Paternal monoenergetic neutron exposure results in abnormal sperm, and embryonal lethality and transgenerational tumorigenesis in mouse F_1 offspring

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Abstract. Experiments were conducted to assay whether monoenergetic neutron-induced genetic damage in parental germline cells can give rise to development of cancer in the offspring. Seven-week-old C3H male mice were irradiated with monoenergetic neutrons with energy levels of 0.2 or 0.6 MeV at doses of 0, 50, 100 or 200 cGy. Two weeks after irradiation, when the male mice showed an increased incidence of sperm abnormalities, they were mated with virgin 9-week-old C57BL females. Litter size was decreased and embryo lethalities were increased in a dose-dependent manner. Furthermore, tumor incidence in male offspring born to male mice irradiated with 25 or 50 cGy at 0.6 MeV showed a tendency for increase as compared to the non-irradiated group value. Liver tumors in the 50 cGy group were significantly increased (P=0.03). It is concluded that the increased hepatic tumor risk in the F_1 generation may have been caused by genetic transmission of some hepatoma-associated trait(s) induced by monoenergetic neutron irradiation.

Introduction

There is now a wealth of information on the transmission of tumor-related genetic traits through germ cells from parents to offspring and research has been performed to address this question not only in man but also experimental animals (1-3). The possible importance of such genetic transmission is evidenced by the finding of increased risk of leukemia and non-Hodgkin lymphoma in children of workers at the Sellafield nuclear plant and in the West Berkshire and North Hampshine nuclear industries (4). Furthermore, experimental evidence for germinal transmission of cancer-related genetic damage has been obtained after parental exposure to ethylnitrosourea (5), X-rays and urethane (6) and neutron irradiation (7-9).

In order to study the radiobiological effects of neutron, the Hiroshima University Radiobiological Research Accelerator (HIRRAC) can be operated under conditions of high proton beam currents of 1 mA and acceleration voltages up to 3 MeV. The biological effects of monoenergetic neutrons are of particular interest to basic science and radiation protection (10). Concern is reflected in *in vitro* assays (11-17) as well as *in vivo* studies (18). To our knowledge, however, there has been relatively little work on the genetic effects of monoenergetic neutrons at various energy levels using *in vivo* systems.

Specifications for biological irradiation are presented in terms of monoenergetic beam conditions, dose rates and deposited energy spectra. High dose rates of monoenergetic neutron fields are useful for studying the neutron energy dependency of biological effects, and also for other radiobiology studies on the basic mechanisms of the effects of neutrons. Monoenergetic neutrons which have a narrow neutron spectrum are the most useful, therefore they were chosen for the present study of whether irradiation-induced genetic damage can be passed to the offspring, causing embryonic lethality and tumor development in the F_1 generation.

Materials and methods

Animals. COBOS male C3H/HeNCrj and female C57BL/6NCrj mice were purchased from Charles River Japan, Inc. (Hino, Japan) and housed in autoclaved cages on sterile wood chips, in a room with controlled temperature (24±2˚C), humidity (55±10%) and a regular 12-h light, 12-h dark cycle, under the guidelines set forth in the 'Guide for the Care and Use of Laboratory Animals' established by Hiroshima University. They were fed a commercial diet MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and were provided with normal tap water *ad libitum*. All experiments used the same lot of animals.

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Table I. Body, testis, epididymis weights and abnormal sperm induced 3 weeks after monoenegic neutron.

	BW	Testis	Epididymis	Testis/BW	Epi/bw	Sperm abnormal
0 cGy	28.9±1.9	0.17±0.02	0.063±0.006	6.01±0.76	2.21±0.20	1.56±0.76
0.2 MeV						
12.5 cGy	27.9±1.0	0.13±0.01[a]	0.062±0.007	4.69±0.47[a]	2.22±0.22	0.96±0.33
25 cGy	28.0±1.0	0.11±0.02[a]	0.056±0.004[a]	3.80±0.55[a]	2.01±0.16	1.93±1.08
50 cGy	27.9±1.3	0.10±0.01[a]	0.054±0.003[a]	3.46±0.17[a]	1.95±0.12[b]	1.92±1.18
100 cGy	26.7±1.2[a]	0.08±0.01[a]	0.053±0.005[a]	2.92±0.34[a]	1.97±0.23[b]	4.06±1.16[a]
		Y=-0.078X+0.14		Y=-0.026X+5.2		Y=0.026X+1.07
		r^2=-0.90		r^2=-0.89		r^2=0.92
		P<0.05		P<0.05		P<0.05
0.6 MeV						
12.5 cGy	27.7±1.1	0.13±0.01[a]	0.061±0.006	4.70±0.35[a]	2.19±0.20	1.50±0.65
25 cGy	29.3±1.9	0.11±0.01[a]	0.061±0.027	3.66±0.37[a]	2.10±0.21	2.36±1.66
50 cGy	28.0±1.3	0.09±0.01[a]	0.057±0.002[b]	3.36±0.33[a]	2.02±0.09	3.07±1.43[b]
100 cGy	27.5±1.1[b]	0.08±0.01[a]	0.055±0.005[a]	2.91±0.18[a]	1.98±0.20[b]	6.18±1.07[a,c]
		Y=-0.078X+0.14		Y=-0.026X+5.1		Y=0.048X+1.14
		r^2=-0.86		r^2=-0.84		r^2=0.98
						P<0.01

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01). [b]Significantly different from 0 cGy value (P<0.05). [c]Significantly different from 0.2 MeV 100 cGy value (P<0.01).

Monoenergetic neutron irradiation. Neutron sources in this study was produced by Hiroshima University Radiobiological Research Accelerator (HIRRAC) as described previously (18). The HIRRAC can generate various monoenergetic neutrons using ^{7}Li(p,n)^{7}Be reaction with maximum accelerated voltage of 3 MV.

The absorbed doses were evaluated using paired ionization chambers IC-17 ATW (FWT, Inc., Goleta, CA, USA) and IC-17G (model GM539, FWT, Inc.). The IC-17ATW, which is made of tissue equivalent materials and filled with propane-base tissue equivalent gas, can measure the sum of neutron and γ-ray dose. The IC-17G, which is made of carbon and filled with carbon dioxide gas, can measure γ-ray dose with a few neutron dose contributions. Using these chambers, separate dose of neutron and γ-ray can be evaluated. The γ-ray contamination was estimated <3% of neutron dose when using 10-μm-thick lithium targets.

Each mouse was put into a box (3 cm x 3 cm x 5 cm) and 5 mice were located 20 cm away from target plane and 10 cm away from beam axis, which means that the mice were placed at 30 degrees direction position.

In order to uniform individual neutron doses, mice were rotated with a speed of 1 rpm. Groups of 5 mice were exposed by monoernergetic neutrons in 0.20 and 0.6 MeV (dose 50 cGy, dose rate 0.5 cGy/min) without anesthesia. The accelerated voltages for their neutron energy were 2.0 and 2.37 MV, respectively.

Experiments. One hundred and ten male mice received a single whole body exposure to monoernergetic neutrons with energy levels of 0.2 or 0.6 MeV at doses of 0, 25, 50, 100 or 200 cGy. Two weeks (spermatid stage) after irradiation, the males were mated with 3 non-irradiated 9-week-old C57BL female mice for a week, and retired males were then sacrificed. Testes were minced in saline and filtered and sperm were stained with Giemsa solution to allow the numbers of normal and abnormal sperm to be counted (19).

A total of 47 successfully mated females in one group were sacrificed 18 days after fertilization and the numbers of surviving and dead embryos were counted. In the remainder, offspring were obtained, the ratio of surviving pups was determined 1 week after birth, and the F_1 mice were maintained until 13.5 months of age.

Pathology. All animals were regularly observed on a daily basis and weighed once a month. At the time of necropsy, full autopsies were carried out under ether anesthesia, and body weights and various organ weights were determined. The number and size of liver tumor nodules were also measured and diseases of the liver and other organs including neoplastic changes were diagnosed by routine histological examination.

Statistical analysis. The significance of differences in numerical data was determined using the χ^2, Student's t-tests and the Dunnett method for multiple comparisons using logarithmic transformation.

Results

Changes in body and organ weights and appearance of abnormal sperm in the irradiated mice. Body weights of

Females mice used.

	Used females	Non-pregnancy	Pregnancy			
			Used for embryo lethality	Non-nursing	Nursing (%)	Total
0 cGy	37	15 (41)	4	1 (6)	17 (94)	18
0.2 MeV						
12.5 cGy	20	6 (30)	8	0	6 (100)	6
25 cGy	15	5 (33)	3	1 (14)	6 (86)	7
50 cGy	16	3 (19)	5	0	8 (100)	8
100 cGy	16	5 (31)	4	2 (29)	5 (71)	7
0.6 MeV						
12.5 cGy	19	7 (37)	7	1 (20)	4 (80)	5
25 cGy	20	4 (20)	8	1 (13)	7 (88)	8
50 cGy	15	5 (33)	3	0	7 (100)	7
100 cGy	37	16 (43)	5	11 (65)	6 (35)	17

100 cGy irradiated males with both energies were significantly decreased as compared with non-irradiated controls. Testis absolute (in 0.2 MeV Y=-0.078X+0.14, r^2=-0.90, P<0.05; in 0.6 MeV Y=-0.078X+0.14, r^2=-0.86) and relative weights (in 0.2 MeV Y=-0.026X+5.2, r^2=-0.89, P<0.05); in 0.6 MeV Y=-0.26X+5.1, r^2=-0.84) were also decreased linearly. The epididymis weights were decreased. Ratios of abnormal sperm were increased and with 100 cGy at 0.6 MeV the value was significantly greater than with 0.2 MeV (Table I) (0.2 MeV Y=0.026X+1.07, r^2=0.92, P<0.05; in 0.6 MeV Y=0.048X+1.14, r^2=0.98, P<0.01).

Survival of embryos. Data for used female mice are shown in Table II. Non-pregnant females accounted for 19-43%. Numbers of implantations per mouse were decreased in a dose-dependent manner (Table III 0.2 MeV Y=-0.035X+8.78, r^2=-0.91, P<0.05; in 0.6 MeV Y=-0.034X+93, r^2=-0.90, P<0.05). Numbers of total embryos in 100 cGy with both energy levels were significantly decreased as compared with other dose groups (Table III). Numbers of surviving embryos were significantly lower with 100 cGy irradiation with the average numbers of surviving embryos per mother were decreased in a dose-dependent manner (in 0.2 MeV Y=-0.058X+7.5, r^2=-0.99, P<0.01; in 0.6 MeV Y=-0.045X+7.6, r^2=-0.97, P<0.01). Conversely, lethality increased with the dose (in 0.2 MeV Y=0.02X+1.57, r^2=0.91, P<0.05; in 0.6 MeV Y=0.01X+1.71, r^2=0.65).

Birth rate and offspring nursing rate. Data for non-nursing mothers are given in Table II. The number was increased with 100 cGy at the 0.6 MeV energy level.

Offspring from mating two weeks after irradiation. Data for litter size and sex ratios are given in Table IV. Mean offspring number per mother was decreased dose-dependently at the 0.2 MeV energy level (total pups Y=-0.05X+8.3, r^2=-0.99, P<0.01; male Y=-0.03X+4.0, r^2=-0.96, P<0.01; female Y=-0.024X+4.2, r^2=-0.94, P<0.05) and with 0.6 MeV (total Y=-0.06X+8.0, r^2=-0.97, P<0.01; female Y=-0.04X+4.4, r^2=-0.86) except in males (Y=-0.01X+2.8, r^2=-0.57). The sex ratio at 0.2 MeV was about 50:50 but at 0.6 MeV differed with 12.5 cGy. In the long-term study, total number of offspring with 100 cGy at both energy levels was small.

Sequential assessment showed significant increase in body weights with 50 cGy at 0.2 MeV during 4-7 months and with 50 cGy at 0.6 MeV during to 12 months in males as

Table III. Mean survival data for embryos.

Group	Survival	Lethal	Total
0 cGy	7.50±1.00[a,c]	1.50±1.00	9.00±1.15a[a,c]
0.2 MeV			
12.5 cGy	6.50±1.20[a,c]	1.38±1.30	7.88±0.99[a,c]
25 cGy	5.00±2.00[b]	2.67±2.52	7.67±0.58[b]
50 cGy	5.20±2.28[a]	2.60±2.07	7.80±1.30[a,d]
100 cGy	1.50±1.29[e]	3.50±1.29	5.00±2.00[e]
	Y=-0.058X+7.5	Y=0.02X+1.57	Y=-0.035X+8.78
	r^2=-0.99, P<0.01	r^2=0.91, P<0.05	r^2=-0.91, P<.0.05
0.6 MeV			
12.5 cGy	7.43±1.72[a,c]	1.43±1.27	8.86±1.57[a,c]
25 cGy	5.75±2.05[a,d]	2.23±1.30	8.13±1.36[a,c]
50 cGy	5.67±0.58[a]	3.00±2.00	8.67±1.53[a,c]
100 cGy	3.00±1.22[e]	2.40±1.52	5.40±0.55[e]
	Y=-0.045X+7.6	Y=0.01X+1.71	Y=-0.034X+9.3
	r^2=0.97, P<0.01	r^2=0.65	r^2=-0.90, P<0.05

(Mean ± SD). [a]Significantly difference from 0.2 MeV 100 cGy value (P<0.01). [b]Significantly difference from 0.2 MeV 100 cGy value (P<0.05). [c]Significantly difference from 0.6 MeV 100 cGy value (P<0.01). [d]Significantly difference from 0.6 MeV 100 cGy value (P<0.05). [e]Significantly difference from 0 cGy value (P<0.01).

Table IV. Sex ratio after birth and effective animals.

Group	Sex ratio			No. of animals		
	Total	Male	Female	Total	Male (%)	Female (%)
0 cGy	8.53±1.37	4.53±1.37	3.88±1.73	138	74 (54)	64 (46)
0.2 MeV						
12.5 cGy	7.33±1.51	3.33±1.63	4.00±1.26	47	24 (51)	23 (49)
25 cGy	7.33±1.21	3.17±0.98	4.17±0.75	43	21 (49)	22 (51)
50 cGy	5.38±1.85[a]	2.63±0.92[a]	2.75±1.28	40	20 (50)	20 (50)
100 cGy	3.20±0.84[a]	1.40±0.89[a]	1.80±0.84[b]	16	7 (44)	9 (56)
	Y=-0.05X+8.3	Y=-0.03X+4.0	Y=-0.024X+4.22			
	r^2=-0.99, P<0.01	r^2=-0.96, P<0.01	r^2=-0.94, P<0.05			
0.6 MeV						
12.5 cGy	7.50±1.9	2.50±2.38[b]	5.00±2.16	29	10 (34)	19 (66)
25 cGy	6.00±1.63[a]	2.29±1.25[a]	3.71±1.60	38	16 (42)	22 (58)
50 cGy	4.43±0.98[a]	3.14±1.21	1.29±1.11[a]	42	22 (52)	20 (48)
100 cGy	2.50±1.22[a]	1.50±0.84[a]	1.00±0.63[a]	17	8 (47)	9 (53)
	Y=-0.06X+8.0	Y=-0.01X+2.8	Y=-0.04X+4.4			
	r^2=-0.97, P<0.01	r^2=-0.57	r^2=-0.86			

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01). [b]Significantly different from 0 cGy value (P<0.05).

Table V. Body weights of F_1 male mice.

Group	3 months	4 months	5 months	6 months	7 months	8 months	9 months	10 months	11 months	12 months	13 months	14.5 months
0 cGy	32.1±2.7	34.2±3.4	38.2±4.0	40.0±3.9	40.8±4.2	42.7±1.9	44.3±3.4	45.2±3.1	46.2±3.3	46.8±3.6	46.8±3.4	46.0±3.4
0.2 MeV												
12.5 cGy	30.7±2.9	32.8±3.5	35.4±4.5[b]	37.1±4.9[b]	39.2±5.1	41.0±5.0	42.3±4.7	43.5±4.3	44.6±4.0	45.8±4.0	45.6±3.1	45.1±3.8
25 cGy	30.8±2.5	33.0±3.4	36.1±4.3	37.0±4.1[b]	38.3±4.3[b]	40.0±4.2[b]	41.3±4.2[a]	42.5±4.0[b]	43.6±4.0[b]	44.4±4.1[b]	45.2±4.1	44.5±4.1
50 cGy	33.8±2.7	37.5±3.6[a]	40.8±3.2[b]	42.1±3.1[b]	43.4±2.9[b]	44.2±2.7	45.2±2.9	46.0±3.0	47.3±3.1	48.1±2.9	48.1±3.9	47.6±3.2
100 cGy	32.5±0.9	34.8±2.0	38.0±3.2	41.6±4.5	44.2±4.5	45.7±2.9	47.0±2.7	47.2±2.2	48.4±1.8	48.6±1.7	46.6±3.9	45.2±2.2
0.6 MeV												
12.5 cGy	31.7±1.4	33.8±2.5	37.4±3.0	38.5±3.1	41.4±4.1	41.9±3.2	43.3±3.1	44.2±2.1	45.8±2.0	46.5±3.0	46.0±3.4	45.0±3.8
25 cGy	31.7±2.3	35.1±3.1	38.5±3.9	40.4±3.0	42.7±3.5	43.9±2.8	45.7±2.8	46.7±2.8	47.9±2.9	48.3±3.3	48.8±3.6	48.1±4.0
50 cGy	32.1±4.2	36.8±4.3[b]	41.1±4.1[b]	42.8±3.8[a]	44.2±3.1[a]	46.1±3.0[a]	47.4±3.4[a]	47.7±4.5[b]	49.6±3.8[a]	49.2±3.7[b]	48.9±3.5	47.8±3.4
100 cGy	31.6±4.1	34.9±5.5	38.6±5.9	40.0±5.7	40.4±5.1	42.2±5.7	43.4±5.5	44.0±6.2	45.6±6.0	45.4±5.3	46.2±5.9	45.3±5.6

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01). [b]Significantly different from 0 cGy value (P<0.05).

compared to control males (Table V), whereas significantly decrease was evident with 25 cGy at 0.2 MeV. Female body weights were significantly heavier than for controls with 50 cGy at 0.2 MeV from 3 to 6 months, with 100 cGy at 0.2 MeV during the whole experiment, with 25 cGy at 0.6 MeV from 5 to 12 months, and with 50 cGy at 0.6 MeV from 5 to 13.5 months, whereas with 25 cGy they were decreased from 8 to 13.5 months as compared with control values (Table VI).

At autopsy, body weights of male F_1 mice of the 0.2 MeV energy level groups were not significantly altered (Table VII). Testis weights with 100 cGy were significantly lower than the non-irradiated group whereas adrenals were heavier. Relative testis weights (organ weight/body weight x1000) with 50 and 100 cGy were also significantly decreased as compared with the non-irradiated group and again adrenal values were increased (Table VIII).

Body weights of F_1 female mice.

Group	3 months	4 months	5 months	6 months	7 months	8 months	9 months	10 months	11 months	12 months	13 months	14.5 months
0 cGy	24.2±1.4	26.0±1.9	27.0±3.2	29.6±3.5	32.0±4.4	34.2±5.1	36.2±5.8	37.6±6.2	40.8±6.0	42.8±6.0	43.6±6.1	43.1±6.4
0.2 MeV												
12.5 cGy	24.7±2.0	26.0±2.3	27.9±2.9	30.2±3.3	31.1±3.6	33.9±4.3	35.8±4.4	38.0±5.1	41.1±6.2	41.9±6.8	42.8±7.2	42.8±7.1
25 cGy	24.1±1.5	25.4±2.1	27.1±2.9	28.3±2.7	30.0±2.8	30.7±3.1[b]	32.7±3.6[b]	33.6±3.7[b]	36.5±4.2[b]	37.9±4.4[a]	37.6±4.7[a]	38.3±4.6[b]
50 cGy	26.0±2.1[b]	28.6±4.2[a]	30.0±3.6[a]	32.6±4.4[b]	34.3±5.2	36.4±5.8	38.2±5.4	40.3±6.7	43.7±6.0	46.2±5.8	47.1±5.6	44.4±9.3
100 cGy	27.8±3.0[a]	31.0±3.2[a]	33.9±3.9[a]	36.6±3.3[a]	38.5±5.2[a]	40.3±4.2[a]	42.3±4.1[a]	44.0±3.4[a]	47.4±3.7[a]	49.9±2.8[a]	50.0±3.0[b]	48.9±3.5[b]
0.6 MeV												
12.5 cGy	24.5±1.8	25.8±3.2	28.4±3.6	29.7±4.2	31.5±4.3	35.1±6.1	38.3±6.3	38.3±6.7	40.7±6.9	41.0±6.7	40.7±6.9	39.6±6.9
25 cGy	24.6±2.3	27.1±2.9	31.3±4.9[a]	33.2±5.2[a]	35.4±5.2[a]	38.4±6.0[a]	40.9±6.0[a]	42.3±6.1[a]	45.3±6.3[a]	46.4±6.2[b]	45.8±10.9	47.4±6.6
50 cGy	24.7±1.3	27.7±2.4	32.3±3.4[a]	35.2±3.4[a]	38.9±3.2[a]	41.4±4.8[a]	44.6±4.5[a]	44.9±4.9[a]	48.8±6.2[a]	49.2±5.3[a]	49.8±5.2[b]	48.6±5.9[b]
100 cGy	26.5±2.5[b]	24.8±11.4	31.9±6.0[a]	34.0±7.1[b]	35.7±7.4	38.0±7.4	40.0±8.4	42.2±8.8	45.7±9.0	46.0±8.1	47.3±9.9	46.3±9.1

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01). [b]Significantly different from 0 cGy value (P<0.05).

Table VII. Body and organ weight for F_1 male.

Group	Body weight	Liver	Kidney	Testis	Adrenal	Spleen
0 cGy	46.0±3.4	2.15±0.36	0.61±0.07	0.21±0.01	0.007±0.002	0.11±0.03
0.2 MeV						
12.5 cGy	45.1±3.8	2.20±0.33	0.62±0.07	0.20±0.02	0.006±0.001	0.10±0.03
25 cGy	44.5±4.1	2.05±0.28	0.59±0.08	0.20±0.03	0.006±0.001	0.10±0.02
50 cGy	47.6±3.2	2.30±0.36	0.63±0.09	0.20±0.002	0.007±0.002	0.12±0.06
100 cGy	45.2±2.2	2.19±0.38	0.58±0.04	0.18±0.05[b]	0.026±0.042[a]	0.13±0.12
0.6 MeV						
12.5 cGy	45.0±3.8	2.00±0.24	0.62±0.09	0.21±0.01	0.007±0.002	0.10±0.01
25 cGy	48.1±4.0	2.52±0.58	0.69±0.07[a]	0.21±0.02	0.009±0.002	0.12±0.05
50 cGy	47.8±3.4	2.27±0.38	0.61±0.05	0.19±0.05[b]	0.008±0.001	0.12±0.03
100 cGy	45.3±5.6	2.22±0.63	0.57±0.12	0.18±0.03[b]	0.008±0.003	0.11±0.03

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01). [b]Significantly different from 0 cGy value (P<0.05).

Body and kidney weights with 25 cGy at the 0.6 MeV energy level were increased, along with the relative liver and kidney weights in 25 cGy were heavier than non-irradiated group but testis in 50 cGy was decreased.

Table IX summarizes data for tumors in male F_1 offspring. Most lesions were liver tumors. Incidences overall were 25.7, 8.3 4.8, 25.0 and 42.9% with 0, 12.5, 25, 50 and 100 cGy at the 0.2 MeV energy level, respectively, and 0, 37.5, 45.5 and 25% at 0.6 MeV. Incidences of liver tumors were 18.9, 8.3, 4.8, 25.0 and 28.6 % at the 0.2 MeV energy level, respectively, and 0, 31.3, 40.1 (P=0.03) and 25% at 0.6 MeV. Sizes and number of liver tumors did not significantly differ among the groups.

Female mouse body and organ weights are shown in Table X. Body weights with 25 cGy at 0.2 MeV were significantly decreased as compared with the non-irradiated group, and ovary and adrenal weights were significantly increased with 100 cGy and liver and kidney weights with 25 and 50 cGy. Relative adrenal weights with 12.5 cGy and liver with 100 cGy were significantly decreased whereas ovary values were elevated at 100 cGy (Table XI).

Regarding incidences of tumors in females, three tumors (4.7%, hemangioma, lymphoma and ovary) appeared in the non-irradiated group, and values were 3/23 (13%, hepatoma, lung and ovary tumors), 3/22 (14%, ovary tumor), 1/20 (5%, ovary tumor) and 0 in the 12.5, 25, 50 and 100 cGy groups at

Table VIII. Relative organ weight for F_1 male mice.

Group	Liver	Kidney	Testis	Adrenal	Spleen
0 cGy	46.7±7.2	13.3±1.1	4.5±0.3	0.16±0.04	2.5±0.7
0.2 MeV					
12.5 cGy	48.8±6.1	13.8±0.9	4.3±0.4	0.14±0.03	2.2±0.6
25 cGy	46.1±3.2	13.3±1.4	4.5±0.6	0.14±0.03	2.3±0.4
50 cGy	48.3±5.8	13.3±1.4	4.1±0.8[b]	0.15±0.03	2.5±1.3
100 cGy	48.5±7.8	12.8±1.1	3.9±1.0[b]	0.59±0.93[a]	3.2±3.0
0.6 MeV					
12.5 cGy	44.3±2.4	13.7±1.7	4.6±0.4	0.17±0.05	2.2±0.3
25 cGy	52.0±9.6[b]	14.5±0.9[a]	4.3±0.4	0.18±0.05	2.5±1.1
50 cGy	47.2±5.7	12.9±0.9	4.0±1.1[a]	0.17±0.03	2.5±0.5
100 cGy	48.2±9.1	12.6±1.4	4.1±0.7	0.17±0.05	2.4±0.5

(Mean ± SD). [a]Significantly different from 0 cGy value ($P<0.01$). [b]Significantly different from 0 cGy value ($P<0.05$).

Table IX. Incidence of tumors for F_1 male mice.

Group	Effective no. of animal	Tumor bearing animal	Incidence	Liver tumor size	No. of liver tumor per mouse	Other tumor
0 cGy	74	19 (25.7)	14 (18.9)	1.59±4.13	0.20±0.40	Lung papilloma
0.2 MeV						
12.5 cGy	24	2 (8.3)	2 (8.3)	1.04±3.53	0.08±0.28	
25 cGy	21	1 (4.8)	1 (4.8)	0.24±1.09	0.05±0.22	
50 cGy	20	5 (25.0)	5 (25.0)	1.57±3.45	0.35±0.67	
100 cGy	7	3 (42.9)	2 (28.6)	2.13±4.16	0.57±0.79	Hemangioma
0.6 MeV						
12.5 cGy	10	0	0	0	0	
25 cGy	16	6 (37.5)	5 (31.3)	4.18±7.03	0.38±0.62	Harderian
50 cGy	22	10 (45.5), P=0.08	9 (40.1)[a], P=0.03	1.23±2.19	0.59±0.85	Hemangioma
100 cGy	8	2 (25)	2 (25)	2.33±4.69	0.38±0.74	

(Mean ± SD).

the 0.2 MeV energy level, respectively. The figures were 0, 5/22 (22.7), 5/20 (25%, ovary tumor) and 1/9 (11.1%, ovary) at the 0.6 MeV energy level (Table XII).

Discussion

The present experiments showed clear increase in the incidence of abnormal sperm in C3H males following monoenergetic neutron irradiation, resulting in increased embryo lethality of F_1 offspring and liver tumors in surviving F_1 males. While the sperm abnormalities were energy dose-dependent, this did not appear to be the case for embryonic death and tumor incidence.

This lack of dose-dependence is in line with the literature. Inverse dose-dependence for fission spectrum neutron induction of somatic hprt deficiency mutations has been reported by Nakamura and Sawada (20) with mouse leukemia L5178Y cells and ^{252}Cf-fission neutrons. Brenner and Hall published an inverse dose effect model for neoplastic transformation *in vitro* following high LET irradiation (21). Furthermore, Zhang *et al* (17) reported different doses of neutrons to produce approximately linear changes in the frequency of micronuclei in root-tip cells of *Allium cepaonion* irradiated as either dry dormant seeds or seedlings. Balcer-Kubiczek *et al* (22) earlier found modification of fission neutron dose-response curves on varying the dose rate to be negligible or

Body and organ weight for F_1 female mice.

Group	Body weight	Liver	Kidney	Ovary	Uterus	Adrenal	Spleen
0 cGy	43.1±6.4	1.60±0.28	0.36±0.03	0.026±0.023	0.478±0.541	0.026±0.023	0.110±0.022
0.2 MeV							
12.5 cGy	42.8±7.1	1.63±0.22	0.37±0.04	0.022±0.005	0.378±0.124	0.022±0.005	0.108±0.024
25 cGy	38.3±4.6[b]	1.46±0.17	0.36±0.04	0.026±0.005	0.580±0.472	0.026±0.006	0.110±0.030
50 cGy	44.4±9.3	1.62±0.37	0.38±0.04	0.026±0.007	0.509±0.176	0.026±0.007	0.120±0.054
100 cGy	48.9±3.5[b]	1.62±0.23	0.40±0.05	0.070±0.138[a]	0.299±0.160	0.070±0.138[a]	0.129±0.052
0.6 MeV							
12.5 cGy	39.6±6.9	1.58±0.30	0.38±0.05	0.029±0.010	0.662±0.509	0.028±0.009	0.119±0.039
25 cGy	47.4±6.6	1.77±0.30[b]	0.40±0.06[a]	0.033±0.033	0.488±0.479	0.033±0.033	0.122±0.029
50 cGy	48.6±5.9[b]	1.95±0.33[a]	0.44±0.04[a]	0.021±0.006	0.742±0.828	0.021±0.006	0.120±0.023
100 cGy	46.3±9.1	1.69±0.48	0.37±0.08	0.058±0.088	0.256±0.171	0.058±0.088	0.103±0.042

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01); [b]Significantly different from 0 cGy value (P<0.05).

Table XI. Relative body weight for F_1 female.

Group	Liver	Kidney	Ovary	Uterus	Adrenal	Spleen
0 cGy	37.3±5.4	8.59±1.22	0.606±0.495	11.85±17.04	0.237±0.186	2.85±1.02
0.2 MeV						
12.5 cGy	38.4±4.6	8.88±1.09[b]	0.525±0.168	9.16±3.75	0.205±0.057[b]	2.60±0.88
25 cGy	38.4±3.7	9.43±1.15	0.683±0.208	15.47±12.24	0.390±0.576	2.88±0.77
50 cGy	35.0±6.4	8.23±0.68	0.553±0.152	11.17±4.02	0.202±0.054	2.56±0.86
100 cGy	33.1±3.0[b]	8.15±0.90	1.375±2.649[b]	6.08±3.13	0.197±0.050	2.61±0.95
0.6 MeV						
12.5 cGy	40.1±4.5	9.74±1.45[a]	0.727±0.234	18.35±17.00	0.267±0.078	3.06±0.95
25 cGy	37.6±3.7	8.52±1.41	0.721±0.734	10.71±10.57	0.235±0.072	2.62±0.71
50 cGy	40.1±3.4	9.06±0.73	0.445±0.134	16.58±20.65	0.171±0.027	2.47±0.36
100 cGy	36.4±5.3	8.05±0.59	1.188±1.756	5.33±3.39	0.192±0.055	2.23±0.65

(Mean ± SD). [a]Significantly different from 0 cGy value (P<0.01). [b]Significantly different from 0 cGy value (P<0.05).

absent. On the other hand, Hill and Williams-Hill (23) observed that reduction of the dose rate of fission neutrons increases their effectiveness for transformation of C3H 10T1/2 cells. Watanabe *et al* (24) reported that a single ^{252}Cf neutron dose resulted in higher incidences of ovarian and Harderian gland tumors than the same total dose given at a low dose rate with B6C3F1 mouse whole body irradiation. Clearly there may be differences between the *in vitro* and *in vivo* situations. It is considered that cells with large chromosomal aberrations or other abnormalities might be able to survive *in vitro*, but *in vivo* they might not, so smaller non-lethal chromosomal changes such as point mutations, frame shifts, as small additions or deletions could be essential for tumor induction *in vivo*. The source of irradiation, strain, sex, age and plants or animals are all clearly factors which need to be taken into account when determining radiation sensitivity. Recently, we reported that there were no significant differences in the tumor induction rate among the different energy such as 0.18, 0.32, 0.6 and 1.0 MeV monoenergetic neutron irradiation (18). Sasaki *et al* (25) also mentioned that induction of chromosome aberrations is not clearly dependent on neutron energy. In conclusion, there have been no consistent differences in tumor incidence among the various energies of neutron irradiation applied.

Goud *et al* (26) reported that exposure of mice to ^{252}Cf neutrons and gamma rays resulted in a decrease in testis weight and a concomitant increase in frequency of abnormal sperm. According to Hugenholtz and Bruce (19) X-ray-

Table XII. Incidence of tumor for F_1 female mice.

Group	Effective no. of animal	Positive (%)	Type of tumor
0 cGy	64	3 (4.7)	Hemangioma, lymphoma, ovary
0.2 MeV			
12.5 cGy	23	3 (13.0)	Hepatoma, lung, ovary
25 cGy	22	3 (13.6)	Ovary 3
50 cGy	20	1 (5.0)	Ovary
100 cGy	9	0	
0.6 MeV			
12.5 cGy	19	0	
25 cGy	22	5 (22.7)	Hepatoma, lymphoma, ovary 2, sarcoma
50 cGy	20	5 (25.0)	Ovary 5
100 cGy	9	1 (11.1)	Ovary

induced abnormalities in sperm are transmissible up to the F_2 generation as dominant mutations. Nomura (27,28) demonstrated an increase in the dominant lethality and congenital malformations in offspring of male or female mice irradiated with X-rays (6) or treated with urethane (27,28). These findings were further confirmed by Kirk and Lyon (29), West *et al* (30) and Lyon and Renshaw (31), using the same dose but different strains of mice. Nomura (6) also reported increased fetal death of F_1 offspring after paternal irradiation at the stage of spermatozoa and spermatids in a dose-dependent manner. Kurishita *et al* (32) demonstrated that external abnormalities are induced in offspring of male mice following treatment of germ cells at the spermatogonia stage with ^{252}Cf neutrons and the dose-response curve was linear up to 0.95 cGy. Streffer (33) similarly observed that a transgenerational transmission occurs for ionizing radiation-induced congenital malformations as well as for genomic instability, the latter measured at the chromosome level. Carls *et al* (34) described that ionizing radiation exposure of the germline can induce delayed DNA deletions in offspring mice. They suggested that DNA deletion events are implicated in the onset of carcinogenesis and a similar phenomenon in humans may account for a portion of childhood cancers. Nomura (6) found the incidence of tumors in F_1 mice of the ICR strain to increased, in this case dose-dependently, after paternal exposure to 36, 216 or 364 cGy of X-rays at the stage of spermatozoa, spermatids or spermatogonia. Of the tumors occurring in the F_1 offspring, 90% were lung tumors. Daher *et al* (35) reported that paternal X-ray irradiation resulted in reduction of litter size and a marginally significant doubling of the leukemia/lymphoma rate in the offspring in N5 strain mice, over a 1 year observation period. Urethane treatment of F_1 offspring derived from irradiated parents caused a 2.4 times greater incidence of tumors than observed in untreated controls (36). Vorobtsova *et al* (37) reported similar results with a different mouse strain. Mewissen *et al* (38) found that repeated administration of ^3H_2O as the drinking water to C57BL/6M males before mating over several generations gave rise to hereditary adenocarcinomas in the small intestine. Essentially comparable effects of chemical carcinogens have been reported (39-41). A high incidence of liver tumors was observed in the F_1 offspring of C3H male mice which had been exposed to 50 cGy of ^{252}Cf neutrons and mated with C57BL/6 females (8,9). In the present experiment, similar results were observed with 50 cGy especially at the 0.6 MeV energy level. Shay *et al* (42) documented that when 35- to 46-day-old Wistar rat females were treated with 3-methylcholanthrene using gastric tubes every day for two months and then mated with untreated males, the incidence of cancer was increased significantly in F_1 and F_2 offspring. Tomatis *et al* (5) subsequently found in the BDV1 rat system that the incidence of nerve tumors was significantly elevated in the F_1 generation when mating occurred two weeks after treatment of 9-week-old male rats with 80 mg/kg of ethylnitrosourea. Dasenbrock *et al* (43) described that maternal preconceptual exposure in C57BL/6J mice to radiation is associated with a moderately increased incidence of liver and lung tumors in the male descendants. The incidence of total tumors in the F_1 offspring, however, was not different from the control value. Lord *et al* (44) reported that with methylnitrosourea following preconceptional paternal contamination with ^{239}plutonium the second generation excess of leukemia appears to be the result of preconceptional paternal irradiation and may be related to inherited changes that affect the development of haemopoietic stem cells. The evidence in humans is most derived from case reports and epidemiological studies of consequences to the progeny of paternal occupational exposure to chemicals, ionizing radiation and electromagnetic fields prior to conception (3,45-47). Dasenbrock *et al* (43) indicated that maternal preconceptual X-ray exposure to radiation is associated with a moderately increased incidence of liver and lung tumors in male descendants in C57BL/6N mice. Thus the fact that genetic damage to parental germ cells can be transmitted to the offspring as an origin of carcinogenesis has been well documented, and this was confirmed in the present experiment.

However, Cattanach *et al* (48) described no significant increase but seasonal changes in the incidence of lung tumors in offspring of BALB/cJ or C3H/Heh mice exposed to X-rays following the experimental protocol of Nomura (6). Evidence for such seasonal changes in tumor incidence has been published and this relates to experiments carried out in insufficiently controlled animal facilities and experimental conditions, e.g., animals exposed to outdoor light. In fact, change of the light-dark interval significantly influences tumor frequencies in mice (49). Cattanach *et al* (48) also reported that reduction in litter size in paternally irradiated groups might be evidence of genetic damage, i.e., dominant lethality, resulting from the radiation exposure.

As a general rule, heavier mice are more likely to develop spontaneous and induced tumors earlier and caloric restriction decreases body weights and tumor incidences and increases longevity. Selby *et al* (50) suggested that induced dominant lethality in mice or rats with increased tumor rates have no relation with induction of dominant tumor mutations. In the experiment numbers of offspring were lower with at both energy levels and the fact that only a few animals survived means that the incidence of liver tumors might not have been accurate. The range of gene damage is presumably very wide, given the sperm abnormalities and the embryo lethality and malformations, and many embryos died, so that surviving animals might have been those less susceptible to induction of tumors. However, if gene damage is limited, tumor-prone animals might survive, resulting in greater causation of tumors. Nomura suggested that germ-line exposure is a very early tumorigenesis by itself. It is possible that the lack of increase in lung tumors reported by Cattanach *et al* (48) may be attributable to increased incidence of embryo lethality caused by high doses of paternal X-ray irradiation.

In conclusion, the results of the present study indicate that paternal exposure to radiation is associated with an increased incidence of liver tumors in the male descendants. While our study was not designed to investigate the mechanism of transmission of increased risk, the results are in keeping with the hypothesis of a germ line-transmitted hereditary effect of monoenergetic neutron irradiation.

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