HERITABILITY OF RADIATION DAMAGE IN MICE¹

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THE genetic hazards of ionizing radiations are the subject of much concern and controversy, and the need for more information on the inheritance of radiation-induced sublethal mutations in mammals has been stressed by the *REPORT OF THE UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION* (1958). There is increasing evidence that many mutations may have slight dominant deleterious effects, which may culminate in a greater population damage than the lethal effects of homozygous mutations (RUSSELL, RUSSELL and OAKBERG 1958). The possibility of reducing the reproductive efficiency of a population to such an extent as to cause its genetic death by accumulation of radiation-induced genetic damage (dominant deleterious effects or a fixation of recessives) through many generations is not beyond comprehension.

The study described here was designed to determine the heritability of radiation damage in terms of reproductive performance in the RFM strain mouse.

METHODS

The population of mice used in this study originated from a single pair of the RFM strain.³ Two males and two females from one litter of the parent generation were paired as shown in Figure 1. These matings were referred to as F₁ generation mice. The male of one sibling pair was given 200 rads of X-rays at 28 days of age and mated to his unexposed sister. Each succeeding generation of male mice in the experimental line was given 200 rads of X-rays at 28 days of age and sib-mated to unexposed sisters. Progeny for continuing the irradiated series were conceived as early as 25 days and as late as 120 days after irradiation of the sire. Males and females in the control line were mated in the same manner as in the experimental line but received no X-ray treatment.

The sixth generation of mice (F₆) was divided into four groups (Figure 1), two from the experimental line and two from the control line. Twenty-five sib-pairs each from the experimental line (Group I) and the control line (Group III, Figure 1) were retained for life span and reproductive performance observations. None of these animals received X-ray exposure; however, Group I accumulated

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³ The nucleus of the colony of RFM mice used at this Laboratory has been brother-sister mated for 40 generations and was furnished by DR. A. C. UPTON of the Biology Division, Oak Ridge National Laboratory.

FIGURE 1.—Heritability of radiation damage in mice (experimental design).

1000 rads per sib-pair through the germ line. Nine breeding characteristics (Table 1) were observed in the experimental and control groups. The other two groups (II and IV, Figure 1) were mated to continue the experiment through the F_{11} generation, at which time the experimental line will have accumulated 2000 rads of X-rays per sib-pair through the germ plasm.

X-rays were provided by a Maxitron 250 with a beryllium window tube under the following conditions: voltage, 250 kvp; filament current, 30 ma; filtration, Thoraeus II; HVL, 2.6 mm Cu; target-to-specimen distance, 60 cm. Dose measurements were made with a Victoreen 100r chamber at the midline of a Lucite mouse phantom. The Victoreen readings were corrected for pressure and temperature and converted to tissue dose in rads by the conversion factor of $1r = 0.96$ rad.

Analyses of the breeding characteristics observed are shown in Table 1. The continuous data (Characteristics 1, 2, 3, 7, 8, and 9, Table 1) were tested by the t test, and the discrete data (Characteristics 4, 5, and 6, Table 1) were tested by the χ^2 (chi-square) contingency table test. Under the t test, the hypothesis tested was the null hypothesis: mean of control group = mean of experimental group ($H_0: \mu_c = \mu_e$) versus the alternate hypothesis: mean of the control group $\neq$ mean of experimental group ($H_a: \mu_c \neq \mu_e$). Under the χ^2 tests, the hypothesis

TABLE 1
Analysis of observed reproductive performance characteristics

Characteristics	Test t or χ^2	Average		Number		Variance	
		Control	Experimental	Control	Experimental	S_o^2	S_e^2
1. Age at first litter (producers)	$t = 0.58$	80.96	83.36	25	22	124.21	293.39*
2. Age at last litter (producers)	$t = 1.04$	263.64	237.59	25	22	6,007.21	8,970.68
3. Reproductive life (producers)	$t = 1.12$	182.64	154.36	25	22	6,103.36	8,988.08
4. Number of conceptions (a) producers	$\chi^2 = 1.12$	5.32	4.91	25	22	4.56	10.45*
(b) all female mice	$\chi^2_3 = 2.36$	5.32	4.32	25	25	4.56	9.14
5. Number mice born (a) producers	$\chi^2_3 = 2.06$	31.96	29.42	25	19	155.64	272.04
(b) all female mice	$\chi^2_3 = 4.66$	31.96	22.56	25	25	155.64	360.17*
6. Number mice weaned (weaners)	$\chi^2_2 = 1.00$	27.60	24.74	25	19	154.42	226.43
7. Average litter size born (producers)	$t = 1.73$	6.90	6.25	25	19	1.09	2.09
8. Average litter size weaned (weaners)	$t' = 1.91$	6.42	5.75	25	19	0.72	1.81*
9. (a) Average weaning weight (weaners)	$t = 4.82^{**}$	11.05	9.77	25	19	0.75	0.76
(b) Total weaning weight (weaners)	$t = 1.56$	305.92	238.81	25	19	19,409.62	20,491.92

* Significant at 5 percent level.

** Significant at 1 percent level.

tested was (H_o):ratio of groupings independent of sample versus (H_a):ratio not independent.

In addition to these tests, sign tests were performed on the change in sign from control to experimental of the mean and variance. An F test was also performed on the ratio $\frac{\text{variance of control}}{\text{variance of experimental}}$ (S_c^2/S_e^2) for all 12 characteristics (Table 1).

RESULTS

For this analysis, the column groupings in the χ^2 test were made with the point in mind of having at least three observations per cell, concurrent with the fact that there was a quantity of missing data, dead or sterile mice, incomplete litters, etc. On looking at Table 1, it can be seen that one (No. 9 average weaning weight) of the 12 t and χ^2 tests and four of the 12 variance ratio tests were significant. Both of the sign tests, mean and variance, were significant. The sign tests showed a decrease in mean and an increase in the variance of the irradiated group as compared to a control group. The increase in variance is statistically significant as shown by the fact that four of the 12 F tests were significant.

Based on the assumption that the present differences between the control and experimental means could be considered significant, the number of pairs of mice that should be tested to detect all significant differences were found to be approximately 200 each per control and experimental groups.

DISCUSSION AND CONCLUSIONS

Although only one of the 12 t and χ^2 tests were statistically significant, by looking at the other t and χ^2 values, using the F and sign tests, and upon examination of the data, it was concluded that 200 rads of X irradiation delivered to the male mouse for five consecutive generations produced heritable genetic damage sufficient to affect the breeding characteristics of their offspring significantly. It was observed that: (a) All control mice were productive, while experimental mice had three nonproductive males and one nonproductive female. In addition, each pair of control mice had at least two litters, while three pairs of experimental mice produced only one litter and three pairs produced no litters ($\chi_1^2 = 6.82$, significant). (b) Every pair of control mice weaned at least one litter, while six pairs of experimental mice weaned no litters ($\chi_1^2 = 6.82$, significant). (c) Experimental mice destroyed more of their litters than control mice ($\chi_1^2 = 5.15$, significant). (d) It was noted that the mean of the experimental group was less than the mean of the control group for all characteristics where a reduced mean value could be construed as a decrement to reproductive performance. (e) It was also noted that the variance of the experimental group was greater than the variance of the control group for all 12 characteristics. This was verified by the sign test to be significant. Four of the 12 increases were found to be significant, and this number is greater than one would normally expect.

A frequent observation in this and other studies at this laboratory is the striking effect of the inheritance of deleterious factors affecting lactation and the mother

instinct of females from irradiated sires. Although no attempt has been made to isolate the loci responsible for these characteristics, it is apparent that the genetic complex involved in lactation is relatively radiation-sensitive. The life span data on the F_6 generation are incomplete at this time; however, there are strong indications that a significantly shorter life span in the experimental group, compared to the control group, might be expected. Assuming that genetic damage increases linearly with increased radiation dosage, one could predict from these data what might be expected from exposures of a lesser or greater magnitude to any generation desired. However, since the 11th generation is now under study, prudence dictates patience.

SUMMARY

The heritability of radiation damage from five generations of X-irradiated male mice was observed in terms of the reproductive performance of their unirradiated F_6 offspring. Nine characteristics of reproductive performance were observed. Although only one of the nine characteristics was statistically significant, the other t and χ^2 values and results of F and sign tests warranted the conclusion that 200 rads of X irradiation, delivered to the male mouse for five consecutive generations, resulted in heritable genetic damage to the unexposed F_6 generation.

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