The cultivation temperature thus has a significant effect on the intensity of blast transformation in tissue culture; the effect of temperature on blast transformation has certain special features which depend on the donors' age.

LITERATURE CITED

1. L. G. Barbaruk, Tsitol. Genet., No. 1, 28 (1974).
2. P. A. Borodkin, Byull. Éksp. Biol. Med., No. 12, 87 (1972).
3. P. A. Borodkin, in: Biological Research in the North-East of the European Part of the USSR [in Russian], Syktyvkar (1974), p. 161.
4. V. P. Voitenko, L. G. Barbaruk, and Yu. V. Pakin, Ter. Arkh., No. 12, 34 (1974).
5. K. M. Geine, R. Klatt, G. German, et al., Probl. Gematol., No. 8, 29 (1970).
6. N. R. Ling, Stimulation of Lymphocytes [in Russian], Moscow (1971), p. 31.
7. P. G. Nazarov, Lab. Delo, No. 2, 74 (1975).
8. Yu. V. Pakin and L. G. Barbaruk, Tsitol. Genet., No. 3, 195 (1976).
9. V. G. Pisarenko, Yu. V. Pakin, and V. N. Starkov, Dokl. Akad. Nauk Ukrainsk. SSR, Ser. B, No. 6, 552 (1976).
10. N. L. Samoilina, Lab. Delo, No. 8, 455 (1970).
11. A. Ya. Fridenshtein and I. L. Chertkov, The Cellular Bases of Immunity [in Russian], Moscow (1969), p. 92.
12. A. S. Coulson and D. G. Chalmers, Nature, 209, 378 (1966).
13. H. M. Hallgren, C. E. Buckley, and E. J. Yunis, Gerontologist, 13, 46 (1973).
14. O. R. McIntyre and A. F. Cole, Int. Arch. Allergy, 35, 105 (1969).
15. M. E. Weksler and T. H. Hutteroth, J. Clin. Invest., 53, 99 (1974).

PROLIFERATIVE ABILITY OF NUCLEATED RAT BONE MARROW CELLS AFTER FREEZING AND THAWING

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The proliferative power of rat bone marrow myelokaryocytes after freezing and thawing under the protection of hydroxyethyl derivative of tetrahydric alcohol (HEDTA) was studied in experiments on mouse—rat radiation chimeras. Rat bone marrow cells were shown to preserve their proliferative power.

KEY WORDS: *bone marrow; proliferative activity; freezing—thawing; radiation chimeras.*

The criterion of effectiveness of low-temperature conservation of bone marrow cells is the assessment of their biological activity, whereby the functional integrity of the cells can be determined. The functional activity of the conserved cells is characterized by their proliferative ability in the recipient.

The object of this investigation was to study the proliferative ability of nucleated rat bone marrow cells after freezing and thawing (to —196°C) under the protection of the hydroxyethyl derivative of tetrahydric alcohol (HEDTA).

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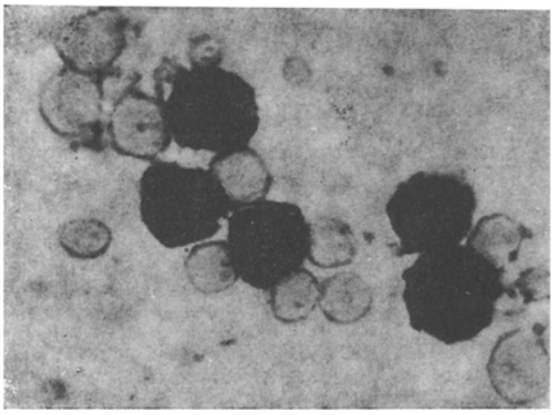

Fig. 1. Phosphatase-positive rat neutrophils in mouse bone marrow. Reaction for alkaline phosphatase by Burstone's method, 900×.

EXPERIMENTAL METHOD

To determine the proliferative ability of the myelokaryocytes, as well as other methods, histochemical labeling is used, by means of which transplanted foreign donor's cells can be detected in the recipient. This method, suggested by Nowell et al. [9], was used as the basis of the present experimental investigation. At the same time, the viability of the transplanted bone marrow cells was estimated by rapid methods: The number of nucleated cells in 1 μl of marrow was counted and the percentage of viable cells determined by the eosin test.

Noninbred albino mice were used as recipients and albino rats as donor. Bone marrow was obtained from the femora of the donors after decapitation by flushing out with TsOLIPK-3 preservative solution. Part of the bone marrow suspension was used as the freshly prepared graft and the rest was frozen. The bone marrow suspension was frozen under the protection of a 15% solution of HEDTA in a two-stage program. After thawing and determination of the absolute number and relative percentage of viable cells, the bone marrow suspension was used as the graft. The freshly prepared conserved bone marrow was injected into irradiated mice in an equal volume.

The recipient mice were irradiated with ^{60}Co γ rays in a lethal dose of 900 R on the Rokus apparatus. A lethal dose of irradiation was necessary because, according to data in the literature [8], injections of bone marrow into mice irradiated with doses in the sublethal range do not give a protective effect and they actually increase the mortality among the animals.

In each series of experiments (four altogether) 50 sexually mature male mice weighing 18-20 g were used. The animals were divided into three groups. Between 2 and 3 h after irradiation, a freshly prepared suspension of rat bone marrow cells was injected into the mice of group 1, the mice of group 2 received a suspension of preserved cells, and the mice of group 3 acted as the control. The suspension of rat bone marrow cells was injected into the caudal vein of lethally irradiated mice in a dose of $4 \cdot 10^5$-$5 \cdot 10^5$ cells in a volume of 0.5 ml. According to the results of the eosin test, the viability of the transplanted bone marrow was about 80%.

The mice were kept under ordinary animal house conditions. To prevent the intestinal form of radiation sickness, the mice were given chloramphenicol with their diet in a dose of 2 mg per mouse.

Mice surviving on the 20th day were decapitated, the bone marrow was removed from their femora, and films were prepared on the basis of which the proliferative ability of the rat bone marrow cells was determined. Data on proliferation of myelokaryocytes of the grafts were obtained by identifying neutrophilic granulocytes of the donors' type with cytochemically detectable alkaline phosphate by Burstone's method [3]. By this method phosphatase-positive neutrophilic granulocytes were quantitatively determined. Investigations by other workers [1, 2, 7, 9] have shown that neutrophilic granulocytes in rat blood and bone marrow react strongly for alkaline phosphatase, whereas this enzyme cannot be demonstrated in the neutrophilic granulocytes of mice.

The numerical results were subjected to statistical analysis [4].

EXPERIMENTAL RESULTS

On the 20th day after transplantation of both freshly prepared and conserved rat bone marrow phosphatase-positive neutrophilic granulocytes were found in the bone marrow of the chimera mice (Fig. 1). Counting the phosphatase-positive cells showed that after transplantation of conserved bone marrow their relative percentage was 41 ± 11.5, compared with only 25 ± 2.24 after transplantation of freshly prepared marrow. However, these differences were not statistically significant ($P > 0.05$). These results suggest that bone marrow cells of donor rats can survive in foreign recipient mice. Meanwhile the presence of the donor's neutrophils in the recipient's bone marrow after such long periods (20 days) indicates that rat hematopoietic cells not only survive, but also proliferate in the chimera mice, for the lifespan of the neutrophils is about 12-14 days [6]. The rat neutrophils found on the 20th day in the mice thus had not entered the bone marrow as a result of transfusion, but were the result of reproduction of the rat cells in the recipient's body.

Throughout the period of the experiment the survival rate of the mice was analyzed in each of the three groups. In the control group the animals began to die on the 5th day and after the 13th day all the mice had died. After transplantation of marrow, whether freshly prepared or frozen under the protection of HEDTA, the animals began to die later, namely on the 8th-10th day after irradiation. The acute period of radiation sickness after transplantation of freshly prepared bone marrow was survived by 32% of the mice (other workers have obtained similar results [5]), whereas after transplantation of bone marrow conserved under the protection of HEDTA 36% survived.

Animals with transplantation of disturbed bone marrow which survived the period of acute radiation sickness began to die mainly at the end of the third week. By this time the mice had developed secondary radiation sickness as a result of the graft versus host reaction [2, 5]. Presumably during transplantation of freshly prepared bone marrow, more immunocompetent cells entered the recipient's blood stream, and these gave rise to stronger immunological conflict between the donor's and recipient's cells, with the result that the survival rate of freshly prepared myelokaryocytes was lower and the mortality of the animals correspondingly higher.

Analysis of the results shows that heterologous myelokarocytes, frozen to −196°C under the protection of a 15% solution of HEDTA, can survive and proliferate in radiation chimeras. This is shown by the presence of phosphatase-positive rat neutrophilic granulocytes with a well marked enzyme reaction in bone marrow of the mice.

It can be concluded from the results of these experiments that nucleated rat bone marrow cells retain their proliferative ability after freezing and thawing under the protection of a 15% solution of HEDTA.

LITERATURE CITED

1. N. V. Butomo, Probl. Gematol., No. 2, 54 (1966).
2. N. V. Butomo, Transplantation of Bone Marrow after Radiation Injuries [in Russian], Leningrad (1970).
3. M. S. Burstone, Enzyme Histochemistry and Its Application in the Study of Neoplasms, Academic Press, New York (1962) [Russian translation, 1965, p. 214].
4. V. S. Genes, Tables of Significant Differences between Groups of Observations Based on Qualitative Indices [in Russian], Moscow (1964), p. 12.
5. K. K. Zaitseva, Arkh. Patol., No. 1, 3 (1976).
6. I. A. Kassirskii and G. A. Alekseev, Clinical Hematology [in Russian], Moscow (1970), p. 32.
7. L. I. Simonova, "Biological characteristics and effectiveness of transplantation of conserved (T −197°C) hematopoietic tissue of fetuses in experimental acute radiation sickness," Author's Abstract of Candidate's Dissertation, Khar'kov (1969).
8. N. Gengozian et al., Cancer Res., 17, 970 (1957).
9. P. Nowell et al., Cancer Res., 16, 258 (1956).