COMPARATIVE RADIOSENSITIVITY OF CFU-s FROM MOUSE BONE MARROW AND FETAL LIVER, FORMING 7- AND 11-DAY COLONIES

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It has recently been shown that splenic colonies in lethally irradiated mice, into which hematopoietic tissue cells have been transplanted, can be formed not only by pleuripotent hematopoietic stem cells, as has hitherto been considered [5, 11], but also by committed (chiefly erythroid) precursor cells with limited proliferative potential [1, 4, 6]. It has therefore been suggested that 7-8-day splenic colonies should be distinguished from colonies formed 11-14 (most frequently 12) days after transplantation of hematopoietic cells, the former being regarded as a mixture of clones formed by precursor cells with different proliferative potential (poly- and unipotent), and the latter as a result mainly of the most primitive cells [4, 10]. Besides other methods of characterizing cells belonging to different CFU-s subpopulations, the method of comparative study of radiosensitivity remains important because it allows the corresponding cell population to be characterized integrally. The aim of the present investigation was accordingly to study the radiosensitivity of CFU-s contained in different hematopoietic tissues (fetal liver on the 14th and 17th days of pregnancy, adult mouse bone marrow), capable of forming colonies in the spleens of lethally irradiated mice 7 and 11 days after transplantation. The use of fetal CFU-s was determined by the fact that it has been considered for several years that they have greater resistance to ionizing radiation than CFU-s from bone marrow of adult animals, although it was often difficult to recognize from the data in the literature which CFU-s subpopulations were being investigated [8, 9]. Meanwhile it has been suggested that the increased radioresistance of CFU-s from fetal liver is largely connected with hypoxia of the fetuses [7]. The method of irradiation of CFU-s from different hematopoietic tissues in suspension was used in this investigation because in that case the cells are well oxygenated and the "oxygen factor" does not influence the evaluation of cellular radiosensitivity.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)F_1 mice aged 6-12 years and fetuses aged 14-17 days. The femoral bone marrow was flushed out with 1-2 ml of medium 199 and suspended by means of a syringe and needle. The fetal liver (FL) was minced in a glass homogenizer (17-day FL), the number of nucleated cells was determined in a hemocytometer, using 3% acetic acid with 0.1% gentian violet. CFU-s were determined by the method of Till and McCulloch [11]. Syngeneic recipient mice were irradiated in a dose of 12-13 Gy on an IPK ^{137}Cs apparatus and 2-5 h after irradiation the necessary number of hematopoietic cells for testing was injected intravenously. Colonies were counted on spleens fixed in Bouin's fluid 7 and 11 days after transplantation of the cells. The mean number of endogenous colonies was 0.24 ± 0.07 colony per spleen. To determine the radiosensitivity of the CFU-s, cell suspensions in medium 199 were irradiated on ice in doses of 0.4, 0.8, 1.2, 1.6, and 2.0 Gy in doses of 1.0, 1.5, 2.0, and 2.5 Gy with γ-rays from the IPK ^{137}Cs source, and were then injected into irradiated recipients. Altogether seven independent series of experiments were carried out. Quantitative characteristics of radiosensitivity of the CFU-s were obtained by analysis of the relationship between dose of irradiation (D) and the surviving fraction of CFU-s (S), by the equation

$$\lg S = a \cdot D + b$$

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TABLE 1. Radiosensitivity of 7- and 11-Day CFU-s from FL at Two Ages (14 and 17 days) and Adult Mouse Bone Marrow (BM)

Source of cells	Parameters of radiosensitivity (M ± m)			
	7-day CFU-s		11-day CFU-s	
	D_0, Gy	n	D_0, Gy	n
FL (14 days)	2,02±0,16	1,0±0,1 (3)	1,45±0,17	1,0±0,1 (3)
FL (17 days)	1,57±0,08	1,0±0,1 (2)	1,08±0,16	1,0±0,2 (2)
BM	0,78±0,05	1,0±0,1 (2)	1,25±0,06	1,0±0,1 (2)

Legend. Number of independent experiments given in parentheses.

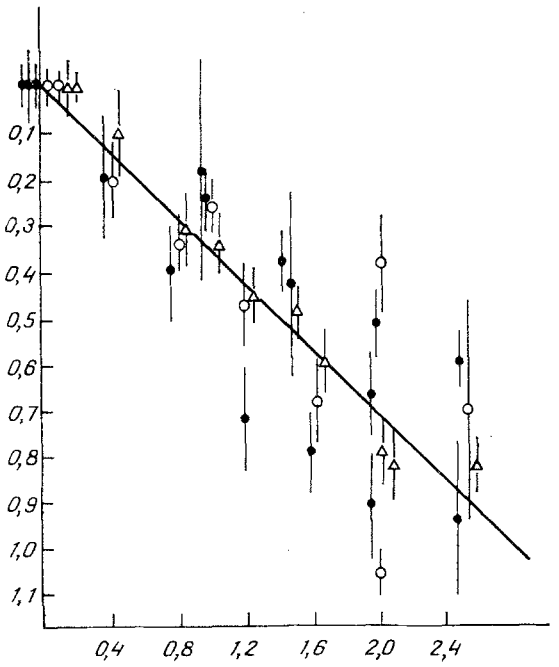

Fig. 1. Dose-dependence of survival rate of 11-day CFU-s. Pooled data for CFU-s from 14-day (filled circles) and 17-day (empty circles) FL, and from BM (triangles). Abscissa, dose of irradiation, in Gy (D); ordinate, logarithm of fraction of surviving CFU-s (lgS); mean values and 95% confidence interval given. General dependence is described by the following regression equation: $\log S = -(0.001 \pm 0.026) - (0.348 \pm 0.022)D$.

with calculation of the mean cellular lethal dose D_0 in each case by the equation

$$D_0 = \frac{\lg e}{a} \approx \frac{0.4343}{a}$$

and of the extrapolation number n by the equation

$$n = \text{antilog}\, b$$

All calculations were carried out on the ES-1023 computer, using a pack of medico-biological programs. To calculate regression equations, the program taking account of the relative weight (the number of repetitions of determination) of each point was used.

EXPERIMENTAL RESULTS

The results of experiments to assess the radiosensitvity of 7- and 11-day CFU-s from different tissues are summarized in Table 1. In all cases the dose dependences of cell survival were close to exponential (extrapolation number 1.0), and differences in radiosensitivity were associated with the unequal value of the mean lethal cell dose D_0. The fetal cells at both times of pregnancy showed considerably less radiosensitivity during the formation of 7-day

than of 11-day colonies. Meanwhile mouse bone marrow CFU-s were more resistant during the formation of 11-day colonies. Thus among 7-day CFU-s considerable differences were found in radiosensitivity depending on the origin of the cells. The high radioresistance of the fetal 7-day CFU-s is evidence that they are not identical to cells of the analogous subpopulation from bone marrow, although the nature of these differences remains largely unexplained. The greater proliferative activity of 7-day CFU-s from fetal liver (about 40% of them were in the S phase of the cell cycle [2]) compared with that from bone marrow [3] may provide an explanation of the increased radioresistance of fetal CFU-s, for we know that the S phase of the cell cycle is relatively resistant to irradiation.

It is an interesting fact that colonies formed on the 11th day after transplantation of both fetal and bone-marrow suspensions are formed by cells which did not differ statistically significantly in their radiosensitivity. The general dose versus effect curve for 11-day CFU-s of all three tissues used is shown in Fig. 1, and it is characterized by the following values of radiosensitivity parameters: $D_0 = 1.25 \pm 0.08$ Gy, and $n = 1.0 \pm 0.1$. Thus in both the pre- and postnatal periods of development radiosensitivity strictly of the hematopoietic stem cells (polypotent 11-day CFU-s) was unchanged.

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