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TISSUE RESPONSES TO LOW PROTRACTED DOSES OF HIGH LET RADIATIONS OR PHOTONS: EARLY AND LATE DAMAGE RELEVANT TO RADIO-PROTECTIVE COUNTERMEASURES

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ABSTRACT

Early and late murine tissue responses to single or fractionated low doses of heavy charged particles, fission-spectrum neutrons or gamma rays are considered. Damage to the hematopoietic system is emphasized, but results on acute lethality, host response to challenge with transplanted leukemia cells and life-shortening are presented. Low dose rates per fraction were used in some neutron experiments. Split-dose lethality studies (LD 50/30) with fission neutrons indicated greater accumulation of injury during a 9 fraction course (over 17 days) than was the case for γ-radiation. When total doses of 96 or 247 cGy of neutrons or γ rays were given as a single dose or in 9 fractions, a significant sparing effect on femur CFU-S depression was observed for both radiation qualities during the first 11 days, but there was not an earlier return to normal with dose fractionation. During the 9 fraction sequence, a significant sparing effect of low dose rate on CFU-S depression was observed in both neutron and γ-irradiated mice. CFU-S content at the end of the fractionation sequence did not correlate with measured LD 50/30. Sustained depression of femur and spleen CFU-S and a significant thrombocytopenia were observed when a total neutron dose of 240 cGy was given in 72 fractions over 24 weeks at low dose rates. The temporal aspects of CFU-S repopulation were different after a single versus fractionated neutron doses. The sustained reduction in the size of the CFU-S population was accompanied by an increase in the fraction in DNA synthesis. The proliferation characteristics and effects of age were different for radial CFU-S population closely associated with bone, compared with the axial population that can be readily aspirated from the femur. In aged irradiated animals, the CFU-S proliferation/redistribution response to typhoid vaccine showed both an age and radiation effect. After high single doses of neutrons or γ rays, a significant age- and radiation-related deficiency in host defense mechanisms was detected by a shorter mean survival time following challenge with transplantable leukemia cells. Comparison of dose-response curves for life shortening after irradiation with fission-spectrum neutrons or high energy silicon particles indicated high initial slopes for both radiation qualities at low doses, but for higher doses of silicon, the effect per Gy decreased to a value similar to that for γ rays. The two component life-shortening curve for silicon particles has implications for the potential efficacy of radioprotectants. Recent studies on protection against early and late effects by aminothiols, prostaglandins, and other compounds are discussed.

INTRODUCTION

The number of humans exposed to space radiations is expected to increase over the next several decades. Ionizing radiation is but one of the potential hazards in space. Interactions between radiation effects, microgravity, and physiological alterations resulting from stress must be considered. Several excellent recent reviews have dealt with the space radiation environment, potential human hazards, and various radioprotective strategies (1,2). The purpose of this contribution is to focus on some tissue responses to fractionated doses of high-or low-LET radiations. Responses of the hematopoietic system are emphasized, and low dose rates were used in fission neutron studies. Many of the data presented here have been presented at meetings, but are documented only in laboratory reports. The purpose is to make these results available to scientists interested in dose fractionation issues relevant to space radiation hazards and radiation protection.

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Acute effects and late occurring cancers are the principle space radiation problems. Acute effects will result from high doses of largely low LET radiations from a solar particle event (SPE) during a deep space mission. SPEs vary, but most of the dose sufficient to produce significant acute effects is experienced over 12–48 hrs. at a variable dose rate. The principle tissues known now to be at risk for acute effects are skin, intestine and marrow. The sensitivity of the central nervous system to acute effects from heavy particles sustained over many hours or days is unknown. An excess risk of cancer and other late effects will result from low-or high-LET radiations experienced in either low earth orbit or in deep space. In addition to an excess cancer risk from an SPE, deep space exploration entails exposure to a mixture of low- and high-LET radiation over months, or perhaps even years. The relatively low frequency of heavy charged particle events during a deep space mission indicates that

the probability of multiple traversals is small, even when a cascade of primary particle fragments results from interactions with spacecraft shielding or tissues (3). Depending on the charges and velocities, the fragments may be more or less carcinogenic than the primary particles. Animal studies on carcinogenesis have been conducted with low-LET radiations and fission-spectrum-neutrons (6), but there is a dearth of information on heavy charged particles (7,8). Most of what is known about non-neoplastic late tissue responses comes from high dose studies in support of radiotherapy (9). Information on late tissue responses, after low doses and dose rates of photon radiation, comes from studies on the hematopoietic system in rodents and canines (10–13). More effort should be devoted to studies on late non-neoplastic tissue responses after low doses. Homeostasis and control systems that regulate tissue proliferation are difficult to study *in vitro*, so additional animal studies at low doses are required.

METHODS AND FACILITIES

Some data presented here have been documented in various reports where details on methodology may be obtained. Also, the methods and facilities used have been described in detail (14–16). Several data concern fission-spectrum neutrons from the JANUS reactor facility at the Argonne National Laboratory. That facility, dosimetry, and procedures for hematological studies have been described (14,17). The BEVALAC Accelerator and procedures have also been described (7,8,16). References to reports and sample sizes are specified as footnotes in tables or in figure legends.

The dose fractionation and dose rate studies with fission neutrons and γ rays were conducted as part of the JANUS program during the 1970s (17–21). Carcinogenesis and life shortening were primary goals, but many fundamental cellular radiobiological studies were conducted *in vivo* to increase understanding of cellular and tissue responses to the dose rate and fractionation paradigms used in the life span study.

RESULTS

Acute Effects: Exposure Time and Dose Fractionation

An experiment was conducted to evaluate the effects of exposure time or dose rate per dose fraction on hematopoietic and intestinal injury produced by fission-spectrum neutrons or γ rays. Table 1 summarizes results

TABLE 1. Split-dose $LD_{50/30}$ in male B_6CF_1 mice. Dose rates were 17 and 48 cGy/min for neutron and gamma radiation, respectively.

Radiation[a]	Group	$LD_{50/30}$, cGy	Net injury, cGy	% of total fractionated dose
Neutron (total fractionated dose 288 cGy)	Static controls	379 (368–391)	—	—
	15 min sham controls	390 (377–402)	—	—
	9 15-min exposures	235 (191–199)	149[b]	52
	9 240-min exposures	240 (215–266)	144[b]	50
Gamma (Total fractionated dose 770 cGy)	Static controls	955 (934–975)	—	—
	240-min sham controls	976 (939–1015)	—	—
	9 15-min exposures	—	—[c]	—
	9 240-min exposures	840	125[d]	16

[a]The factors used to convert from exposure to midline tissue cGy were 0.8 and 0.96 for [illegible]

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	15 min sham controls	**390** (377–402)	—	—
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	9 15-min exposures	—	—[c]	—
	9 240-min exposures	840	125[d]	16

[a]The factors used to convert from exposure to midline tissue cGy were 0.8 and 0.96 for neutron and gamma rays, respectively. The 95% confidence limits are shown in parentheses.

[b]Static and sham control $LD_{50/30}$ values do not differ significantly, so the pooled value of 384 cGy was used to compute net injury, viz., 384–235 = 149.

[c]The $LD_{50/30}$ experiment was inconclusive; a minimum estimate is 750 cGy, with residual injury of 215 cGy or 28% of the fractionated dose. A maximum estimate is ~900 cGy with residual injury of 65 cGy or 8% of the total fractionated dose.

[d]Since no significant difference was detected in $LD_{50/30}$ for sham and static controls, a pooled value of 965 cGy was used, viz., 965-840 = 125.

The original documentation of these data was: E.J. Ainsworth, R.J.M. Fry, F.S. Williamson, W.E. Kisieleski, D.L. Jordan, M.P. O'Malley, M. Miller, E.M. Cooke, A. Sallese, and P.C. Brennan. Relative Biological Effectiveness of Neutrons from the JANUS Reactor. Argonne National Laboratory Division of Biological and Medical Research Annual Report, 1971 (ANL-7870), p. 1926.

from a split-dose lethality study where mice were given 9 fractions over 17 days and challenged 2 days later at a high dose rate. This method measured "unrecovered injury," presumably primarily in the hematopoietic system, at the time the graded single challenge doses were given. Fractionated neutron doses of 32 cGy and fractionated γ doses of 86 cGy were given in either 15 or 240 minutes. The selection of fractionated doses was based upon the RBE of 2.1 measured for survival of marrow CFU-S after brief exposures. The value of 2.1 was based on neutron exposure expressed at that time in kerma (rad), compared with midline tissue dose for gamma rays. In terms of midline tissue dose for both radiations, the RBE was 2.6. Table 1 shows the LD 50/30's measured for groups of approximately 100-150 "control" mice that were either sham irradiated 9 times or remained unhandled in a mouse room. Also shown are the LD 50/30's for mice that had received fractionated doses. The effects of γ ray exposure time/fraction could not be assessed, because the amount of recovery expected for 9–15 minute exposures was overestimated, and graded mortality was not produced by the challenge doses selected. The results show: (1) no effect of neutron exposure time on the injury detected, (2) of the order of 50% of the neutron injury remained at the end of the fractionation sequence, and (3) about 8–28% of the γ injury remained. This indicates that under conditions of fractionated exposure, the neutron RBE was greater than 2.6, probably due to less repair and recovery during and after fission neutron irradiation.

Table 2 (first column) shows the femur CFU-S content in animals sacrificed at 2 days after the third, sixth, or ninth fraction of neutron or γ–radiation. The results show a dose-dependent decrease in CFU-S content in all irradiated groups and a statistically significant effect of exposure time for both neutrons and γ rays at some times. When the neutron dose rate was decreased 16-fold from 2.1 to 0.14 cGy/min., there was no effect on CFU-S content after 96 cGy had been accumulated, but after the 2 higher doses, the CFU-S content was significantly higher at the lower dose rate. In γ irradiated animals, no significant difference was observed after 510 cGy, but after 255–770 cGy, decreasing the dose rate from 5.7 to 0.36 cGy/min. resulted in a higher CFU-S content. It should be noted that CFU-S content did not correlate with radiosensitivity of the mouse as determined by the split-dose technique. For example, the femur CFU-S content was about 2-fold greater in neutron irradiated animals at the low dose rate, but the LD 50/30's values differed by only 5 cGy. It has been reported previously that CFU-S content did not correlate with split-dose radiosensitivity (22).

Results on peripheral blood leukocyte counts from the same animals are shown in Table 2 (second column). The counts were also largely dose-dependent, but due to the small sample sizes and high variability, no significant differences related to dose rate were detected.

Results of tritiated thymidine incorporation in intestinal jejunal segments are present in Table 2 (third column). These results show no dose-dependent decrease in uptake of ^{3}HTdr, but rather, increased incorporation which suggests a compensatory proliferation in response to radiation injury.

Effect of Neutron- or γ-Dose Fractionation on Femur CFU-S Repopulation

The objective was to evaluate CFU-S depression and repopulation after single or fractionated doses of fission neutrons, heavy charged particles or photons. Figure 1 shows results of the initial study on 9 neutron or γ

TABLE 2 Effect of Fractionated Fission Neutrons or ^{60}Co Gamma Exposures, at Two Dose Rates, on Femur CFU-S Content, Total Leukocytes in Brachial Artery Blood and Tritiated Thymide Incorporation in the Jejunum in B_6CF_1 Male Mice. Samples Were Taken About 48 Hrs After 3,6 or 9 Doses of Neutron or Gamma Radiation

Group	Femur CFU-S[a]	Leukocytes/mm^3 X 10^3[b]	Gut ^{3}H Content[c]	Femur CFU-S	Leukocytes/mm^3 X 10^3	Gut ^{3}H Content	Femur CFU-S	Leukocytes/mm^3 X 10^3	Gut ^{3}H Content
	Neutron 96 cGy Gamma 255 cGy			Neutron 192 cGy Gamma 510 cGy			Neutron 288 cGy Gamma 770 cGy		
Controls[b]									
Shammed 15 min	7021 (6381–7660)[d]	4616 (3401–5830)[d]	1.31 ± 0.28[e]	7543 (6716–8370)	5485 (3787–7231)	1.77 ± 0.22	6778 (5886–7669)	3781 (3305–4256)	1.16 ± 0.1
Shammed 240 min	6381 (5485–7277)	6455 (4988–7921)	1.54 ± 0.24	7087 (6141–8033)	4918 (2984–6851)	1.84 ± 0.33	8405 (7300–9510)	5239 (2355–8124)	---
Static	6783 (5964–7602)	5944 (4064–7825)	1.61 ± 0.13	6143 (5246–7039)	5212 (4473–5450)	1.83 ± 0.3	6935 (6003–7867)	6690 (4897–8483)	1.60 ± 0.33
Neutron									
15 Min	1639 (1305–1973)	1556 (916–2195)	1.79 ± 0.19	636 (496–776)	1288 (577–1999)	2.98 ± 0.34	338 (275–401)	806 (697–915)	1.87 ± 0.19
240 Min	1788 (1521–2056)	2142 (1654–2630)	1.98 ± 0.43	1106 (868–1344)	1383 (644–2121)	1.76 ± 0.17	600 (439–761)	1214 (888–1539)	2.10 ± 0.45
Gamma									
15 Min	1275	2699	1.72 ± 0.16	875	1401	2.10 ± 0.42	361	1180	2.17 ± 0.43

Group	Neutron 96 cGy Gamma 255 cGy: Femur CFU-S[a]	Neutron 96 cGy Gamma 255 cGy: Leukocytes/mm^3 X 10^3[b]	Neutron 96 cGy Gamma 255 cGy: Gut ^{3}H Content[c]	Neutron 192 cGy Gamma 510 cGy: Femur CFU-S	Neutron 192 cGy Gamma 510 cGy: Leukocytes/mm^3 X 10^3	Neutron 192 cGy Gamma 510 cGy: Gut ^{3}H Content	Neutron 288 cGy Gamma 770 cGy: Femur CFU-S	Neutron 288 cGy Gamma 770 cGy: Leukocytes/mm^3 X 10^3	Neutron 288 cGy Gamma 770 cGy: Gut ^{3}H Content
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Gamma									
15 Min	1275 (953–1597)	2699 (1657–3742)	1.72 ± 0.16	875 (577–1173)	1401 (441–2361)	2.10 ± 0.42	361 (250–473)	1180 (744–1615)	2.17 ± 0.43
240 Min	2028 (1633–2423)	2922 (1803–4040)	1.99 ± 0.48	767 (505–1029)	1378 (971–1786)	2.12 ± 0.14	685 (567–803)	1594 (811–2377)	1.70 ± 0.31

[a]CFU-S were harvested by grinding the femurs with mortar and pestle. At each sample time marrow from 5 donor mice was pooled, diluted as needed, and injected into 15–25 recipients. Since approximately 8 hrs of laboratory work was required to process all the animals at each sacrifice time, diurnal variations were controlled. No significant difference in femur CFU-S content was detected at (0930 and 1430, the values are 8952 (7568–10,337) and 8111 (6882–9340), respectively. The neutron data for CFU-S have appeared elsewhere (reference 15)

[b]Pooled values for the mice shammed 15 or 240 min, or static controls were 4627 (3480–5889), 5537 (3442–7632) and 5948 (4029–8762), respectively. The pooled values for 9 control samples was 5429 (3894–7232).

[c]μ Ci/mg dry wt X 10^{-3} from 5 mice from each group was sampled 45 min after injection of 0.5 μ Ci/gm ^{3}HTdR (Spec. Act. 0.36 Ci/mM).

[d]95% confidence limits of the mean.

[e]Mean and standard deviation.

These results were originally documented as were those in Table 1.

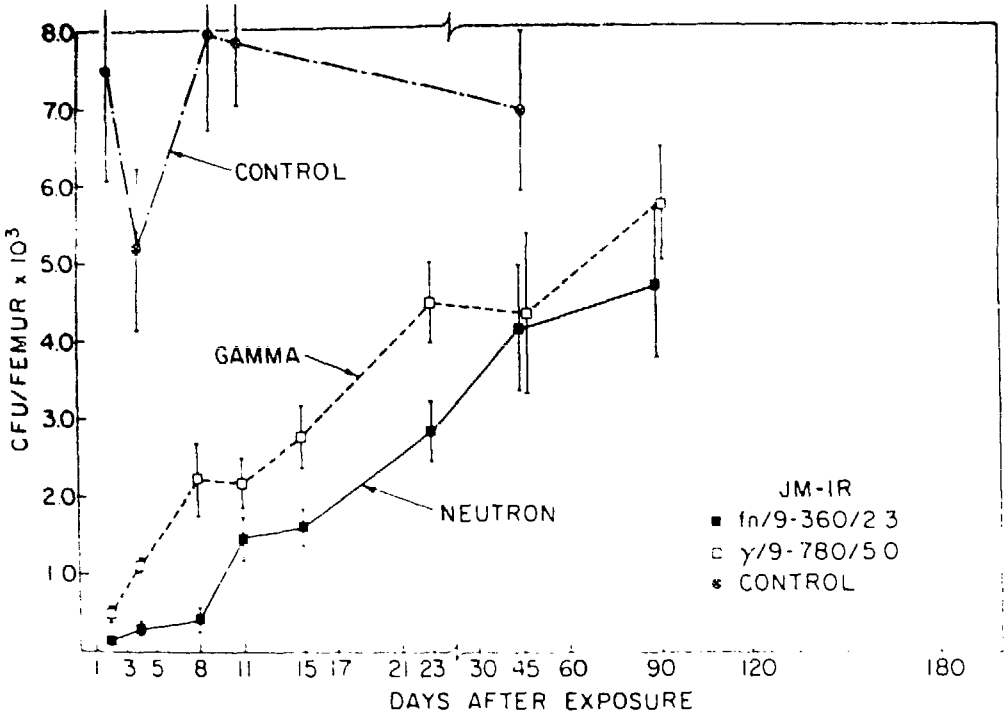
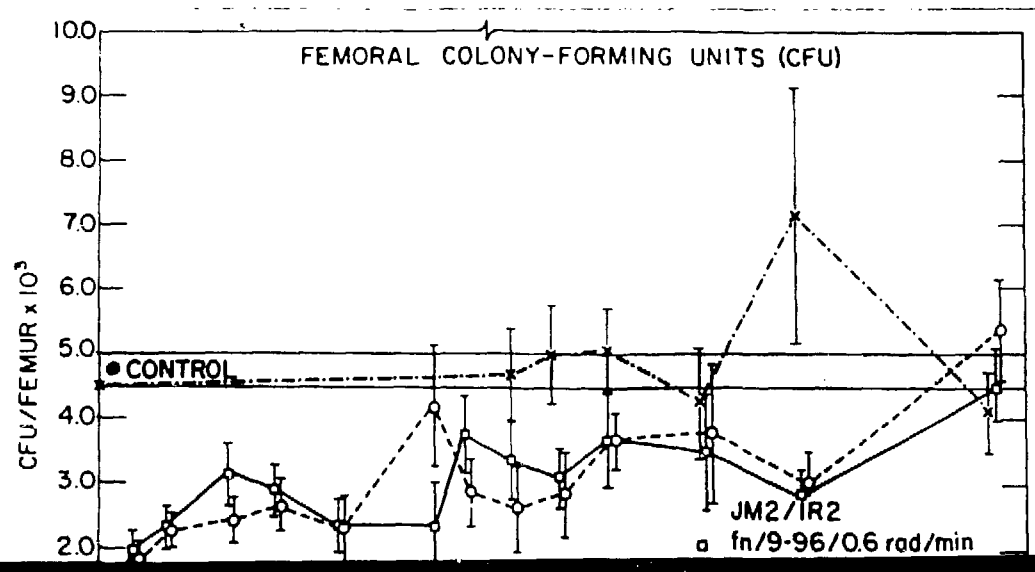


Fig. 1. Femur CFU-S repopulation in B_6CF_1 male mice. The inset in the lower right indicates experiment number JM-1R, the radiation quality, total exposure [for neutrons (Fn)] in kerma or R [for gamma rays (γ)] and the exposure rate per fraction. Conversion from Kerma to midline cGy is 0.8 and from R to midline cGy 0.96. Nine dose fractions were given over 17 days (see text). Pooled marrow from 5-10 donor mice was collected by grinding femurs, and appropriate dilutions were injected into 15–25 supralethally-irradiated female recipents. Values plotted indicate means and 95% confidence limits. The original documentation of these data was: Progress of JM-2 and related neutron- and gamma-radiation toxicity studies. E.J. Ainsworth, R.J. M. Fry, D. Grahn, F.S. Williamson, J.H. Rust, P.C. Brennan, A.V. Carrano, D.L. Jordan, M. Miller, K.H. Allen, M.P. Nielsen, E. Cooke, E. Staffeldt and A. Sallese. Argonne National Laboratory, Division of Biological and Medical Research Annual Report, 1972 (ANL 7970) pp. 13–27; and E.J. Ainsworth, Effects of single or fractionated doses of neutron or gamma-irradiation on hematopoietic stem cells. Radiat. Res. 59: 49 (1974).

fractions (over 3 wks.) totaling 280 neutron or 749 γ cGy. At this high dose, a pronounced delay in repopulation occurred after neutron fractionation (18). Figure 2 shows repopulation after single doses of 96 neutron or 247 γ cGy. Note that femoral CFU-S content tended to plateau in both groups between 3 and 8 days, and between 8 and about 15 days, the contents more than doubled. Thereafter, there was a trend toward a second plateau at about 90 days when the counts ranged from 60–80% of control values.



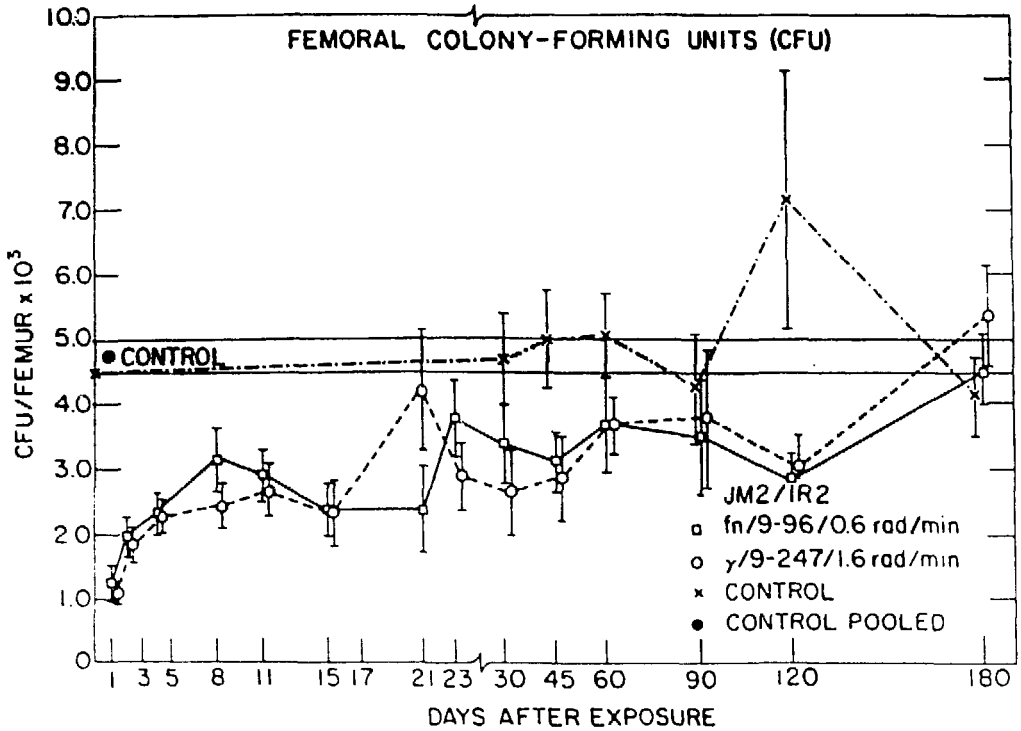


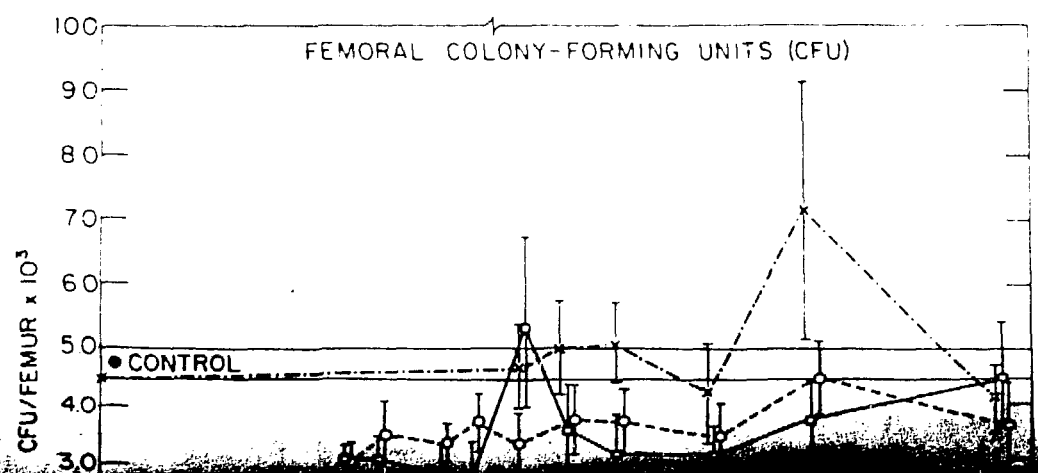
Fig. 2. Femur CFU-S repopulation in B_6CF_1 male mice given a single midline dose of 96 neutron or 247 gamma cGy. Sample sizes and methods were as in Fig. 1 and reference 15. The inset shows the experiment number, number of fractions, dose in midline cGy, and the dose rate. The exposure times were about 17 minutes. These results were origionally documented as were those in Table 1.

Figure 3 shows repopulation results when the same total doses were given in 9 fractions, but at about a 9-fold lower dose rate. Comparison of the results in Figure 2 and Figure 3 indicates that dose fractionation, at low dose rates, produced a marked early sparing effect for both neutron- and γ-irradiated animals during the first 11 days following dose fractionation. Following this 9 fraction sequence, the femur CFU-S depression was not nearly as marked as after the single dose, and the RBE of about 2.6 again obtained. Of particular interest was the observation that the diminished depression during the first 8–11 days after dose fractionation, and after higher doses, was not accompanied by an earlier overall return to normal or near normal femur CFU-S counts (18). Note that there was a tendency for the counts to plateau at 2000–3000 between 5 and 15–21 days after the fractionated exposures. At subsequent times, most of the femur CFU-S counts were significantly below control levels.

CFU-S repopulation was evaluated for 32 days following completion of an 8 fraction sequence (T,W,Th,F) of ^{60}Co γ–radiation, or neon or silicon particles (Figure 4). The purpose was to determine if the delay in CFU-S repopulation observed with 288 cGy of fission neutrons following a 9 fraction sequence (Figure 1) would also occur with neon or silicon particles. Daily doses of 72 cGy of stopping neon or high energy silicon particles were expected to produce approximately the same CFU-S inactivation as was produced by 113 cGy of γ rays (8). Assuming equal inactivation for each fraction, the extent of depression and repopulation would also be expected to be similar if repair and proliferation between fractions were the same for the 3 radiations. Measurements of CFU-S content at 3 and 5 days following completion of the 72 cGy per fraction regimen indicated significantly higher counts after exposure to stopping neon particles than to high energy silicon particles (Fig. 4). Estimates of RBE for CFU-S inactivation after a single dose were similar for stopping neon (1.6) and high-energy silicon particles (1.7), but there was apparently greater accumulation of injury and slower repopulation with exposure to silicon. The LET for silicon is approximately 50 keV/μm and about 130 keV/μm for neon. The LET at which peak effectiveness for CFU-S inactivation was observed was in the range of 50-100 keV/μm (8). In general, the extensive repopulation observed in γ–irradiated mice at 17 and 20 days was considerably greater than for silicon or neon particles. The number of CFU-S in animals that received silicon doses of 72 cGy per fraction was significantly below those for γ–irradiated animals at all but 2 time points. The early phase of CFU repopulation, say, within the first 10 days, probably reflects the net effect of CFU-S inactivation and "injury accumulation"; whereas, in the next 10 days, factors that regulate CFU-S proliferation, repopulation, and differentiation come into play. During this phase, as well as the earlier phase there may be differences between the effect of particles and photons on CFU-S repopulation. Comparison of these repopulation results with the earlier neutron data is tenuous, because different mouse strains were used. These studies should be repeated and extended using lower doses per fraction.

Long-Term Fractionated Exposures: Hematologic Changes

The extent of repopulation was influenced by the number of radiation fractions and/or the total period over which they are administered. Figure 5 shows results on femur and spleen CFU-S content, and blood platelets and leukocytes at 3 times after a 24-week fractionation sequence with fission-spectrum neutrons from the JANUS reactor. The animals received 72–3.36 cGy fractions (3 times per week for 24 weeks at a dose-rate of 0.22 cGy/min). This fractionation regimen was used in the initial sequence of life-shortening and carcinogenesis experiments in JANUS Program at Argonne National Laboratory (17–20). In this experiment, the first samples were taken 4 weeks after the last fractionated exposure and at two 8-week intervals thereafter. While there may have been oscillations between the samples collected at 28, 36, and 48 weeks after the first fractionated exposure,



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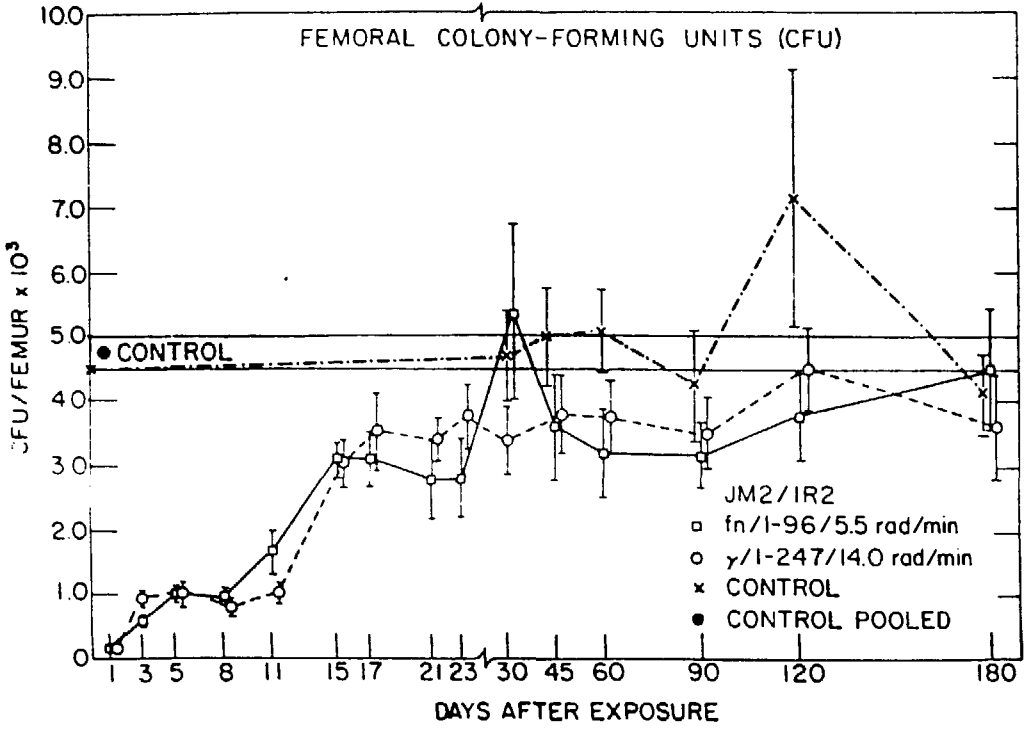


Fig. 3. Femur CFU-S repopulation in B_6CF_1 male mice given 9 fractions totaling 96 neutrons or 247 gamma cGy over 17 days. Sample sizes and methods as in Fig. 1 and Reference 15. These results were originally documented as were those in Table 1.

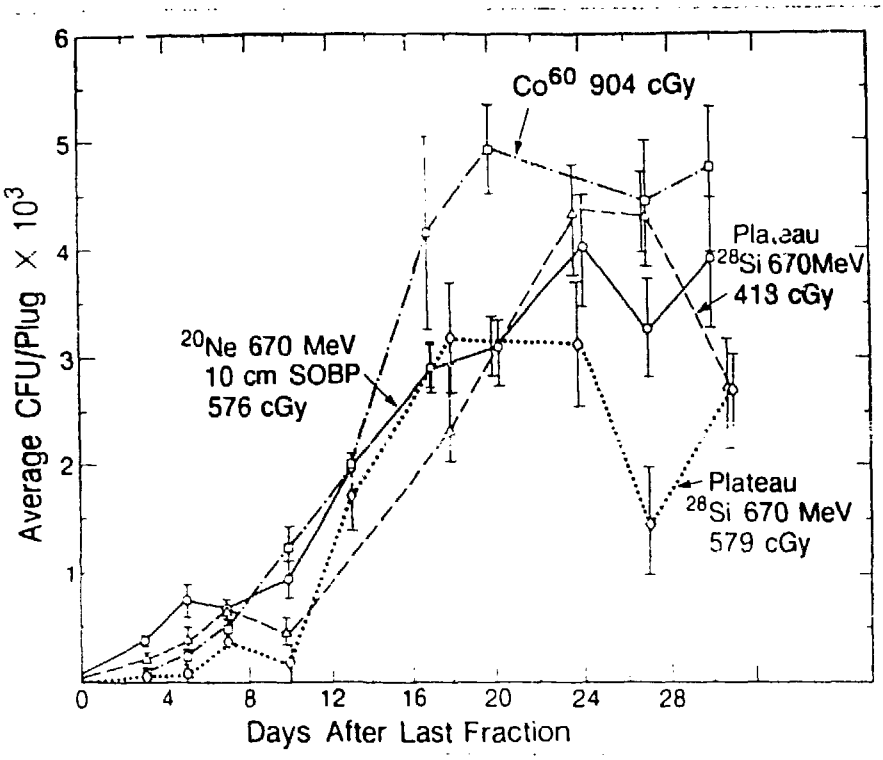
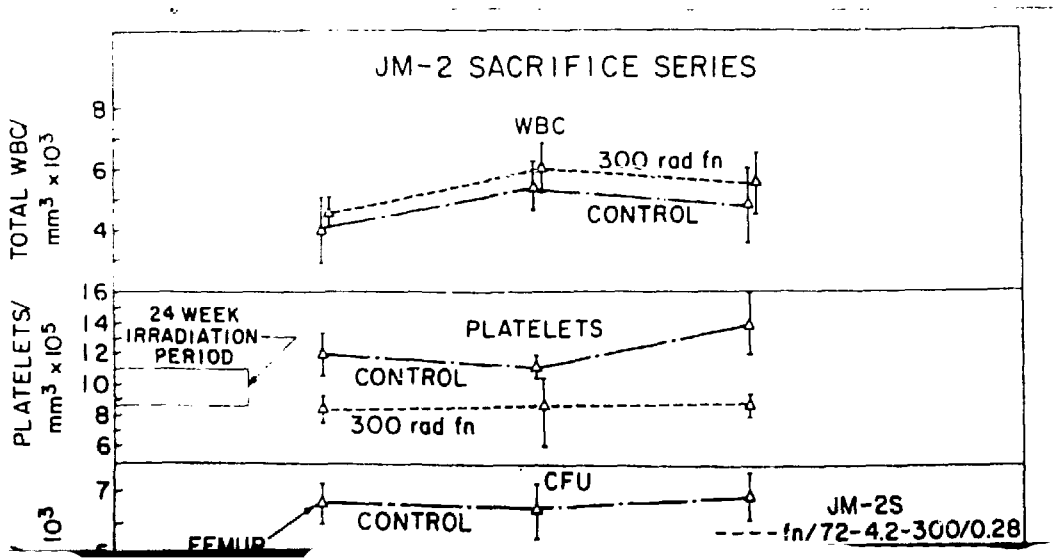


Fig. 4. Femur CFU-S repopulation in CB_6F_1 female mice given 8 fractionated total body doses of gamma rays, exposed to 670 MeV/A silicon ions in the plateau portion of the Bragg, or exposed to stopping 670 MeV/A neon ions in the distal 4 cm of a 10 cm spread Bragg peak. See reference 40 for methods and conditions. Marrow 5–10 donors was injected into 12–16 recipients. Values shown are means and 95% confidence limits.

the results indicate a significant and sustained depression in spleen and femur CFU-S counts and in circulating platelets. In contrast, there was no evidence that total leukocyte counts were depressed; evidently, compensation had taken place.

CFU-S Cycling in Young, Aged, and Aged-Irradiated Mice

The objective was to determine changes in the number of CFU-S in cycle, as a function of age and of prior radiation experience. The issue of "transplantability" in relation to proliferative status of CFU-S may impact on the interpretation of these results (23). Table 3 shows results of the thymidine suicide technique, whereby cells in the S-phase of the cell cycle are killed by incorporation of high specific activity thymidine into DNA. The reciprocal of the percent survival indicates the percentage of CFU-S in the S-phase of the cell cycle. In the marrow and spleen, age- and radiation-related alterations in regulation of CFU-S proliferation were detected



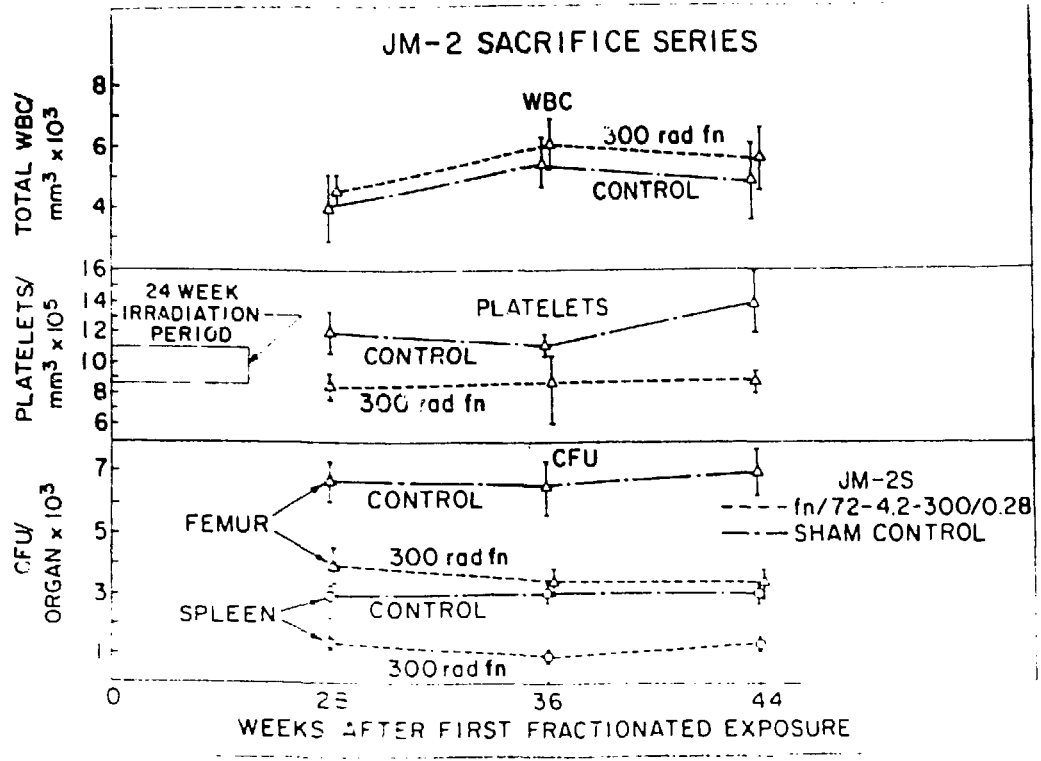


Fig.5. Late hematopoietic responses of mice B_6CF_1 male mice to 72 neutron fractions given 3 times per week over 24 weeks. Leukocyte and platelet counts were done on 15-20 mice (see reference 15 for methods) and marrow "plugs" were flushed from femurs of 5-10 donor mice and injected into 15-25 supralethally irradiated female recipients. See Fig. 1 for source of data.

(Table 3.) The fraction of CFU-S in cycle more than doubled between 105 and 408 days of age. A further alteration was found in mice that received 24 weekly neutron doses of 3.3 cGy commencing at 110 days of age; the fraction of CFU-S in cycle was increased by about 50%, compared with nonirradiated control animals 408 days of age. Quite a different situation prevailed in the spleen, since about 80% of the CFU-S appeared to be in cycle in young mice. The effect of age was the reverse of that observed in the marrow, because at 408 days of age, the fraction of CFU-S in cycle decreased to about 5%. The effect of fractionated doses of neutron radiation was to increase the fraction in cell cycle to about 60%.

There are differences in the CFU-S that can be aspirated and those that remain more firmly associated with the bone. Table 4 presents data on thymidine suicide results in the "residual and aspirated" marrow compartments, respectively. In young animals 109 days old, it is clear that the cycling characteristics of the 2 putative CFU-S populations are different. At 109 days of age, only about 10% of the residual CFU-S were in cycle; whereas, about 50% of the aspirated CFU-S were killed by thymidine suicide. As in the previous study (Table 3), the fraction of CFU-S in cycle increased as a function of age (alone) in both populations. These data indicate that the relative increase was greater in the residual population than in the aspirated populations.

When the data for whole marrow preparations (Table 3) are compared with values obtained by summing the contents of the aspirated and residual compartments (Table 4), one may speculate that interaction or flow occurs between compartments. This matter warrants further study. Different radiation effects were observed in the 2 compartments when the animals were given a single 80 cGy dose of fission neutrons at 113 days of age. The fraction of CFU-S in cycle decreased by about 30% in the "aspirated" compartment compared with 513 day old

TABLE 3. ^{3}H-Thymidine Suicide of CFU-S *in vivo* .[a]

Group	Control CFU-S Femur	^{3}HTdR Suicide CFU-S/Femur Femur	% Survival	Control CFU-S/Spleen	^{3}HTdR Suicide CFU-S/Spleen	% Survival
Young (105 days old)	7627[b] (6885-8368)	6585 (5350-7820)	86	1600[b] (1242-1958)	340 (255-425)	21
Old (408 days old)	7120 (5798-8441)	4686 (4055-5316)	66	662 (402-918)	636 (546-725)	96
Old Irradiated[c] (408 days old; 298 days after first exposure)	3314 (2851-3778)	1601 (1270-1930)	48	427 (274-580)	157 (118-196)	37

[a]Half of mice (B_6CF_1) in each group were injected (i.p.) with 1 mCi of high specific activity ^{3}H-thymidine (65 Ci/mM) 24 hours before CFU-S assay. The intact femur was ground with mortar and pestle to obtain the total CFU-S population.

[b]Data are expressed as the mean and the 95% confidence limits.

[c]Mice were exposed to 80 cGy of fission spectrum neutrons given in 24 weekly fractions of 3.3 cGy (in 45 min.) beginning at 110 days of age. These results were originally presented/documented as follows: D.A. Crouse, E.J. Ainsworth, J.S. Hulsech. Thymidine suicide studies on hematopoietic stem cells in aging irradiated mice, Anat. Rec. 189: 539 (1977).

TABLE 4. ^{3}H-Thymidine Suicide of CFU-S *in vivo* [a]: Aspirated and Residual Marrow Compartments.

	Aspirated Marrow			Residual Marrow		
Group	Control CFU-S/ Survival Marrow Plug	^{3}HTdR Suicide CFU-S/Marrow Plug	% Survival	Control CFU-S/ Hollow Femur Shaft	CFU-S/Hollow Femur Shaft	%
Young (109 days old)	6200[b] (5273–7127)	3185 (2651–3716)	51	1075[b] (960–1190)	958 (818–1097)	90
Old (513 days old)	9893 (9054–10733)	3700 (3226–4174)	37	1466 (1380–1552)	1055 (947–1162)	72

assay. The intact femur was ground with mortar and pestle to obtain the total CFU-S population.

[b]Data are expressed as the mean and the 95% confidence limits.

[c]Mice were exposed to 80 cGy of fission spectrum neutrons given in 24 weekly fractions of 3.3 cGy (in 45 min.) beginning at 110 days of age. These results were originally presented/documented as follows: D.A. Crouse, E.J. Ainsworth, J.S. Hulsech. Thymidine suicide studies on hematopoietic stem cells in aging irradiated mice, Anat. Rec. 189: 539 (1977).

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Old (513 days old)	9893 (9054–10733)	3700 (3226–4174)	37	1466 (1380–1552)	1055 (947–1162)	72
Old Irradiated[c] (513 days old; 400 days post-exposure)	5036 (4006–6065)	2903 (2710–3050)	57	951 (739–1164)	291 (242–340)	31

[a]Half of the mice ($B6CF_1$) in each group were injected (i.p.) with 1 mCi of high specific activity ^{3}H-thymidine (65 Ci/mM) 24 hours before cell preparation. The marrow plug was removed by gentle aspiration; the remaining hollow femur shaft was ground, filtered and appropriately diluted for injection.

[b]Data are expressed as the mean and the 95% confidence limits.

[c]Mice were exposed to 80 cGy of fission spectrum neutrons given as a single dose (in 20 min.) at 113 days of age.

These results were originally documented as follows and as in Table 3: E.J. Ainsworth and M.P. O'Malley. Properties of colony-forming units-studies in progress. Argonne National Laboratory, Division of Biological and Medical Research Annual Report, 1971 (ANL-7770), pp. 34–36.

controls. In contrast, in the small "residual" compartment, the fraction of CFU-S in cycle increased by approximately 2-fold. It should also be noted that these comparatively low total neutron doses, given in either a single or in multiple fractions, produced a significant decrease in the size of the "residual" and "aspirated" compartments in comparison with aged controls. Sampling errors may be a significant factor in relation to the data presented in Table 4, since the results are based on pooled "donor" groups of 3-5 animals. Nevertheless, the results are of considerable interest, and experiments of this nature should be repeated and extended to lower single and protracted doses. When these experiments were done, measurements were also made of peripheral blood CFU-S; in comparison with aged controls, no decrease in blood CFU-S content was detected in aged-irradiated animals."[1]

Late Alterations in Immuno-Hematopoietic Responsiveness

Late effects on immunological competence were detected based on a reduced mean survival time following injection (challenge) with a syngenic leukemia cell line (Table 5). Cell-mediated immunity showed significant late alterations in neutron- and γ-irradiated mice, and an increased susceptibility to bacterial infection was also observed (20,24). Studies on host defense mechanisms should be extended to low doses with emphasis on late effects of long term exposure.

Changes in CFU-S content after injection of typhoid vaccine showed a diminished responsiveness in irradiated mice (Tables 6 and 7). Even when no deficiency in CFU-S content was observed, the spleen and/or femur response to vaccine was different than in aged-irradiation survivors. The observation that a greater reduction of CFU-S content was produced by fractionated neutron or gamma doses, compared with a single dose, should be confirmed. Altered responsiveness and/or disregulation persists as a late form of radiation injury.

Life-Shortening Effects of Heavy Charged Particles or Photons

Various tissues are at risk for radiation carcinogenesis. At low doses, life-shortening is thought to represent the composite of radiation-induced neoplastic disease. Figure 6 presents results from a recent analysis of our life-shortening data. Gamma rays, high energy silicon and iron particles are compared with our previous results for fission-spectrum neutrons (25,26). Weighted linear regressions were fitted to the results. The slopes of the dose-response curves for fission neutrons and silicon particles were quite different. The slope of the γ-radiation curve was similar to that for silicon particles. Only 3 doses were used for high energy iron particles, and at only one, 160 cGy, was there life-shortening. While fission neutrons and high energy silicon particles are characterized by similar dose-averaged LET values, namely about 70–80 keV/μm, the RBE for silicon particles compared with gamma rays, at 50 days of life shortening, is about 4. The value for fission neutrons is about 10. The silicon curve could have two components; namely, a high initial slope, at doses lower than 50 cGy, followed by a more shallow slope at higher doses. Curves of this general nature have been described previously for several endpoints including tumorigenesis, chromosomal abberations, and induction of cataracts (6,27). The implications of the apparent high initial slope for silicon with respect to sparing effects or enhancement of damage resulting from low doses, and for that matter, radioprotection (28), is open to speculation.

TABLE 5. Susceptibility to Transmissible Leukemia: Effects of Age and Prior Irradiation

Challenge Cell Dose	Aged Controls			Irradiated Suvivors		
	Age (Days)	Dead/Total	MST[a]	Age (Days)	Dead/Total	MST[a]
16×10^6	128	20/20	79 (55-105)	—	—	—
	310	20/21	62[b]	300[c]	37/37	23 (15-31)
9×10^6	117	6/29	87[b]	—	—	—
	243	17/29	63[b]	245[c]	39/39	19 (7–31)
	313	26/30	39[b]	—	—	—
	621	25/25	25 (11–39)	—	—	—

[a]Mean suvival time and 95% confidence limits following [illegible]

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16×10^6	128	20/20	79 (55-105)	—	—	—
	310	20/21	62[b]	300[c]	37/37	23 (15-31)
9×10^6	117	6/29	87[b]	—	—	—
	243	17/29	63[b]	245[c]	39/39	19 (7–31)
	313	26/30	39[b]	—	—	—
	621	25/25	25 (11–39)	—	—	—

[a]Mean suvival time and 95% confidence limits following i.p. injection.
[b]Incomplete because mice remained alive when the original report was written.
[c]288 neutron or 741 gamma rad at 110–120 days.
These results were originally documented as follows: E.J. Ainsworth, M.P. O'Malley, T.N. Tahmisian, R.J.M. Fry, M.M. Miller, E.M. Cooke, and P.C. Brennan. Argonne National Laboratory, Division of Biological and Medical Research Annual Report 1971 (ANL-7870) pp. 32–34 and E.J. Ainsworth, P.C. Brennan, R.J.M. Fry, A.V. Carrano, and W.T. Kickels. Argonne National Laboratory, Division of Biological and Medical Research Annual Report 1973 (ANL 8070) pp. 15–17.

*[1]Late effects of radiation on the hematopoietic stem cell compartment. D.A. Crouse, E.J. Ainsworth, J.S. Hulesch, M. Miller, and E.M. Cooke. Radiat. Res. 74: 467 (1978).

TABLE 6. CFU-S Content after 0.1 ml Typhoid Vaccine Injection in Irradiated Survivors and Aged Controls at 260–270 Days After (Sham) Irradiation.

	CFU-S Count in Femur and Spleen[a]					
	Aged Controls		Neutron		Gamma	
Days after Injection	Femur	Spleen	Femur	Spleen	Femur	Spleen
0	6100	1600	8225	1160	5571	1400
	(5111–7088)	(1235–1965)	(7020–9429)	(954–1366)	(4318–6825)	(1132–1668)
1	7607	2500	7600	2683	7933	2233
	(6135–9080)	(2059–2941)	(6101–9099)	(20037–3330)	(6606–9261)	(1741–2726)
3	10033	11500	6392	3446	14781	6901
	(8529–11573)	(9856–13144)	(4638–8147)	(2788–4105)	(12800–16763)	(5635–8198)
5	6803	6546	7640	4173	5525	5640
	(5832–7725)	(5778–7316)	(6513–8768)	(3641–4752)	(4524–6526)	(4940–6340)
7	3500	1653	3429	1771	2800	1280
	(23368–4632)	(1246–2060)	(2545–4312)	(1317–2226)	(2148–3452)	(816–1744)

[a]Male B_6CF_1 mice received a single dose of 96 neutron or 247 gamma cGy in 20 minutes at 110–120 days of age. Groups of 3–5 vaccine-injected (i.p.) mice were sacrificed, femurs or spleens were pooled and injected into 15–20 recipents. Colonies were counted at 8 days. Femurs were ground to prepare cell suspensions.

[b]Data are expressed as the mean and 95% confidence limits.

These results were originally documented as follows: E.J. Ainsworth, M. Miller, E.M. Cooke, J.S. Hulesch, and W.T. Kickels. Hematopoietic injury and recovery. Argonne National Laboratory, Division of Biological and Medical Research Annual Report 1973 (ANL 8070) pp. 13–15. Also, E.J. Ainsworth, E.M. Cooke, D.L. Jordan, J.S. Hulesch, W.T. Kickels, and M. Miller. Earth and late injury to the hematopoietic system: Influence of dose rate and dose fractionation. Argonne National Laboratory, Division of Biological and Medical Research Annual Report 1974 (ANL 7530) pp. 50–55.

DISCUSSION

Radiation hazards related to space station and deep space missions involve mixed radiation fields at low dose rates over long periods of time. In deep space, infrequent SPE's contribute doses at higher rates over 8–48 hours. Presented here are results on short- or long-term fractionation protocols with low-LET photons or high- LET neutrons or charged particles to illustrate how organized tissues respond to protracted low doses.

The hematopoietic system has been used as a model for the study of both early and late effects of low doses. The advantage of this system is that CFU-S inactivation, population size, fraction of cells in DNA synthesis, repopulation kinetics, and differentiated end cells can be quantitated. Results obtained with the hematopoietic system do not necessarily reflect effects on other proliferative tissues quantitatively, but a significant degree of concordance between tissues would be expected.

The dose equivalent to the bone marrow on a 3-year mission to Mars has been estimated at about 1 Sv. This estimate does not include exposures to SPEs. It is of interest that low daily doses of photons or neutrons produce sustained and significant reductions in mouse marrow CFU-S content. Carsten et al. reported that daily doses of 0.7 cGy from either tritiated drinking water (3μCi/ml) or ^{137}Cs γ rays produced a 20% depression of CFU-S after 11 weeks and a total of about 55 cGy (29). Thereafter, the CFU-S level remained between 60–80% of the age controls throughout 80 weeks of radiation exposure. Cronkite et al. have studied various doses and fractionation schedules with 250 kVp X-rays and reported a significant depression of femur CFU-S when doses of 1.25 cGy were given on alterntive days for a total of 31 cGy (30). Werts reported a decline in marine blood platelets by nearly 30% when a total dose of between 45–60 cGy was accumulated during the course of twice weekly X-ray doses of 15 cGy separated by 2 days (31). With increasing doses, the platelet counts tended to plateau, and following cessation of irradiation, there was some increase in counts, but they remain below values observed in

TABLE 7. CFU-S Content After 0.1 ml Typhoid Vaccine Injection in Irradiated Suvivors and Aged Controls at 350-360 Days After (Sham) Irradiation[a]

Days after	Aged Controls		Neutron		Gamma	
	Femur	Spleen	Femur	Spleen	Femur	Spleen

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Days after Injection	Aged Controls		Neutron		Gamma	
	Femur	Spleen	Femur	Spleen	Femur	Spleen
0	7071 (5934–8208)	2900 (2332–3468)	4500 (3201–5799)[b]	3883 (3330–4437)	4692 (3493–5892)[b]	1468 (1222–1714)[b]
1	5179 (4039–6318)	2383 (1840–2927)	5700 (4485–6915)	2300 (1877–2723)	4167 (3901–5243)	2288 (1792–2785)
2	5046 (3655–6478)	5600 (4668–6532)	3653 (2778–4529)	3813 (3259–4368)[b]	3666 (2187–5146)	4615 (3349–5882)
3	6143 (4745–7541)	5627 (4468–6785)	3700 (2871–4529)	6213 (5003–7426)	2167 (1437–2897)[b]	5200 (4132–6268)
4	5333 (4382–6285)	5464 (4141–6787)	2733 (2089–3377)	4800 (3740–5860)	3153 (2389–3918)[b]	4420 (3729–5110)
5	4321 (3163–5479)	4343 (3549–5137)	3071 (2153–3990)	4627 (3213–4840)	2808 (2013–3603)	2400 (1610–3190)

[a]The fractionated irradiation schedule consisted of 9 doses of 10.7 neutron cGy or 27.4 gamma cGy administered over 3 weeks. The dose rates were 0.6 and 1.5 cGy/min for neutron and gamma irradiation, respectively. Total doses were 96 neutron and 247 gamma cGy. The first radiation fraction was administered to male B_6CF_1 mice at 110–120 days of age. Groups of 3–5 vaccine-injected (i.p.)mice were sacrificed, femurs or spleen were pooled and injected into 15–20 recipients. Colonies were counted at 8 days.

[b]Significantly different from aged controls; the mice were 460–470 days of age when sacrificed.

[c]Data are expressed as the mean and 95% confidence limits.

These results were originally documented as in Table 6.

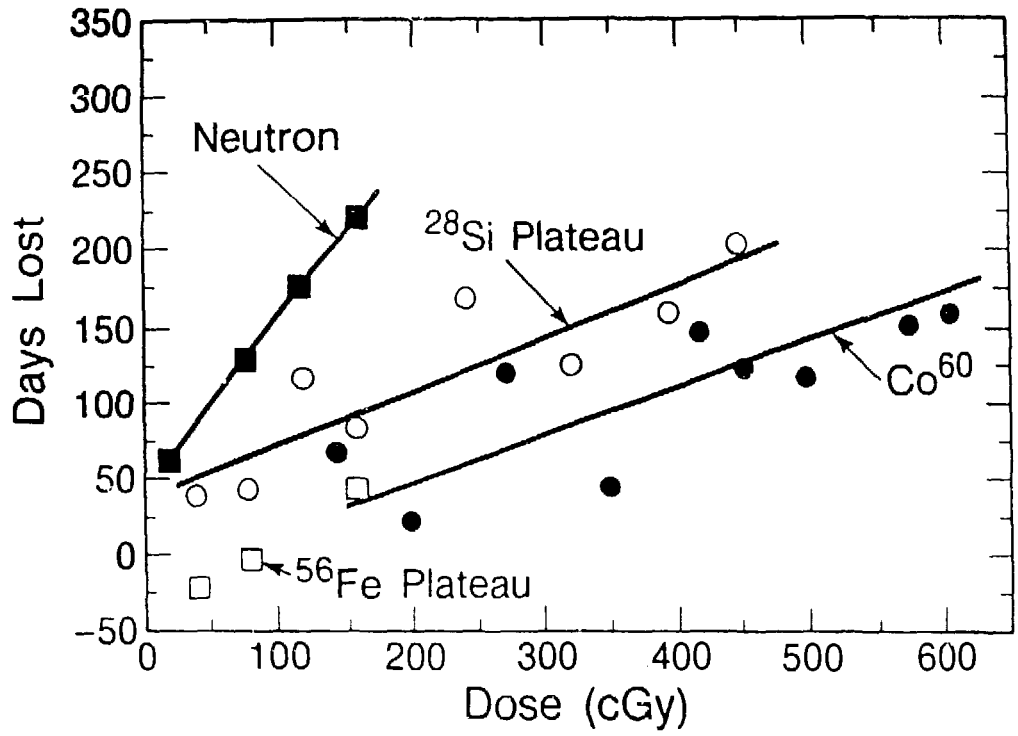

Fig. 6. Life shortening in CB_6F_1 male mice exposed to a single dose of gamma rays, iron (600 MeV/A) or silicon (320 MeV/A) particles compared with life shortening in B_6CF_1 male mice given a single dose of fission-spectrum neutrons from the JANUS reactor (see reference 25). Age at irradiation was about 110–120 days for neutrons and 90–150 days for particles or gamma rays. Sample sizes were from about 600–850 for controls, 120–240 for neutrons, and 40–120 for heavy charged particles. Dose rates for neutrons were exposure time dependent (about 20 min) about 50 cGy/min for gamma rays and ranged from about 50 to several hundred cGy/min for iron or silicon particles. Days lost indicates the difference in mean survival time between irradiated and unirradiated groups. See reference 8 and 18. The fitted lines are weighted linear regressions. The extrapolated Y intercept values were about 35 days lost for neutrons and silicon particles and are consistent with a high initial slope at low doses. The intercept value of minus 20 days for gamma rays is consistent with a low initial slope.

aged controls for 6 weeks. A single X-ray dose of 30 cGy produced a 20% decline in blood platelets at day 11, and after a single dose of 30 cGy, the femur CFU-S content was decreased by about 35%. However, single or fractionated doses of this magnitude produced no significant effects on circulating leukocyte counts (31). These results on platelets and leukocytes are similar to those we obtained with 72 fractions of 3.3 neutron cGy, protracted over 24 weeks (Figure 5). At the high total neutron dose of 240 cGy, femur and spleen CFU-S content remained depressed at approximately 50% of the control level. There was a persistent thrombocytopenia, but circulating leukocyte counts were not depressed. Cronkite et al. have also presented evidence for incomplete repopulation after much lower doses (30). It is clear that significant effects on CFU-S and platelets are produced by comparatively low doses or dose fractions of ionizing radiations.

The sustained depression of CFU-S produced by low-fractionated doses of γ– or neutron-radiation was unexpected and appears to differ from the situation at 300–350 days following a single dose (18,32). Recovery to near normal levels occurred between 90 and 250 days, but there was a subsequent decline by 300-350 days to a similar level of the femur CFU-S that was observed at 1 year after a single or fractionated dose of 240 neutron cGy (18). Results presented here indicate that the fraction of CFU-S in cycle was increased in both aged- and aged-irradiated mice, indicating a compensatory adjustment whereby femur cellularity and the population size of some end cells are maintained at normal levels. Cronkite et al. and Hendry and Lord have made a similar observation (30,32). The mechanism whereby radiation damage results in a sustained alteration in hematopoietic regulation influencing the number of cells in DNA synthesis is a subject of considerable interest. Proliferative state might influence susceptibility to or expression of hematopoietic neoplasias. We previously reported that after single hind leg irradiation, the CFU-S content was decreased in both femurs, and the fraction of cells in DNA synthesis was increased late in life in both the irradiated and non-irradiated femur;[*2,*3] thus, the regulatory process is not only local (33).

Unexpected dose-rate effects were observed when fractionated neutron or gamma doses were administered over 17 days at 2 different dose rates. Decreased dose rate resulted in a higher femur CFU-S content for both fission-

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Unexpected dose-rate effects were observed when fractionated neutron or gamma doses were administered over 17 days at 2 different dose rates. Decreased dose rate resulted in a higher femur CFU-S content for both fission-spectrum neutrons and γ rays. Elsewhere, we reported a small but reproducible effect of fission neutron dose rate/exposure time on LD 50/30 and hematopoietic recovery, but no effect on CFU-S inactivation (15). Griffin and Hornsey have also reported a neutron dose-rate effect on jejunal crypt survival (34).

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*2Response of mouse marrow colony-forming units (CFU-S) to heavy charged particles. E.J. Ainsworth, L.J. Mahlmann and J.C. Prioleau. Lawrence Berkeley Laboratory, Biology and Medicine Division (1981–1982) Annual Report (LBL-14986 UC-48), p. 68–72, April 1983.

*3Late effects of radiation on the hematopoietic system. E.J. Ainsworth, L.J. Mahlmann, and J.C. Prioleau. Lawrence Berkeley Laboratory, Biology and Medicine Division (1982–1983) Annual Report (LBL-16840 UC-48), pp. 65–67, April 1984.

A significant sparing effect of either fission neutron or γ-dose fractionation on CFU-S depression and repopulation was observed during the first 8 days following irradiation (Figures 2 and 3). Interestingly, the decreased effect in animals that received fractionated neutron or γ exposures was not sustained, and the expected earlier overall return to normal levels did not occur. What this means concerning regulation of CFU-S repopulation is open to speculation.

The results from the split-dose lethality study indicate that repair/recovery in the hematopoietic system was less complete between neutron fractions than between γ fractions, resulting in a far greater injury accumulation. Those results also show that femur CFU-S content is not a meaningful predictor of the animal's radiation sensitivity under circumstances where the marrow has been perturbed by prior irradiation, as reported previously (22). In contrast, in unperturbed (normal) marrow, CFU-S content and survival predicted $LD_{50/30}$ in mice treated with an aminothiol protectant, but not with endotoxin (35).

The space radiation hazard issue has two main facets; namely the risk of acute affects, such as the prodromal syndrome, performance decrement (36,37), skin injury, and possibly even lethality due to an unexpectedly large solar particle event (SPE), and the risk of significant late or chronic affects cancer, mutation and cataracts. Combinations of radioprotective drugs may have to be used, and in the event of an SPE, a storm cellar with extra shielding will be important. An estimate of a hypothetical worst case SPE was that the dose rate would be 94 rem/hour with no shielding and 22 rem/hour with 30 cm aluminum shielding (5). Protection from radiation during an SPE must be sustained over 12–48 hours. This poses a challenge. There is information on chemical protection against acute radiation exposures lasting a few minutes, but there is little or no information on protection against longer exposures. A relevant study has been reported by Travis et al. where the protection factor for WR2721 was measured for CFU-S survival and 30-day lethality when 200 mg/Kg was administered before a single dose or after 4 fractions of photons given at 6 hour intervals (38). The protection factor (PF) decreased from 1.8 to 1.3 for 30-day lethality and from 2.3 to 1.3 for CFU-S survival. The basis for the reduced effectiveness with fractionation is not clear, but in earlier split-dose studies, mice treated with AET before a conditioning dose shows a higher than expected radiation sensitivity to subsequent challenge doses (39). It was as if the drug either did not protect the "recovery system" to the extent expected, or the drug somehow interfered with recovery processes.

We have recently explored the ability of WR-2721 to protect mouse marrow CFU-S against damage produced by various heavy charged particles (40). Because of interest in the potential mechanism of action, prostaglandin compounds were studied alone and in combination with WR-2721 using γ rays (41–43). The results showed that WR-2721 alone protected effectively against γ rays and against neon and silicon particles, characterized by LETs of about 35 and 50 keV/μm where protection factors were 2.1 and 2.3, respectively. A small but significant degree of protection (1.2) was afforded against argon particles at 130 keV/μm. A receptor-site specific prostaglandin compound, misprostol, provided a small degree of protection (PF 1.2) against both γ rays and neon particles (25 keV/μm). Misprostol given 2 hours before irradiation produced a protective effect in addition to that produced by 300 mg/Kg of WR2721 given 30 minutes before radiation; this additional effect was related to an increase in the apparent extrapolation number, rather than a change in the slope of the CFU-S survival curve.*4 It would seem particularly important to assess prostaglandin protection at low radiation doses and at low dose rates. Hanson (unpublished data) has observed upregulation of receptor sites and augmented protection of intestinal microcolonies when a different synthetic prostaglandin compound was given before 3 radiation fractions separated by 8 hrs.

Where a high total dose in incurred as a consequence of protracted low-LET irradiation from an SPE is the issue, one might speculate that combined treatment involving agents that work by different mechanisms will be the strategy of choice (2). Maisin showed combinations of drugs with acceptable toxicity were quite effective for protection against both early and late effects in mice (44). Other agents such as prostaglandin compound and perhaps an immunomodulator and/or cytokines might be used in addition to some relatively non-toxic future aminothiol (45). There is a need to develop an experimental protocol, whereby the radioprotective effects of various agents or combinations can be assessed quantitatively under circumstances where the radiation doses are sustained over a period of 12-48 hours. Following completion of rodent studies, it would seem prudent to extend the experiments using larger animals (45).

Conklin and Hagan have speculated that long-term effects such as cancer and life- shortening are the most important facets for space radioprotection (1). Considerable information exists considering carcinogenic effects of low-LET photons, but information is only now emerging concerning the carcinogenic effect *in vivo* of the heavy charged particle component of the space radiation dose (7,8). Life-shortening results presented here compare fission neutrons with silicon and iron particles. The dose-response curve for silicon particles appears consist of a high initial slope, and at higher doses, a lower slope similar to that observed in γ-irradiated animals. The response to iron particles, on the other hand, does not show such a high intial slope. One might speculate that if the initial slope for silicon and for some other particles is high, due to direct rather than indirect damage mechanisms, it

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*[4]CFU-S survival in mice treated with a synthetic prostaglandin E_1 alone or in combination with WR-2721. E.J. Ainsworth, S.M.J. Afzal, and W.R. Hanson, presented at the Radiation Research Society Meeting, Philadelphia, PA, April 1988.

Also, the LET or charge dependence of enhancement or sparing is not yet known for charged particles *in vivo*. Life-shortening results with particles indicate a significant sparing effect with 24 weekly doses of high energy neon ions (about 30 keV/μm), but the results for stopping carbon ions (about 80 keV/μm) over 24 weeks are equivocal (8). However, murine cataractogenesis was enhanced by 24 weekly doses of stopping carbon particle fractions, and recent data indicate enhancement of rat cataracts with high energy argon particles when 4 fractions were given over 12 hours (8,50). The enhancement phenomenon was first noted by Searle and Phillips for neutron mutagenesis in mice (51) and subsequently for life-shortening and appearance of some murine tumors (18–20,47). The *in vivo* results were subsequently confirmed and extended using *in vitro* transformation systems (52–54). Enhancement of damage by fractionated neutron doses has also been observed for chromosomal translocations in spermatogenia (55), damage to the immune system (20) and to the vasculature (21).

The fluence of high-LET heavy charged particles in deep space will be low, and the dose rate will be high. Because of the low fluence, damage interactions between high- and low-LET radiation events are not expected to be of significance. The dose or number of particles necessary to trigger cells into proliferation should be determined (33,56). Alterations in the proliferative status of tissues produced by radiation, stress, microgravity, or a combination of these factors could influence susceptibility to subsequent irradiation or to expression of neoplastic or non-neoplastic damage. Effective chemical protection against the acute effects of high-LET radiations *in vivo* have been reported (40,57,58). Protection against tumorigenesis is of great interest (59). Maisin et al. have demonstrated projection against long-term effects of photons (44,60). Chemical protection against photon induced carcinogenesis has been reported by Milas using WR-1065 and by Werts using superoxide dismutase (61,62). An experiment is in progress at Argonne National Laboratory with WR2721 to determine the extent to which this animothiol compound will protect against life-shortening and tumorigenesis after irradiation with fission neutrons or γ rays (63). Innovative combined strategies will be needed to protect against the carcinogenic and other late effects associated with low doses of low- and high-LET irradiation that will be encountered during ventures to deep space (8).

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