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The Influence of Sex on Life Shortening and Tumor Induction in CBA/Cne Mice Exposed to X Rays or Fission Neutrons

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An experimental study of male and female CBA/Cne mice was set up at Casaccia primarily to investigate the influence of sex on long-term survival and tumor induction after exposure to high- and low-LET radiation. Mice were whole-body-irradiated at 3 months of age with fission-neutron doses of 0.1, 0.2, 0.4, 0.8, 1.2 and 1.8 Gy at the RSV-TAPIRO reactor (mean neutron energy 0.4 MeV, in terms of kerma, $\bar{y}_D$ = 51.5 keV/μm), or with 250 kVp X-ray doses of 1, 3, 5 and 7 Gy. Control and irradiated animals were then followed for their entire life span. As a general finding, male CBA/Cne mice appear more susceptible to tumorigenesis than females. In particular, the incidences of induced acute myeloid leukemia and malignant lymphomas are significant only in male mice. Benign and malignant solid tumors of many types are observed in mice of both sexes, the most frequent being in the lung, liver and ovary. However, evidence for a radiation response is limited to the case of Harderian gland neoplasms. In addition, a comparison of the observed frequency of all irradiated compared to unirradiated animals bearing solid tumors shows that the total tumor occurrence is not altered markedly by radiation exposure. A decrease in survival time is observed for both sexes and radiation types and correlates well with increasing dose. Moreover, both sex and radiation quality appear to influence the life shortening. A similar dose dependence of survival time is found when tumor-free animals alone are considered, suggesting a non-specific component of life-shortening. © 1996 by Radiation Research Society

INTRODUCTION

Sex is one of the biological variables that can influence the susceptibility to tumor induction. A clear example of sex-related difference in the response of some tissues to radiation is provided by the results of an experiment by Ullrich and Storer on RFM/Un mice showing that the rates of tumor induction after irradiation with ^{137}Cs γ rays as well as the natural incidence of thymic lymphoma and myeloid leukemia are quite distinct between males and females (*1*, *2*). Some years ago, we compared the effectiveness of fission neutrons in the induction of solid tumors, excluding ovarian tumors, in male and female (C57BL/Cne × C3H/HeCne)F_1 ($BC3F_1$) mice. We found a sex-dependent difference in radiosensitivity, females being two to three times more susceptible than males in the range of doses where tumor induction was significant (*3*). This observation, coupled with the scarcity of specifically designed studies, prompted us to carry out an *ad hoc* experiment using the more susceptible inbred CBA/Cne mouse strain (*4*), with the hope of getting larger differences between the two sexes.

On the other hand, the determination of RBE has often led to considerable discrepancies, as radiation quality influences both the biological response to the radiation as well as the shape of the dose–response curves.

We were therefore interested in studying the interplay of a biological variable, the sex of animals, with a physical one, radiation quality, in a single experiment. Large dose ranges of two radiation beams (1–7 Gy of 250 kVp X rays, 0.1–1.8 Gy of fission neutrons), and observations over the complete life span and examination of the pathology in relevant mouse populations were considered necessary.

MATERIALS AND METHODS

Mice

The animals were young adult CBA/Cne mice bred and kept in our animal house. This subline was derived from CBA/H mice given to the Casaccia laboratories by M. F. Lyon (Medical Research Council, Radiobiology Unit, Harwell, UK) in 1968. They were whole-body-irradiated at 3 months of age. Animal husbandry and care complied with Italian law, and every effort was made to reduce animal stress and discomfort to a minimum.

Irradiation Conditions

Fission-neutron irradiation was carried out at the experimental fast nuclear reactor RSV-TAPIRO of CR-Casaccia (Rome). The facility as well as the irradiation procedures have been described elsewhere (*5*). The calculated average energy of the neutron spectrum at the site of irradiation was about 0.4 MeV, in terms of kerma. The dose-averaged lineal energy measured at d = 2 μm was $\bar{y}_D$ = 51.5 keV/μm for the total radiation field (*6*). The γ-ray contamination of the field was estimated at about 12% of the total dose. Biological effects due to the γ-ray component of the total dose were expected to add negligibly to those of neutrons. Therefore, no attempt was made to subtract the γ-ray contribution from

TABLE I
Survival and Pathology Data for Male and Female CBA/Cne Mice Whole-Body-Irradiated with Doses of 250 kVp X Rays or Fission Neutrons

Sex, radiation and dose (Gy)	Mean after survival ± SD (days)	Number of autopsied mice	Number of non-neoplastic lesions		Number of neoplastic lesions						
			Pneumonia	Nephrosclerosis	Acute myeloid leukemia	Malignant lymphoma	Harderian gland	Lung	Liver	Ovary	Others[a]
♂, X rays											
0.0	710 ± 194	60	13	4	—	—	10	7	40	na[b]	8
1.0	693 ± 118	60	16	—	6	2	17	13	42	na	3
3.0	600 ± 184	55	18	2	8	2	24	7	33	na	9
5.0	575 ± 180	58	21	5	2	8	16	3	35	na	9
7.0	569 ± 142	56	14	9	1	1	21	6	22	na	7
♂, Neutrons											
0.0	852 ± 156	50	29	3	—	2	1	3	28	na	2
0.1	676 ± 149	79	32	5	3	8	7	8	52	na	2
0.2	722 ± 173	71	16	1	6	4	21	6	40	na	4
0.4	662 ± 150	56	10	2	4	8	12	2	36	na	1
0.8	523 ± 152	57	7	—	13	12	13	2	24	na	2
1.2	564 ± 145	65	13	4	3	12	6	2	32	na	4
1.8	571 ± 129	63	17	4	1	8	8	4	25	na	6
♀, X rays											
0.0	896 ± 170	50	10	—	—	7	5	3	8	6	8
1.0	774 ± 201	50	5	1	—	15	7	—	11	11	4
3.0	705 ± 221	49	1	3	1	12	9	3	9	8	5
5.0	611 ± 188	61	5	11	—	11	18	5	23	4	7
7.0	530 ± 126	49	9	21	—	8	12	1	8	3	6
♀, Neutrons											
0.0	849 ± 176	58	21	1	—	6	7	4	14	12	2
0.1	804 ± 173	70	26	1	—	14	12	3	24	7	2
0.2	749 ± 130	68	18	—	—	6	20	3	20	17	—
0.4	658 ± 181	59	12	1	—	9	12	3	19	9	6
0.8	608 ± 133	57	14	1	—	10	19	5	17	5	4
1.2	572 ± 158	69	18	2	—	7	21	3	14	13	12
1.8	528 ± 166	70	13	—	—	12	19	3	11	11	9

[a]Adrenal gland, bone, brain, GI tract, lacrimal gland, mammary gland, prostate, salivary gland, skin, soft tissue, urinary bladder, uterus and vascular system.
[b]Not applicable.

the total effects, and the indicated neutron doses thus correspond to total doses. Mice were whole-body-irradiated with doses of 0.1, 0.2, 0.4, 0.8, 1.2 and 1.8 Gy. Doses of 0.1 and 0.2 Gy were given at a dose rate of 0.08 Gy/min, and larger doses at a dose rate of 0.18 Gy/min.

The X rays were produced by a deep-therapy unit operated at 250 kV peak and 15 mA (half-value layer = 1.5 mm Cu). Female mice were whole-body-irradiated with doses of 1, 3, 5 and 7 Gy at a dose rate of 1.26 Gy/min. In addition to the results of this new experiment, a different set of data from a previous experiment (*7*, *8*), in which male CBA/Cne mice were irradiated in a similar X-ray field, was also considered in the present paper for comparison.

Follow-up and Pathology

All mice were housed five or fewer to a cage and followed for their entire life span with daily inspection (six per week). Soon after spontaneous death, autopsies were performed on 1439 (90%) of the 1597 mice under observation which survived 30 days after treatment. The necropsy included external and internal gross examination. Tissue masses, as well as sections of the major organs, were taken and processed for histological analysis.

Data Analysis

The reduction of life expectancies in animal groups exposed to different doses was compared on the basis of the mean number of days of life lost with respect to the mean life span of the nonirradiated animals. The same analysis was performed on tumor-free mice to evaluate the possible existence of a life-shortening component not associated with the presence of neoplasm at death. In addition, to make the information from this study comparable to the effects in other strains and species (*9*), we have also calculated the percentage of life shortening relative to the mean life span of the nonirradiated group.

A complete observation of tumors occurring at death was carried out and the actual number of tumors observed is reported. However, tumor occurrence, hereafter referred to as incidence, was evaluated in terms of percentage of tumor-bearing animals. Raw observation results were corrected for reduction of life using a well-documented technique (*5*).

Our results were analyzed further by the statistical method described by Peto *et al.* (*10*) to establish the existence of a positive trend with dose. This method involves a basic comparison between the number of tumors observed at death in a particular treatment group and the number that would have been expected had the age-specific tumor-onset rates been similar in all groups after correction for differences in longevity.

RESULTS

Longevity

The decrease in mean survival time correlates reasonably well with the dose for both sexes and types of radiation used in the present study (Table I). To compare the effects of sex and radiation quality, we have calculated the mean number of days of life lost (LL) for each irradiated group with respect to the life span of the corresponding nonirradiated animals. The results for each group were then fitted to a simple linear dose response, i.e. $LL = aD$, limiting the fit

to 0.4 Gy in the case of neutron exposure. The results are reported in Fig. 1A. A similar analysis was performed for tumor-free mice to detect a radiation-induced life shortening not associated with the presence of neoplasms at death (Fig. 1B). The numerical values of the resulting best-fitting parameters are reported in the upper part of Table II. From the comparison of the linear coefficients of the fits values of RBE were determined and are also indicated.

In addition, the percentage life-span shortening (%LSS) relative to the life span of the unirradiated groups has been calculated. This was also repeated for the tumor-free mice. The different sets of survival data have been fitted empirically with the parametric expression

$$\%\mathrm{LSS}(D) = c(1 - e^{-\lambda D}),$$

which appears to yield a good description of the experimental points throughout the range of doses tested. The numerical values of the resulting best-fitting parameters are reported in the lower part of Table II. Using this expression, we have also derived the initial slopes at the dose $D = 0$, given numerically by $a \cdot b$, and from these we have calculated the maximum RBE values also shown in Table II. These proved to be equal to the values obtained by the simple linearity assumption at the lower doses, within the errors.

Pathology

Data for the pathology of the non-neoplastic and neoplastic lesions are reported in Table I. Pneumonia is a frequent disease affecting CBA/Cne mice and is distributed homogeneously throughout experiments and dose groups, without any trend with radiation type, dose or clustering in

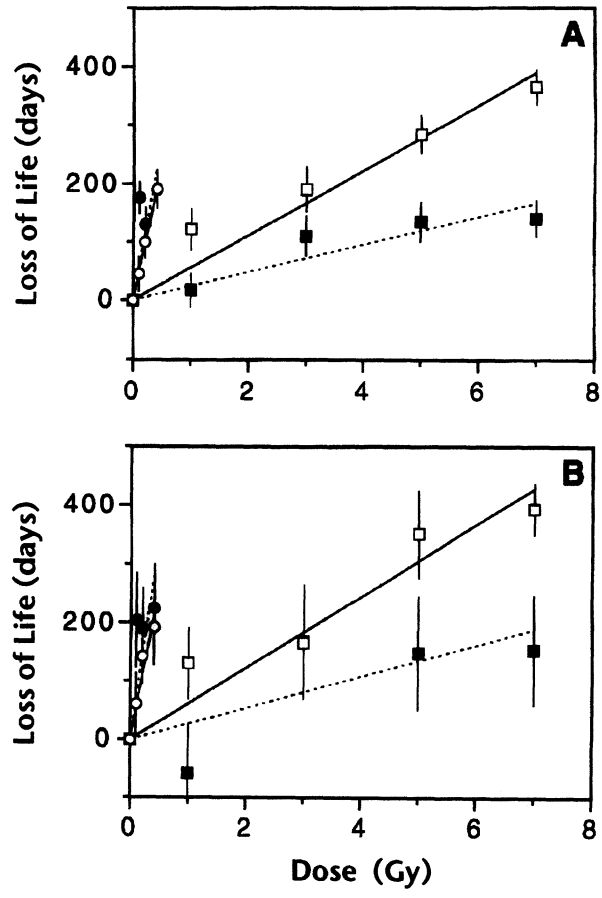

FIG. 1. Dose–effect relationships for loss of life in CBA/Cne mice irradiated with graded doses of 250 kVp X rays (■ males, □ females) or fission-spectrum neutrons (● males, ○ females) (panel A), and in tumor-free animals (panel B). Bars are SE. Fitted curves correspond to the equation $LL = aD$.

TABLE II
Numerical Values (±SE) of Best-Fitting Parameters for the Life-Span Shortening after Exposure to 250 kVp X Rays and Fission Neutrons in CBA/Cne Mice

End point	Radiation type	Sex	Assumed dose response	c	a (Gy^{-1})	λ (Gy^{-1})	R	RBE
Loss of life (days)	X rays	♂	aD		24 ± 3		0.93	
		♀			56 ± 4		0.96	
	Neutrons (0–0.4 Gy)	♂			570 ± 159		0.54	23.7 ± 7.3
		♀			480 ± 6		0.99	8.6 ± 0.6
Loss of life in tumor-free mice (days)	X rays	♂	aD		27 ± 7		0.79	
		♀			61 ± 5		0.95	
	Neutrons (0–0.4 Gy)	♂			707 ± 193		0.53	26.2 ± 9.9
		♀			530 ± 53		0.96	8.7 ± 1.1
Relative life shortening (%)	X rays	♂	$c(1 - e^{-\lambda D})$	25.1 ± 12.3		0.25 ± 0.25	0.97	
		♀		57.1 ± 18.6		0.17 ± 0.10	0.99	
	Neutrons	♂		34.2 ± 1.59		4.42 ± 0.81	0.91	23.9 ± 27.0
		♀		38.0 ± 3.27		1.85 ± 0.44	0.99	7.1 ± 4.9
Relative life-shortening in tumor-free mice (%)	X rays	♂		29.7 ± 38.1		0.25 ± 0.63	0.81	
		♀		59.0 ± 29.9		0.18 ± 0.17	0.99	
	Neutrons	♂		37.2 ± 3.15		5.34 ± 2.28	0.91	27.3 ± 79.0
		♀		37.5 ± 3.79		2.61 ± 0.95	0.99	9.2 ± 10.5

TABLE III
Numerical Values (±SE) of Best-Fitting Parameters for the Adjusted Percentage Incidences of Myeloid Leukemia, Malignant Lymphoma and Harderian Gland Tumors after Exposure to 250 kVp X Rays and Fission Neutrons in CBA/Cne Mice

End point	Radiation type	Sex	Assumed dose response	a_0	a (Gy^{-1})	b (Gy^{-2})	λ (Gy^{-1})	R	RBE
Myeloid leukemia	X rays	♂	$aDe^{-\lambda D}$		28.7 ± 12.3		0.62 ± 0.12	0.99	
		♂	$(aD + bD^2)e^{-\lambda D}$		19.4 ± 11.5	–2.59 ± 1.39	0.23 ± 0.23	0.99	
		♂	$bD^2e^{-\lambda D}$			35.2 ± 16.9	0.96 ± 0.15	0.99	
	Neutrons	♂	$aDe^{-\lambda D}$		67.0 ± 21.4		2.14 ± 0.41	0.94	2.3 ± 1.2
Malignant lymphoma	X rays	♂	$(a_0 + aD)e^{-\lambda D}$	–0.07 ± 1.68	4.91 ± 3.62		0.36 ± 0.16	0.84	
		♂	$(a_0 + aD + bD^2)e^{-\lambda D}$	0.04 ± 1.70	2.48 ± 8.97	2.59 ± 7.63	0.57 ± 0.37	0.84	
		♂	$(a_0 + bD^2)e^{-\lambda D}$	0.17 ± 1.65		4.31 ± 3.55	0.63 ± 0.18	0.84	
	Neutrons	♂	$(a_0 + aD)e^{-\lambda D}$	3.85 ± 2.63	55.7 ± 25.8		0.81 ± 0.34	0.95	11.3 ± 9.9
Harderian gland tumors	X rays	♂	$a_0 + aD$	18.7 ± 2.74	9.23 ± 1.46			0.97	
		♀		10.3 ± 3.90	13.2 ± 2.63			0.99	
	Neutrons	♂	$a_0 + aD^a$	2.50 ± 1.98	186.3 ± 28.9			0.98	20.2 ± 4.5
		♀		12.9 ± 3.68	125.4 ± 32.2			0.94	9.5 ± 3.1
		♂	$(a_0 + aD)e^{-\lambda D}$	1.96 ± 1.99	373.5 ± 68.7		2.17 ± 0.26	0.99	
		♀		12.3 ± 3.72	168.8 ± 42.5		0.79 ± 0.21	0.98	

[a]In the dose range 0–0.4 Gy.

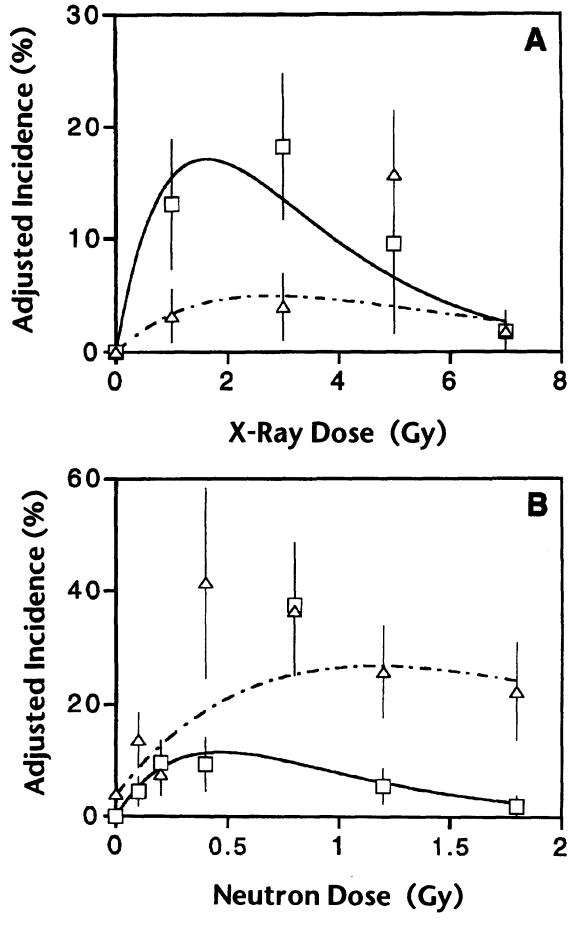

FIG. 2. Dose–effect relationships for percentage adjusted incidences of myeloid leukemia (□) and malignant lymphoma (△) in CBA/Cne male mice irradiated with graded doses of X rays (panel A) or neutrons (panel B). Bars are SE. Fitted curves correspond to equations AAI = $aDe^{-\lambda D}$ for myeloid leukemia, and AAI = $(a_0 + aD)e^{-\lambda D}$ for malignant lymphoma.

time of appearance. As far as degenerative diseases are concerned, severe nephrosclerosis, which appeared at a relatively low frequency in the untreated mice late in life, increased significantly in comparison with the control values only after 7 Gy X rays in both sexes.

Systemic neoplasms observed in this study were acute myeloid leukemia and malignant lymphomas (Table I). Myeloid leukemia was not observed in any of the untreated controls, as in a previous experiment (*7*), and was also practically absent in irradiated females, i.e. 1 case in 602 females. Conversely, a distinct dose–effect curve was identified in irradiated males and appeared highly curvilinear. Age-adjusted data for myeloid leukemia after X-ray exposure have been fitted to various mathematical expressions, i.e. with linear, quadratic or linear-quadratic dose dependence, all including a cell killing term. For neutrons, only a linear fit with a term for cell killing was considered. The numerical values of the resulting best-fitting parameters are reported in Table III. The curves fitted using the linear dose dependence are shown as solid curves in Fig. 2A and B, and from the values of the linear coefficients the neutron RBE was derived.

Malignant lymphoma included mostly mixed-cell and histiocytic lymphomas, and rarely lymphoblastic lymphomas. They were not expressed by irradiated female mice in excess compared to the untreated controls. The age-adjusted incidences in male mice were fitted applying the same models used for myeloid leukemia, but adding a parameter a_0 to account for the possibility of a non-null spontaneous incidence ($D = 0$) in the control animals, as this was observed in other mouse experiments (*11*). The numerical values of the best-fitting parameters are also reported in Table III, showing that an a_0 different from zero, but small, is obtained only for the group of male mice

in the experiment with neutrons. Using the parameters of the linear fits, the RBE was calculated.

Benign and malignant solid tumors of many types were also found in mice of both sexes, the most frequent being in the Harderian gland, lung, liver and ovary, as was observed by Grahn *et al.* in a large series of experiments carried out on $B6CF_1$ mice of both sexes irradiated with ^{60}Co γ rays or fission neutrons produced by the Janus reactor at the Argonne National Laboratory (*12*).

The high effectiveness of radiation doses in the induction of both benign and malignant neoplasms of the Harderian gland, observed in CBA/Cne mice (*7*), is confirmed by the present data. The age-adjusted incidence data suggest that for X rays a linear dose dependence is likely to hold for both sexes in the whole dose region of 0 to 7 Gy tested (Fig. 3A); however, linearity holds only in the range of approximately 0 to 0.4 Gy in the neutron experiment. With this limitation, the values of the coefficients reported in Table III were obtained, and from these the neutron RBE was calculated. To describe the totality of results obtained with neutrons, a correction for cell killing had to be introduced as indicated by the last expression in Table III and shown in Fig. 3B.

The results of the trend analysis are reported in Table IV. They indicate a significant positive trend with dose for both X rays and neutrons for all tumor types considered, apart from the lung and liver tumors in male mice irradiated with X rays.

DISCUSSION

The present study was designed to complete and enlarge previous studies on CBA/Cne male mice (*7*, *8*) carried out in our laboratory. In particular, we were interested in knowing the interplay of a biological variable, the sex of animals, with a physical one, radiation quality, in a unique experimental run using large dose ranges (1 to 7 Gy of X rays, 0.1 to 1.8 Gy of neutrons).

In general, at low to intermediate doses, life shortening has been considered almost entirely a tumor-specific phenomenon; however, the presence of an increasing dose-dependent non-specific component that at high doses is

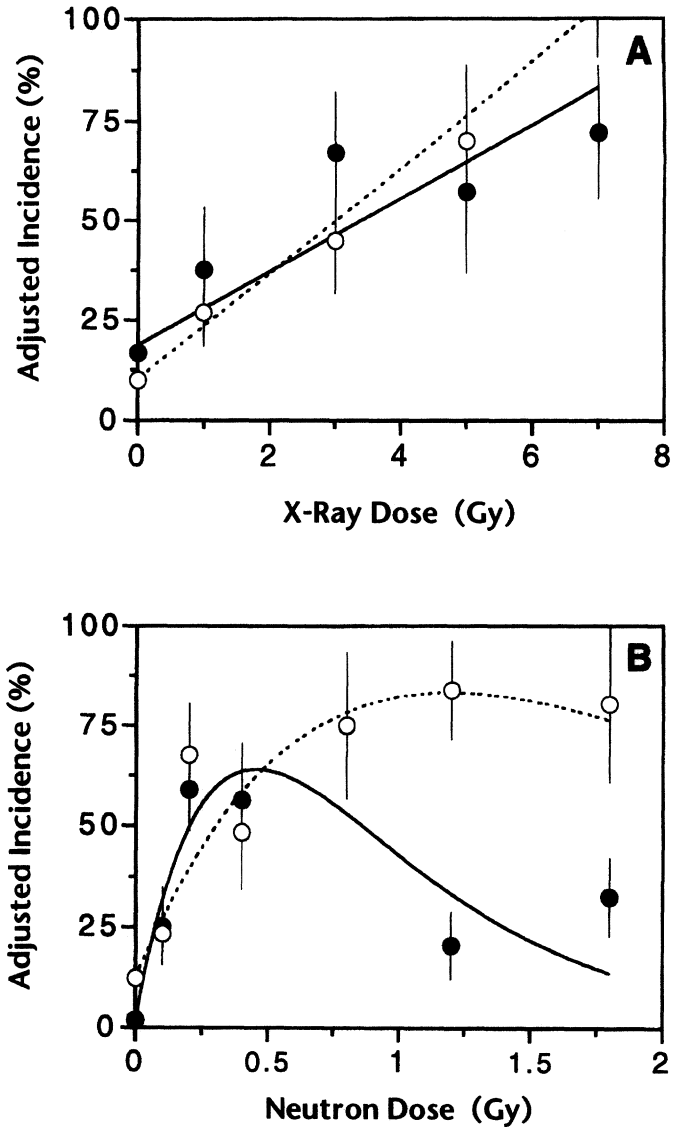

FIG. 3. Dose–effect relationships for percentage adjusted incidences of Harderian gland tumors in CBA/Cne male (●) and female (○) mice irradiated with graded doses of X rays (panel A) or neutrons (panel B). Bars are SE. Fitted curves correspond to equations $AAI = a_0 + aD$ for X rays, and $AAI = (a_0 + aD)e^{-\lambda D}$ for neutrons.

TABLE IV

One-Tailed *P* Values from Positive Trend Analysis on the Observed Incidence of Selected Tumors in CBA/Cne Mice after Exposure to 250 kVp X Rays or Fission Neutrons

Radiation type (dose range)	Sex	Myeloid leukemia	Malignant lymphoma	Harderian gland	Lung	Liver	Ovary
X rays (0–7 Gy)	♂	0.05[a]	0.03	<0.001	ns[b]	ns	—
	♀	—	0.02	<0.001	0.001	<0.001	0.03
Neutrons (0–1.8 Gy)	♂	0.003[c]	<0.001	0.005	0.02	<0.001	—
	♀	—	<0.001	<0.001	<0.001	<0.001	<0.001

[a]In the dose range 0–5 Gy.
[b]ns (not significant) indicates a *P* value ≥0.1.
[c]In the dose range 0–1.2 Gy.

TABLE V
Results of the χ^2 Test on the Raw Number of CBA/Cne Mice with Tumors at Spontaneous Death Compared to Untreated Controls Concerning Sex or Radiation Quality

Category	♂ Irradiated[a]	♀ Irradiated	X rays ♂ and ♀[b]	Neutrons ♂ and ♀
Myeloid leukemia	++[c]	–	+[d]	+
Malignant lymphoma	++	ns[e]	+	+
Harderian gland tumor	++	++	++	++
Lung tumor	ns	ns	ns	ns
Liver tumor	ns	ns	ns	ns
Solid tumors	ns	ns	ns	ns

[a]X- or neutron-irradiated animals pooled.
[b]Male and female animals pooled.
[c]The difference is significant at $P \leq 0.01$.
[d]The difference is significant at $0.05 \geq P \geq 0.01$.
[e]ns: not significant.

likely due to long-term damage to bone marrow and severe glomerulosclerosis has also been recognized (*13*). The present study also shows that, after exposure to radiation of different LETs and for both sexes, the life expectancy in tumor-free animals is reduced with a dose dependence similar to that found for the irradiated populations. This is true not only for the mean survival times but also for the Kaplan and Meier survivorship functions (data not shown). Similar indications were obtained when examining the results of other long-term studies carried out in our laboratory over the last two decades. In particular, we have examined the survivorship functions of (1) young adult $BC3F_1$ male mice acutely irradiated with fission-neutron doses from 0.17 to 1.79 Gy (*11*); (2) $BC3F_1$ mice of both sexes irradiated 17.5 days post coitum with fission-neutron doses from 0.09 to 0.62 Gy (*13*); (3) $BC3F_1$ female mice irradiated at 1 month of age with 14 MeV neutron doses from 0.05 to 0.16 Gy or 250 kVp X-ray doses from 0.16 to 2.56 Gy (*14*). This analysis confirms that in CBA/Cne and $BC3F_1$ mice, under our experimental conditions, radiation can also cause a reduction in life span by a mechanism independent of the induction of neoplasms in the whole range of doses examined.

The influence of radiation quality on life-shortening is larger for male than for female CBA/Cne mice, both in the whole and in the tumor-free populations. This appears to be due to a larger effectiveness of the X rays and a smaller effectiveness of fission neutrons for female mice compared to males (Table II).

Our results show a low susceptibility of female CBA/Cne mice to induction of myeloid leukemia, which agrees with the observations by Humphreys *et al.* (*15*) after injecting female CBA/H mice with ^{239}Pu. It should be said that internal dosimetry complications related to plutonium make the conclusion that the low incidence is a result of low sensitivity of female mice not fully warranted. However, this seems to be a more general phenomenon in accordance with the observations of Upton *et al.* in RF mice treated with fast neutrons or γ rays (*16*), and of Ullrich and Storer in RFM/Un mice treated with γ rays (*1*). In this respect, it is worth mentioning that Fennelly *et al.* (*17*) have stressed the role of the rearrangements of the Y-chromosome sequences in activating or suppressing the expression of genes involved in myeloid leukemia.

To analyze the variables sex and quality of radiation globally, we have constructed a series of simple 2×2 contingency tables in which we compare the observed frequency of the most relevant neoplastic lesions after pooling irradiated male or female mice, disregarding radiation quality and dose, or X- and neutron-irradiated mice, disregarding sex, compared to the frequency observed in each related control group. The results of these comparisons are summarized in Table V.

Although the conclusions of such an analysis cannot be generalized, we consider it interesting that, on the basis of the observed final incidences, radiation appears to be effective as a carcinogen only for tumors of the lymphohemopoietic and Harderian gland tissues, at least in the CBA/Cne mouse. In fact, the overall frequency of mice bearing solid tumors is not altered significantly by exposure to low- or high-LET radiation.

However, the life span is shortened in all groups of treated animals and in all experiments and, interestingly, is also reduced to a similar extent when only tumor-free animals are considered. Therefore, one might be tempted to conclude that the effect of exposure to radiation can be attributed more to acceleration of the neoplastic and non-neoplastic pathological processes leading to death of the animal than to the induction of an increase in neoplasms. In this respect the use of age-adjustment procedures may cause overcorrection, as in fact observed sometimes in our experience and in the work of others (*18*).

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