REPRODUCTIVITY AND LIFE SPAN OF MOUSE POPULATIONS FROM 25 GENERATIONS OF IRRADIATED SIRES[1]

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THE possible effects of heritable radiation-induced mutations in mammalian populations have been among the experimental objectives of several laboratories for a number of years. The majority of investigations on mammalian populations with more than one generation under exposure to ionizing radiation were reviewed at a symposium on "The Effects of Radiation on the Hereditary Fitness of Mammalian Populations," edited by RODERICK (1964). Although suggestions of radiation-induced population decrements were made throughout the symposium, no definite genetic effect accumulating with an increase in the genetic burden theoretically imposed by successive generations of irradiation was demonstrated. In general, the words of DR. JAY L. LUSH, "We do expect there will be mutations, but we haven't got our techniques to where we can corner the evidence at least to the extent of making it of statistical significance," captioned the symposium summary. Cognizant of the difficulties in recognizing and enumerating the genetic effects of exposure to ionizing radiation, the International Commission on radiological protection (1964) conservatively suggested a Maximum Permissible Genetic Dose to the whole population of 5 rems above background, and medical exposures to be distributed over the years between conception and the mean age of childbearing (30 years). Aware of the need, concern, and interest in this radiation discipline, our laboratory started a research program in which mice were used to propagate irradiated and nonirradiated lines for comparative studies. The following paper conveys the results of studies on reproductive efficiency and life span in offspring from as many as 25 generations of X-irradiated progenitors.

EXPERIMENTAL METHODS

One sib pair of mice was used to propagate the populations in this program. The parents (sib pair) were second generation progeny of RFM No. 25–5 from the RFM pedigreed line maintained by DR. A. C. UPTON at the Oak Ridge National Laboratory. Two sibs from the parent pair were used to propagate three basic populations. In one population line all male mice were exposed to whole-body X rays (250 kv; filament current 30 ma; Thoraeus II filter; HVL 2.6 mm Copper; target-to-specimen distance 60 cm; dose rate 50 rads per min) totaling 200 rads each consecutive generation. Exposures were made at 26 ± 2 days of age, and irradiated males were then caged with their sisters as progenitors of the succeeding generation. During the first five generations each line was expanded from one to 25 sib pairs. From the fifth through the

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40th generation 75 sib pairs were maintained as progenitors of each succeeding generation. Development of a number of sublines is inevitable with this method of propagation. In all lines under study, as many sibs were allowed to contribute to each succeeding generation as time would permit (four to five generations per year were produced). However, a few sublines were terminated each generation, and likewise an equal number of new sublines were started each succeeding generation.

This brings up the question of selection pressure in favor of one line or another. To check probabilities that two litters taken at random trace back to different ancestral litters in the first, second, or third previous generation (35th, 36th, and 37th generations), E. R. DEMPSTER (personal communication, 1965) found convincing evidence of greater selection in irradiated line mice than in the control line. It is then quite possible that irradiation damage was eliminated to an undeterminable amount through selection of propagative breeders.

At 26 days of age, when mice were exposed to X rays, spermatogonia were predominant in the seminiferous tubules; however, early spermatid stages were also present at this age. Conceptions occurred as early as 25 days after irradiation of the male, but about 34 days was the general rule. Only nonirradiated second litter mice were used in comparative studies so that the direct effects of postspermatogonial irradiation from the last generation exposed were excluded.

Sister-brother matings were used in continuing lines, although this mating scheme does have the disadvantage of eliminating recessive and semidominant lethal characteristics from the population at an increased rate.

After ten generations of X-irradiation, a line designated as the irradiated subline was started from the irradiated line. This line has been contnued with no further X-ray exposure. The third line (control) has been maintained with the same inbreeding as the irradiated lines but has had no history of progenitor X-ray exposure.

The experiment reported here relates to reproductive fitness (through the entire life) and life span of nonirradiated offspring (F_{26}) of progenitors with 10 and 25 generations of X-irradiated males. In an effort to detect the significance of subline development within the lines, both sib littermates and nonsib matings from the above progenitors were used. An attempt was made to determine the possible influence of litter size on litter characteristics by setting up one F_{26} control group in which all of their litters were culled to four at birth. Reicprocal crosses between one irradiated line and control line were made to determine sex influence in the irradiated line. Five extra pairs per group were set up to use as replacements should one or more mate of the first 50 pairs die. If either mate died, the pair was replaced. Three pairs each were replaced in the control sib and nonsib groups and one each in all of the remaining groups.

Mice involved in this program were housed on wood shavings in stainless steel cages and given fresh water and Wayne Lab Blox *ad libitum*. Since its inception in 1957, animals in this study have enjoyed a clean, comfortable, and disease-free environment.

Breeding characteristics were analyzed by a nested analysis of variance procedure which removed possible cage influences from the test criteria.

RESULTS

Comparative breeding and litter data are shown in Table 1. Three of seven characteristics tested showed significant differences among the nine groups. Although breeding groups with control line females had average maturation ages a few days younger than groups with irradiated line females, the early maturity of nonsib controls (67.5 days) and late maturity of irradiated line females bred to control line males (75.4 days) was primarily responsible for the significant difference among the nine groups. Reproductive life ranged from 281 days in control line females bred to irradiated line males to 249 days in irradiated line females bred to control line males; however, differences were not significant. The number of conceptions was greatest for the irradiated sibs (8.5) and least for the

TABLE 1

Characteristics of reproductive fitness of F_{26} nonirradiated progeny from irradiated progenitors

Characteristic (50 pairs/group)	Control		Irradiated line		Irradiated subline		Irradiated control cross		Culled group*	Test of significance (.05)
	Sibs	Nonsibs	Sibs	Nonsibs	Sibs	Nonsibs	I♀×C♂	C♀×I♂	Sibs	
Age at first litter (days)	69.5± 7.5	67.5± 4.6	70.4± 8.9	70.4± 8.5	72.4±13.9	70.3±10.9	75.4±18.0	69.7± 9.4	69.5± 5.4	Yes
Reproductive life (days)	267±71	272±83	263±84	271±90	253±84	252±92	249±83	281±81	262±87	No
Number of conceptions	7.6± 3.2	7.6± 3.0	8.5± 2.7	7.9± 2.6	7.4± 2.8	6.9± 3.4	6.6± 2.4	8.0± 3.4	8.2± 2.9	No
Number of mice born	34.4±14.6	32.9±13.2	26.6± 9.6	27.7± 9.9	30.7±11.0	28.8±12.0	28.3±10.6	36.1±13.2	36.7±12.4	No
Number weaned	24.4± 9.9	24.1± 9.7	21.8± 9.3	22.5± 9.9	26.4± 9.2	25.6± 9.4	26.0± 8.8	29.2± 9.5	(15.8± 6.8)†	No
Weaning weight (g)	10.5	10.7	10.3	10.6	11.3	10.9	11.2	10.8	11.9	Yes
Litters cannibalized	114	93	182	147	97	94	105	95	99	Yes
Nonproductive pairs	0	0	1	3	0	0	3	2	0	..
Number of mice stillborn per group‡	32	28	47	53	34	34	39	24	26	..

* F_{26} control line sibs; only their litters were culled.

† Not included in significance test.

‡ Subject to human error.

NOTE: All figures reported are distribution mean and standard deviation values. Standard error of the mean may be computed by dividing the standard deviation by the square root of 50.

irradiated line females bred to control line males (6.6), but differences among the nine groups were not significant.

The average litter interval ranged between 37.7 and 30.9 days; however, there appeared to be no relationship between the interval between litters and ancestral radiation history. Weaning weights were significantly different among the nine groups. The smallest weaning weight was in the irradiated line sibs (10.3 g), and the greatest was in the control line sib culled litter group (Table 1). Significance in this characteristic was not related to control versus irradiated lines, nor was there any apparent correlation between litter size at birth or weaning with weaning weight. Cannibalism was significantly greater in the irradiated line sib and nonsib groups than in any of the other groups. Irradiated line sib and nonsib groups also produced more stillbirths than did other groups tested. None of the control line or irradiated subline matings was sterile; however, all groups with irradiated line mice showed some sterility (Table 1). Differences in reproductive performance of reciprocal crosses between irradiated and control lines (Table 1) were due primarily to a general improvement in reproductive fitness in the group with irradiated line sires. This may have been due, to some extent, to heterozygosis introduced by the irradiated line males.

All mated pairs in this experiment remained together from birth throughout their natural lives. The possible effects of irradiation to progenitors on their non-irradiated progeny subjected to the stress of bearing and rearing offspring to weaning age throughout their reproductive lives were tested through life span studies. When the average life span of the nine groups in Table 1 were tested, only the control line sibs with culled litters showed a significantly shorter mean life span. Therefore, mice were pooled according to sex and line origin, and their survival curves are shown in Figure 1. The control sib group with culled litters had the shortest mean life span in both sexes (549 ± 19 days for females and 627 ± 20 days for males). The mean life spans and standard error of the mean for control, irradiated, and irradiated subline females were 597 ± 11, 575 ± 10, and 589 ± 10 days, respectively, and did not differ significantly from one another. Mean life spans for males in the same order as above were 682 ± 13, 692 ± 10, and 669 ± 11 days and again did not differ significantly from one another. No explanation is apparent for the significantly shorter life span of the control group with culled litters; however, it was apparent that the non-irradiated survivors of as many as 25 generations of irradiated progenitors suffered no inherited radiation damage which significantly affected their survival time. This finding is in agreement with earlier studies (SPALDING and BROOKS 1965) on life span as a measure of radiation-induced genetic damage.

DISCUSSION

Age at maturity (first litter) varied significantly among the nine mating groups tested. However, one group (irradiated line females bred to control line males) was primarily responsible for the significance, and two pairs in this group with first litter ages of 130 and 173 days increased the average from 72.2 to 75.4

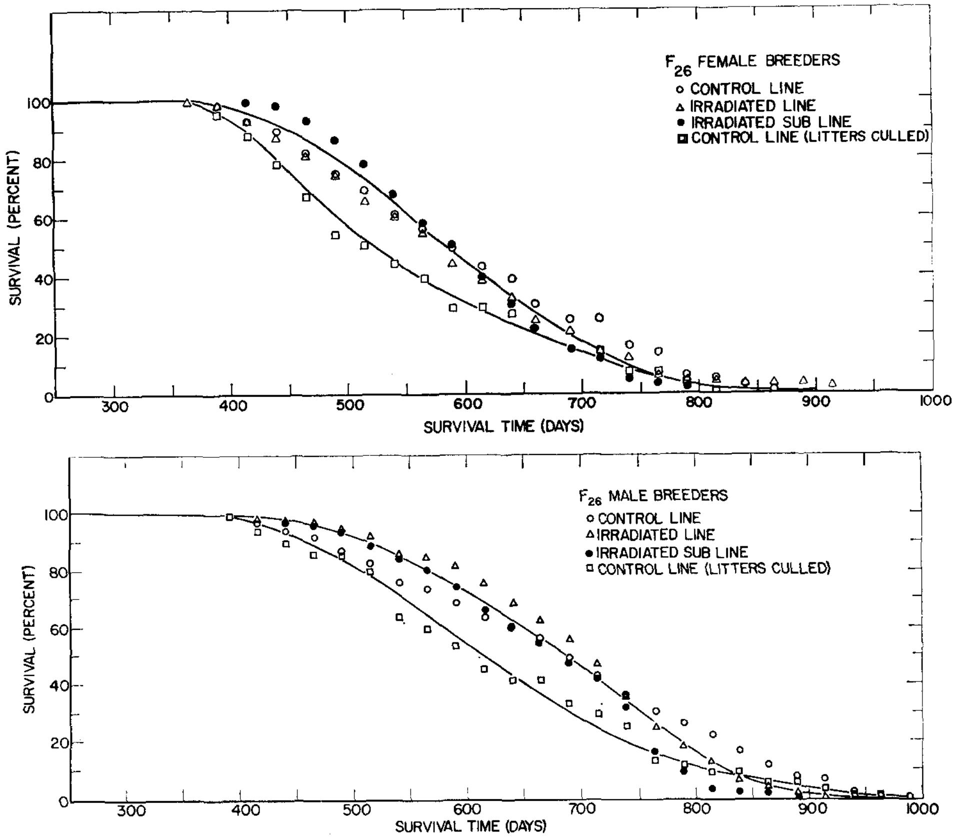

FIGURE 1.—Percent survival plots of 26th generation breeding females (above) and males (below) from control and irradiated progenitors.

days. In addition, this characteristic has not shown consistent differences in earlier tests (SPALDING *et al.* 1961, 1963, 1964) and what, if any, influence radiation to progenitors may exert on this characteristic is questionable.

Reproductive life after five generations of irradiated sires was significantly less than control (SPALDING 1961); however, the differences reversed after ten generations of irradiated male progenitors and, at five-generation intervals, favored the irradiated line through 20 generations of irradiation (SPALDING 1963, 1964). As evidenced in this study, reproductive life has returned to control levels after 25 generations of irradiated ancestry.

GREEN (1964) reported a real reduction in rpproductive life after nine generations of X-irradiated spermatogonia at 100r per generation. He reportedly found no influence in level of inbreeding on radiation effect of this characteristic. This being true, it is of interest to note the lack of agreement between these two studies. This characteristic appears to vary greatly from one generation to another in nonirradiated lines, as evidenced by a range of nearly 200 days in six generations in GREEN's "hybrid" DX-RP line (1964) and 84 days in 25 generations in our study (SPALDING *et al.* 1961). It will be most interesting to follow GREEN's study to see if his "cost" of irradiating spermatogonia increases with additional irradiated generations or follows the pattern of this program.

The number of conceptions during the entire reproductive life was actually greatest, although not significant, in the irradiated line sib mated group (Table 1). However, this included all conceptions regardless of their productivity. When litters born but not weaned were excluded, groups in which irradiated line females were used showed a decrement of 0.5 litter compared to control line groups. Irradiated subline groups were equal to controls, and the control line culled group was superior to unculled controls by 0.5 litter. The average number of mice weaned per productive breeding pair ranged from a high of 28 (irradiated line males bred to control line females) to a low of 20 (nonsib mated control group). There was no consistent decrement in this characteristic associated with radiation history of the progenitors. There did appear to be a radiation-associated decrement in cannibalism, sterility, and stillbirths which has been observed in earlier generations (SPALDING 1964). Although our earliest generations (SPALDING *et al.* 1961, 1962) forecast radiation-induced genetic damage in both reproductive fitness and life span, subsequent generations have shown these earlier manifestations to be unreliable indicators. If such radiation-induced genetic damage has been accumulating in relation to the number of generations irradiated, selection has effectively eliminated mutations measurable in terms of reproductive fitness and life span. To refer to the word of LUSH in the introduction, mutations may be accumulating in irradiated line mice, but if they are, the end points being used are inadequate to assess the damage.

SUMMARY

Reproductive fitness (comparative litter data studies) and life span of nonirrairradiated offspring from 25 generations of X-irradiated progenitors was studied. Irradiated line mice showed decrements in fitness by producing and weaning fewer progeny, by exhibiting a greater tendency toward cannibalism and sterility, and by producing stillbirths in greater numbers than control line mice. Life span was not significantly changed by progenitor irradiation.

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