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Lifespan and autopsy findings in the first-generation offspring of X-irradiated male mice

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Summary

Male mice of the C3Hf strain were exposed to 600 R of acute X-rays and, along with unirradiated control males, mated with 101-strain females. The offspring of the treated males were all conceived more than 7 weeks after irradiation, thereby ensuring that they were derived from germ cells exposed as stem-cell spermatogonia. After weaning, the offspring were caged individually and allowed to live their normal lifespan. Tumors and other major pathological disorders were recorded at a careful post-mortem examination. The lesions encountered were typical of those characteristically seen in aging $(101 \times C3Hf)F_1$ mice. The results showed no significant differences in lifespan between experimentals and controls. This held true when allowance was made for littermate correlations and for other factors that might contribute to differences among litters. Likewise, there were no significant differences between experimentals and controls in the frequency, severity, or age distribution of neoplasms and other diseases.

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The results of this experiment, which was started in 1956 and completed in 1959, have not been published before. They showed no clear-cut effect on either the lifespan or the autopsy findings in the offspring of X-irradiated male mice. Since induction of specific-locus gene mutations by X-rays had by that time been demonstrated in mice, and at rates considerably higher than in Drosophila (Russell, 1951), we had speculated that it might not be difficult to demonstrate life-shortening and other deleterious effects in the offspring of irradiated mice. Furthermore, evidence of F_1 lifespan reduction had already been obtained following exposure of a relatively limited number of male mice to neutron irradiation

from an above-ground atomic bomb explosion (Russell, 1957). It was, therefore, something of a surprise not to obtain a positive result in the experiment described here, the first exploratory investigation of this kind with X-rays. We hoped that a larger and, if possible, more definitive experiment could be conducted to determine whether a significant positive effect could be obtained on which to base a quantitative estimate of genetic risk in humans.

We learned that investigations of this type were being made by other laboratories that were looking for deleterious effects in the first-generation offspring of irradiated animals, and even in descendants of multi-generation exposures. However, the results again turned out to be negative (see review by Green, 1968). With this much confirmation of the findings of our experiment, and with the demonstrated difficulty of detecting genetic damage with the end-points chosen, we decided to concentrate on disorders induced in one of the major body systems, namely the skeleton, as a measure of genetic damage in the first-generation descendants of irradiated mice.

What makes the publication of the early research cogent now is that, in experimental work, new controversy has arisen over the degree of risk, particularly from cancer, to the offspring of irradiated males (see reviews by Selby, 1990; UNSCEAR, 1986; BEIR V, 1990). Furthermore, a recent epidemiological study has suggested a possible deleterious effect in the children of men exposed to what appear to have been very low doses of radiation (Gardner et al., 1990). Therefore, it is clearly desirable to make available the early data that fail to show a statistically significant effect of high-dose X-irradiation of male mice on the lifespan or causes of death of their offspring.

Materials and methods

Young adult C3Hf strain male mice were exposed to 600 R of X-radiation (250 kvp; 15 mA; inherent filtration 3 mm Al; H.V.L. 0.4 mm Cu; dose rate ca. 85 R/min). These and unexposed male controls were mated to 101-strain females, thus producing, from the inbred parents, F_1-hybrid offspring that were vigorous and genetically highly uniform with the exception of any new mutations. The offspring were conceived from matings made more than 7 weeks after treatment, thereby ensuring that they were derived from germ cells irradiated as stem-cell spermatogonia. The sensitivity of this cell stage is of major importance in the estimation of genetic risk to the offspring of human males, because these cells can accumulate induced mutations over the entire reproductive lifespan of the exposed individual. At weaning age, all offspring were caged individually, fed pelleted food and water ad libitum, and

TABLE 1

EXPERIMENTAL DETAILS

Detail listed	Irradiated group	Control group
Number of fathers	12	7
Number of mothers (all unirradiated)	25	13
Maximum mothers per father (minimum = 1 for each group)	4	2
Total offspring recorded [a]	377	173
Total offspring that died before weaning	26	11
Percent of offspring dying before weaning	6.9% [b]	6.4%
Number of animals weaned and used in study	351	162 [c]

[a] Excludes all litters (two in experimental group, containing 8 and 9 offspring) in which all offspring died before weaning — this was assumed to be a "poor mother" effect.

[b] Chi-square for comparison of early death rates = 0.055, degrees of freedom = 1, *P*-value (1-tailed) = 0.48.

[c] Two mice (both in control) that drowned, owing to a broken water bottle, were excluded from the mortality and autopsy studies because they did not die a natural death.

allowed to live their natural lifespan. Experimental details are shown in Table 1. After dying, each animal was subjected to a complete autopsy, which included gross examination of all organs, including the cranial contents. Any tissue showing evidence of neoplasm or other abnormality was sec-

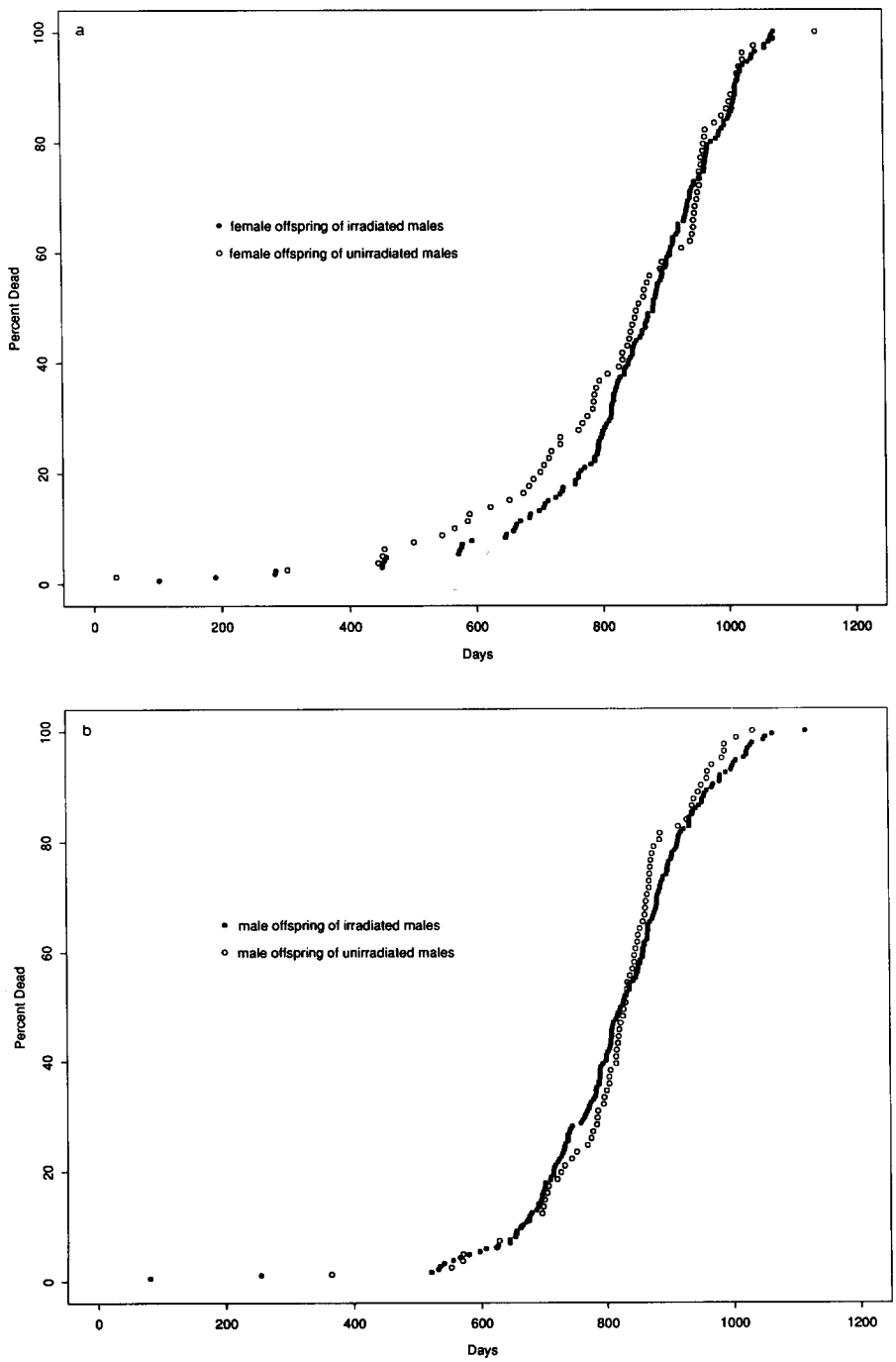

Fig. 1. Cumulative distribution of lifespan for (a) female offspring and (b) male offspring of irradiated and unirradiated fathers.

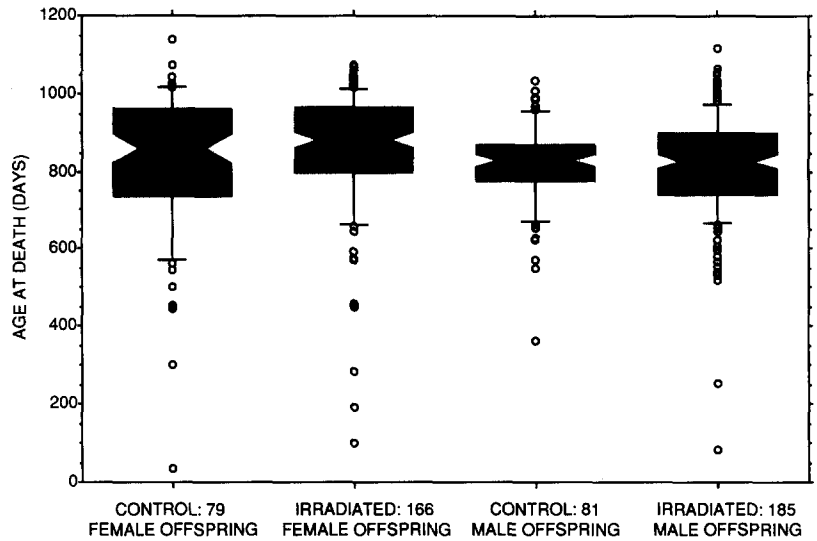

Fig. 2. Box plots, produced using StatView™ II by Abacus Concepts, show the distribution of the ages at death (days) for each of the 4 groups of offspring studied. The 10th, 25th, 50th, 75th and 90th percentiles are shown by the lower bar, bottom of box, bar in box, top of box and upper bar, respectively. Circles represent actual data points for the extreme 20% of the observed values. There appears to be little difference among the groups; note, for example, the 95% confidence intervals for the median lifespans, which are shown by the notches in the boxes.

tioned for histological examination, and the findings were carefully recorded. Details of the methods used have been described elsewhere (Cosgrove et al., 1964). Deaths before weaning age are not dealt with in any detail in this paper because they are so much more likely to be due

TABLE 2

OBSERVED MEAN AGE AT DEATH (IN DAYS) FOR THE 4 GROUPS OF OFFSPRING, TOGETHER WITH STANDARD ERRORS OF THE OBSERVED MEANS AND 95% CONFIDENCE INTERVALS FOR THE TRUE MEANS

Also given are the observed differences between the mean age at death for the offspring of control fathers and the mean age at death for offspring of irradiated fathers, separately for each sex, with standard errors and 95% confidence intervals.

Offspring	Father	*N*	Mean	Standard error	95% confidence limits
Part A (in which each mouse is treated as an experimental unit)					
Female	Control	79	826.3	21.4	784.3, 868.2
Female	Irradiated	166	849.6	12.8	824.5, 874.7
Difference			−23.3	24.9	−72.1, 25.5
Male	Control	81	815.6	12.6	790.9, 840.3
Male	Irradiated	185	815.2	9.9	795.8, 834.6
Difference			0.4	16.1	−31.2, 32.0
Part B (in which the mean for mice of like sex in a litter is treated as an experimental unit)					
Female	Control	29	805.9	35.0	737.2, 874.6
Female	Irradiated	66	841.6	15.9	810.4, 872.8
Difference			−35.7	38.5	−111.2, 39.8
Male	Control	33	809.1	9.2	791.2, 827.1
Male	Irradiated	64	822.9	10.6	802.2, 843.6
Difference			−13.8	14.0	−41.2, 13.6

to the quality of maternal care than to new mutations. The loss of a whole litter before weaning is a clear example of this. Any meaningful study of death prior to weaning would require much larger sample sizes; see Selby and Russell (1985) for another publication that deals specifically with this.

The research was carried out in the Biology Division of Oak Ridge National Laboratory; the strains of mice, the irradiations and the life-time care of the F_1 offspring were provided by the Mammalian Genetics Section under the direction of W.L.R.; the autopsies were performed by G.E.C. in the Pathology and Physiology Section directed by A.C.U. Before his death, G.E.C. provided a table on autopsy findings as well as detailed printouts, displayed in several different formats, of his records, which had been computerized years ago. His data were reentered into a computer to facilitate their analysis and to check the accuracy of his table. The few slight errors found were corrected.

95% confidence limits of the mean age at death were calculated as $\pm$ (1.96) (standard error of the mean).

Results

Lifespan

The distribution of the ages at death in each of the four groups is shown by means of mortality curves in Fig. 1 and by boxplots in Fig. 2. There appears to be little difference among the groups; note, for example, the confidence intervals for the median lifespans shown by notches in Fig. 2.

The mean age at death and its standard error is given in Part A of Table 2 for each group, together with a 95% confidence interval for the true mean age at death. Part A of Table 2 also gives the difference between the mean age at death for offspring of control males and the mean age at death for offspring of irradiated males, within each sex, together with the standard error of that difference and a 95% confidence interval for the true mean. In each case, any hypothesized value of mean life shortening that falls within the confidence interval is not refuted by these data. In particular, since 0 is within both intervals, one cannot reject the hypothesis that there is no mean life shortening either for males or for females. The appearance of more early deaths for the offspring of irradiated males in Figs. 1 and 2 is due to the greater number of offspring in these two groups.

It must be recognized that the standard errors and confidence limits in Part A of Table 2 are based on the assumption that each entry of an age of death represents an independent event. However, one might expect to see positive littermate correlations, since littermates are born of the same pair of parents and are raised together. *F*-tests of the hypothesis of equality of mean lifespans across litters within each group provide somewhat inconsistent evidence of positive littermate correlations. The *P*-values for this test are 0.008 for female controls, 0.29 for female experimentals, 0.9995 for male controls, and 0.28 for male experimentals.

If, however, positive littermate correlations are assumed, it seems reasonable to conclude that the difference between experimentals and controls (which did not approach significance under the hypothesis of independent events) would not be raised to a significant level. (The assumption of positive correlations among animals in a group increases the estimated variance of the mean of that group.) To support this conclusion, an analysis was conducted, separately for male and female offspring, in which each litter was treated as an experimental unit and the mean lifespan for the animals (of the appropriate sex) in that litter was the response. Calculation of the difference between experimentals and controls for these analyses gave the results shown in Part B of Table 2. Again there is no significant difference, and it happens that the point estimates of the lifespans for both sexes of the offspring of irradiated fathers are greater than for their counterpart controls.

Factors that might contribute to differences among litters, e.g., size of litter, litter order, pedigree subline, and parent identity, were also analyzed in varying degrees of detail. The basic conclusion of no significant difference between controls and experimentals was not affected.

Autopsy findings

The post-mortem changes exhibited by the offspring of irradiated mice did not differ signifi-

cantly in frequency, severity, or age distribution from those seen in concurrent or historical controls (Table 3). Lesions of various types were encountered, such as are typical for aging (101 × C3Hf)F_1 mice, each of which has been described in detail elsewhere (Cosgrove et al., 1964). The commonest of the lesions was pyelonephritis, which manifested itself as a focal, chronic inflammatory process in more than two-thirds of the animals (Table 3).

Of the different types of neoplasms that were encountered in both sexes, pulmonary adenomas were the most prevalent, occurring in 10–20% of all animals (Table 3). Other commonly observed

TABLE 3

LONGEVITY AND MAJOR DISEASES IN (101 × C3Hf)F_1 OFFSPRING OF IRRADIATED AND NONIRRADIATED MALE MICE [a]

Category	Male offspring		Female offspring		Historical female controls [b]
	Irradiated parent	Control parent	Irradiated parent	Control parent	
Number of offspring	185	81	166	79	–
Mean age at death (days)	815.2	815.6	849.6	826.3	866
Number autopsied [c]	180	80	159	74	192
Mean age at death (days) of those autopsied	825	821	873	859	–
% of mice with neoplastic disease (Some had more than one)	30.0	28.8	45.9	62.2	66
Mean age at death (days) of mice with one or more neoplasm	821.6	814.0	879.5	861.4	871
Mice with specific neoplasms or other major diseases:					
Total leukemia or lymphoma (%)	4.4	0	7.5	16.2	9.5
thymic (%)	0.6	0	0	1.4	0.5
myeloid (%)	0	0	1.9	2.7	0
other (%)	3.9	0	5.7	12.2	9
Other tumors					
ovary (%)	–	–	20.1	20.3	30
breast (%)	0	1.2	6.3	6.8	4
uterus (%)	–	–	10.7	9.5	11
lung (%)	16.7	17.5	10.1	20.3	21
liver (%)	8.3	7.5	1.3	0	5
other (%)	5.0 [d]	5.0 [e]	4.4 [f]	6.8 [g]	–
Ovarian cyst (%)	–	–	5.0	6.8	1
Uterine hypertrophy (%)	–	–	54.1	45.9	47
Pyelonephritis (%)	73.3	83.8	80.5	68.9	66
Volvulus or intussusception (%)	2.8	6.2	6.3	4.1	1
Pneumonia (%)	0	1.2	0.6	1.4	2

[a] Percentages are presented to one place beyond the decimal point. Often this suggests more precision than the data permit, but by presenting the numbers in this way, it becomes possible for the reader to calculate the actual numbers of mice found with each disease.

[b] Data from Cosgrove et al. (1964).

[c] A few animals were not autopsied for the following reasons: (a) the decision to include the autopsy part of the experiment was made after the longevity study had begun, by which time a few animals had already died and been discarded, or (b) precluded by autolysis of tissue.

[d] Tumors of following types: clavicle (1), kidney (1), occiput (2), pelvic (1), peritoneum (1), seminal vesicle (1), testis (1), head carcinoma (1).

[e] Tumors of following types: adrenal (1), kidney (1), mesenteric (1), pleura (1).

[f] Tumors of following types: bone sarcoma (1), carcinoma peritoneum (1), pituitary (2), sarcoma lumbar vertebrae (1), squamous cell carcinoma ear (1), squamous cell carcinoma stomach (1).

[g] Tumors of following types: peritoneum (1), sacrumosteosarcoma (1), shoulder (2), thigh (1).

growths included tumors of the ovary, uterus, and mammary gland, which occurred in 20%, 9–11%, and 6% of females, respectively; hepatocellular tumors of the liver, which occurred in 8% of males; and lymphomas/leukemias of various types, which occurred in 0–4% of males and in 8–16% of females. In no case did the frequency of neoplasms in offspring of irradiated mice significantly exceed that in controls. In the case of lymphomas/leukemias in males, the neoplasms were observed only in the experimental group, but the difference is of doubtful statistical significance and in the opposite direction from that observed in females, where the frequency of lymphomas/leukemias in control offspring (16.2%) greatly exceeded that in female offspring of irradiated males (7.5%). Averaged over both sexes combined, the point estimate of the frequency of lymphomas/leukemias in controls (7.8%) exceeded that in the offspring of irradiated mice (5.9%).

None of the various neoplasms that were observed occurred in mice younger than 500 days of age, and the mean age at death with each type of neoplasm tended to be slightly later than the mean age at death from all causes, as reflected in the mean ages at time of death shown in Table 3. This later death in mice with tumors is characteristic in mice of the $(101 \times C3Hf)F_1$ strain (Cosgrove et al., 1964). It is noteworthy in this connection that the few animals not autopsied, which died in the initial phase of the experiment, died predominantly before 500 days of age, which was the youngest age at which neoplasms were observed in any of the animals.

Discussion

Lifespan

The introduction to this paper mentions that, at the time when the results of this experiment were obtained, the finding of no significant effect of a large dose of X-irradiation of male mice on the lifespan of their progeny was a surprise. However, subsequent X-ray studies by other investigators, reviewed by Green (1968), all support the finding reported here, in showing no significant difference in length of life between offspring of control and irradiated fathers. A neutron experiment by Spalding (1964) also detected no effect on lifespan in the offspring of irradiated male mice.

With several investigations unable to demonstrate a significant effect of high doses of paternal irradiation on the lifespan of the offspring, the question remains of why this is so. A dose of 600 R of acute X-rays delivered to stem-cell spermatogonia in male mice induces a total mutation rate at 7 specific loci of approximately one mutation in a thousand offspring (Russell, 1963), or a mean mutation rate per locus of one mutation per 7000 offspring. Assuming a total number of loci per mouse genome of between 10 000 and 100 000, one would expect, in the experiment described here, that the average offspring of the irradiated males would carry, in round numbers, between one and ten new mutations. A significant proportion of the mutations induced at the 7 loci (taken as a group) are recessive lethals, and many of these have some dominant deleterious effects, the most easily noticeable being reduced body size. These calculations provided some basis for expecting a detectable effect of paternal irradiation on the lifespan of the offspring in the kind of experiment reported here. Why was none found?

One possibility is that the per-locus mutation rate averaged for the entire genome is lower than that for the 7 loci used in the standard specific-locus test. Another is that most mutations, including recessive lethals that have dominant effects, do not cause damages that affect post-weaning survival. It is also conceivable that the structural spectrum of mutations induced by radiation — which includes a significant proportion of small deletions and other rearrangements — is not optimum for producing the phenotypic effects that are associated with reduced lifespan of weaned mice.

Autopsy findings

The results of this investigation, which disclosed no significant differences in disease frequencies between the offspring of irradiated mice and those of nonirradiated controls, are consistent with the findings of Kohn et al. (1965). In this respect they differ from results published by Nomura (1978, 1982, 1986, 1988) and Vorobtsova

and Kitaev (1988), who have reported increased susceptibility to lung tumors, lymphoid leukemias, or both in offspring conceived by male or female mice exposed previously to 36–504 cGy acute or fractionated whole-body X-radiation. The results of this study are also at variance with the findings of Takahashi et al. (1992), who reported the incidence of liver tumors to be greatly increased in male, but not at all in female, (C3H × C57BL)F_1 mice sired by males mated 2 weeks after 50 cGy whole-body ^{252}Cf neutron and γ-ray irradiation.

The basis for the discrepancies between studies remains to be determined. In view of the relatively high background incidence of lung tumors, liver tumors, and lymphomas/leukemias in control (101 × C3Hf)F_1 mice, however, it is clear that animals of this strain are not inherently resistant to these diseases. Nomura (1978, 1988) and Vorobtsova and Kitaev (1988) have interpreted their findings to mean that postnatal exposure of offspring of irradiated males to the promoting agent urethane considerably increases the expressivity of induced tumor mutations. Although that phenomenon is not well established (Selby, 1990), it is important to note that the present study has no bearing on that particular argument since it involved no additional exposure to a promoting agent such as urethane.

The inter-group differences in lymphoma/leukemia incidence observed in the present study, although possibly suggestive of a radiation effect in males, are within the range of control variation and cannot be interpreted as treatment-induced. A concern when comparing our results with those of Nomura on leukemia is whether some of the 18 non-autopsied animals, most of which died earlier than most of the mice autopsied, may have had leukemia that thus went undetected. The ages at death (in days) of the non-autopsied mice in the 4 groups were as follows: female control (34, 302, 444, 451 and 454), female experimental (101, 190, 283, 284, 450, 451, 457), male control (364), and male irradiated (81, 254, 532, 541, 858). Nomura (1986) noted that "most leukemia bearing offspring died within 3 month after birth" in his studies. Since only one experimental animal died between the time of weaning and three months of age, it seems unlikely that our experiment would have seriously underestimated the number of leukemias of the type detected by Nomura. Leukemias and lymphomas diagnosed in our study were found in mice that died much later than three months of age. The mean was 838 days, with a range from 589 to 1012 days.

While tumor development has been reported to be enhanced in the offspring of certain other strains of mice by treatment with chemical carcinogens before conception, the nature and reproducibility of the results, the degree to which they are generalizable to animals of other species and strains, and their potential significance for human health remain to be determined (Selby, 1990; Turusov et al., 1992) Not only have the studies of Nomura, Vorobtsova and Kitaev, and Takahashi et al., noted above, been interpreted by their authors to indicate that dominant tumor mutations are induced, but the results of these studies suggest that offspring of heavily irradiated males should have a high frequency of induced tumors, especially if one conducts detailed autopsies looking for all major types of disease. The present results lend no support to this notion for neoplastic diseases, and indeed they provide no evidence for induction of any detectable effects on disease incidences or length of life.

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