of basophilic and polychromatophilic erythroblasts. These changes persisted rather longer than in the preceding experiments. The further removal of a large volume of bone marrow led in one case to intensive stimulation of the reticulo-endothelial system. Numerous reticulum cells and cells resembling monocytes (up to 20-25%) appeared in the bone marrow and peripheral blood, while in 2 other cases the appearance of numerous mononuclear cells resembling micromyeloblasts (20-25%) was observed in the marrow. After the third or fourth aspiration of marrow, all the animals of this group began to lose weight and to develop anemia (the hemoglobin concentration fell in the first case from 78 to 40 units, in the 2nd from 65 to 37 units, in the 3rd, from 62 to 34 units, and in the 4th, from 72 to 44 units). The number of erythrocytes per mm³ blood fell correspondingly from 5,100,000 to 2,750,000, from 4,500,000 to 2,000,000, from 4,700,000 to 2,200,000, and from 5,680,000 to 2,050,000, and all the animals later developed septicemia (multiple abscesses, osteomyelitis of several bones with sequestration of bone tissue, and marked leukocytosis with a marked shift of the formula to the left were observed).

The depression of the reactivity of the donor was also revealed by a progressive fall in the reticulocyte count. Instead of the usual reaction (an increase in the reticulocyte count) to removal of bone marrow, after each successive extraction a depression of erythropoiesis was observed and an anemia of hyporegenerative character developed. The

number of cells of the myeloid series in the bone marrow increased, with a predominance of neutrophils (metamyelocytes, stab cells, segmented polymorphs). As a result of these changes 2 rabbits died, while 2 more were sacrificed in a serious condition. At autopsy, multiple abscesses were found in the internal organs of these animals.

In group 4 the repeated extractions of bone marrow were not essentially reflected in the general condition of the experimental animals. In the rabbits of this group the changes in hemopoiesis differed only slightly from those observed in the animals of groups 1 and 2. However, it may be concluded from the results of the experiments in group 3 that the extraction of marrow in a volume of 20 ml monthly for several months caused significant changes in the animal organism.

SUMMARY

It was shown in an experiment on 17 rabbits that animals from which bone marrow had been extracted in amounts of 10-20 ml one time, 2-3 times at intervals of 4-5 months or 7-8 times at intervals of 1-1.5 months were in good condition during the entire experiment and even put on weight. Four animals from which bone marrow was taken in large quantities (25-50 ml 3-4 times at 4-5 months intervals) developed anemia of a hyporegenerative character 12-16 months after the beginning of the experiment, which was followed by a septic conditions with multiple abscesses in the visceral organs. Hence, repeated extractions of large quantities of bone marrow may bring about severe disorders in hemopoietic processes and a reduction in the body resistance.

LITERATURE CITED

1. I. V. Il'inskii and T. N. Astakhova, In book: Problems in Hematology and in the Conservation of Blood and Tissues [in Russian], Leningrad (1961), p. 127.
2. M. A. Kotovshchikova, L. K. Nikolaeva, and N. M. Ivanova, et al., Probl. gematol., 10, 35 (1963).
3. D. I. Rafal'son, Z. F. Veikher, and L. M. Rozanova, et al., Probl. gematol., 10, 29 (1963).
4. C. T. Nicolau, G. Grigoriu, and V. Apatianu, et al., Probl. gematol., 5, 35 (1962).
5. G. Mathé, Sang, 30 (1959), p. 747.
6. W. McFarland, N. Granville, and W. Dameshek, Blood, 14 (1959), p. 503.
7. R. Schwartz, D. Misra, and W. Dameshek, Ibid., 15 (1960), p. 137.

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.
