Longevity and Mortality Distributions of Mice With and Without X-Ray Exposure to 45 Generations of Male Progenitors[1] (36066)

J. F. SPALDING AND MARY R. BROOKS

Biomedical Research Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544

An extensive multigeneration radiation genetics program was initiated over a decade ago to investigate the possible genetic impact on future generations from whole-body exposure to large doses of ionizing radiation. The results of life-span studies (from this program) on animals from the 45th and final generation to be observed are presented.

Experimental Methods. The mice used in this investigation were from four populations. The four lines from which the populations were developed originated from a single pair of strain RFM mice. Two basic lines of mice were propagated from two sibling pairs of littermates taken from the original RFM parent pair and expanded to 75 sib-pairs/line. Thus, 75 sublines were established in each of the two basic lines and were maintained with one sib-pair from each subline. When a subline was lost from death of a parent, sterility, cannibalism, *etc.*, the subline was replaced with a sib-pair taken at random from another subline within the basic line. Less than 1 subline/basic line was lost per generation.

In one line, all male mice in each of 45 successive generations were exposed to 200 rads of x-irradiation (about 5000 times their natural background dose) to the whole body a few days after weaning (26 ± 2 days of age) and were then sibling-mated (offspring were derived mostly but not exclusively from irradiated spermatogonia). The X-ray exposure factors were 250 kV peak, 30 mA, Thoraeus II filter, 2.6-mm copper HVL, 60-cm target-to-specimen distance, 45–50 rads/min dose rate, and 200 rads total midbody air dose. The second line was a nonirradiated control and, except for not being exposed to X-irradiation, was propagated in the same manner.

Fortieth generation mice in both lines were serotyped by the hemagglutination method of Gorer and Mikulska (1). The serotype test showed that homozygous and heterozygous H-2^k and H-2^f genotypes were segregating in both of the basic lines but with control sublines being predominantly H-2^k and irradiated sublines predominantly H-2^f. Control- and irradiated-line mice homozygous for H-2^k or H-2^f were selected and bred to propagate sublines H-2^f and H-2^k within each of the two basic lines. Relatively few of the 75 sib-pairs in each of the two basic lines were homozygous for either H-2^f or H-2^k and, although all homozygous sib-pairs were used, some selective elimination may have occurred with genetic characteristics which may have been peculiar to H-2 heterozygotes.

At the 45th generation, four lines of mice, X-irradiated H-2^f and H-2^k and control H-2^f and H-2^k, were under investigation. Mice used in the present study were obtained from these four populations. Control-line mice were from the 47th nonirradiated generation. One hundred and forty H-2^f genotype, C (H-2^f), and 82 H-2^k genotype, C (H-2^k), mice were used. Experimental-line mice were from nonirradiated 46th generation progenitors that were, in turn, the progeny of 45 generations of X-irradiated males. Thus, the experimental line mice were grandprogeny of 45 generations of X-irradiated males. One hundred and twelve irradiated-line mice of the H-2^f genotype, I (H-2^f), and 117 irradiated-line H-2^k genotype, I (H-2^k), mice were used. Except for X-irradiation of the progenitors of irradiated-line mice, all groups had received identical treatment.

[1] Work performed under the auspices of the U.S. Atomic Energy Commission.

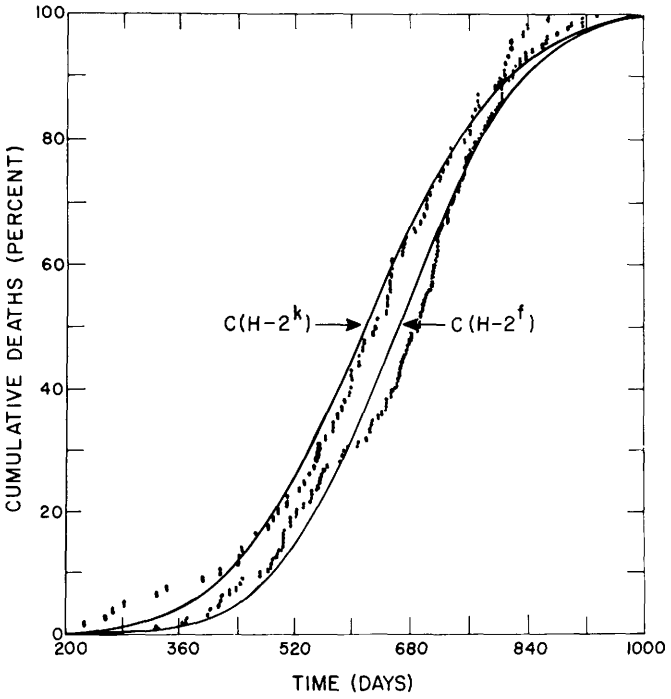

FIG. 1. Cumulative deaths (%) plotted against life-span (days) for two H-2 genotypes (H-2^k and H-2^f) in control-line mice. The normal distribution curve was obtained by a nonlinear least-squares fit.

Animals in this investigation were virgin females selected at weaning exclusively for life-span observations. They were housed two to six per cage on wood shavings in stainless steel 5 × 8 × 12-in. box-type cages and were provided fresh bedding and water weekly and fed Rockland-Teklad rodent food *ad libitum*. The mice were observed daily for proper animal husbandry, and deaths were recorded for mortality distribution and mean life-span analysis.

Results and Discussion. Mortality distributions and corresponding normal distribution curves for the two genotypes in the control line are plotted in Fig. 1, and those for the two genotypes within the irradiated line are plotted in Fig. 2. These indicate the underlying normality of the data. Mortality distributions within each line (Figs. 1 and 2) do not indicate abnormal death patterns but, rather, suggest that life-span differences between the two H-2 genotypes are due to a general shift in death distributions.

The mean life-spans of the four populations are shown in Table I. There is no indication that the variances within the four groups differ significantly. A two-way analysis of variance of the mortality data (with unequal subclass numbers) is presented in Table II. Interaction between genotypes and treatments is negligible. The H-2 genotypes differ significantly, with the C (H-2^f) genotype being longer-lived than genotype C (H-2^k).

TABLE I. Mean Survival Times of F_{47} Mice of Two Genotypes from 46 Generations of X-Irradiated or Nonirradiated Progenitors.

Group and H-2 genotype	No. of animals	Mean survival time ± SE[a] (days)
Nonirradiated line		
C (H-2^f)	140	662 ± 11.5
C (H-2^k)	82	621 ± 16.5
Irradiated line		
I (H-2^f)	112	647 ± 11.7
I (H-2^k)	117	592 ± 12.5

[a] Standard error = square root of (variance divided by the number of animals per group).

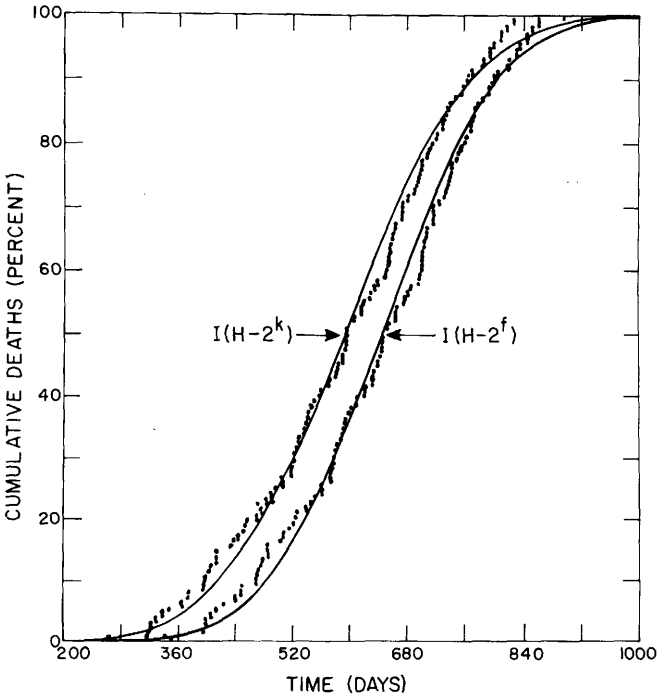

FIG. 2. Cumulative deaths (%) plotted against life-span (days) for two H-2 genotypes ($H\text{-}2^k$ and $H\text{-}2^f$) in irradiated-line mice with 45 generations of X-irradiated male progenitors. The normal distribution curve was obtained by a nonlinear least-squares fit.

Note that, although the different genotypes within the control and irradiated lines differ significantly from each other, control- and irradiated-line populations do not have significantly different life spans. The absence of interaction indicates that these results hold within each genotype. Furthermore, significant differences in genotype appear within both the control and irradiated groups.

The results of this study have shown that radiation-induced genetic injury does not accumulate and shorten the lives of progeny of inbred irradiated progenitors. The data also suggest that a single gene locus may play a significant role in determining species longevity—an observation that, if proven to be true, would cast considerable doubt on the usefulness of life shortening as a measure of possible genetic decrement from radiation-induced multiple minor mutations. This observation finalizes life-span studies in a genetics program in which 45 successive generations of mice have been exposed to approximately one-third of a lethal dose of X-

TABLE II. Analysis of Variance of Survival Time Data of Four Groups of F_{47} Mice Shown in Table I.

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F* ratio
Genotypes	1	248,388	248,388	13.56[a]
Treatment (irradiated vs control)	1	51,403	51,403	2.81
Genotype–treatment interaction	1	4889	4889	0.27
Error	447	8,188,101	18,318	
Total	450			

[a] Significant at the 0.01 level.

irradiation. Life-span has been investigated at five-generation intervals; therefore, nine life-span replications have been completed and most documented (2–6).

The negative nature of this long-term study and other reports on the genetic effects of electromagnetic (7–11) and particulate (8) ionizing radiations provides strong evidence that cumulative radiation-induced genetic injury affecting life-span is insignificant. If life-span shortening from genetic injury cannot be detected in mice after 45 generations, with each generation exposed to over 4500 times the natural background radiation dose, it seems extremely unlikely that exposure of man to twice his natural background radiation level, conforming to recommended exposure guidelines set by the various national and international committees, would have a detectable life-shortening impact on future generations. A mortality distribution study of children of irradiated parents in Japan by Kato *et al.* (12) agrees with findings on experimental mammals.

Summary. Longevity and mortality distributions were observed on two H-2 genotype substrains of mice within each of two lines. Two substrains ($H\text{-}2^f$ and $H\text{-}2^k$) were from 45 generations of X-irradiated male progenitors, and two were from nonirradiated progenitors with the same degree of inbreeding. Lifespan differed significantly for H-2 genotypes within the two lines but not for the same genotypes between lines. It was concluded that life shortening was not a genetic consequence of 45 generations of exposure to over 4500 times the background radiation level. The data also suggest a single-gene effect on longevity.

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