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Tumor Induction and Life Shortening in BC3F$_1$ Female Mice at Low Doses of Fast Neutrons and X Rays

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Extension of previous investigations at this laboratory regarding life shortening and tumor induction in the mouse has provided more complete dose–response information in the low dose region of X rays and neutrons. A complete observation of survival and late pathology has been carried out on over 2000 BC3F$_1$ female mice irradiated with single doses of 1.5 MeV neutrons (0.5, 1, 2, 4, 8, 16 cGy) and, for comparison, of X rays (4, 8, 16, 32, 64, 128, 256 cGy). Data analysis has shown that a significant life shortening is observable only for individual neutron doses not lower than 8 cGy. Nevertheless, assuming a linear nonthreshold form for the overall dose–effect relationships of both radiation qualities, an RBE value of 12.3 is obtained for the 1.5 MeV neutrons. The induction of solid tumors by neutrons becomes statistically significant at individual doses from 8 cGy and by X rays for doses larger than 1 Gy. Linear dependence on neutron dose appears adequate to interpret the data at low doses. A separate analysis of ovarian tumor induction substantiates the hypothesis of a threshold dose for the X rays, while this is not strictly needed to interpret the neutron data. A trend analysis conducted on the neoplasm incidence confirms the above findings. Death rates have been analyzed, and a general agreement between the shift to earlier times of these curves and tumor induction was found.

INTRODUCTION

In the recent past, new emphasis has been placed on the study of the effects induced by low radiation doses and dose rates to clarify important problems related to risk estimates, particularly for high-LET radiation (*1–6*). The development of *in vitro* cell transformation systems now enables a better understanding of the fundamental processes which underlie radiation-induced carcinogenesis, particularly in the critical region of low doses (*7–9*). Furthermore, *in vitro* models yield results in a shorter period of time using samples large enough to obtain statistically convincing information about transformation phenomena after low doses.

It is clear, however, that a substantial improvement of risk estimates at doses of concern to radiation protection relies on the investigation of the *in vivo* effects of doses as low as 1 cGy or less. In the absence of direct human data, no substitutes to tumor induction and life shortening in the experimental animal could easily be found. However, data from *in vivo* experimentation in the so-called low-dose region, i.e., below 20 and 5 cGy for low- and high-LET radiation, respectively (*6*), do not appear to be adequate as yet, so that the problem of the shape of the dose–effect relationships at very low doses still remains unsolved, particularly for high-LET radi-

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ation. This was considered a sound reason to carry out, at our laboratory, a complete observation of life span and late pathology through a large scale experiment on mice irradiated with graded doses from 4 to 256 cGy of 250 kVp X rays and from 0.5 to 16 cGy of 1.5 MeV neutrons. In particular, because previous experiments (*10, 11*) have shown that ovarian tissues in female mice are very susceptible to neoplastic transformation by low-LET radiation, we planned the present study in such a way as to obtain more information on the shape of the dose–response curves at low doses of X rays and neutrons for tumor induction in the ovary.

MATERIALS AND METHODS

Mice. The animals used were female hybrid mice resulting from the crossing of C57Bl/Cne females and C3H/HeCne males (hereafter referred to as $BC3F_1$). They were irradiated at 4 to 6 weeks of age, bred, kept in the conventional facilities of our animal house, and maintained on pelleted food *ad libitum.* Animal quarters were conditioned at 20°C and 60% relative humidity.

The experimental animals were divided into two groups. Those of the first group were taken to the Joint Research Center (JRC)–EURATOM (Ispra, Italy) by car and housed in animal quarters where environmental conditions and care were provided so as to meet the standards of our animal house in Casaccia (Rome) as closely as possible. Some of these animals were not treated and were used as unirradiated controls. The day after exposure to 1.5 MeV neutrons (0.5, 1, 2, 4, 8, 16 cGy), they were sent back to Casaccia by car. Here they were kept in a special isolation room, checked, weighed, and housed randomly five to a cage. When the quarantine period was over, all the animal cages were transferred to our animal house. The second experimental group, receiving X-ray irradiation and including its own unirradiated control, were not transported by car. These mice were irradiated with single doses of 4, 8, 16, 32, 64, 128, or 256 cGy in a facility located in our laboratory.

Follow-up and pathology. The two control groups were always considered separately because of their different handling history. All mice were followed for their entire life span with daily inspections (six per week). Soon after spontaneous death, a complete autopsy was performed on 1683 (96%) of the 1745 mice under observation which survived 30 days after treatment. The necropsy included a complete external and internal gross examination. Tissue masses, as well as sections of the major organs, were taken and processed for histological analysis. Tissues were fixed in Bouin's fluid and processed for paraffin embedding and sectioning. Sections were stained with hematoxylin and eosin.

The histological diagnoses were coded and entered in a computer for statistical analyses.

Data analysis. The statistical analyses for life shortening and age-adjusted incidence (Figs. 1A and 1B) included an additional 408 female $BC3F_1$ mice from a previous experiment (*11*). Disease occurrence was evaluated in terms of percentage of tumor-bearing animals (hereafter referred to as incidence) with age adjustment for the differences in mortality rates of the treated groups, in accordance with the method described in detail by Ullrich *et al.* (*12*), modified to account for accidental losses during the lifetime of the animals (*13*). Furthermore, age-related death rates for tumors diagnosed at death were computed and plotted as cumulative probabilities as a function of time according to the model of Rosenblatt *et al.* (*14*). In essence, this method analyzes both the frequency of a disease at death and the time of its appearance by means of a single set of statistics, taking competing risks and losses into account, and is particularly useful for the comparison of different treatment groups. Death-rate data were then fitted with the generalized Weibull distribution for time dependence (*15*),

$$D(t) = 1 - \exp[-\lambda^{\gamma}(t - G)^{\gamma}], \tag{1}$$

where γ and λ are the shape and scale parameters, respectively, and G is the guarantee time. The maximum likelihood estimates of γ and λ have been obtained for each dose by means of an iterative procedure. As the γ parameters for the various groups were very close in each series, finally single average values within each series were used. The time G was assumed to be 200 days in all cases.

The statistical method, extensively described by Peto *et al.* (*16*), to estimate the carcinogenic effect in long-term animal experiments and a computer program supplied by J. Wahrendorf, Division of Epidemiology and Biostatistics, International Agency for Research on Cancer, Lyon, France, were applied to the data sets. This method tests for positive trend with dose and involves a basic comparison between the number

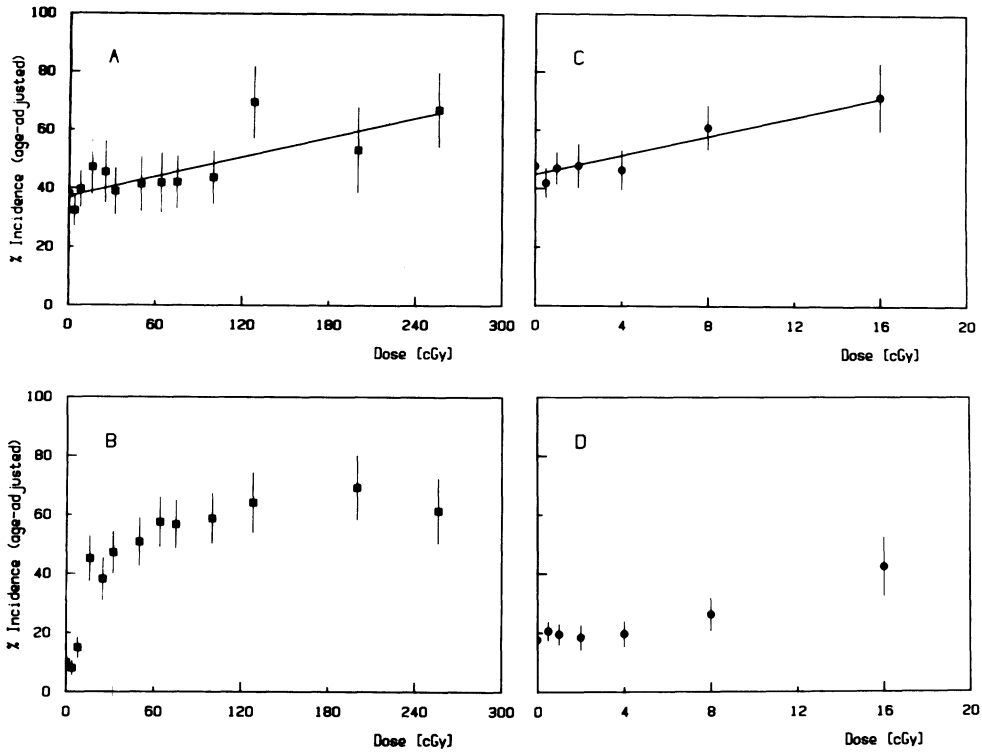

FIG. 1. Percentage incidences of solid tumors induced in mice irradiated with X rays (A) and neutrons (C). Fitted curves correspond to the linear dose–response model (see text). B and D show data of ovarian tumor induction after X and neutron irradiation, respectively. Bars are standard errors.

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. The application of this method requires that each neoplastic lesion be classified according to its "observation context." To this end, neoplasms have been classified as fatal or incidental, taking into account the histopathologic criteria, the extent of dissemination and/or metastases, and the occurrence of other competitive causes of death. Different analyses were performed for each of these two classes of neoplasms, and the results were combined to give the overall P values for differences among treatment groups. This method was applied to progressively reduced dose ranges, i.e., each time excluding the highest dose, to ascertain whether a positive trend still existed in the lower dose region (*17*).

Irradiation procedures. X rays were produced by a deep therapy unit operated at 250 kVp (HVL = 1.5 mm Cu). The X-ray dose rate was 6 cGy/min at the lower doses and 68 cGy/min at doses from 64 cGy up. Neutron irradiations were carried out at the JRC-EURATOM, Ispra. Neutrons were produced by a 3 MeV van de Graaff accelerator from the p-T reaction. A 2 mg/cm^2 tritiated titanium target bombarded by 2.5 MeV protons produced neutrons of average 1.5 MeV energy at angles between 10° and 30° where the mice were positioned. Irradiation monitoring was provided by a tissue-equivalent transmission chamber, operated with air, which had been dose calibrated with a spherical 1 cm^3 tissue-equivalent chamber flushed with tissue-equivalent gas. Mean dose rate was 11 cGy/h. Quoted neutron dose values include a γ-ray contamination of around 5%.

RESULTS

Longevity. Although differences in the mean survival times for the controls of the mouse groups irradiated with either X rays or neutrons could not be statistically as-

certained, comparison of the cumulative mortality curves has shown a small but constant shift of one curve with respect to the other; therefore, life span and pathology data for the two control groups have not been pooled (Tables I–III).

The longevity of irradiated mice appears to be significantly affected only at individual X-ray doses from 64 cGy up ($P < 0.02$), while irradiation with neutrons does not reveal any clear effect at doses below 8 cGy ($P < 0.001$). A comparison of the data of mean survival times after X irradiation in this and in a previous experiment (*11*) on the same hybrid female mice has shown the same values for the two control groups. In addition, the dose rates used in the two experiments are within a region where their influence is expected to be minor, if any. Therefore, the points from the older experiment were also included in the present analysis.

As far as the shape of the dose–response curve for life shortening at low doses is concerned, the experimental points for sparsely ionizing radiation are generally in agreement with a linear expression for doses up to a few grays (*18*). The same has been observed for radiation such as fission neutrons for single exposures at doses lower than 0.2 Gy (*4*). Therefore, nonthreshold linearity was assumed to hold within the dose range examined and good fits were obtained ($P = 0.99$ for X rays and for neutrons). Neutrons turned out to be more efficient than X rays, and an RBE value of 12.3 (±1.8) was calculated from the ratio of the slopes (-7.07 ± 0.51 days/cGy for neutrons and -0.58 ± 0.07 days/cGy for X rays).

Incidence and rates of lymphomas and tumors. The incidence of malignant lymphomas does not appear to change appreciably with the X-ray dose, except above 200 cGy (Table I). This is in agreement with the pattern observed in a previous study on the same subject (*11*). A limited number of myeloid leukemias is seen with both types of radiation, and there is no trend as a function of dose.

As regards all solid tumors of different organs and sites, excluding ovaries, the highest contribution was from the soft-tissue tumors, mainly subcutaneous fibrosarcomas but with no apparent trend as a function of dose. This finding is in line with previous observations in female mice of this and different strains (*11, 19, 20*). Very few neoplasms were seen in other sites, so the analysis was carried out by pooling all the tumor cases found in each experimental group. The incidences of all solid tumors are also reported in Tables I and II and plotted in Fig. 1. A significantly increased incidence is observed only above 100 cGy of X rays (Fig. 1A) and from 8 cGy of neutrons (Fig. 1C). If a threshold hypothesis is not considered, a linear dose–response model fits both the neutron data over the whole 0–16 cGy dose range ($P = 0.88$) and the X-ray data over the 0–256 cGy dose range ($P = 0.93$), but an expression of the form $y = a + cD^2$ (hereafter called quadratic) also gives good fits ($P = 0.84$ for neutrons and $P = 0.85$ for X rays). The use of an equation of the form $y = a + bD + cD^2$ (hereafter called linear–quadratic) yields coefficients for the quadratic terms which are not significantly different from zero. A comparison of the coefficients of the linear fits gives a neutron RBE value of 14.7 ± 4.7. This is not distinguishable from that obtained using the quadratic expressions (15.3 ± 2.8).

The age-related death-rate curves for solid tumors are shown in Fig. 2 for X rays (2A) and neutrons (2C). The curves fitted by Eq. (1) show that the time-to-tumor expression at a level of 25% is significantly shortened with respect to the unirradiated controls at the doses of 128–256 cGy for X rays and 8–16 cGy for neutrons, at a confidence level of 95%.

TABLE I

Survival and Neoplasms in Whole-Body X-Irradiated Mice

Dose (cGy)	0	4	8	16	32	64	128	256
No. of mice per group	353	100	84	53	58	57	60	55
Mean survival	887	912	893	854	874	833[c]	707[b]	697[b]
(±SD, days)	(±158)	(±170)	(±168)	(±159)	(±187)	(±159)	(±178)	(±186)
No. of autopsied mice	335	97	79	52	56	56	59	51
Malignant lymphoma	114	25	23	15	15	10	14	15
Percentage incidence[a]	34 (34)	26 (25)	29 (29)	29 (35)	27 (29)	18 (29)	24 (43)	29 (49)[d]
Myeloid leukemia						1		1
Solid tumors: Site and type								
Lung								
Alveolar adenoma	7		3	1	5		1	
Alveolar adenocarcinoma	5	2	2			2	3	1
Liver								
Hepatocellular adenoma	11	1	3	1	3	1	5	4
Hepatocellular carcinoma	1	3			1		1	1
Adrenal gland								
Cortical adenoma	3		1	1		1	2	1
Cortical carcinoma				1			1	
Kidney								
Carcinoma	1							3
Skin								
Squamous cell carcinoma	2	1			1			1

TABLE I—*Continued*

Soft tissues								
Fibrosarcoma	79	19	24	16	12	11	11	7
Rhabdomyosarcoma	4			1			1	
Vascular system								
Malignant hemangioendothelioma	2		1	1		2	1	
Bone								
Osteogenic sarcoma	3		2		1	1	3	3
Pancreas								
Adenocarcinoma		2						
Harderian gland								
Adenocarcinoma								2
Uterus								
Adenoma	1							
Adenocarcinoma	2		1					1
Leiomyofibroma	4	3					1	
Leiomyosarcoma	4	1		1				
Mammary gland								
Adenoma	2							
Adenocarcinoma	9	2	2	2	2		1	2
Total	140	34	39	25	25	18	31	26
Percentage incidence[a]	38 (38)	34 (32)	44 (40)	46 (47)	39 (39)	32 (42)	51 (69)[b]	49 (67)[b]

[a] Numbers in parentheses are age-adjusted incidences (see text for details).
[b] $P < 0.001$.
[c] $0.02 > P > 0.01$.
[d] $0.050 > P > 0.025$.

TABLE II

Survival and Neoplasms in Whole-Body Neutron-Irradiated Mice

Dose (cGy)	0	0.50	1	2	4	8	16
No. of mice per group	279	165	150	95	96	92	48
Mean survival	865	870	859	852	843	794[b]	758[b]
(±SD, days)	(±143)	(±163)	(±144)	(±160)	(±161)	(±174)	(±143)
No. of autopsied mice	267	163	144	94	92	90	48
Malignant lymphoma	98	70	50	33	32	19	9
Percentage incidence[a]	37 (37)	43 (40)	35 (37)	35 (38)	35 (38)	21 (28)	19 (38)
Myeloid leukemia		1	2				
Solid tumors: Site and type							
Lung							
Alveolar adenoma	7	4	5		3	4	4
Alveolar adenocarcinoma	9	2	3	3	2	6	1
Liver							
Hepatocellular adenoma	3	3	3	2	4	4	3
Hepatocellular adenocarcinoma	4	4	2	3	3	7	6
Histiocytic sarcoma		1					
G.I. tract							
Adenocarcinoma	1		1			1	1
Leiomyosarcoma	2						
Adrenal gland							
Cortical adenoma	1	4	1	1	1	1	1
Cortical adenocarcinoma	1		4	2			3
Kidney							
Carcinoma	1		1				1
Skin							
Squamous cell carcinoma	5	1			2		
Soft tissues							
Lipoma							1
Fibrosarcoma	76	33	39	21	17	17	8
Rhabdomyosarcoma		1					
Vascular system							
Malignant hemangioendothelioma	4	4	2	3		1	
Bone							
Osteogenic sarcoma	3	2	1	2	1	5	1
Pancreas							
Cystadenoma	1						
Adenocarcinoma	1						
Harderian gland							
Adenoma			1				
Adenocarcinoma	1			1	7	2	2
Uterus							
Adenocarcinoma	4	3	2	2			1
Leiomyofibroma	13	6	3	3	6	6	5
Leiomyosarcoma	2	2			1	1	1
Mammary gland							
Adenoma			1			1	
Adenocarcinoma	6	9	3	3	7	7	1
Adenoacanthoma	1		3			3	
Carcinosarcoma	1						

TABLE II—*Continued*

Solid tumors: Site and type							
Submaxillary gland							
Adenocarcinoma			1	1			
Pituitary gland							
Eosinophil adenoma							1
Total	147	79	76	47	54	66	41
Percentage incidence[a]	48 (48)	41 (42)	44 (47)	44 (48)	45 (46)	59 (61)[d]	67 (72)[c]

[a] Numbers in parentheses are age-adjusted incidences (see text for details).
[b] $P < 0.001$.
[c] $0.010 > P > 0.001$.
[d] $0.050 > P > 0.025$.

Tumors of the ovary are shown separately in Table III. The predominant histotypes are tubular adenomas and granulosa cell tumors. Some of the latter tumors show a poorly differentiated trabecular or follicular pattern, are composed of cells with large and irregular nuclei and mitotic figures, and closely resemble the undifferentiated carcinomas. Other tumor types, including luteomas, mixed tumors, papillary cystadenomas, and teratomas, are relatively rare.

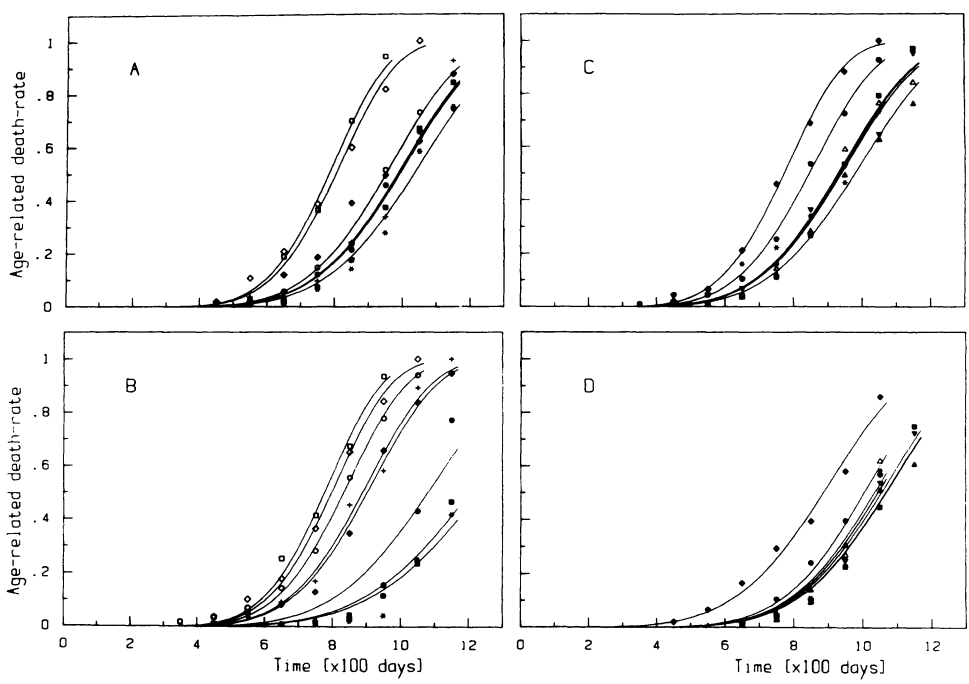

FIG. 2. Cumulative age-related death rate for solid tumors in mice: (A) X rays at 0 (■), 4 (*), 8 (●), 16 (♦), 32 (+), 64 (○), 128 (□), and 256 (◇) cGy. (C) Neutrons at 0 (■), 0.5 (▲), 1 (△), 2 (▼), 4 (*), 8 (●), and 16 (♦) cGy. The symbols are the same for ovarian tumors in B (X rays) and D (neutrons). Fitted curves correspond to Eq. (1) (see text).

TABLE III

Incidence of Ovarian Tumors in Whole-Body Irradiated Mice

Type of radiation	*Dose (cGy)*	*No. of mice*	*Tubular adenoma*	*Granulosa cell tumor*[a]	*Luteoma*	*Mixed tumor*	*Papillary cyst-adenoma*	*Tera-toma*	*Total*[a]	*Percentage incidence*[b]
X rays	0	335	27	4 (1)	3				34 (1)	10 (10)
	4	97	5	4 (2)					9 (2)	9 (8)
	8	79	13	2 (1)	1	1			17 (1)	20 (15)[e]
	16	52	21	6 (3)	3	4			34 (3)	60 (45)[c]
	32	56	22	25 (11)	2	3			52 (11)	80 (47)[c]
	64	56	19	27 (12)		1			47 (12)	73 (58)[c]
	128	59	7	18 (7)	5	3			33 (7)	54 (64)[c]
	256	51	6	15 (3)	5	3			29 (3)	51 (61)[c]
Neutrons	0	267	29	6 (1)	4	7	2		48 (1)	18 (18)
	0.50	163	28	4 (1)	2	2	2		38 (1)	23 (21)
	1	144	15	5 (1)	1	2	2	1	26 (2)	18 (20)
	2	94	12		2	3			17	18 (19)
	4	92	13	2 (1)	2	1	2		20 (1)	20 (20)
	8	90	11	3	2	2		1	19 (1)	21 (26)[d]
	16	48	15	1					16	33 (42)[c]

[a] Numbers in parentheses refer to malignant tumors.
[b] Numbers in parentheses are age-adjusted incidences (see text for details).
[c] $P < 0.01$.
[d] $0.10 > P > 0.05$.
[e] $P = 0.16$.

The incidences of all ovarian tumors are given in the last column of Table III and plotted in Figs. 1B and 1D. For neutrons, a significant increase of the incidence with respect to the control animals is first observed in mice receiving a dose of 8 cGy. The application of a quadratic dose–response model provides the best fit to the experimental points ($P = 0.98$), although a linear or a linear–quadratic expression fits the data equally well ($P = 0.92$ and $P = 0.96$, respectively). As far as X rays are concerned, a significantly increased incidence is observed at doses from 8 cGy with a rapid rise at doses slightly above. In addition, the current models of radiation action (*6*) do not appear adequate to describe the data at low doses. In fact, the best fit in the 0–16 cGy dose region, obtained with the linear model, corresponds to only $P = 0.012$. Therefore, the existence of a threshold dose within the dose region below 8 cGy cannot be excluded. If this is the case, the current dose–effect relationships at low doses can still be used, provided that the value of the absorbed dose D is replaced by $D - D_{th}$, where D_{th} is the threshold dose. In particular, the following expressions for either the linear or quadratic dose dependence of the incidence should be applied (10), for $D \geqslant D_{th}$,

$$y = y_c + a_1(D - D_{th}) \tag{2}$$

or

$$y = y_c + a_2(D - D_{th}), \tag{3}$$

where y_c is the incidence in the control group. Such an analysis shows that the linear relationship fits the X-ray data ($P = 0.65$) and the neutron data ($P = 0.58$) best, using an estimated threshold dose of 6 cGy, although the quadratic expression is still

TABLE IV

Trend Analysis on the Observed Incidence of Tumors and Lymphomas

		One-tailed P values for positive trend		
Type of radiation	*Dose range (cGy)*	*Solid tumors*	*Malignant lymphomas*	*Ovarian tumors*
X rays	0–256	<0.001	<0.001	<0.001
	0–128	<0.001	0.018	<0.001
	0–64	ns[a]	ns	<0.001
	0–32	ns	ns	<0.001
	0–16	0.075	ns	<0.001
	0–8	ns	ns	0.024
Neutrons	0–16	<0.001	ns	<0.001
	0–8	<0.001	ns	0.096
	0–4	ns	ns	ns
	0–2	ns	ns	ns
	0–1	ns	ns	ns

[a] The abbreviation ns (not significant) indicates a P value ≥ 0.1.

acceptable ($P = 0.35$ and $P = 0.21$, respectively). As far as the overall shape of the incidence curve for X rays is concerned, the data in Fig. 1B suggest that, after the fast rise, an approximately linear increase of the incidence is present for doses between 16 and 100 cGy followed by a plateau region.

In the event that a threshold dose for the induction of ovarian tumors exists, calculation of neutron RBE could appear meaningless. However, to obtain information with regard to the different effectivenesses of the two radiation qualities in this experiment, in the dose regions where the effect is most pronounced, the slopes of the two linear fits (2) have been compared and yielded a value close to unity (0.8 ± 0.2) for the ratio. This is in agreement with the RBE value around 1 mentioned by Dobson and Straume (*21*) for ovarian tumors in the mouse in a comparable dose region.

The high efficiency of low radiation doses in eliciting ovarian tumors is confirmed by the age-related death-rate analysis (Figs. 2B and 2D). For X rays, the latency is already significantly shortened at 8 cGy (confidence level of 95%) and decreases with increasing dose. Also for neutrons a dose of 8 cGy is sufficient to cause an appreciable displacement of the fitted curve to earlier times (confidence level of 95%) compared with that of unirradiated controls.

Analysis of trend. The trend of the observed final incidences has been analyzed for all tumor classes as a function of the dose, and the results are reported in Table IV. These indicate that for X rays a positive trend is present for the ovarian tumors in dose ranges from 0 to 8 cGy and higher. The same analysis, when applied to mice bearing other solid tumors or malignant lymphomas, indicates that the trend becomes significant only when doses from 128 cGy are included. In the neutron series, a positive trend for both solid and ovarian tumors is present when doses from 8 cGy up are included.

DISCUSSION

The main object of the present study is to extend the previous results of this laboratory regarding tumor induction in female $BC3F_1$ mice (*11*) by providing more complete dose–response information in the low dose region of X rays and neutrons.

With regard to longevity, the dose–response curve for life shortening after neutron irradiation does not present any steep rise at low doses followed by a decreasing slope with increasing dose. The RBE value of 12.3 for 1.5 MeV neutrons at low doses, estimated on the basis of linear dose–response relationships for both neutrons and X rays, is consistent with our previous value of 15 obtained at a dose of 17 cGy on male $BC3F_1$ mice for fission neutrons (average energy 0.4 MeV) (*22*), and also with the results of larger experimental series carried out at the Oak Ridge and Argonne National Laboratories (*4, 23*). However, since in these studies γ rays were used as the baseline radiation to obtain the neutron RBE at low doses, one might have expected from the present data a value relative to X rays lower than the one actually derived, if general considerations based on microdosimetry arguments apply (*24*). This might suggest that the RBE for life shortening in female mice is either markedly strain dependent or not so sensitive to differences in radiation quality of the baseline radiation.

It has already been pointed out (*13*) that, at doses in the same range as those of the present experiment, the life shortening effect on irradiated mice appears to be associated primarily with an increased incidence of tumors and/or an acceleration of time of appearance. This conclusion can be substantiated by the results of the present study (Tables I–III).

At the doses used in the present experiment, malignant lymphoma induction is not apparent in $BC3F_1$ mice after irradiation with either neutrons or X rays (except at 256 cGy). This seems to correspond to a more general pattern, as shown by the results of previous work on mice of $BC3F_1$ (*11, 22*) and CBA (*25, 26*) strains. Myeloid leukemia is not seen in our strain of mice, which is in contrast with the results reported for CBA mice (*25, 26*), indicating that the susceptibility to this type of leukemia is strain dependent.

The relatively small number of solid tumors observed in each site and organ has urged the pooling of all cases, regardless of histotype and site (*27*). As a consequence the shape of the dose–effect relationship is not representative of the various tissue responses, since the induction mechanism of different tumor types can be different (*5*). However, the combined effect can still be described in terms of the current models of radiation action, and the present data for solid tumors support possible linear or quadratic dose–response relationships for X rays and neutrons. In particular, within the limits represented by the size of this experiment, neutron effectiveness does not appear to be increased at very low doses at least for the long-term parameters examined and in the strain of mice used.

Previous studies in this laboratory on ovarian carcinogenesis in $BC3F_1$ mice after X rays suggested that increasing the dose of radiation resulted in an increased frequency of ovarian tumors to a maximum of over 50% following 75 cGy (*11*). The present data, which include points at very low X-ray doses, support the idea of a threshold dose model, in line with the hypothesis formulated with convincing arguments by Ullrich (*3*), which is based on the assumption that a certain degree of oocyte killing is essential to begin the sequence of events leading to the development of ovar-

ian tumors. Thus the hypothesis of a threshold dose seems to be valid independently of the mouse strain.

To further investigate this point, the contributions of the two major ovarian tumor histotypes have been considered separately. This has shown that for X rays, the tubular adenomas have a threshold-like dose–response curve and are the most responsible for the pronounced early onset of the total ovarian tumors, as also observed in RF/Un mice (*28*). Incidence of granulosa cell tumors has a less rapid increase at low doses but continues to rise with dose in the whole dose range of the present experimental series without a clear indication of a threshold dose. Similar patterns are observed for the ovarian tumors in the neutron-irradiated series, where a rapid rise in the incidence of tubular adenomas is observed from the dose of 8 cGy, while the granulosa cell tumors offer only a very minor contribution to the overall incidence of ovarian tumors at the doses used in this experiment. If we assume that a threshold dose for the induction of tubular adenomas exists, fitting the experimental points at doses up to 16 cGy with expressions (2) and (3) indicates that the linear model agrees with the data for both X rays and neutrons ($P = 0.75$ and $P = 0.99$) better than the quadratic model ($P = 0.41$ and $P = 0.37$).

In conclusion, the shape of the dose–response curve for the overall ovarian tumors appears to be determined predominantly by the induction of tubular adenomas; however, the complexity of the induction mechanism is not compatible with the description provided by a simple model of dose response.

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