Growth and Senescence of the Bone Marrow Stem Cell Pool in RFM/Un Mice (35809)

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The maturation, function, and regulation of hemopoietic progenitor cells, most commonly referred to as "stem" cells, is not completely understood. During fetal and early postnatal development of the mouse, significant numbers of these cells are found in the liver and spleen (1), while in the adult the richest organ source is the bone marrow (2, 3). As primitive precursors, hemopoietic stem cells perform at least two major functions (4): (i) they give rise to cells which differentiate into mature blood elements, probably in response to humoral stimulation; and (ii) they maintain, by self-renewal, their own population.

The bulk of available information concerning hemopoietic stem cells deals primarily with their development and functional capabilities during the first third of the animal life-span. Such studies have provided information concerning stem cell radiation sensitivity (5, 6), potential for differentiation into mature blood elements (7), and relationship to the cells comprising the immune system (8). Few, if any of these properties, however, have been investigated in middle-aged or old animals.

In the present studies, we have (i) evaluated the stem cell content of mouse femoral marrow throughout the animal life-span, (ii) compared the radiation sensitivity of the marrow stem cell compartment in mice exposed to a sublethal dose of X-radiation during fetal life with that in mice irradiated at 10–12 weeks of age, and (iii) ascertained possible residual injury to the bone marrow stem cell compartment caused by radiation damage during fetal development or as young adults.

In each of these studies, femoral bone marrow was tested for its relative stem cell content and hemopoiesis function by its ability to give rise to spleen nodules of hemopoiesis when transplanted into lethally irradiated young adult, syngeneic recipients. Previous studies have shown that a linear relationship exists between the number of nucleated bone marrow cells injected and the number of nodules observed in the host spleen (9, 10). Considerable evidence has also been presented to establish the view that the majority of cells in each nodule are derived from a single hemopoietic precursor cell (11–13). Therefore, such nodules are considered clonal in nature, and the cell responsible for one of these colonies is commonly referred to as a colony-forming unit (CFU) (9).

Throughout this paper, cells comprising the bone marrow hemopoietic progenitor pool will be referred to as CFU or hemopoietic stem cells, interchangeably.

Materials and Methods. Bone marrow donors and recipients were inbred RFM/Un (RF) male and female mice produced at Oak Ridge National Laboratory. All recipients were 12–14 weeks of age and were exposed to 750 R[1] of whole-body X-radiation 1 or 2 hr prior to marrow cell injection.

Irradiation techniques. Whole-body X-radi-

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ation was administered by means of a General Electric Maxitron 300 operated with the following factors: 300 kV, 20 mA, 0.1 mm of aluminum inherent filtration, 3 mm of aluminum added filtration, 0.45 mm copper HVL, 75-cm target-to-mouse distance, exposure rate of approximately 150 R/min. The mice were irradiated in a perforated Lucite exposure cage rotated axially in the radiation field at 12 rpm.

Irradiation of fetal and young adult bone marrow donor mice. In some experiments, pregnant females were exposed to 300 R of whole-body X-radiation at 17.5 days gestation. Their offspring were then used as bone marrow donors at various age intervals. Radiation controls were represented by comparable age, nontreated animals. The gestational age of the fetus was determined by checking females twice daily (morning and evening) for vaginal plugs during mating. The maximum error in this method is no greater than 12 hr. Therefore, treatment times are indicated in terms of 0.5 days.

Ten-week-old RF male and female mice were exposed to 150 or 300 R of X-rays and their femoral marrow was assayed for relative CFU potential at selected time intervals after treatment.

Bone marrow preparation and injection. Bone marrow donor mice were sacrificed by cervical dislocation. Their femurs were then removed; the ends of the bones were clipped; and the marrow contents were aspirated into chilled Tyrode solution using a 23-gauge needle. A single cell suspension was attained by repeated passage or marrow plugs through a 27-gauge needle. The resulting cell suspension was then filtered through a 200-mesh/in. stainless steel screen, and nucleated cell values were determined by means of a Fischer Autocytometer. Final dilutions of cell suspensions, made to contain 10^5 nucleated cells/ml of Tyrode solution, were injected in 0.5-ml volumes into the lateral tail veins of the lethally irradiated, syngeneic recipients.

Assay for bone marrow colony-forming units (CFU). Bone marrow injected mice and irradiated controls were sacrificed 6 days after treatment; and their spleens were removed and fixed in Bouin's solution. A 5-μ-thick longitudinal section was cut from the middle region of each spleen, stained with hematoxylin and eosin, and scored microscopically for contained colonies of hemopoiesis. Hemopoietic colony types were recorded as erythroid, undifferentiated, granulocytic, megakaryocytic, or mixed. The criteria used for colony identification and time of assay were those developed by Curry and Trentin (7).

Results. Bone marrow CFU potential as influenced by donor age. Repeated estimates of bone marrow CFU were made using RF donor mice whose ages ranged from 6 days to 28 months. The results are given in Fig. 1. Bone marrow CFU potential was seen to change throughout the life span of these mice. During the first 3 weeks of age the number of CFU increased rapidly until it reached a maximum mean value of 20.9 CFU/10^5 nucleated marrow cells. This value was maintained through the first half of the mouse life (12–13 months). Thereafter, a progressive decline of marrow CFU potential was associated with increasing age of the donor.

Differential evaluation of spleen colony types derived from these marrow donors of various ages showed all cell lines of hemopoiesis to follow the same general pattern of development and senescence (Table I).

Radiosensitivity of stem cells in fetal mice. Figure 2 shows the depletion and recovery of bone marrow CFU potential in mice exposed to 300 R of X-rays at 17.5 days gestation. When examined 6 days after birth, the mean femoral marrow CFU value in these mice was less than 30% of the value found in sham-irradiated, comparable-age controls. Ensuing stem cell recovery reached control values by 5 weeks of age and did not deviate from controls over the following 6 weeks. However, when examined at 58 and 78 weeks of age, the number of CFU in the bone marrow of *in-utero*-irradiated mice was markedly below that in nonirradiated, comparable-age controls.

Differential evaluation of spleen colonies derived from bone marrow of *in-utero*-irradiated mice showed no one cell line of hemopoietic differentiation to be depressed

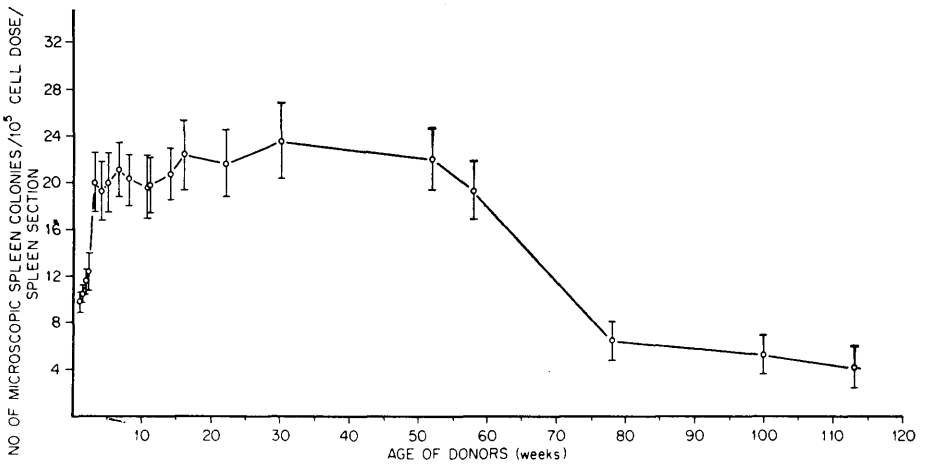

FIG. 1. Influence of donor age on spleen colony formation by transplantation bone marrow cells: All recipients were 12–14 weeks old. Each point represents results for 15–20 recipients injected with a pool of marrow cells from 5 donors. Results are presented as the mean number of microscopic colonies per spleen section per 10^5 nucleated cell dose ± 1 SE.

any more severely or to recover any earlier after treatment than any other cell line (Table II).

Radiosensitivity of stem cells in young adult mice. The depletion and recovery of the bone marrow stem cell pool in mice exposed to either 150 or 300 R is shown in Fig. 3. Following an immediate stem cell depletion, the 300 R dose group returned to control levels by day 14 and was observed at supranormal levels on day 17 after radiation exposure. This accelerated recovery was seen to be temporary and to be followed by a fall to subnormal levels persisting between days 19 and 30. A second and more permanent recovery began on day 30 which lasted through day 95.

Continued observations of 300-R-irradiated mice revealed a progressive loss of bone marrow stem cell function beginning at about 120 days and continuing through 79 weeks after exposure. Although stem cell levels of

TABLE I. Numbers and Types of Microscopic Spleen Colonies in Irradiated Mice[a] as Influenced by Age of Marrow Donors.[b]

Age of donor (weeks)	Types of spleen colonies (mean no. of colonies/spleen section ± SE)[c]					
	Undifferentiated	Erythroid	Granuloid	Megakaryocytic	Mixed	Total
1	2.10 ± 0.56	6.00 ± 0.86	0.54 ± 0.20	1.10 ± 0.32	0	9.76 ± 0.92
2	4.20 ± 1.02	6.40 ± 1.02	0.80 ± 0.30	0.80 ± 0.30	0	12.40 ± 1.64
3	7.60 ± 1.80	7.60 ± 1.26	1.60 ± 1.12	3.20 ± 0.90	0	20.00 ± 2.56
11	6.60 ± 1.44	6.80 ± 1.27	3.20 ± 1.13	3.20 ± 0.70	0	19.80 ± 2.30
52	8.00 ± 1.10	7.70 ± 0.80	4.22 ± 0.40	2.22 ± 0.30	0	22.10 ± 2.60
58	7.20 ± 0.80	7.60 ± 1.00	3.65 ± 0.40	0.95 ± 0.30	0	19.40 ± 2.50
78	1.07 ± 0.27	2.68 ± 0.48	2.00 ± 0.46	0.87 ± 0.30	0	6.62 ± 1.51
100	1.35 ± 0.28	1.76 ± 0.31	1.60 ± 0.88	0.54 ± 0.20	0	5.25 ± 1.67
113	0.90 ± 0.10	1.32 ± 0.70	1.38 ± 0.60	0.60 ± 0.40	0	4.20 ± 1.80

[a] Recipients were 10- to 12-week-old RF males and females given 750 R and 5 × 10^4 syngeneic femoral marrow cells iv and sacrificed on day 6 postinjection; 15 to 20 animals/group.

[b] Donors were RF males and females; 2 to 5 animals/group.

[c] Data corrected to 10^5 cell dose.

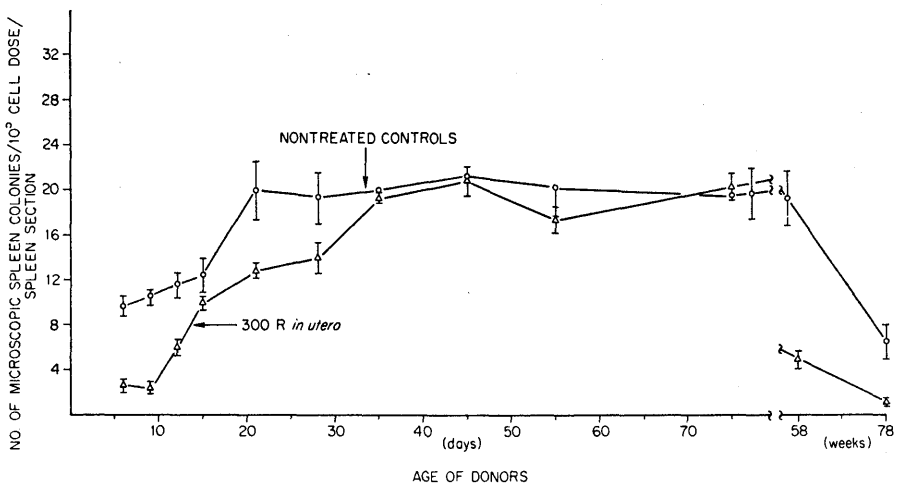

FIG. 2. Recovery pattern of the bone marrow stem cell compartment in RFM/Un mice following a 300-R X-radiation exposure *in utero* at 17 days gestation: (△) 300 R; (○) same age controls.

nontreated controls were declining after 49 weeks of age, they were significantly above those observed for 300-R-irradiated mice of comparable age.

Mice exposed to 150 R of X-rays showed less initial stem cell depletion but a more delayed recovery to control levels than that seen for 300-R-irradiated mice. The bone marrow stem cell recovery pattern in these mice showed no overshooting of control levels

TABLE II. Numbers and Types of Spleen Colonies Derived from Bone Marrow of Mice Exposed to 300 R of X-Radiation *in Utero*.[a]

Age of donor[b]	Treatment	Types of spleen colonies (mean no. of colonies/spleen section ± SE)[c]					
		Undifferentiated	Erythroid	Granuloid	Megakaryocytic	Mixed	Total
6 days	None	2.10 ± 0.56	6.00 ± 0.86	0.54 ± 0.20	1.10 ± 0.32	0	9.76 ± 0.92
	300 R	0.30 ± 0.14	1.20 ± 0.30	0.40 ± 0.16	0.20 ± 0.12	0	2.70 ± 0.56
12 days	None	3.78 ± 0.60	5.88 ± 0.64	0.84 ± 0.34	1.14 ± 0.34	0	11.56 ± 1.08
	300 R	2.00 ± 0.36	3.46 ± 0.38	0.42 ± 0.18	0.22 ± 0.14	0	6.00 ± 0.66
15 days	None	4.20 ± 1.02	6.40 ± 1.02	0.81 ± 0.30	0.80 ± 0.30	0	12.30 ± 1.64
	300 R	3.46 ± 0.54	5.56 ± 0.72	0.54 ± 0.20	0.32 ± 0.16	0	9.88 ± 0.62
28 days	None	6.41 ± 0.51	7.12 ± 0.61	1.98 ± 0.62	3.81 ± 0.70	0	19.32 ± 2.50
	300 R	4.32 ± 0.58	6.22 ± 0.98	1.54 ± 0.44	1.76 ± 0.56	0	14.00 ± 1.50
35 days	None	7.26 ± 0.54	8.32 ± 1.06	3.19 ± 0.43	1.23 ± 0.34	0	20.00 ± 2.47
	300 R	7.44 ± 0.98	8.00 ± 1.00	2.44 ± 0.48	1.54 ± 0.50	0	19.44 ± 1.32
75 days	None	7.70 ± 1.03	8.31 ± 0.90	2.50 ± 0.43	1.09 ± 0.36	0	19.60 ± 2.72
	300 R	5.05 ± 0.68	11.78 ± 1.18	2.94 ± 0.54	0.66 ± 0.28	0	20.42 ± 1.21
58 weeks	None	7.20 ± 0.80	7.60 ± 1.00	3.65 ± 0.40	0.95 ± 0.30	0	19.40 ± 2.50
	300 R	1.89 ± 0.20	2.10 ± 0.21	0.90 ± 0.13	0.21 ± 0.12	0	5.10 ± 0.76
78 weeks	None	1.07 ± 0.27	2.68 ± 0.48	2.00 ± 0.46	0.87 ± 0.30	0	6.62 ± 1.51
	300 R	0.12 ± 0.09	0.33 ± 0.13	0.44 ± 0.11	0.33 ± 0.05	0	1.22 ± 0.38

[a] Recipients were 10- to 12-week-old RF males and females given 750 R and 5×10^4 syngeneic femoral marrow cells iv and sacrificed on day 6 postinjection; 15 to 20 animals/group.

[b] Donors were RF males and females; 2 to 5 animals/group.

[c] Spleen colony numbers corrected to 10^5 nucleated bone marrow cell dose.

TABLE III. Numbers and Types of Spleen Colonies Derived from Bone Marrow of Mice[a] Exposed to 150 or 300 R of X Radiation as Young Adults

Treatment	Time After Treatment	Types of Spleen Colonies (Mean No. of Colonies per Spleen Section, ±S.E.)[b]					
		Undifferentiated	Erythroid	Granuloid	Megakaryocytic	Mixed	Total
None	1 day	6.60 ± 1.44	6.80 ± 1.27	3.20 ± 1.13	3.20 ± 0.70	0	19.80 ± 2.30
150 R	1 day	1.21 ± 0.33	0.73 ± 0.30	0.94 ± 0.27	0.84 ± 0.30	0	3.78 ± 0.59
300 R	1 day	0.21 ± 0.04	0	0.19 ± 0.07	0.33 ± 0.07	0	0.73 ± 0.08
None	7 days	6.60 ± 1.44	6.80 ± 1.27	3.20 ± 1.13	3.20 ± 0.70	0	19.80 ± 2.30
150 R	7 days	1.61 ± 0.25	2.66 ± 0.41	0.94 ± 0.19	1.11 ± 0.25	0	6.22 ± 0.58
300 R	7 days	1.57 ± 0.43	1.98 ± 0.45	1.25 ± 0.35	1.47 ± 0.33	0	6.40 ± 0.74
None	14 days	8.00 ± 0.60	7.42 ± 1.00	1.97 ± 0.10	3.10 ± 0.24	0	20.80 ± 2.20
150 R	14 days	2.66 ± 0.47	3.77 ± 0.64	2.55 ± 0.56	0.88 ± 0.20	0	10.11 ± 1.17
300 R	14 days	6.56 ± 0.79	8.25 ± 1.27	3.50 ± 0.54	2.41 ± 0.61	0.50 ± 0.19	21.00 ± 2.37
None	17 days	8.00 ± 0.60	7.42 ± 1.00	1.97 ± 0.10	3.10 ± 0.24	0	20.80 ± 2.70
150 R	17 days	4.06 ± 0.43	6.87 ± 0.76	2.75 ± 0.42	1.37 ± 0.45	0	15.12 ± 1.10
300 R	17 days	7.30 ± 0.81	9.90 ± 0.67	3.90 ± 0.55	2.20 ± 0.37	0	23.30 ± 0.73
None	30 days	7.90 ± 0.70	7.50 ± 0.50	3.26 ± 0.81	3.30 ± 0.62	0	22.40 ± 2.90
150 R	30 days	2.57 ± 0.54	10.00 ± 1.50	2.00 ± 0.40	1.80 ± 0.52	0	16.81 ± 1.52
300 R	30 days	4.02 ± 0.71	6.05 ± 0.55	1.70 ± 0.41	1.76 ± 0.37	0	13.51 ± 0.85
None	53 days	8.20 ± 0.91	8.60 ± 1.13	1.76 ± 0.55	3.11 ± 0.30	0	21.67 ± 2.89
150 R	53 days	7.78 ± 0.69	9.78 ± 1.23	2.84 ± 0.50	2.87 ± 0.60	0	22.94 ± 1.55
300 R	53 days	7.00 ± 0.64	8.10 ± 0.72	2.50 ± 0.39	2.30 ± 0.25	0	21.10 ± 0.85
None	120 days	8.60 ± 0.94	8.00 ± 1.00	4.60 ± 0.60	2.40 ± 0.73	0	23.60 ± 3.27
150 R	120 days	7.46 ± 0.47	8.30 ± 0.58	3.41 ± 0.27	0.43 ± 0.11	0	19.60 ± 1.30
300 R	120 days	3.89 ± 0.56	6.31 ± 0.77	1.70 ± 0.38	0.84 ± 0.27	0	12.63 ± 1.38
None	39 weeks	8.00 ± 1.10	7.70 ± 0.80	4.22 ± 0.40	2.22 ± 0.30	0	22.10 ± 2.60
150 R	39 weeks	5.10 ± 0.73	8.30 ± 0.78	2.00 ± 0.32	1.00 ± 0.40	0	16.40 ± 1.08
300 R	39 weeks	3.97 ± 0.51	4.32 ± 0.42	1.63 ± 0.39	0.68 ± 0.30	0	10.60 ± 1.62
None	52 weeks	7.20 ± 0.80	7.60 ± 1.00	3.65 ± 0.40	0.95 ± 0.30	0	19.40 ± 2.50
150 R	52 weeks	3.61 ± 0.43	6.41 ± 0.76	3.19 ± 0.42	0.76 ± 0.30	0	13.97 ± 1.10
300 R	52 weeks	2.23 ± 0.29	3.20 ± 0.31	1.10 ± 0.21	0.60 ± 0.33	0	7.13 ± 1.14
None	65 weeks	1.07 ± 0.27	2.68 ± 0.48	2.00 ± 0.46	0.87 ± 0.30	0	6.62 ± 1.51
150 R	65 weeks	2.38 ± 0.31	4.59 ± 0.35	1.72 ± 0.26	0.41 ± 0.20	0	9.10 ± 1.00
300 R	65 weeks	1.00 ± 0.31	2.90 ± 0.39	1.98 ± 0.51	0.66 ± 0.31	0	6.44 ± 1.52
None	83 weeks	1.35 ± 0.28	1.76 ± 0.31	1.60 ± 0.88	0.54 ± 0.20	0	5.25 ± 1.67
150 R	83 weeks	1.02 ± 0.11	2.22 ± 0.41	0.80 ± 0.20	0.36 ± 0.10	0	4.40 ± 0.48
300 R	83 weeks	0.15 ± 0.10	0.81 ± 0.19	1.04 ± 0.48	0.60 ± 0.29	0	2.60 ± 0.80

[a] Recipients were 10 to 12-week-old RF males and females given 750 R and 5 x 10^4 syngeneic femoral marrow cells i.v. and sacrificed on day 6 post-injection; 15 to 20 animals per group. Donors were RF males and females; 2 to 5 animals per group.

[b] Spleen colony numbers corrected to 10^5 nucleated bone marrow cell dose.

like that observed with the 300-R-dose group. Although there were significant increases in bone marrow stem cell numbers on days 17 and 30, control levels were not attained until day 53 after radiation exposure. When examined at 81 days, stem cell values were noticeably below normal in these mice. However, after this point stem cell values were observed to level off through day 180 after treatment.

Continued observations of 150-R-irradiated mice showed a depression of stem cell function below control values when examined 39 weeks after radiation exposure. Loss of femoral marrow stem cell function was seen to be progressive with increasing age of the irradiated donors through 77 weeks after treatment.

Differential evaluation of spleen colony types derived from bone marrow of mice exposed to either 150 or 300 R as young adults revealed no one cell line of hemopoiesis to be depressed any more severely or to recover any faster after radiation exposure than any other cell line (Table III). This same trend was observed during delayed observations of residual damage.

Discussion. In this study the bone marrow stem cell compartment of RF mice was seen to undergo changes during the normal mouse

life-span. The first occurs during the first 3 weeks of postnatal life and involves a rapid accumulation of stem cells in the bone marrow. Once the marrow stem cell compartment reaches maximum levels, it remains essentially unchanged until approximately middle age. At this time a second change takes place involving a depletion of the marrow stem cell pool. This depletion was seen to be progressive with increasing age of the animal.

The stem cell pool appears to be less radiation sensitive during early stages of growth and development of the mouse than after maturity. Mice exposed *in utero* to 300 R of X-rays recovered their normal stem cell complement by 35 days of age. In contrast, mice exposed to 150 R as young adults required 55 days to regain normal stem cell numbers. The pattern of recovery was also different in these two experimental groups. Mice irradiated *in utero* showed a steadily progressive recovery to normal stem cell levels, while young adult mice give the same radiation exposure showed irregular and fluctuating increases in stem cell number. Stem cell recovery in 300-R-irradiated young adults rose to supranormal levels on days 14 through 19 after radiation exposure. This overshooting of recovery, comparable to that observed by others (14, 15), was found to be temporary and was not observed with fetal irradiated mice.

The senescent changes also appeared to be radiation dose dependent with respect to time of onset and severity of hemopoietic dysfunction (Fig. 3). They are reminiscent of those noted to Albright and Makinodan (16) in (C57Bl/6 × C3H) F_1 hybrid mice, which were seen to lose stem cell function and ability to make antibodies with increasing age, and by Yuhas and Storer (17) in C57Bl mice, which showed depletion of marrow CFU with advancing age.

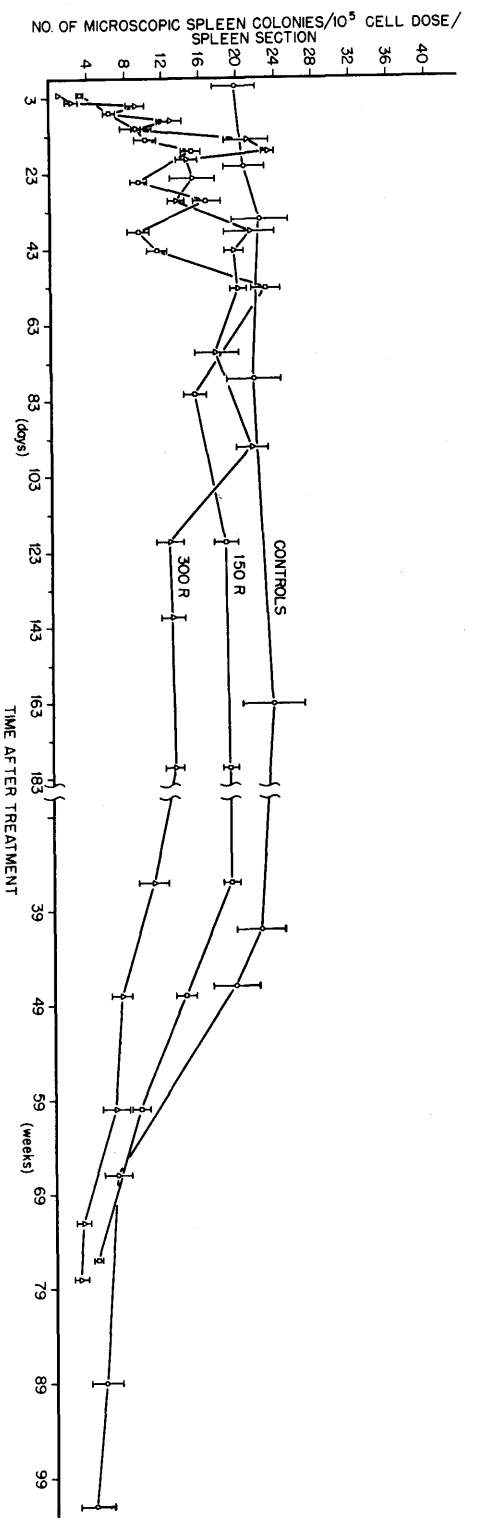

FIG. 3. Early and delayed effects of either 150 or 300 R of X-rays on the bone marrow stem cell pool of RFM/Un mice: Donors were 10-12 weeks old at the time of radiation exposure. Each point represents results for 15–20 recipients injected with a pool of marrow cells from 5 donors; (△) 300-R-irradiated donors, (□) 150-R-irradiated donors, (○) nontreated controls.

The observed age differences remain to be explained. Whether the decline in relative CFU during old age reflects impairment of hemostasis or some other process cannot be decided without further data. Our observations are consistent, however, with those cited in the foregoing paragraph, which suggest that the stem cell pool becomes gradually depleted during senescence. If this interpretation is borne out, the acceleration of the process by irradiation, which also causes shortening of the life-span, may provide an experimental approach for investigating the mechanisms of the decline. The possible relation between the alterations in CFU content and other lesions associated with aging, such as neoplasia, loss of vigor, and diminution in immunological responsiveness, calls for further study.

Summary. The exogenous spleen colony assay procedure has been used in RF mice to: (i) measure growth and senescence of the bone marrow stem cell compartment, (ii) compare the radiation sensitivity and recovery capacity of marrow stem cell compartments in fetal vs young adult mice, and (iii) determine residual radiation injury to tissues giving rise to hemopoietic progenitor cells in mice irradiated during fetal development or as young adults. The bone marrow of normal RF mice reached maximum hemopoiesis potential (20.9 CFU/10^5 nucleated marrow cells) within the first 3 weeks of postnatal life. This value was maintained through the first half of the mouse life. Thereafter, a progressive decline of marrow CFU potential was associated with increasing age of the animals. The stem cell pool in fetal mice appears to be less radiation sensitive and more capable of early recovery from radiation injury than that of 10- to 21-week-old young adults. Senescence of bone marrow stem cell function was accentuated in mice exposed to radiation either during fetal development or as young adults.

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