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SOME OBSERVATIONS ON THE COMPARATIVE EFFECTS OF INBREEDING AND WHOLE-BODY X-RAY EXPOSURE TO SEVENTY GENERATIONS OF MICE

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ABSTRACT

Male mice in each of 70 generations were sibling-mated after being exposed (whole-body) to 200 rad of x irradiation at 26 $\pm$ 2 days of age. A control line was sibling-mated for the same number of generations but received no x-ray exposure. Reproductive characteristics of importance to the continuance of the species were studied to determine the genetic impact of 70 generations of inbreeding alone and in combination with 200 rad of x irradiation to the male mice in each generation. The irradiated line had fewer conceptions (with correspondingly longer intervals between conceptions) and less mice born per breeding pair than the control line; however, irradiated line mice were significantly less cannibalistic and contributed 32% more progeny to the next generation than did an equal number of breeding pairs of their control counterparts. The major effect of inbreeding *per se* was in the born-to-weaned ratio. Control line mice with 70 generations of sibling matings weaned only 38% of the mice born, while control line mice, 65 generations earlier, weaned 86% of the mice born. Radiation, in combination with inbreeding for 70 generations, did not appear to produce genetic injury detrimental to survival of the species.

I. INTRODUCTION

Public concern over the possible genetic impact of a general or limited population exposure on future generations has existed since the war-time detonation of a nuclear weapon. This concern is now directed toward the possible contamination of the environment with radioactive materials which may result in increased internal radiation levels from internal emitters and whole-body exposures from external sources. A formal population genetics program, initiated in the late 1950s, addressed itself to many of the broad areas of concern. This program produced extensive data[1-16] which, to some extent, alleviated our concerns for the problem and showed that, if a radiation-induced genetic decrement was caused from whole-body exposure to ionizing radiation, it was insufficient in magnitude to show statistically significant effects in the offspring of 45 generations of x-irradiated male progenitors.[16]

Four populations of mice (two sublines and two treatment groups) from this formal program have been maintained as a population monitor for some of the more obvious genetic effects of whole-body exposure to acute doses of x irradiation. These are phenotypic mutations (visible to the eye on examination), genotypic mutations that result in significant changes in reproductive characteristics (observed by careful scrutiny of breeding production records), and trends toward complete genetic extinction of a population by increasing sterility and physiological incompetency through a degradation of the natural parental instincts or physiological inability of the female to nurse her young.

II. METHODS

The mammals used in this report were obtained from populations produced by lines originating from two sibling pairs of RFM strain mice. The male mice in each of 70 generations in the experimental line were sibling-mated after being exposed to 200 rad of whole-body x irradiation at 26 $\pm$ 2 days of age. The 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 (the $H\text{-}2^f$ and $H\text{-}2^k$ sublines) were segregating in both lines. Control and irradiated line mice were separated into $H\text{-}2^f$ and $H\text{-}2^k$ lines. Thus, the mice used in this study were from these four populations. Litter mates (34 to 40 pairs) from each of the four populations were observed for 1 yr. Experimental mice were $H\text{-}2^f$ and $H\text{-}2^k$ sublines and progeny of 70 generations of x-irradiated males. Control mice were also $H\text{-}2^f$ and $H\text{-}2^k$ sublines and progeny of 70 generations of progenitor nonirradiated sibling matings. Both groups received identical treatment except for radiation exposure in the experimental line.

The animals in this investigation were used exclusively for comparative studies on the characteristics listed in Table I. Characteristics observed were those that should indicate possible trends detrimental to the genetic well-being of the population in question. Each pair was housed on wood shavings in stainless steel box-type (127 by 203 by 304 mm) 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 data.

Radiation conditions for the experimental lines were 250 KVP, 30 mA, Thoraeus II filter, 2.55 mm

TABLE I

CHARACTERISTICS OF REPRODUCTIVE FITNESS OF IRRADIATED AND NONIRRADIATED LINES OF MICE

	Control		Irradiated		Significant
Characteristic	Group I ($H\text{-}2^f$)	Group II ($H\text{-}2^k$)	Group III ($H\text{-}2^f$)	Group IV ($H\text{-}2^k$)	at 0.05 Level
1. Total Number of Breeding Pairs Observed	37	37	40	34	--
2. Conceptions per Pair (steriles excluded)	6.92 ± 0.38[a] (36)[b]	7.38 ± 0.37 (37)	4.74 ± 0.36 (39)	6.82 ± 0.39 (33)	Yes
3. Litter Interval (days)	36.89 ± 1.10 (213)	32.54 ± 1.05 (236)	43.04 ± 1.32 (149)	36.98 ± 1.16 (192)	Yes
4. Age of Dam at First Parturition	76.11 ± 1.55 (36)	80.83 ± 1.92 (37)	80.43 ± 1.87 (39)	76.76 ± 2.03 (33)	No
5. Number of Mice Born per Litter	5.41 ± 0.17 (215)	5.18 ± 0.16 (222)	5.50 ± 0.19 (172)	5.13 ± 0.18 (183)	No
6. Number of Mice Weaned per Litter (only litters with weanlings)	5.91 ± 0.25 (69)	4.93 ± 0.28 (55)	5.22 ± 0.21 (91)	5.60 ± 0.24 (75)	Yes
7. Number of Mice Born per Pair	32.31 ± 1.86 (36)	31.92 ± 1.86 (36)	24.26 ± 1.79 (39)	28.45 ± 1.95 (33)	Yes
8. Number of Mice Weaned per Pair	15.11 ± 1.68 (27)	9.68 ± 1.65 (28)	12.84 ± 1.44 (37)	14.00 ± 1.60 (30)	No
9. Number of Pairs not Weaning Litters (nonproductive)	10	9	3	4	--
10. Number of Litters Cannibalized per Pair	4.97 ± 0.40 (35)	6.03 ± 0.40 (36)	2.85 ± 0.42 (33)	4.63 ± 0.42 (32)	Yes
11. Sex Ratio at Weaning (male : total)	0.57 ± 0.03	0.56 ± 0.03	0.55 ± 0.02	0.52 ± 0.03	No
12. Number of Pairs (one of which was sterile)	1	0	1	1	--
13. Number of Phenotypic Mutations (visible to observer) Detected during 70 Generations of Exposure	1	0	1	0	--
14. Number of Weanlings Contributed to Next Filial Generation	408	271	475	420	Yes

[a]Standard error of the mean. [b]Sample size.

copper HVL, 60 cm target-to-specimen distance, 50 rad/min dose rate, and 200 rad total mid-body air dose.

III. RESULTS AND DISCUSSION

Comparative reproductive characteristics of the two sublines within the control and irradiated line are shown in Table I. Of the 10 characteristics tested, six showed significant differences among the four groups.

The breeding pairs remained together during the course of this study. The average numbers of conceptions among the groups studied were significantly different. This difference was due to the small number of conceptions in the H-2^f group of the irradiated line which differed from the other three groups. The significant difference was attributed to the longer interval between litters or conceptions shown for Group III (see characteristic 2, Table I). Thus, the irradiated H-2^f group showed a longer interval between litters than the other three groups. There were no differences in age of the dam at first parturition among the groups. We have observed in earlier studies that this characteristic is a measure of maturity in the male mouse, rather than in the female, because the female mouse matures at an earlier age than does the male.

There were no significant differences among the four groups in litter size at birth (number of mice born per litter, characteristic 5, Table I); however, the number of mice weaned per litter did show a significant difference which was attributable to the low value for Group II (H-2^k control). The number of mice born per pair (characteristic 7, Table I) was lowest in Group III, the H-2^f group in the irradiated line. This value was less than either control but not significantly different than the H-2^k group of the irradiated line. Although the H-2^k group of the control line weaned less mice (per breeding pair of pairs that weaned at least one) than the other three groups, the difference was not significant. The number of breeding pairs not weaning any litters was a factor of about 2 to 3 greater in both control line groups than in either of the irradiated line groups (characteristic 9, Table I). Group H-2^f in the irradiated line cannibalized fewer litters than did the other three groups which resulted in the significant difference among groups shown in Table I.

There was no significant difference in the sex ratio of mice weaned among the four groups tested. One breeding pair in the H-2^f group control line and one pair each in the H-2^f and H-2^k irradiated lines failed to conceive, presumably because at least one member of each pair was sterile. During the 70 generations covered in this study, only two phenotypic mutations have been observed. A viable recessive mutation[17] for a recurrent hairless condition occurred in a control line (F_{21} pair), and a self-limiting mutation for dystrophia muscularis was observed in an irradiated line with 23 generations of exposure.

Viable mutations causing diseases which impair an individual's ability to function normally in human society may result in personal hardship and mental anguish. However, perhaps the most serious threat to the continuance of a mammalian species is the genetic injury which may result in the gradual genetic death of the species. The total number of phenotypically normal weanlings contributed to the next filial generation are shown as characteristic 14 in Table I. The H-2^f and H-2^k groups in the control line with 37 breeding pairs each contributed 408 ($\bar{x}$ = 11.0 per pair) and 271 ($\bar{x}$ = 7.3 per pair), respectively. The H-2^f and H-2^k groups in the irradiated line with 40 and 34 breeding pairs contributed 475 ($\bar{x}$ = 11.9 per pair) and 420 ($\bar{x}$ = 12.4 per pair), respectively. When these data are analyzed by the chi-square test, we find significant differences between the observed and expected number of weanlings produced among the four groups. The H-2^f control line group produced less weanlings than the H-2^f irradiated line, the H-2^k control line group produced less weanlings than the H-2^k irradiated line group, and the two control line groups pooled produced 216 (31.8%) less (phenotypically normal) weanlings than did the two irradiated line groups pooled.

Two practices presumably detrimental to the genetic well-being of a population have been employed in this study. The control line was subjected to the deleterious effects of many generations of intense inbreeding, while the experimental line was subjected to the same inbreeding plus the additional genetic injury 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, have failed to show any radiation-induced genetic injury detrimental to the genetic well-being of the progeny of x-irradiated male progenitors. In fact, the 74 breeding pairs from 70 generations of x-irradiated progenitor males weaned 216 (31.8%) more mice for the next generation than did the 74 pairs with no history of exposure to ionizing radiation.

When compared with the performance of control line mice 65 generations earlier,[1] we find that the age of the dam at first litter, number of conceptions, and number of mice born have not been adversely affected by 65 generations of inbreeding; however, the number of mice weaned by control line breeding pairs ($H\text{-}2^f$ and $H\text{-}2^k$) is about half of what was reported 65 generations earlier.[1] Thus, the greatest genetic impact of intense inbreeding on this mammalian population seems to be the degradation of the natural parental instinct to care for the young and/or physiological inability of the female to nurse her offspring. If viable mutations have been induced and accumulated in the irradiated line, they have not appeared to compound but, if anything, seem to have ameliorated the detrimental effects of inbreeding.

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