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Effects of Low-Level Radiation upon the Hematopoietic Stem Cell: Implications for Leukemogenesis *

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Abstract

These studies have addressed firstly the effect of single small doses of x-ray upon murine hematopoietic stem cells to obtain a better estimate of the D_q. It is small, of the order of 20 rads.

Secondly, a dose fractionation schedule that does not kill or perturb the kinetics of hemopoietic cell proliferation was sought in order to investigate the leukemogenic potential of low level radiation upon an unperturbed hemopoietic system. Doses used by others in past radiation leukemogenesis studies clearly perturb hemopoiesis and kill a detectable fraction of stem cells. The studies reported herein show that 1.25 rads every other day decrease the CFU-S content of bone marrow by the time 40 rads are accumulated. Higher daily doses as used in published studies on radiation leukemogenesis produce greater effects.

Studies on the effect of 0.5, 1.0, 2.0, and 3.0 rads 3 times per week are under way. Two rads 3 times per week produced a modest decrease in CFU-S content of bone marrow after an accumulation of 68 rads. With 3.0 rads 3 times per week an accumulation of 102 rads produces a significant decrease in CFU-S content of bone marrow. Dose fractionation at 0.5 and 1.0 rad 3 times per week has not produced a CFU-S depression after accumulation of 17 and 34 rads.

Radiation leukemogenesis studies published to date have utilized single doses and chronic exposure schedules that probably have significantly perturbed the kinetics of hematopoietic stem cells. Whether radiation will produce leukemia in animal models with dose schedules that do not perturb kinetics of hematopoietic stem cells remains to be seen.

Introduction:

Leukemia induction in man by large single or repeated doses of radiation (> 50 rad) is well-documented (1,2). These doses perturb the bone marrow, kill a large fraction of hemopoietic stem and stromal cells, It is not known whether the marrow ever returns to its pre-irradiation state. The murine hemopoietic stem cell has a D_o of about 90 rads and a D_q of about 20 rads. Recovery after 50 or more rads may never be complete, as will be discussed later. It is not known whether levels of radiation that do not detectably perturb marrow function will induce leukemia. The first step in experimentally trying to answer this question is to establish levels of intermittent exposure that will not kill significant numbers of hemopoietic stem cells or produce aberrations of marrow function. This research directs attention to the effects of intermittent small doses of radiation upon the bone marrow content of murine hematopoietic stem cells, the presumed target cell for induction of leukemia and to an estimate of the minimum number of stem cells initiated into the leukemogenic process by single doses of radiation that increase the incidence of acute myelo-blastic leukemia in the CBA/Ca male mouse.

The induction of leukemia may well be a multistep process irrespective of whether the initiating agent is ionizing radiation, a chemical, or a virus. The net excess incidence of radiogenic leukemia must depend upon at least two independent competing processes--the initiation of the multistep leukemogenic process and inactivation of the target cell by radiation so that the incidence of leukemia will increase with dose up to a maximum and thereafter decline as the killing process reduces the number of cells that have been initiated. The influence of cell killing upon incidence of radiation-induced tumors was first analyzed by Gray (3). The

target cell for leukemia, which lies within the hematopoietic system, is most likely the pluripotent stem cell that has been termed the colony-forming cell spleen (CFC-S). The assay for the CFC-S is based on the production of macroscopic spleen colonies in the fatally irradiated mouse that has been given a transfusion of syngeneic bone marrow cells (4). Each visible colony is a clone produced by a single cell (5). The CFC-S has the properties of self-renewal, extensive proliferation and differentiation into at least three hematopoietic cell types (erythrocytic, granulocytic, and megakaryocytic). Colonies may be one cell type or mixed. Only a fraction (f) of the CFC-S actually colonize the spleen. This subset is called colony-forming unit spleen (CFU-S) and is assumed but not proved to be a random sample of the total body pluripotent hematopoietic stem cell pool. The f-fraction (6) is experimentally determined by assaying the spleen at different times after large bone marrow transfusions for the CFU-S content and it is assumed that the splenic homing properties of the CFU-S have not been changed by the sojourn in the spleen. Monette and DeMello (7), however, have shown that CFU-S in DNA synthesis (S) have about a 50% reduction in splenic seeding from 11.3 ± 2.4 to 6.2 ± 0.4 percent. Thus, if any treatment increases the fraction of CFU-S in S there will be a decrease in number of spleen colonies counted but not necessarily a decrease in the total CFC-S. For example, Lahiri and Feinendegen (8) injected lethally irradiated mice with 0.5-1 femur of bone marrow cells (circa 10^7 cells, i.e., 1500-4000 CFU-S). The fraction in S in bone marrow and spleen was determined at regular intervals commencing 1 1/2 hours after the transfusion. Within 5 hours 60% of the CFU-S in the spleen were in S and near 100% of CFU-S in bone marrow were in S as determined by means of tritiated thymidine cytocide (9) method.

Many studies led Schofield (10) to embrace the concept that there are a defined number of niches within the body for stem cells, and when these are filled further stem cells cannot be accepted from transfusion or through regenerative processes after induction of marrow hypoplasia. Carsten et al. (11,12) and Cronkite et al. (13) showed that 70 weeks after 550 rads CFU-S in the bone marrow of Hale-Stoner mice are not fully repopulated. Furthermore, transfusions of 2 x 10^8 bone marrow cells did not fill up the bone marrow of mice given 525 rads 12 weeks earlier or mice that had been on 3.0 μCi of ^{3}HOH per ml of drinking water for 10 weeks. These studies suggested that irradiation had altered the marrow stroma perhaps reducing the number of niches or capability of niches to accept transfused stem cells. With the above caveats on the only existing in vivo assay for stem cells, a brief reference to some publications on the long-term effects of radiation upon the CFU-S is indicated.

Schofield and Dexter (14) have studied the effect of a single dose of 50 rads and its repetition seven days after the first dose. Their observations were made for 15 days after initial exposure. After a single dose of 50 rads there was nearly complete recovery at 60 days in CFU-S. After a second dose of 50 rads, recovery leveled off at 50% of age-matched controls from 22-65 days. In our studies (11-13) on tritiated water (3 μCi/ml of drinking water) or chronic ^{137}Cs at a comparable daily dose of 0.7 rads per day, there was a depression in CFU-S to 80% of the age-matched controls for 80 weeks although absolute marrow cellularity had returned to control levels in the mice that had received 550 rads x ray, chronic ^{137}Cs and ^{3}HOH. This return was accomplished by a greater fraction of stem cells being in S (11-13).

The studies undertaken and reported now were aimed at establishing the dose and dose fractionation at which there would be no detectable

perturbation of the CFU-S population of the C57Bl/6J and the CBA/Ca male mice. The studies are still in progress.

Methods and Materials:

Mice are C57Bl/6J males and females raised in the animal colony at BNL or CBA/Ca purchased from Jackson Laboratories. They are irradiated with a 250 Kvp Maxitron X-ray machine, dose rate 3-30 rads/min, 1 mm Al and 0.5 mm Cu filtration.

The CFU-S assay was performed according to the Till and McCulloch (4) procedure. The radiation survival curve for CFU-S was modified so that a constant number of viable plus inactivated bone marrow cells was injected. Since one has to inject enough bone marrow cells to form roughly 10 spleen colonies, the number of bone marrow cells injected increases as the dose of radiation increases and the surviving CFU-S decreases. For example, at a 0.01 radiation survival dose the number of bone marrow cells required to produce about 10 colonies is 100 times the number of nonirradiated bone marrow cells, in which case only 30,000 are needed. We, therefore, gave bone marrow 2000 rads to inactivate the CFU-S and added sufficient irradiated bone marrow cells for a constant intravenous injection of 2.4×10^6 cells.

Results:

Figure 1 shows the dose survival curve after a single dose of 250 Kvp x rays. D_o = 94.3 rads, γ = -0.0106, n = 1,16, and D_q ~ 20 rads. The estimation of D_q is shown on an expanded scale in Figure 2, for which doses of 5, 10, 20, 40, 80 and 160 rads were given. At doses of 20 and

below, there is little if any detectable effect setting D_q close to 20 rads.

Since 40 rads is on the exponential part of the dose survival curve, the effect of 5.25 rads/day for a total of 42 rads was compared to a single dose of 42 rads. The data are shown in Table I. The CFU-S per leg was reduced in 6 successive experiments from 68-86 percent of the CFU-S in nonirradiated controls with an average reduction of 78 percent. In four successive experiments with fractionation at 5.25 rads/day, the reduction was 71-88 percent of the nonirradiated controls for an average of 80.7 percent. With fractionation at 5.25 rads per day, there appeared to be little if any reduction in the killing of CFU-S.

Table II compares the recovery of CFU-S after a single dose of 42 rads and 42 rads fractionated at 5.25 rads/day at 10, 14, 21 and 28 days after the end of radiation exposure. With a single dose the recovery is not complete at 28 days. After the fractionated doses recovery is complete by 14 days and there appears to be an overshoot.

Since 5.25 rads/day clearly killed CFU-S, we reduced the dose to 1.25 rads/day. Mice were exposed for 32 days (40 rads) and 64 days (80 rads). Table III presents the CFU-S content after single doses of 40 and 80 rads which resulted in a reduction to 77 and 31 percent respectively. With a dose of 1.25 rads/day, there was no detectable decrease after an accumulation of 40 rads (109% of controls), whereas after accumulation of 80 rads there was a reduction to 83 percent of controls in terms of total CFU-S per leg.

The mice were then exposed to 1.25 rads/day on alternate days for 32, 50 and 64 days. The results are shown in Table IV. With a daily dose of 1.25 rads there was a reduction to 82 and 85 percent of controls after 50 days (62 rads) and 64 days (80 rads). With alternate day irradiation after 32 days (20 rads), there was no detectable diminution in CFU-S content.

After 50 days (31 rads) and 64 days (40 rads) there was a small, possibly not significant reduction in CFU-S to 92 and 96 percent of the controls.

CBA/CaJ male mice were exposed to 50, 100, 200 and 300 rads and the cellularity and CFU-S were assayed at 1, 14, 28 and 63 days after this single exposure. The data are shown in Table V. The CFU-S had returned to normal levels in the mice exposed to 50 rads but not in the 100, 200, and 300 rads exposed mice. These studies are continuing for longer intervals.

CBA/CaJ male mice in long-term leukemogenesis studies are being exposed to 0.5, 1, 2 and 3 rads three times per week. The data to date are shown in Table VI. The apparent increase in CFU-S content after 17 rads and 34 rads accumulated at 0.5 rads and 1.0 rad three times per week of 11 percent was not expected and until confirmed will be considered insignificant. After 2 rads three times per week for a total of 68 rads, the modest diminution to 96 percent of control may not be significant whereas the decrease to 79 percent of controls after 3 rads three times per week for an accumulation of 102 r is significant.

Discussion:

The data presented herein and in the literature (11-17) show that there is a major and prolonged perturbation of the CFU-S population after exposure to modest single doses of radiation and repeated small doses of radiation. As little as 0.7 rad/day continuously from ^{137}Cs or continuous ingestion of 3 μCi/ml $^{3}H_2O$ in drinking water of Hale-Stoner mice results in a diminution in CFU-S content of the femur detectable after 11 weeks of exposure for a total of 53 rads. The CFU-S level remains between 60

and 80 percent of the age-matched controls through 80 weeks of exposure. No leukemia was observed in this strain during 80 weeks of exposure.

The studies presented in this paper resulted in a D_q of 20 rads, a small value for a single dose of X-ray. After this exposure there is no detectable diminution in the CFU-S content. Our studies clearly show that 5.25rads/day, the lowest dose used by Upton et al. (18) in their studies on radiation leukemogenesis in RFM mouse, kills a significant fraction of CFU-S after an accumulation of 42 rads. Our studies also show that as little as 1.25 rad/day kills CFU-S after an accumulation of 62.5 rads, and results in a depression of CFU-S while 1.25 rads on alternate days for a total of 31 rads also produces a depression in leg CFU-S. With 1.25 rads on alternate days it appears as if recovery of the total number of CFU-S is nearly complete after accumulation of 40 rads, but incomplete with 1.25 rads daily for 64 days (80 rads). Whether there will be a sustained recovery with continued irradiation on daily or alternate days remains to be seen.

In the series of CBA/Ca mice receiving 0.5, 1.0, 2.0, and 3.0 rad three times a week, a depression in CFU-S is evident with 2.0 rads and 3.0 rads after accumulation of 68 and 102 rad respectively. With 0.5 and 1.0 rad three times per week, there may be an increase in CFU-S content of the leg. In these continuing studies later results will answer the question of whether this regimen will reduce the CFU-S population.

It is interesting to make calculations on the number of surviving CFU-S and the incidence of leukemia. After 300 rads the incidence of leukemia is 25 percent and the CFU-S survival fraction is 0.047. Since one leg is about 0.07 of the entire bone marrow and contains an average of 11,169 CFU-S, the total skeleton will thus contain 159,560 CFU-S. The 2-hour seeding factor for CFC-S has not been determined for the

10-week-old CBA/Ca male mouse. If it is assumed to be 0.15, the average mouse will have 159,560/0.15 or 1.06×10^6 CFC-S per mouse, a number almost identical to that estimated by Schofield and Lajtha (21) in the C57 x DBA_2F_1 mouse. Thus, after 300 rads 0.047 of the CFC-S survive; this amounts to 49,800 per mouse or 4.98×10^6 in 100 mice. The incidence of acute myeloblastic leukemia (AML) after 300 rads is 16 per 100 mice exposed (20). Thus, there must be a minimum of 16 stem cells initiated, at least 1 initiated cell per mouse that develops AML, thus at least 1 in 0.31×10^6 surviving stem cells is initiated. How many more is a matter of conjecture. Table VII is constructed from our CFU-S survival curves for CBA/Ca male mice and the incidence of AML from Major and Mole (20). Column 5 of Table VII clearly shows that as the dose increases the minimum fraction of surviving CFC-S initiated, increases from 1 per 1.06×10^9 CFC-S to 1 per 5×10^4 surviving CFC-S after 600 rads.

The lowest dose that is known to produce an excess incidence of leukemia in the CBA/H male mouse is 25 rads of low LET radiation. Twenty-five rem produce 175 single strand breaks, 25 double strand breaks, 400 DNA base changes with near 100% stem cell survival. Single strand breaks are repaired with a high efficiency and fidelity and can perhaps be discounted as inducing leukemia. Double strand breaks are repaired less efficiently and may result in chromosomal rearrangements. Reciprocal translocations may be associated with leukemia. DNA base pair changes and double strand breaks may occur on any chromosome and at any place in the

* The original CBA/Ca mouse renamed CBA/H where it is now bred at AERE, Harwell, England.

DNA helix. There are then two factors to be considered--one, the injury to the DNA and, two, its position in DNA. If one accepts that the gene for the transforming protein must be activated for leukemogenesis, there is a low probability that a single dose of radiation will induce the DNA lesion at this site and thus act as initiator and promoter. If the DNA lesion(s) are remote from the transforming gene, a relocation is required by an unspecified mechanism to serve as the promoter for leukemogenesis. This hypothetical mechanism may account for the highly variable latent period after irradiation.

Above 20 rads exposure and fractionation at 1.25 rads/day, stem cells are killed reducing the number of target cells at risk and perturbing the kinetics of the hematopoietic stem cell. Since normal bone marrow cellularity is maintained along with normal production rates of blood cells, compensation has taken place at the hematopoietic stem cell. The population is reduced in size. By an increase in the fraction in DNA synthesis (13), production rate and flow into the differentiated lines is maintained thus increasing the probability that an initiated stem cell will differentiate, produce functional cells, and thus be removed from the stem cells at risk for a later promotional event and reduce the probability of developing leukemia. One can propose mechanisms for removal of initiated cells as above which would appear to reduce the probability of developing a leukemic clone but experimental proof would be extremely difficult if not impossible to obtain.

After larger single doses of radiation, Table VII shows that the minimum number of stem cells that must have been initiated decreases, but as a fraction of stem cells surviving there is a progressive increase with dose of radiation. After 600 rads the stem cell survival is 0.002 and the minimum number initiated is four. We will assume that the CFU-S will

regenerate to 0.8 normal size (12,13). If the initiated and non-initiated CFU-S expanded in parallel at the same rate to 0.8 the normal stem cell population, the initiated cell population would have expanded to 1600 in 100 mice (16 per mouse) whereas the initiated stem cell population after 200 rads (highest leukemia incidence) would have expanded only to 129 under the same assumptions. It would thus appear that the initiated stem cells proliferate at a much slower rate than the non-initiated cells or perhaps remain in a prolonged "G_0". This would allow time for genetic repair mechanisms to operate before cell division can expand the population of initiated cells and/or fix the lesion. These arguments point out the need for extensive studies on effects of low single doses and fractionated radiation upon the stem cell population and its kinetic behavior.

Radiation unquestionably produces single and double strand breaks and DNA base pair changes. To varying extents these effects can be identified and repaired by a remarkable series of enzymes. Diseases exist in which repair enzyme defects are present impairing capability of "unhooking" DNA strand-strand crosslinks (22), repairing base damage (23), prereplication repair (24) and post-replication repair (25). One can then hypothesize at least that alteration of the gene(s) for DNA repair may be involved in radiation leukemogenesis thus implicating injury to two sites on DNA of the cell that ultimately becomes leukemic.

The need for concentrating and separating stem cell populations harboring initiated cells for further <u>in vitro</u> and <u>in vivo</u> studies is evident.

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TABLE I--EFFECT OF FRACTIONATION OF 42 RADS X-RAY DOSE[1] ON CFU-S (C57 MICE)

	CFU-S/LEG x 10^3		
	Control	Single Dose	5.25 Rads Daily
March 1982 Male Donors Female Recipients	11.2	9.6 (86)[2]	----------
April 1982 Male Donors Female Recipients	12.6	9.3 (74)	----------
April 1982 Male Donors Male Recipients	10.5	7.2 (68)	9.1 (86)
May 1982 Male Donors Female Recipients	11.7	8.9 (76)	10.3 (88)
June 1982 Male Donors Male Recipients	11.5	9.9 (86)	8.9 (78)
June 1982 Male Donors Male Recipients	15.6	12.3 (79)	11.0 (71)

1 250 KvP, 0.5 mm Cu + 1.0 mm Al filter, dose rate 5 rads/min.
2 ( ) = % of control value

TABLE II--CFU-S[1] RECOVERY AFTER 42 RADS (5.25 RADS/DAY OF 250 KVP X RAY)

		TIME P IRRADIATION (DAYS)				
		0	10	14	21	28
CONTROL	CFU-S/LEG	15,592	19,999	17,641	18,782	19,608
	COLS/SPLEEN[2]	15.9 ± 1.1	20.5 ± 1.5	19.3 ± 1.0	19.8 ± 1.2	20.3 ± 1.0
SINGLE DOSE	CFU-S/LEG	12,348 (79)[3]	13,933 (70)	13,514 (77)	15,357 (82)	18,320 (93)
	COLS/SPLEEN	12.3 + 1.1 (77)	13.6 ± 1.2 (66)	13.9 ± 1.4 (72)	16.6 ± 1.0 (84)	18.8 ± 1.3 (92)
FRACTIONATED DOSE	CFU-S/LEG	11,038 (71)	18,806 (94)	18,243 (103)	24,038 (128)	20,980 (107)
	COLS/SPLEEN	10.9 ± 0.9 (69)	18.4 ± 0.9 (90)	17.1 ± 1.2 (89)	24.7 ± 0.8 (125)	24.0 ± 1.1 (118)

1 10-wk-old male C57 (BNL) BM Donors, 10-16-wk-old male recipients.
2 Mean ± SE, 40,000 BM cells injected.
3 ( ) = % of control value.

TABLE III--CFU-S[1] DURING DAILY 1.25 RAD 250 KVP X-RAY EXPOSURES

	Time (Days) 32 (40 rads)	64 (80 rads)
CONTROL CFU-S/LEG	5,203	8,222
CONTROL COLS/SPLEEN[2]	8.2 ± 0.4	10.8 ± 0.9
SINGLE DOSE CFU-S/LEG	4,000[3] (77)[4]	2,510 (31)
SINGLE DOSE COLS/SPLEEN	6.4 ± 0.7[3] (77)	3.3 ± 0.8 (31)
DAILY DOSE CFU-S/LEG	5,673 (109)	6,809 (83)
DAILY DOSE COLS/SPLEEN	8.3 ± 0.7 (102)	9.5 ± 0.6 (79)

1 10-wk-old male CBA/CaJ BM donors.
2 Mean ± SE 40,000 BM cells injected 10-12-wk-old male recipients.
3 These recipients sac'd. on D. 7 (vs. D.10 for all others).
4 ( ) = % of control value.

TABLE IV--CFU-S[1] DURING DAILY AND ALTERNATE-DAY IRRADIATIONS (1.25RAD/DAY)[2]

	Time (Days)		
	32	50	64
CONTROL			
CFU-S/LEG	8,027	9,637	9,957
COLS/SPLEEN[3]	11.7 ± 0.5	12.5 ± 0.7	12.6 ± 0.6
DAILY TOTAL DOSE			
CFU-S/LEG	8,059 (100)[4]	6,954 (72)	8,198 (82)
	40 rads	62 rads	80 rads
COLS/SPLEEN	11.7 ± 0.8 (100)	10.3 ± 0.6 (82)	10.8 ± 0.6 (85)
ALTER DAYS TOTAL DOSE			
CFU-S/LEG	7,878 (98)	6,888 (71)	9,397 (97)
	20 rads	31 rads	40 rads
COLS/SPLEEN	12.3 ± 0.7 (105)	11.5 ± 0.7 (92)	12.0 ± 0.5 (96)

1 8-wk-old female C57 (BNL BM donors).
2 250 kVp x-ray dose rate 2.5 R/min.
3 Mean ± S.E., 40,000 BM cells injected, 9-11-wk-old male recipients.
4 ( ) = % of control value.

TABLE V--CFU-S CONTENT AND MARROW CELLULARITY IN CBA/CaJ MALE MICE AFTER GRADED DOSES OF 250 KV X-RAY

CELLULARITY X 10^6

TIME AFTER EXPOSURE	OR	50 RADS	100 RADS	200 RADS	300 RADS
1 DAY	30.8 ± 1.3	21.8 ± 1.4	17.3 ± 1.4	11.9 ± 1.1	11.7 ± 0.6
14 DAYS	29.3 ± 0.8	24.7 ± 2.0	20.2 ± 1.1	23.8 ± 1.9	22.6 ± 1.9
28 DAYS	26.5 ± 1.3	27.1 ± 1.7	30.8 ± 1.7	27.4 ± 0.7	30.1 ± 1.7
63 DAYS	37.6 ± 1.1	37.6 ± 1.1	34.9 ± 2.9	33.1 ± 2.0	31.7 ± 1.5

CFU-S CONTENT PER LEG (FEMUR AND TIBIA)

1 DAY	11,495	4,095 (36)*	1,889 (16)	332 (3)	87 (1)
14 DAYS	9,534	7,753 (81)	7,161 (75)	6,710 (70)	5,693 (60)
28 DAYS	12,353	8,136 (66)	8,311 (67)	8,577 (69)	9,458 (77)
63 DAYS	13,325	18,626 (102)	11,405 (86)	9,644 (72)	9,960 (75)

* ( ) percent of CFU-S in control nonirradiated mice

TABLE VI--CFU-S CONTENT AND BONE MARROW CELLULARITY IN MICE AFTER GRADED INTERMITTENT EXPOSURE TO 250 KVP X-RAY COMPARED TO AGE-MATCHED NONIRRADIATED CONTROL MICE

DOSE SCHEDULE	TOTAL DOSE RADS	CELLS/LEG*	CFU-S/LEG
0	0	27.6 ± 0.4	10,144**
0.5 rad x 3/wk.	17	25.2 ± 1.1	11,273 (111)
1.0 rad x 3/wk.	34	24.4 ± 0.6	11,224 (111)
2.0 radsx 3/wk.	68	26.0 ± 0.8	9,734 (96)
3.0 rads x 3/wk.	102	26.6 ± 0.6	8,000 (79)

* $\bar{x}$ ± SE (x 10)

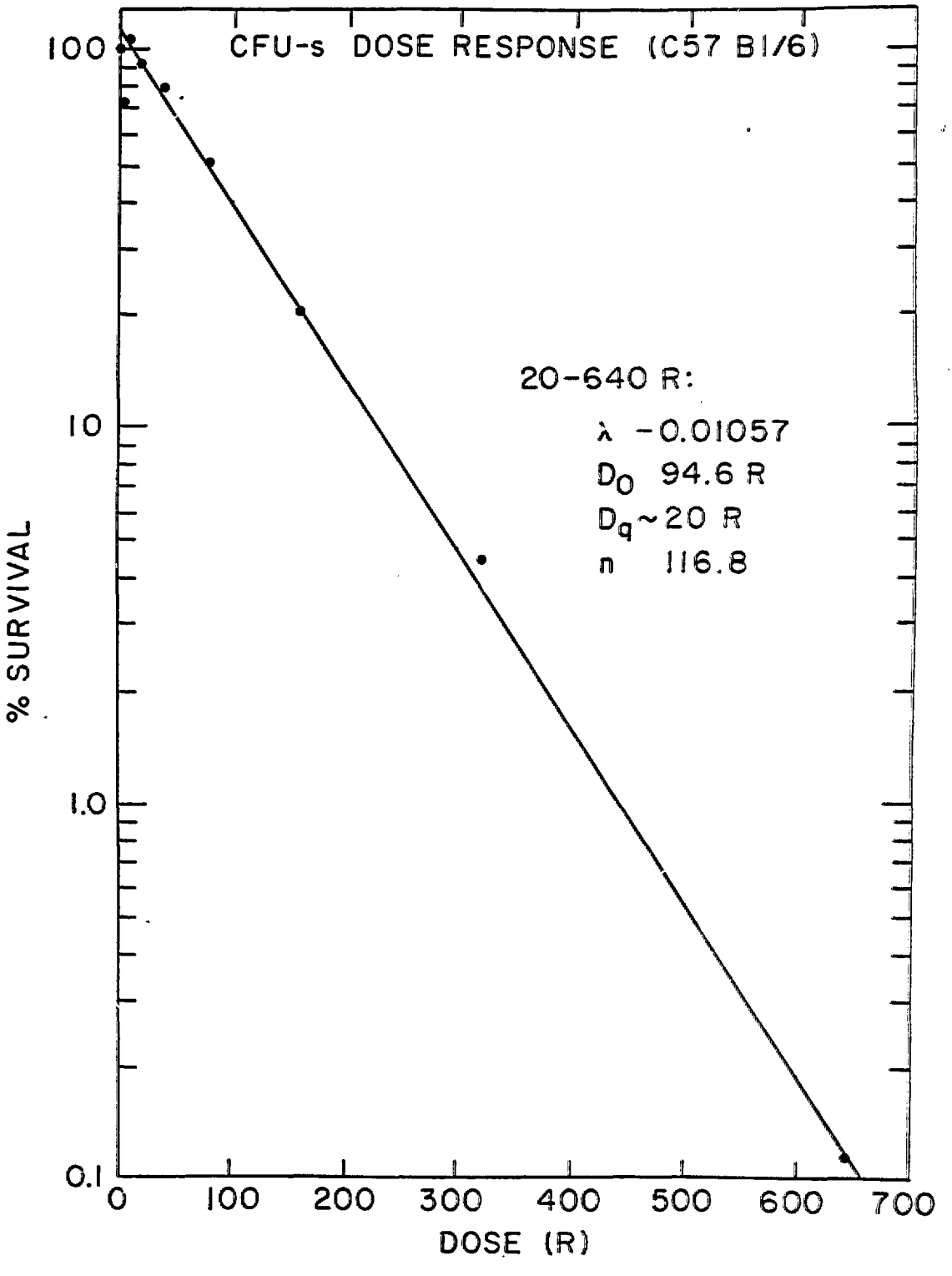
** ( ) percent of nonirradiated age-matched control mice

TABLE VII--HEMOPOIETIC STEM CELL KILLING, NUMBER OF STEM CELLS SURVIVING AND INCIDENCE OF ACUTE MYELOBLASTIC LEUKEMIA (AML) AS A FUNCTION OF DOSE OF 250 KVP X RAY

	1	2	3	4	5
DOSE IN RAD	FRACTION WITH AML	FRACTION OF CFC-S SURVIVING	NO. CFC-S SURVIVING PER 100 MICE x 10^8	MINIMUM NO. CFC-INITIATED AND SURVIVING PER 100 MICE	RATIO OF MINIMUM NO. OF CFC-S INITIATED TO NO. OF CFC-S SURVIVING
0	< 0.001	1.0	1.06	0.1	$1/1.06 \times 10^9$
25	0.02	~ 1.0	~ 1.06	2.0	$1/5.3 \times 10^7$
100	0.13	0.40	0.42	13.0	$1/3.0 \times 10^6$
200	0.21	0.13	0.14	21.0	$1/6 \times 10^5$
300	0.16	0.047	0.05	16.0	$1/3 \times 10^5$
400	0.10	0.017	0.02	10.0	$1/2 \times 10^5$
500	0.075	0.006	0.006	7.5	$1/8 \times 10^4$
600	0.04	0.002	0.002	4.0	$1/5 \times 10^4$

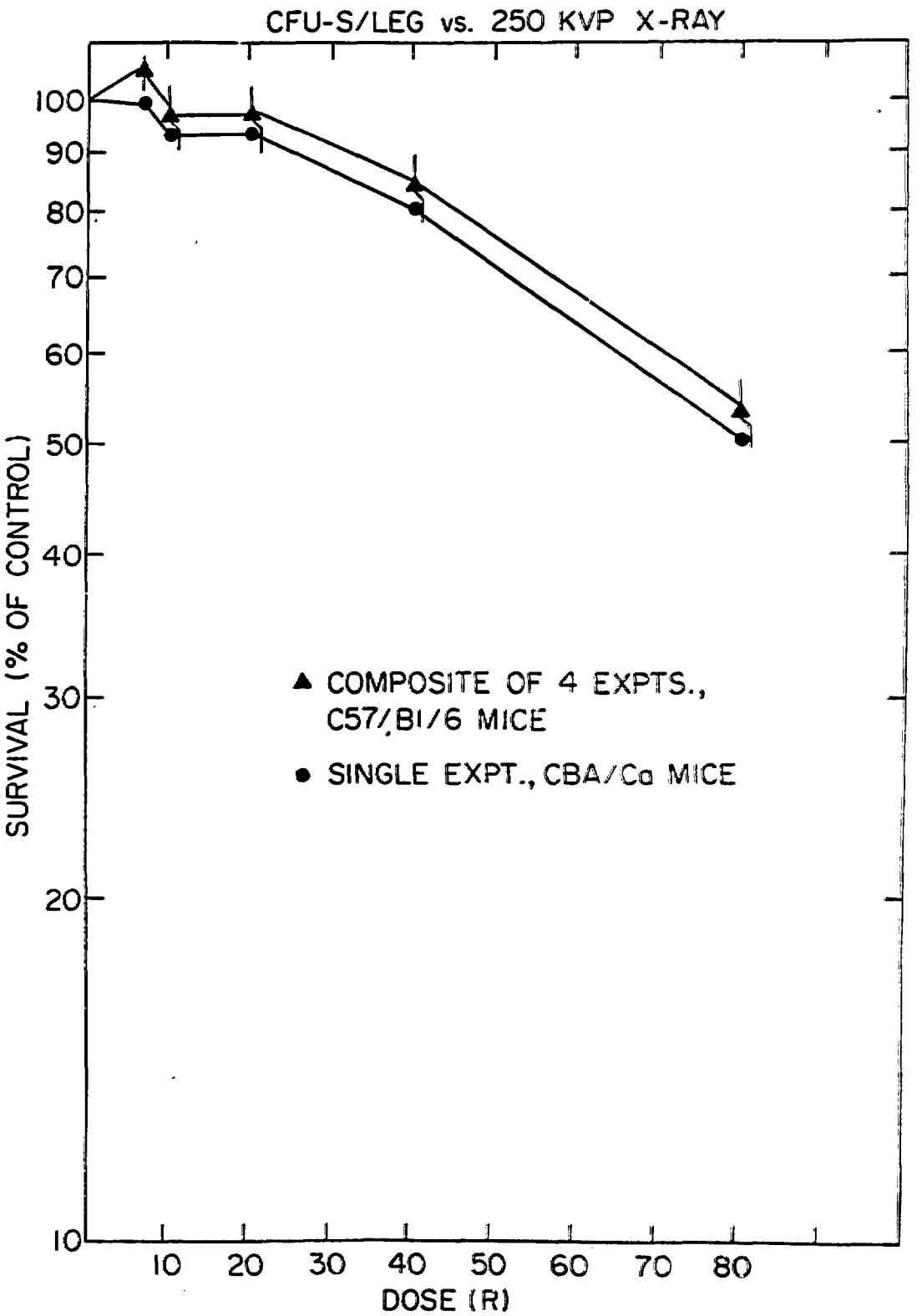
Legend for Figure 1

Radiation dose survival curve of CFU-S in C57/Bl/6J mice


CFU-s DOSE RESPONSE (C57 B1/6)
100
10
1.0
0.1
% SURVIVAL
20-640 R:
λ -0.01057
D_0 94.6 R
D_q ~20 R
n 116.8
0
100
200
300
400
500
600
700
DOSE (R)

Legend for Figure 2

Estimation of D_q for C57Bl/6J and CBA/Ca male mice using small single doses of X ray to determine extent of the shoulder.


CFU-S/LEG vs. 250 KVP X-RAY
SURVIVAL (% OF CONTROL)
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
DOSE (R)
▲ COMPOSITE OF 4 EXPTS., C57/BI/6 MICE
● SINGLE EXPT., CBA/Ca MICE