such foci, repopulated twice with hematopoietic cells of the CBF_1 hybrid, stem cells of the C57BL genotype regenerated the same way as in syngeneic hematopoietic tissue. These results show that cells of hematopoietic origin capable of repopulation, including lymphocytes and macrophages, do not participate in the mechanism of hybrid resistance.

Another important conclusion is that regulation of regeneration of hematopoietic stem cells is local in character and is due to cooperate interaction between the injected hematopoietic stem cells and the stromal microenvironment of the hematopoietic organs and not to their interaction with cells of myeloid origin or their progeny, capable of repopulation.

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RADIOSENSITIVITY OF COLONY-FORMING UNITS OF DOG BONE MARROW IN AGAR CULTURES

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The radiosensitivity of colony-forming units in dog bone marrow was determined by a modified method of cloning hematopoietic cells in semisolid agar gel in diffusion chambers in vivo. The dose of radiation whose action was followed by preservation of 37% of the objects (D_o) was 144 ± 14.8 rad ($n = 0.8$) for committed precursor cells of granulocytopoiesis (CFUc) and 468 ± 35.8 rad ($n = 0.9$) for precursor cells forming "stellate" colonies of fibroblast-like cells (CFUf). It is concluded that the CFUf belong to the class of stromal precursors of hematopoietic organs. This system is suitable for the simultaneous study of hematopoietic and stromal precursor cells in dogs.

KEY WORDS: hematopoietic precursor cells; stromal precursor cells; bone marrow; radiosensitivity.

The agar method of culture of mammalian bone marrow cells can be used to obtain information about colony-forming units, i.e., about precursor cells committed in the granulocytic direction (CFUc).

During culture of dogs' bone marrow in an agar medium, besides colonies of granulocytes and monocytes, "stellate" colonies consisting of elongated cells resembling fibroblasts also were observed [1]. However, it was not clear whether these colonies were formed by stromal mechanocytes (fibroblasts) or by hematopoietic cells (histiocytes, for example). Since analysis of the morphological data and the kinetics of the "stellate" colonies [1] did not answer this question, the investigation described below was undertaken to determine

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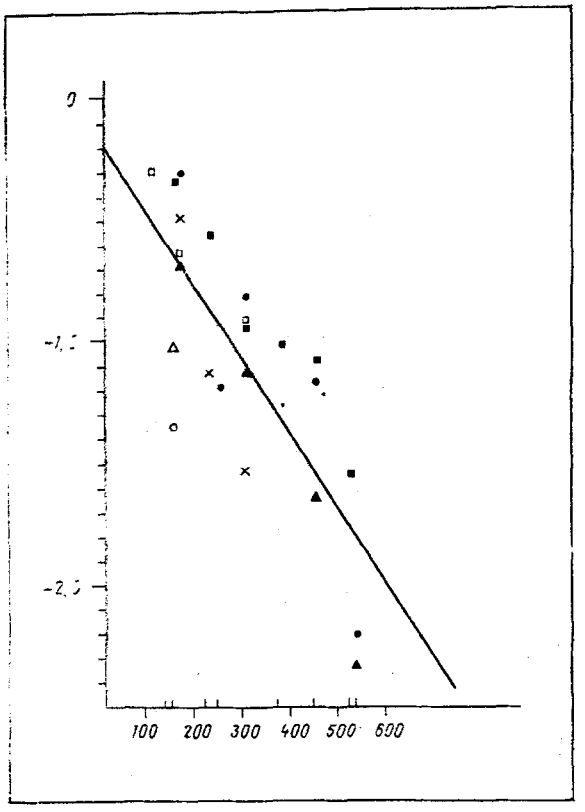

Fig. 1

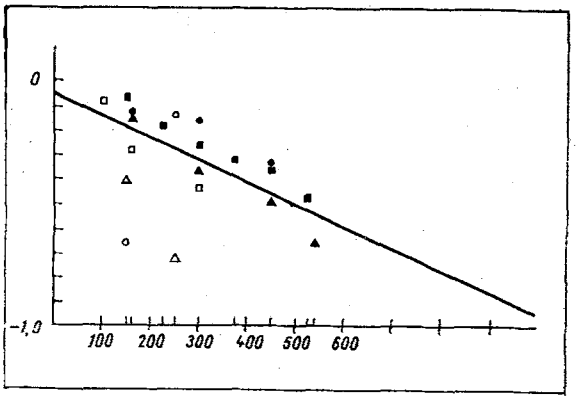

Fig. 2

Fig. 1. Curves of survival of CFUc of dogs' bone marrow after irradiation in vitro. Different symbols denote separate experiments. Abscissa, dose of irradiation (in rad); ordinate, logarithm of surviving fraction.

Fig. 2. Curve of radiosensitivity of CFUf of dogs' bone marrow after irradiation in vitro. Legend as in Fig. 1.

comparative radiosensitivity of CFUc and colony-forming units of fibroblast-like colonies (CFUf) in dogs' bone marrow by using a modified agar culture technique in vivo in diffusion chambers.

EXPERIMENTAL METHOD

Adult mongrel dogs of both sexes weighing 8-12 kg and female (CBA × C57BL/F_1) mice aged 6-8 weeks were used.

Dogs' bone-marrow cells were obtained by the method described in [2]. The nutrient medium consisted of 75% single medium No. 199 (salt base and Hanks's solution), 16.7% calf embryonic serum, and 8.3% Bacto-agar (4% solution) to which penicillin and streptomycin were added in concentrations of 100 units/ml of each.

Bone marrow cells in a concentration of 1.5×10^6/ml were irradiated in the medium in vitro by ^{137}Cs γ-rays in a dose of 100-540 rads with a dose rate of 250-500 rad/min.

The cells were suspended in basic medium and introduced into diffusion chambers (millipore filters with pores 0.3 μ in diameter); the cell concentration was 2.1×10^5 in 0.14 ml. Pairs of chambers were introduced into the peritoneal cavity of mice previously irradiated in a dose of 900 rad.

On the 7th day of culture the number of granulocytic and "stellate" colonies in the chambers was counted. Radiosensitivity was calculated by the method of linear regression analysis [3].

EXPERIMENTAL RESULTS

Curves showing survival rates of CFUc and CFUf of dogs' bone marrow are shown in Figs. 1 and 2. Clearly the radiosensitivity of the CFUc and CFUf differed sharply. For CFUc,

the value of Do was 144 ± 14.8 rad (extrapolation number n = 0.8), whereas for CFUf, Do was 468 ± 35.8 (n = 0.9).

High radiosensitivity of CFUc is characteristic of hematopoietic precursor cells: All categories of the latter have a Do value of about 100 rad [4]. Meanwhile the radioresistance of CFUf is very close to that of the stromal cells of hematopoietic organs, namely 325 rad [7], whereas for cells tolerating a hematopoietic microenvironment it is about 400 rad [5]. Cells of the pleural cavity of mice forming "stellate" colonies in agar culture have radioresistance close to that of CFUf, namely 280 rad [6].

These results are a weighty argument in support of the view that the CFUf belong to one category of stromal precursor cells contained in bone marrow. They can evidently form colonies during growth in culture not only in vivo, but also in agar in vitro [1]; other investigators [8] have given preliminary reports of having obtained similar colonies by culturing dogs' bone marrow in methylcellulose; the radiosensitivity of the cells forming them, although it was not precisely determined, was reflected in a Do value of about 400 R.

It can be concluded on the whole that during culture of dogs' bone marrow cells in semisolid agar gel in vitro (in Leighton's tubes) and in vivo (in diffusion chambers), besides hematopoietic precursor cells, stromal precursors are also cloned, and the "stellate" colonies of fibroblast-like cells are evidently formed by stomal mechanocytes.

The system of culture of bone marrow in semisolid media can be used to study hematopoietic (CFUc) and stromal (CFUf) precursor cells simultaneously in dogs.

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