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Comparative Litter and Reproduction Characteristics of Mouse Populations for 82 Generations of X-Irradiated Male Progenitors[1] (41052)

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Abstract. Male mice have been exposed to 200 rad of X rays (acute whole-body exposures) for 82 consecutive generations. Comparative studies on the reproductive capacity of two sublines ($H2^k$ and $H2^f$) from the X-ray exposed line with the control line with no exposure to ionizing radiation above background were done. Irradiated line $H2^f$ mice were significantly affected by 82 generations of X-ray exposure. However, irradiated line $H2^k$ mice were no different than control line mice.

Since 1927 when Dr. Muller reported that massive X-ray doses could induce lethal mutations in fruit flies, many questions have been posed about possible genetic hazards from medical X-ray exposures. Then with the advent of nuclear power as a source of energy, the impact of possible genetic injury or changes from radiation exposures became a major concern of environmentalists, proponents of nuclear energy, and national and international political leaders. The lack of adequate research data and the fact that experimental human-exposure data cannot be obtained have led to dire theoretical predictions on the genetic fate of a population exposed to ionizing radiation in successive generations. Predictions have ranged from radiation-induced genetic damage resulting in bizarre phenotypic abnormalities and life shortening to the complete genetic death of a species.

A formal population genetics program initiated in the mid-1950s was designed to provide some insight into the problem. To date, this program has produced extensive data on the possible inheritance of radiation injury resulting from large X-ray exposures to successive generations. From these data, quantitative estimates of radiation-induced genetic injury, if significant, would be measurable as a decrement in one or more of the following: reproduction and litter characteristics (1–4), life span (4–8), resistance to abnormal stress (9–13), lifetime growth, body and organ weights (13, 14), physical activity (15), phenotypic mutations (16, 17), and translocation induction (18). However, these attempts to quantify possible radiation-induced heritable injury experimentally have been unsuccessful. During the first 45 generations of X-ray exposure, there was no evidence of genetic injury (8, 19).

Experimental Methods. The mice used in our investigation were from four populations that originated from a single pair of strain RFM mice from 25 generations of inbreeding. The first two population lines were propagated from two sibling pairs from the original RFM parents. In one population line, all male mice for 82 successive generations were exposed to 200 rad of X irradiation (about 5000 times their natural background dose) to the whole body a few days after weaning (26 ± 2 days of age). They were then sibmated so that offspring were derived mostly but not exclusively from irradiated spermatogonia. At 5-generation intervals (through the 45th irradiated generation) characteristics of nonirradiated mice from the irradiated line were compared with control line mice to assess the possible genetic impact of X irradiation on the experimental population. The X-ray exposure factors were: 250 kV peak, 30 mA, Thoraeus II filter, 2.6-mm copper

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half-value layer (HVL), 60-cm target to specimen distance, 45–50 rad/min dose rate, and 200 rad total midbody air dose. The second population line was a nonirradiated control. Serotype tests of 40th-generation (F_{40}) mice in both lines showed that two H2 locus genotypes ($H2^f$ and $H2^k$) were segregating in both lines (11). Approximately 25 F_{83}-mated pairs from each of these four populations were studied for 1 year which is longer than the normal reproductive life of the female.

The experimental mice were nonirradiated progeny of 82 generations of X-irradiated males and were either subline $H2^f$ or subline $H2^k$. The control mice were progeny of 82 generations of nonirradiated control line sibling matings. Both groups received identical treatment except for radiation exposure in the experimental line and were used exclusively for comparative observations on reproduction characteristics. Each pair was housed separately 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.

Results and Discussion. Comparative litter and breeding characteristics and their standard errors are listed in Table I. In order to take account of the dependencies among the variables studied, Hotelling's T^2 test was used to determine whether radiation had any effect on breeding pairs from the irradiated line. At the 5% level of significance, the irradiated line $H2^f$ group mean (which consists of 11 variables) differs significantly from the $H2^f$ control mean and from the $H2^k$ control mean ($T^2 = 45.7$ with 11 and 25 df and $T^2 = 42.6$ with 11 and 28 df), but the $H2^k$-irradiated line mean does

TABLE I. CHARACTERISTICS OF REPRODUCTIVE FITNESS OF F_{83}-NONIRRADIATED PROGENY FROM IRRADIATED PROGENITORS AND F_{83} CONTROL MICE

	Mean values			
	Control line		Irradiated line	
Characteristic	$H2^f$	$H2^k$	$H2^f$	$H2^k$
Conceptions/pair	6.37 (19) ± 0.31	5.12 (26) ± 0.29	3.73* (26) ± 0.34	5.17 (24) ± 0.37
Births/pair	33.58 (19) ± 1.63	28.92 (26) ± 2.03	22.58* (26) ± 2.15	30.43 (23) ± 2.46
Weanlings/pair	24.89 (19) ± 2.12	18.23 (21) ± 1.96	19.50 (26) ± 1.94	21.32 (22) ± 2.53
Births/litter	5.32 (19) ± 0.15	5.67 (26) ± 0.26	6.39* (26) ± 0.33	5.51 (24) ± 0.43
Weanlings/litter	5.42 (19) ± 0.24	5.63 (21) ± 0.39	6.41* (26) ± 0.31	5.85 (22) ± 0.36
Weaning weight	9.37 (19) ± 0.34	8.61 (21) ± 0.34	9.21 (26) ± 0.35	8.94 (22) ± 0.29
Sex ratio	0.509 (19) ± 0.02	0.510 (21) ± 0.03	0.521 (26) ± 0.03	0.539 (22) ± 0.03
Age at first parturition	74.16 (19) ± 2.23	73.96 (25) ± 2.13	82.65 (26) ± 3.78	81.71 (24) ± 3.12
Age at last parturition	285.31 (19) ± 9.85	266.27 (26) ± 9.55	235.44* (25) ± 17.17	259.42 (24) ± 11.79
Litter interval	40.63 (19) ± 1.90	51.19 (26) ± 4.01	57.28* (22) ± 2.22	48.30 (23) ± 5.43
Reproductive life	211.10 (19) ± 9.48	193.23 (26) ± 10.25	167.09* (23) ± 15.87	185.43 (23) ± 11.70
Nonproductive pairs	0	5	0	2
Sterile pairs	0	0	1	0

Note. Number of pairs involved in mean is shown in parentheses. Mean ± SE.
* Mean value differs significantly from control mean at the 5% level of significance.

not differ from the $H2^k$ control mean and there are no other differences. (The T^2 tests were performed by omitting pairs for which one or more of the measured characteristics was missing.) In order to give some indication of the particular variables which cause the difference, we have marked with an asterisk in Table I those variables in the $H2^f$-irradiated line which would differ (at the 0.05 level of significance) from the $H2^f$ control line if independence of the variables were assumed. As a result of these tests, we see that the $H2^f$-irradiated line begins their reproductive life about 8 days later than the controls and ceases about 50 days earlier than the controls. The litter interval for the irradiated line is also about 17 days longer than for the control line. As a consequence, there are an average of 2.64 fewer conceptions per pair, 11 fewer births per pair, and 5 fewer weanlings per pair in the $H2^f$-irradiated line. However, there is on the average, about 1 more birth per litter and 1 more weanling per litter in the irradiated group. The irradiated lines also show a slightly (but not significantly) larger proportion of males at weaning. The only indication of sterility was in one $H2^f$-irradiated breeding pair that failed to conceive during the full year of being caged together. The number of breeding pairs not weaning any mice was greatest in the $H2^k$ control line. Most of the observed characteristics varied among control and irradiated lines from generation to generation. The only significant difference in the F_{46} litter data was that fewer mice were weaned in the $H2^k$ control line than in the other three lines (19). Comparative litter data after 70 generations of exposure showed that the $H2^f$-irradiated line had many of the same differences that were observed for the 83rd generation, such as fewer conceptions and births per pair and a longer time between litters. However, this line still contributed a significantly greater number of weanlings to the next generation than any of the other three, irradiated or control, lines (16).

There was no significant difference in the sex ratio of weanlings among the four lines, and only two phenotypic mutations were observed. A viable recessive mutation (16) for a recurrent hairless condition occurred in an F_{31} control pair, and a self-limiting mutation for a muscle distonia was observed in an F_{33}-irradiated pair.

The total number of phenotypically normal weanlings contributed to the next filial generation by 45 breeding pairs of $H2^f$ and $H2^k$ control line mice was 856. The $H2^f$- and $H2^k$-irradiated lines had 50 breeding pairs that contributed 916 weanlings. When corrected to an equal number of breeding pairs the two control lines produced only 4% more phenotypically normal weanlings than did the two irradiated lines. Two practices presumably detrimental to the genetic well being of a population were used in this study. The control line was subjected to many generations of intense inbreeding. The experimental line was subjected to the same inbreeding plus the additional genetic stress imposed by 200 rad of whole-body X-ray exposure to all male mice in each generation.

The comparative effects of intense inbreeding alone and with inbreeding and X irradiation in combination, as measured by characteristics of reproductive performance, failed to show a consistent radiation-induced genetic detriment to the well being of the species. In fact, the breeding pairs from 82 generations of X-irradiated progenitor males weaned only 4% less mice for the next generation than did the breeding pairs with no unnatural exposure to ionizing radiation. Four percent is a relatively small difference after 82 generations of exposed male progenitors and, after 45 (19) and 70 generations (17) of exposure, the irradiated mice produced more phenotypically normal weanlings than did the control mice.

If radiation-induced genetic injury is assumed to be directly related to a decrease in heterozygosity of the genome, exposure to radiation beyond the 21st generation in this study would not be expected to produce observable genetic injury as a balance of new vs eliminated old genetic injury would theoretically be achieved by this time (20). Sixty-day body weights in nonirradiated progeny from an irradiated population with 14 generations of exposure (theoretical accumulated genetic exposures from 1501 to 4494 R) were reportedly reduced at the rate

of 6.75×10^{-4} g/R in male progeny and 2.00×10^{-4} g/R in female progeny (20). These single-age weight differences reported in Green's study (20) do not appear to be significant. In an early report from our program Chaffee (14) reported significantly lower body weights in 6- to 8-month-old nonirradiated female progeny from 25 to 35 generations of irradiated progenitors. However, in a more extensive study in which body weights were observed throughout the life span on nonirradiated female progeny from 10 to 25 generations of irradiated progenitors (200 rad per generation) weights were not significantly different from controls at any age (13). Thus, body weight changes as an end-point effect for any accumulated genetic exposure to ionizing radiation are questionable. The only phenotypic mutations observed in this program occurred after 30 generations of inbreeding (16, 17).

If lethal mutations have been provoked during one or more of the 82 generations studied, they have not been evidenced in any of our observations. If viable mutations have been induced and accumulated during the course of this study, they have not been expressed as a decrement in any of our observations nor have they had a significant effect on the continuance of the species. Eighty-two generations in man would cover a time span of approximately 2400 years.

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