OBSERVATIONS ON LIFESPAN, RADIORESISTANCE, AND PRODUCTIVITY IN OFFSPRING FROM 5 TO 25 GENERATIONS OF X-IRRADIATED MALE MICE

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THE requirement for research to determine some of the general and specific genetic effects of X irradiation to consecutive generations of mammals was stressed by The UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION (1958). In response to this need a program was started to investigate possible nonspecific effects of X irradiation to consecutive generations of male mice. This program was designed with full awareness that there are as many approaches to the problem as there are investigators willing to undertake such a task, and with appreciation of our then present and projected facilities and personnel.

One of the primary objectives of this program was to do comparative studies on control-line and X-irradiated-line mouse populations after several generations of X-ray exposure. For this reason it was desirable to start the two lines with the smallest amount of heterozygosity possible. No attempt has been made to preserve "recessive" mutations or to estimate the frequency of lethals, and although X-ray exposures were given primarily at the prespermatid stage of germ cell development, a few offspring may have been propagated from sperm irradiated as spermatids.

The following are observations on lifespan of mice from 5, 10, and 15 generations of X-irradiated parentage, productivity of mice from 20 generations of X-irradiated parentage, and radioresistance of mice from 25 generations of X-irradiated ancestors.

MATERIALS AND METHODS

The experimental design is illustrated in Figure 1. The origin of the population was a brother-sister pair of RFM strain mice from the inbred colony of Dr. A. C. UPTON at the Oak Ridge National Laboratory. Experimental methods have been described in earlier reports (SPALDING and STRANG 1961, 1962; SPALDING, STRANG and LeSTOURGEON 1961, 1963a,b; SPALDING, BROOKS and ARCHULETA 1964). Two lines of mice were propagated from the single sib mating as illustrated in Figure 1. Male mice siring the irradiated line were exposed to 200r of whole-body X irradiation at 26 ± 2 days of age and were then caged with their sisters. At five-generation intervals, mice from this line and from a parallel unirradiated control line were used for comparative studies. After ten generations, a third line was started from the irradiated line. This line (irradiated subline, Figure 1) has received no subsequent X-ray exposure and was established to determine to what degree radiation-induced genetic damage may be eliminated through natural processes. This line may also serve as a second control line, since it eliminates to a great extent the possibility of chance segregation of genes favoring a lower resistance to radiation exposure.

Irradiation to the males in the experimental line was given to the whole body with the follow-

* 200 RADS OF WHOLE BODY X-IRRADIATION TO EACH GENERATION OF MALES ONLY.

** NONIRRADIATED LINE WITH THE SAME DEGREE OF INBREEDING.

FIGURE 1.—Schematic outline of experimental design of radiation genetics program.

ing factors: 250kvp, 30ma, Thoraeus II filter, 2.6 mm Cu HVL, 60 cm target-to-specimen distance, and 50 rads/min dose rate.

Comparative productivity was studied among F_{21} control-line mice (offspring from 20 generations of sib-mating) F_{21} irradiated-subline mice (nonirradiated mice from 10 generations of irradiated males followed by 11 generations with no irradiation), and F_{21} irradiated-line mice (nonirradiated offspring from 20 generations of irradiated males). One hundred second-litter sib-pairs in each of the above groups were caged together at weaning and remained together until natural death.

Breeding characteristics were analyzed by a one-way nested AOV (unequal replications [GRAYBILL 1961]). The nested AOV method provided for a possible cage effect within the groups considered. If there were indeed a cage effect it was taken into consideration in forming the proper F test.

Comparative lifespan studies on breeding sibs were done. These mice were nonirradiated offspring from 5, 10, and 15 generations of X-irradiated sires and their control counterparts. Comparative lifespan studies were also done on second litter virgin females which were nonirradiated grandoffspring from 10 and 20 generations of X-irradiated sires and their control equivalents.

Comparative radioresistance was studied in F_{27} (virgin females, 6 weeks old) control-line mice, grandoffspring from 25 generations of X irradiation, and crosses between the two lines. In these studies, mice were exposed continuously (except for short periods required for animal care

and feeding) in a Co^{60} gamma-ray field at approximately 4 rads/hr until dead. Resistance was measured in terms of mean survival time.

Lifespan and radioresistance data were analyzed by the standard AOV (unequal replication) method (SNEDECOR 1956), and the multiple range test (DUNCAN 1955; KRAMER 1956) was used to make pairwise comparisons between groups.

RESULTS AND DISCUSSION

Productivity of F_{21} sibs: Some of the breeding and litter data used to evaluate reproductive performance are shown in Table 1. Characteristic 1, age at first litter, was greater in the irradiated subline than in control or irradiated-line mice (Table 1). This slight but significant difference in maturation age was also observed in the same group in the 16th generation (SPALDING *et al.*, 1964). The fertile life of irradiated-line mice has, for the past ten generations (SPALDING *et al.*, 1963a,b) been longer than that of control or irradiated-subline mice. The average period of fertility is again greatest in irradiated-line F_{21} mice (Table 1); however, because of the rather wide variance in this characteristic in all three groups, the mean differences are not significant. Irradiated-line mice had the largest number of conceptions, smallest number of mice born, and smallest average litter size. Characteristic 6, mice born-to-weaned ratio (Table 1), indicates that irradiated-line mice were equally capable of rearing their offspring when they were born alive and not cannibalized. The number of litters cannibalized was different with each group and was consistent with the total number of conceptions. After producing five litters, there is a rapid rise in cannibalism. A decrease in fitness manifested by an increase in stillbirths in the irradiated line was apparent. This has been a consistent observation over the past 15 generations (SPALDING, STRANG, and LESTOURGEON 1963a,b). All sib-pairs in the control line

TABLE 1

Reproductivity of three lines of F_{21} mice with different genetic burdens of X irradiation

Characteristic (100 sib-pairs)	Group I Control line	Group II Irradiated line	Group III Irradiated subline	Test of significance† (0.05)
Age at first litter	70	72	76	yes
Length of fertile life (days)	267	290	258	no
Number of conceptions	708	851	683	yes
Number of mice produced	3004	2856	3247	yes
females	1490	1339	1534	..
males	1514	1517	1713	..
Sex ratio at birth (male/total)	50.4	53.1	52.8	..
Mice born to weaned ratio*	0.72	0.81	0.92	..
Litter size at birth	6.16	5.79	6.18	..
Litters cannibalized	345	427	187	yes
Number mice stillborn	4	102	9	..
Nonproductive sibs	0	2	1	..

* Only mice seen and sexed at birth were included.

† A dash indicates that a test of significance was not performed.

Group I = F_{21} control line. Group II = F_{21} offspring of 20 generations of X-irradiated males. Group III = F_{21} mice in which only the first ten generations of males were X-irradiated.

were productive, as compared with a 2 percent decrement in the irradiated line and a 1 percent decrement in the irradiated subline for this characteristic.

The sex ratio (males to total born) increased slightly with the number of generations exposed; however, the significance of this difference was not tested. It is, of course, not possible to assess the total genetic damage with the characteristics tabulated in Table 1. Cannibalism, litter size, stillbirths, and sterility appear to be indicators of genetic damage induced by X irradiation, but the apparent increase in such characteristics as fertile life and number of conceptions has in part compensated for these decrements. The value of litter data from inbred populations as described here is limited in most respects to quantitative rather than qualitative effects, and with this inbreeding, recessive lethals (detrimental when heterozygous) would be eliminated more rapidly than in a panmictic population. To obtain qualitative effect data, comparative studies on resistance to various specific and nonspecific stresses are necessary.

Comparative lifespan studies: Lifespan has been studied on breeding males and females one generation removed from irradiated-line mice and on virgin females two and seven generations removed from irradiated-line mice. Comparative lifespan on control and irradiated-line mice from 5, 10, and 15 generations of X-irradiated males is illustrated in Figure 2. In an earlier report (SPALDING and STRANG 1962), lifespan in male and female breeders was significantly less in irradiated-line mice than in control-line mice. The sample size in this earlier study was small (25 mice of each sex per line), and all data were included. Figure 2 excludes all mice with a total lifespan of less than 180 days. The elimination of these data did not affect differences in any groups except F_6 females. In this case, where the sample size was small it did reduce the difference between control

FIGURE 2.—Comparative lifespan on control and irradiated-line male and female breeding mice from 5, 10, and 15 generations of X-irradiated males.

and irradiated-line female mice to insignificance. It can be seen from Figure 2 that survival time in F_6 irradiated-line male mice was significantly less than control-line mice with the same amount of inbreeding. However, survival time in F_{11} irradiated-line male mice was somewhat greater (not a significant amount) than control-line mice with the same inbreeding. The only other difference was in survival time in F_{16} irradiated-subline mice, which was less than both control-line mice with the same inbreeding and irradiated-line mice with the same inbreeding but with five additional generations of X-irradiated sires.

Lifespan for virgin females of control-line mice, mice two generations removed from ten generations of X-irradiated males, and offspring from F_1 crossbreedings between the two lines is illustrated in Figure 3. This same figure also shows comparative lifespan of F_{17} virgin females from the control line, seven generations removed from ten generations of X-irradiated sires and two generations removed from 15 generations of X-irradiated sires. There were significant differences among the five groups of F_{12} mice (Figure 3), but these differences did not indicate a decrement in lifespan of irradiated-line mice. For example, F_{12} females from control-line sib-matings had survival times not significantly different from F_{12} females from irradiated-line sib-matings. Mice from nonsib-mated controls did not survive as long as offspring from crosses between the control and irradiated lines, and this difference was significantly less than offspring of control-line

FIGURE 3.—Comparative lifespan on F_{12} virgin females of control-line, mice, mice two generations removed from ten generations of X-irradiated males, and offspring from F_1 cross breedings between the two lines. Comparative lifespan of F_{17} virgin females from control line, seven generations removed from ten generations of X-irradiated sires.

females mated to irradiated-line males. The lifespan picture becomes even less clear when comparisons are made on F_{17} mice. This generation showed all three groups to be different from each other, with control-line mice having a shorter lifespan than either the grandoffspring from 15 generations of X-ray exposure or the mice seven generations removed from ten generations of X-irradiated males.

Lifespan studies in this program, in general, have been unable to show a decrement which can be attributed to X irradiation of up to 15 generations of male ancestry. Although RUSSELL (1957) first indicated that lifespan in offspring of neutron-irradiated sires might be reduced, no subsequent studies have been able to confirm his suspicions and, in fact, a more recent study (SPALDING 1964) showed no decrement in lifespan from offspring or grandoffspring from sires exposed to graded doses of neutrons or gamma rays.

Radioresistance studies: Earlier in this program it was noted that grand-offspring from the irradiated line were less resistant to continuous low intensity Co^{60} gamma-ray exposure (4r/hr) than were control-line mice (SPALDING and STRANG 1961). It was also noted that this difference in resistance did not prevail with acute X-ray exposures (SPALDING *et al.*, 1963b). However, the inherent difference in resistance to continuous low intensity gamma rays has persisted through the last 25 generations. Radioresistance in F_{27} mice was tested and is shown in Figure 4. All mice tested were virgin females 6 weeks $\pm$ 3 days of age and, if from the X-irradiated line, were two generations removed from X-irradiated sires. Offspring from sib and nonsib-matings in the irradiated line (P_2 , Figure 4) had the same degree of resistance, and their mean survival time (MST) was 715 hours. Control-line offspring from sibs and nonsibs (P_1) were also equal in resistance with an MST of 906 hours. The F_1 cross between P_1 and P_2 had an

FIGURE 4.—Radioresistance of control lines, X-irradiated lines, and test crosses after 25 generations of X irradiation to the sire.

MST value of 834 hours when the males were from the control line (F_1^1) and 846 hours when the females were from the control line (F_1^2). Backcrosses between F_1^1 and P_1 and P_2 (Figure 4) took resistance positions somewhat intermediate to their parents. It has not been possible to establish the inheritance pattern of this radioresistance difference between the two lines, and neither can the number of loci involved be established. Existing data do establish the facts that the two lines involved do have a real difference in radioresistance, that this difference is a genetic one, and that some additive genetic system is at least partially involved. When this characteristic difference between the two lines was first observed, it was attributed to a radiation-induced decrement in irradiated-line mice. There is some question now as to whether or not chance genetic segregation in the F_1 generation (Figure 1) may be responsible for this difference. Further studies are now in progress which may clarify this question.

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SUMMARY

Male mice have been exposed to 200 rads of X rays (acute whole-body exposures) for 30 consecutive generations. Comparative studies on productivity of mice from 5, 10, 15, and 20 generations of X-irradiated males have shown a consistent decrement in many litter and breeding characteristics but have also shown that irradiated-line mice produce more litters and have a longer fertile life. Life-span studies of offspring and grandoffspring from 5 through 15 generations of X-irradiated sires have shown no reduction in survival which can be attributed to irradiated ancestry. A radioresistance decrement in irradiated-line mice after 10 generations of X-irradiated sires has persisted through the 25th generation, and the genetic nature of this decrement is still in question.

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