Comparative Litter and Reproduction Characteristics of Mouse Populations with X-Ray Exposure, Including 45 Generations of Male Progenitors[1] (36794)

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Recent expressed concern for possible genetic consequences to future generations of man from increased environmental levels of ionizing radiation is understandable. These concerns were anticipated by scientists as soon as the potential of atomic power for peaceful uses became apparent. Experimental efforts to determine and quantitate possible radiation-induced heritable injury have proved unrewarding (1). Radiation genetics programs initiated over a decade ago at the Los Alamos Scientific Laboratory (LASL) have included studies on the possible heritable effects of radiation-induced injury on reproduction and litter characteristics (2–5), life span (5–9), resistance to ionizing radiation (10–14), lifetime body weights, (14), lifetime activity (15), and phenotypic mutations (16). Some of these earlier reports suggested that genetic injury may affect the generation subsequent to exposure. There has been no evidence of cumulative radiation-induced genetic injury resulting from several generations of X-ray exposure.

An earlier communication (9) reported the investigations on longevity and mortality distributions of mice from male progenitors with 45 generations of X-ray exposure. In this paper we present results of comparative studies on litters and on reproductive characteristics of mice from 45 generations of X-irradiated male progenitors. These observations represent the final generation to be studied in this program at LASL.

Experimental Methods. The mammals used in this study were obtained from populations produced from lines propagated from two sibling pairs of RFM strain mice. Male mice in each of 45 generations in the experimental line were sibling-mated after being exposed to 200 rads of whole-body X-irradiation at the age of 26 ± 2 days. Control line mice were sibling-mated for the same number of generations but received no X-ray exposure. Serotype tests of 40th generation mice in both lines showed that two H-2 genotypes (H-2^f and H-2^k) were segregating in both lines. Control and irradiated line mice were then separated into H-2^f and H-2^k sublines. Thus, mice used in this study were obtained from these four populations. Lifetime observations were made on approximately 50 mated pairs from each of the four populations. Experimental mice were progeny of 45 generations of X-irradiated males and were either subline H-2^f, designated I(H-2^f), or subline H-2^k, designated I(H-2^k). Control mice were progeny of 45 generations of nonirradiated sibling-matings and were designated as C(H-2^f) and C(H-2^f). Both groups received identical treatment except for radiation exposure in the experimental line.

Animals in this investigation were used exclusively for comparative reproduction observations. Each pair was housed on wood shavings in stainless steel 5 × 8 × 12-in. box-type cages. Fresh bedding and water were provided weekly, and Rockland-Teklad rodent food was provided *ad libitum*. Daily observations were made for proper animal husbandry and for recording litter data.

Radiation conditions for the experimental lines were 250 kVp, 30 mA, Thoraeus II filter, 2.55 mm copper HVL, 60 cm target-to-specimen distance, 45–50 rads/min dose rate, and 200 rads total midbody air dose.

[1] This work was performed under the auspices of the U.S. Atomic Energy Commission.

Results and Discussion. Comparative litter and breeding characteristics are shown in Table I. Two out of the eight characteristics tested showed significant differences among the four groups. The significant difference in the number of mice weaned was caused by the small number weaned by group C(H-2^k). The few litters cannibalized in group I(H-2^f) caused the significant difference among the four groups for that characteristic. What may appear to be a high rate of cannibalism in the four groups observed is due primarily to the fact that these are lifetime observations and that the last few litters produced by aging mates are generally cannibalized in this inbred RFM strain. Therefore, neither of these differences among the four groups tested was the result of a radiation-induced decrement. Maturity, as indicated by age at first litter, and period of fertility were quite similar in irradiated and nonirradiated groups. No significant differences were noted in the number of conceptions, conception interval, number of mice born or weaned, or sex ratios among the four groups. Although none of the mated pairs was sterile, five pairs in the two irradiated groups were nonproductive in contributing viable offspring to the next generation. Lifetime body weights were examined in detail for earlier generations (14) and, although average weaning weights are presented in this paper, they have not been analyzed for significant differences. There appear to be differences in both sexes between substrains within irradiated and control groups but not within substrains between irradiated and control groups. This suggests a single locus difference affecting birth weights in both sexes. Single locus differences affecting radiation sensitivity (12), activity (13), and life-span (9) have been demonstrated in these two H-2 sublines. The possible effects of radiation-induced genetic injury on body weight have been studied by other groups (17–20) with results varying from greater, through lesser, to no difference in weights of progeny from irradiated progenitors. Data analyzed and presented in Table I reveal no significant differences in litter and reproduction characteristics that can be attributed to a radiation-induced decrement. The relatively high percentage of nonproductive pairs in the two irradiated lines may be due to radiation-induced genetic injury. However, the mating scheme used in this program does not permit identification of a specific genetic decrement. Lüning (21) and Sheridan and Wardell (22) reported on the dominant effects and frequency of recessive lethals in irradiated mouse populations by using mating methods designed specifically for these observations.

The objective of this long-term radiation genetics program was to obtain an estimate of the magnitude of the genetic impact of whole-body irradiation exposure on future generations. The sibling-mating system used would be expected to eliminate recessive, lethal, and deleterious mutations at a greater rate than would be the case in man. Thus, one might expect a genetic effect to reach a maximum and plateau after a few generations or to result in increased lethality either during various stages of embryonic and fetal development or soon after birth. Comparative values in Table I indicate that there has been no cumulative radiation-induced genetic decrement over 45 generations of exposure that can be measured by any of the characteristics tested. Because this study has included more generations of X-ray exposure than any other mammalian investigation, it is interesting to compare the mean values of characteristics of the first generation studies (2) with the last generation observed. The 25 mated pairs of the F_6 mice (offspring from five generations of exposure) had no nonproductive pairs in the control line, whereas six pairs (24%) in the irradiated line were nonproductive. This compares with zero and 5 pairs (5%) in F_{46} mice. Other observations from the F_6 experimental line were: age at first litter, 83.4 days; period of fertility, 154 days; number of conceptions, 4.91; number of mice born, 29.4; number of mice weaned, 24.7; and weaning weight, 9.44 g. Comparing the F_6 values with the F_{46} values in Table I shows that there was no general degradation expressed in reproductive performance during the last 40 generations of X-ray exposure.

Summary. Observations were made of com-

TABLE I. Characteristics of Reproductive Fitness of F_{46} Nonirradiated Progeny from Irradiated Progenitors.

Characteristic	Control		Irradiated		Significant at 0.05 level[a]
	H-2^f	H-2^k	H-2^f	H-2^k	
No. of pairs	50	51	50	50	—
Age at first litter	69.6 ± 1.0	67.5 ± 0.8	69.6 ± 1.1	69.7 ± 0.9	No
Period of fertility	259 ± 11.4	271 ± 9.2	256 ± 8.8	265 ± 11.2	No
No. of conceptions	8.6 ± 0.4	9.4 ± 0.3	7.8 ± 0.0	8.2 ± 0.4	No
Conception interval	30.9 ± 0.9	32.5 ± 0.9	32.9 ± 1.2	30.8 ± 0.8	No
No. of mice weaned	26.1 ± 1.1	21.7 ± 1.2	26.4 ± 1.3	25.9 ± 1.4	Yes
Sex ratio at birth	52 ± 0.13	51 ± 0.04	51 ± 0.42	54 ± 0.43	No
No. of mice born	34.1 ± 1.1	35.0 ± 1.6	32.6 ± 2.4	35.0 ± 1.8	No
Weanling wt					
(females)	10.19	9.58	10.47	9.14	—
(males)	10.42	9.83	10.60	9.39	—
Litters cannibalized	3.26 ± 0.27	2.97 ± 0.25	2.43 ± 0.21	3.14 ± 0.20	Yes
Cannibalistic pairs	42	38	41	42	—
Nonproductive pairs[b]	0	0	1	4	—
Sterile pairs	0	0	0	0	—

[a] Using a one-way analysis of variance with four groups.

[b] Produced but did not wean progeny.

parative litter and reproduction characteristics of two H-2 genotype substrains of mice within each of two lines. Two substrains (H-2^f and H-2^k) were from 45 generations of X-irradiated male progenitors, and two were from F_{45} nonirradiated progenitors. No cumulative radiation-induced genetic decrement was noted in irradiated line mice of either H-2 substrains. A comparison of F_6 with F_{46} data showed no degradation in reproductive fitness during 40 generations of X-ray exposure. The F_{46} data suggest that a single gene effect on weaning weights may exist.

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