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Influence of Paternal ^{252}Cf Neutron Exposure on Abnormal Sperm, Embryonal Lethality, and Liver Tumorigenesis in the F_1 Offspring of Mice

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Experiments were conducted to determine whether neutron-induced genetic damage in parental germline cells can lead to the development of cancer in the offspring. Seven-week-old C3H male mice were irradiated with ^{252}Cf neutrons at a dose of 0, 50, 100, or 200 cGy. Two weeks or 3 months after irradiation, the male mice were mated with virgin 9-week-old C57BL females. Two weeks after irradiation, the irradiated male mice showed an increased incidence of sperm abnormalities, which led to embryo lethalities in a dose-dependent manner when they were mated with unirradiated female mice. Furthermore, liver tumors in male offspring of male mice in the 50 cGy group were significantly increased in 19 of 44 (43.2%) animals, in clear contrast to the unirradiated group (1 of 31; 3.2%) ($P < 0.01$). In the 100 cGy group, 6 of 39 (15%) mice had lesions. At 3 months after irradiation abnormal sperm and embryonal lethality were not significantly increased. The incidences of liver tumors in male offspring from the 50 cGy, 100 cGy and 200 cGy groups were 6 of 20 (30%), 5 of 22 (23%) and 1 of 19 (5%), respectively, which are not significantly increased compared with the control. It is concluded that increased hepatic tumor risk in the F_1 generation may be caused by genetic transmission of hepatoma-associated trait(s) induced by ^{252}Cf neutron irradiation.

Key words: ^{252}Cf neutron — Mouse — Paternal exposure — Offspring — Liver tumorigenesis

There is a wealth of information on the transmission of induced tumor-related genetic traits through germ cells from parents to offspring, not only in man, but also in experimental animals.[1–3] The possible importance of such genetic transmission is evidenced by the finding of the development of leukemia and non-Hodgkin's lymphoma in children of workers at the Sellafield nuclear plant and at the West Berkshire and North Hampshire nuclear industries (UK).[4–6] Furthermore, there is experimental evidence for the germinal transmission of cancer-related genetic damage after parental exposure to ethylnitrosourea,[7] X-rays and urethane.[8] In the present report, we show that irradiation-induced genetic damage can be passed to the offspring, causing embryonic lethality and the occurrence of liver tumors in the F_1 generation after paternal exposure to ^{252}Cf neutrons in mice.

MATERIALS AND METHODS

Animals Male C3H/HeNCrj and female C57BL/6NCrj mice were purchased from Charles River Japan, Inc. (Hino) 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) and were provided with normal tap water *ad libitum*.

^{252}Cf neutron irradiation A ^{252}Cf source (71.4 GBq) at the Research Institute for Radiation Biology and Medicine, Hiroshima University was used for neutron irradiation. The emitted radiation from the ^{252}Cf source consisted of 67% fission neutrons and 33% γ-rays. Neutron doses were measured as described previously.[9] Seven-week-old C3H mice were exposed to whole-body irradiation at a dose rate of 0.8 cGy/min. Individual animals including unirradiated controls were put into acrylate mesh containers, that allowed free movement within the space. The ferris wheel was rotated slowly during irradiation. All irradiation procedures were carried out at room temperature.

Experiments The mice received a single whole-body exposure to ^{252}Cf neutrons at a dose of 0, 50 (pure neutron dose=33.5 cGy), 100 (67 cGy), or 200 cGy (134 cGy). Total irradiation time was 0, 62.5, 125, or 250 min, respectively. Two weeks (spermatid stage) or 3 months (spermatogonia stage) after irradiation, a male was mated with 3 unirradiated 9-week-old C57BL female mice for a week, and then killed. The testes were minced in saline and

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the mince was filtered. Sperm were stained with Giemsa solution. The numbers of normal sperm and abnormal sperm with one head and two tails were counted.

Successfully mated females in one group were killed 18 days after fertilization and the numbers of surviving and dead embryos were counted. In another group, offspring were obtained, the ratio of surviving pups was determined 1 week after birth, and the F_1 mice were maintained until 14.5 months of age.

Pathology All animals were observed daily and weighed once a month. At termination, autopsy was carried out, and body weight and various organ weights were determined. The number and mean size (mean of larger and shorter diameters) of liver tumor nodules were also measured and diseases of the liver and other organs, including neoplastic changes, were diagnosed from routine histological examination.

Statistical analysis The significance of differences in numerical data was determined using the χ^2 and Student's t tests. The confidence interval was obtained from the theory of likelihood ratio statistics.

RESULTS

Changes in body and organ weights and appearance of abnormal sperm in the irradiated mice Seven-week-old male C3H mice were exposed to ^{252}Cf neutrons, and 2 weeks thereafter, were mated with normal 9-week-old C57BL females for a week. Body and liver weights in 200 cGy-irradiated males were significantly decreased as compared to unirradiated control values ($P<0.05$). Testis and spleen weights were decreased at doses of 50 cGy and above. The ratio of abnormal sperm increased linearly with increasing dose ($y=0.04x+0.5$, $r=0.98$, x is dose in cGy) (Table I). In the irradiated male C3H mice which were mated 3 months after irradiation, body, liver, kidney and spleen weights were not significantly different among groups as measured 1 week after mating. Testis weights, however, decreased with increasing dose ($y=-0.33x+102$, $r=-0.98$) and the incidence of abnormal sperm remained increased ($y=0.009x+36$, $r=0.97$) (Table I).

Effects on embryos Seven to nine pregnant C57BL mice which had been mated with males 2 weeks after irradiation were killed on day 18 of pregnancy and the numbers of living and dead embryos were counted. Numbers of implantations per mouse (4.9–6.9/mouse) were not significantly different among the groups but lethality in the 100 and 200 cGy groups was significantly increased as compared to that in the 0 or 50 cGy group. The number of surviving embryos was significantly lowered by high-dose irradiation with the average number of surviving embryos per mother being decreased in a dose-dependent manner ($y=-0.28x+90$, $r=-0.99$). Conversely, embryonal lethality increased dose-dependently ($y=0.28x+10$, $r=0.99$) (Table II).

At 3 months after irradiation, average numbers of implantations as well as living and dead embryos did not significantly differ from those in the controls (Table II).

Birth rate and nursing offspring rate Seven of 10 females (70%) which had been mated with males given 0 cGy had pups, 15 of 20 (75%) in the 50 cGy case, 33 of 60 (55%) in the 100 cGy case and 9 of 20 (45%) in the 200 cGy case. Survival of litters was decreased at the dose of 100 cGy (18/33, 54%), and there was no survival (0/9) at the dose of 200 cGy (significant difference between 0 or 50 cGy vs. 200 cGy, $P<0.01$) in mating groups 2 weeks after irradiation (Table III).

In mating groups from 3 months after irradiation, 8 of 10 females (80%) in the 50 cGy group had pups, 6

Table I. Body and Organ Weights and Abnormal Sperm in Male C3H Mice

Dose (cGy)	N	Weight (g)				Ratio of abnormal sperm (%)
		Body	Liver	Spleen	Testis	
(a) Three weeks after irradiation						
0	13	27.4±1.7	1.44±0.16	0.14±0.03	0.20±0.01	0.4±0.5
50	16	27.3±1.2	1.55±0.22	0.10±0.01**	0.11±0.01**	3.4±1.4**
100	30	26.7±1.5	1.54±0.18	0.08±0.01**	0.08±0.01**	3.9±2.8**
200	20	26.0±1.3*	1.33±0.11**	0.09±0.03**	0.08±0.01**	9.4±4.1
						$y=0.04x+0.5$ ($r=0.98$)
(b) Three months after irradiation						
0	27	30.7±2.7	1.71±0.28	0.17±0.03	0.10±0.01	0.5±0.5
50	25	33.6±2.7	1.98±0.07	0.17±0.2	0.09±0.01	0.8±0.7
100	18	31.9±1.4	1.89±0.27	0.13±0.03	0.07±0.01	1.0±0.8
200	30	32.1±2.2	1.95±0.11	0.16±0.04	0.03±0.08	2.3±1.6
					$y=-0.33x+102$ ($r=-0.98$)	$y=0.009x+36$ ($r=0.97$)

Significantly different from 0 cGy (* $P<0.05$, ** $P<0.01$ by t test).

Table II. Embryonal Lethality in Embryos of Irradiated Male Mice

Dose (cGy)	2 weeks after irradiation				3 months after irradiation			
	No. of mothers with embryo(s)	Implantation	Dead embryos	Surviving embryos	No. of mothers with embryos	Implantation	Dead embryos	Surviving embryos
0	14	96	9 (9%)	87 (91%)[a, b, c]	18	147	17 (12%)	130 (88%)
50	18	124	28 (23%)	96 (77%)[a, b]	7	54	8 (15%)	46 (85%)
100	19	121	51 (42%)	70 (58%)[b]	9	60	11 (18%)	49 (82%)
200	16	78	51 (65%)	27 (35%)	18	154	17 (11%)	137 (89%)
			$y=0.28x+10$ ($r=0.99$)	$y=-0.28x+90$ ($r=-0.99$)			$y=-0.007x+14.6$ ($r=-0.199$)	$y=0.007x+85.4$ ($r=0.19$)

a) Significantly different from 100 cGy at $P<0.01$ (by χ^2 test), *b*) Significantly different from 200 cGy at $P<0.01$ (by χ^2 test), *c*) Significantly different from 50 cGy at $P<0.01$ (by χ^2 test).

Dose (cGy)	2 weeks after irradiation	3 months after irradiation
	Dead embryos, mean (95% confidence interval)	Dead embryos, mean (95% confidence interval)
0	0.094 (0.046–0.163)	0.116 (0.071–0.174)
50	0.226 (0.159–0.305)	0.131 (0.062–0.231)
100	0.421 (0.336–0.511)[d]	0.159 (0.086–0.257)
200	0.654 (0.545–0.753)[d]	0.110 (0.068–0.166)

d) Significantly different from 0 cGy at $P<0.05$ (by the theory of likelihood ratio statistics).

of 16 (38%) at 100 cGy and 5 of 10 (50%) at 200 cGy (Table III).

Offspring from mating 2 weeks after irradiation In the long-term study, mean offspring numbers per mother were 2.4–3.0 for both sexes. At autopsy, body weights of both sexes of the F_1 generation from the 50 and 100 cGy groups were significantly heavier than those from the unirradiated group. The liver in female offspring from the 50 cGy group weighed significantly more than in the unirradiated group ($P<0.01$). The kidney and testis weights in male offspring of the 100 cGy group were significantly decreased as compared with the unirradiated group ($P<0.01$, Table IV).

Table V summarizes data for liver tumors in F_1 offspring. In males of the 50 cGy group, 19 of 44 (43%) mice had tumors as compared to 1 of 31 (3%) in the unirradiated group ($P<0.01$) and 6 of 39 (15%) in the 100 cGy case. Average numbers and sizes of hepatic tumors were also high in male offspring of high-dose irradiated mice. In female offspring, the incidence of liver tumors did not differ among the groups. Tumors at other sites than the liver are also shown in Table VI, but no significant inter-group variation was apparent.

The incidence of fatty liver in offspring of the 50 cGy (82%) and 100 cGy groups (82%) was increased as compared with that in the 0 cGy group (59%).

Offspring from mating 3 months after irradiation In the long-term study, mean offspring numbers per mouse were 2.4–4.3. The body weights of males of the 200 cGy group and F_1 females from fathers given 50–200 cGy were

Table III. Birth Rate and Litter Survival

Dose (cGy)	Birth rate 2 weeks	Surviving litter 2 weeks	Birth rate 3 months
0	7/10 (70%)	7/7 (100%)[a]	
50	15/20 (75%)	15/15 (100%)[a]	8/10 (80%)
100	33/60 (55%)	18/33 (54%)	6/16 (38%)
200	9/20 (45%)	0/9 (0%)[b]	5/10 (50%)

a) vs. *b*) $P<0.01$ (by χ^2 test).

significantly lower than in the unirradiated group. The liver weights of males of the 200 cGy group and females of the 100–200 cGy groups were also significantly decreased as compared to the controls (Table VII).

Incidences of liver tumors in male offspring from the 50 cGy, 100 cGy, and 200 cGy groups were 6 of 20 (30%), 5 of 22 (23%), and 1 of 19 (5%), respectively (Table V), and were not significantly different from the 3 of 33 (9%) observed for controls. The incidence of fatty liver in the offspring of the 200 cGy group (32%) was significantly decreased as compared to that in the 0 and 50 cGy cases (74%).

DISCUSSION

The present results clearly show that irradiation of spermatids gives rise to abnormal sperm in C3H males, resulting in dose-dependent lethality to embryos and liver tumors in surviving offspring. On the other hand, in the

Table IV. Body and Organ Weights in F_1 Offspring from Irradiated and Non-irradiated Male Mice 2 Weeks after Irradiation

Father's radiation dose (cGy)	Sex of offspring	Effective No.	Weight (g)				
			Body	Liver	Spleen	Kidney	Testis Ovary
0	M	31	41.9±3.6	2.26±0.29	0.136±0.032	0.645±0.066	0.226±0.015
50		44	45.1±5.4**	2.27±0.40	0.133±0.036	0.631±0.079	0.224±0.023
100		39	44.9±3.8**	2.09±0.63	0.115±0.027*	0.562±0.072**	0.199±0.047**
0	F	30	33.0±4.9	1.50±0.16	0.124±0.018	0.385±0.028	0.017±0.009
50		58	46.2±6.2**	1.71±0.26**	0.121±0.034	0.396±0.042	0.019±0.013
100		35	41.5±8.2**	1.37±0.23	0.123±0.034**	0.377±0.041	0.013±0.011

** $P<0.01$ compared to same sex 0 cGy (by t test).
* $P<0.05$ compared to same sex 0 cGy (by t test).

Table V. Incidence of Liver Tumors in F_1 Offspring

Father's radiation dose (cGy)	Sex of offspring	2 weeks[a]				3 months[a]			
		No. of mice	Liver tumor (%)	No./mouse (95% confidence interval)	Tumor size (mm)[b] (95% confidence interval)	No. of mice	Liver tumor (%)	No./mouse (95% confidence interval)	Tumor size (mm)[b] (95% confidence interval)
0	M	31	1 (3)	1.08 (1.00–1.39)	10	33	3 (9)	1.23 (1.05–1.72)	9.14 (0.28–303.39)
50		44	19 (43)**	6.49 (3.84–12.45)*	3.94 (2.10–7.40)	20	6 (30)	3.16 (1.78–7.55)*	4.46 (1.68–11.80)
100		39	6 (15)	2.28 (1.61–3.72)*	7.73 (4.69–12.74)	22	5 (23)	5.93 (2.90–15.89)*	3.31 (1.01–11.12)
200		0				19	1 (5)	1.13 (1.07–1.71)	13
0	F	30	1 (3)						
50		58	1 (2)			18	1 (6)		
100		35	0			24	0		
200		0				14	0		

a) Exposed C3H male mice were mated with unexposed C57BL mice 2 weeks or 3 months after irradiation.
b) Average of the larger and shorter diameters.
* Significantly different from 0 cGy at $P<0.05$ (by the theory of likelihood ratio statistics).
** Significantly different from 0 cGy at $P<0.01$ (by χ^2 test).

case of spermatogonia irradiated 3 months before mating, these effects were far less prominent, when present. Goud *et al.*[10] reported that exposure of mice to ^{252}Cf neutrons and γ-rays resulted in a decrease in testis weight and a concomitant increase in frequency of abnormal sperm. According to Hugenholtz and Bruce[11] X-ray-induced abnormalities in sperm are transmissible up to the F_2 generation as dominant mutations. Nomura[12] demonstrated an increase in dominant lethality and congenital malformations in offspring of male or female mice irradiated with X-rays. These findings were confirmed by Kirk and Lyon,[13,14] West *et al.*[15,16] and Lyon and Renshaw,[17] using the same dose but different strains of mice. Nomura[12] also reported increased fetal death of F_1 offspring after paternal irradiation at the stage of spermatozoa and spermatids in a dose-dependent manner, but, as in the present study, this did not occur with exposure of spermatogonia. Kurishita *et al.*[18] 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 Gy. Nomura[8] investigated the incidence of tumors in F_1 mice of ICR strain after paternal exposures 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. Urethane treatment of F_1 offspring derived from irradiated parents caused a 2.4 times greater incidence of tumors than that observed in untreated controls.[19] Vorobtsova and Kitaev[20] reported similar results with a different mouse strain. Mewissen *et al.*[21] 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.[22–24] Shay *et al.*[25] reported in 1952 that when 35- to 46-day-old Wistar rat females were treated with 3-methylcholanthrene by gastric intubation every day for 2 months and then mated with untreated males, the incidence of cancer was signifi-

cantly increased in F_1 and F_2 offspring. Tomatis *et al.*[7] found in the BDV1 rat system that the incidence of nerve tumors was significantly elevated in the F_1 generation when mating was conducted 2 weeks after treatment of 9-week-old male rats with 80 mg/kg of ethylnitrosourea. The incidence of total tumors in the F_1 offspring was not different from the control.

Thus, the fact that genetic damage to parental germ cells can be transmitted to the offspring, leading to carcinogenesis, has been well documented, and this was confirmed in the present experiment. As the relative biological effectiveness of ^{252}Cf neutrons is supposed to be about 3.4 for sperm abnormality,[10] 2.4 for embryo abnormality[18] and 15.2 for liver tumorigenesis,[9] the radiation doses and sources are quite different between Nomura's experiment and this experiment. The prevalence of lung tumors in Nomura's experiments[8] and liver tumors in the present experiment requires explanation. Nomura considered that F_1 offspring would have many mutations which would be insufficient to cause cancer in themselves. Exposure after birth to various factors, including aging, acted to promote lesion development, as indicated by the appearance of clustering tumors in F_1 offspring after postnatal exposure to urethane.[26] However, it must be born in mind that strain difference plays a major role in mice. In the F_1 offspring of $B6C3F_1$ derived from male C3H and female C57BL mice, 20–60% of males develop liver tumors over 2 years, but the tumor incidence remains low in females.[27,28] We have therefore argued that sex hormones have a crucial influence on the development of liver tumors.[29,30] Since all mice were killed at the age of 14.5 months in the present experiment, the incidence of liver tumors in the control group was low (3%). In contrast, that in the male F_1 offspring obtained from irradiated fathers was significantly higher (42%). If aging is a causative factor for liver tumorigenesis, the radiation treatment may enhance this process, and any genetic change which accelerates the aging process would be transmitted. Drinkwater *et al.*[31] have proposed the possible existence of an *Hcs* (hepatocarcinogen-sensitive) locus which might be involved in the induction of liver tumors in C3H mice. Irradiation of C3H mice may increase the expression of related genes, or may delete genes whose products suppress their expression (*Hcs* locus).

To investigate other possibilities, many transgenic mice, such as TGF mice[32,33] or hepatitis B virus mice,[34,35] have been prepared and shown to exhibit enhanced hepatic tumorigenesis. Thus, there is ample evidence that genetic change in germ cells can be transmitted to give rise to

Table VI. Tumors at Sites Other than Liver in F_1 Offspring from Irradiated and Unirradiated Male Mice

Father's radiation dose (cGy)	Sex of offspring	No. of mice	Other tumors
(a) Two weeks after irradiation			
0	M	31	Mesenteric 3, Spleen 2
50		44	Thyroid 2, Adrenal 1, Mesenteric 1, Pituitary 1
100		39	Testis 3
0	F	30	Ovary 6, Adrenal 1
50		58	Ovary 5, Spleen 1, Pituitary 3, Salivary gland 1, Adrenal 1
100		35	Ovary 1
(b) Three months after irradiation			
50	M	20	No
100		22	No
200		19	No
50	F	18	Sarcoma 1
100		24	No
200		14	No

Table VII. Body and Organ Weights in F_1 Offspring from Irradiated and Unirradiated Male Mice 3 Months after Irradiation

Father's radiation dose (cGy)	Sex of offspring	Effective No.	Weight (g)				
			Body	Liver	Spleen	Kidney	Testis Ovary
0	M	33	41.9±3.5	2.03±0.25	0.121±0.071	0.564±0.062	0.214±0.016
50		20	43.5±3.6	2.13±0.22	0.135±0.144	0.584±0.066	0.241±0.108
100		22	40.5±5.4	1.91±0.26	0.102±0.022	0.532±0.090	0.208±0.016
200		19	37.8±5.5**	1.66±0.25**	0.108±0.032	0.531±0.045*	0.213±0.023
0	F	27	37.0±5.5	1.48±0.17	0.113±0.025	0.358±0.025	0.025±0.074
50		18	32.3±5.7[a]	1.60±0.75	0.125±0.056	0.353±0.028	0.038±0.072
100		24	30.6±5.3[a]	1.37±0.21[a]	0.104±0.023	0.346±0.031	0.022±0.033
200		14	30.0±3.0[a]	1.31±0.15[a]	0.101±0.021	0.340±0.026	0.021±0.019

*$P<0.05$ compared to same sex 0 or 50 cGy (by t test).
** $P<0.01$ compared to same sex 0 or 50 cGy (by t test).
a) $P<0.01$ compared to same sex 0 cGy (by t test).

neoplasia in progeny. It remains unknown what kind of genetic mutation might be responsible for the increased susceptibility of F_1 offspring to liver tumorigenesis in the present case. The range of gene damage is presumably very wide, given the sperm abnormalities and the embryo lethality. However, it is not known why the number of liver tumors per mouse was significantly higher for the 3 months-50 or 100 cGy group than for unirradiated controls or why the incidence of ovarian tumors in female offspring did not increase after paternal exposure to ^{252}Cf neutrons. Further studies are required.

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REFERENCES

1) Napalkov, N. P., Rice, J. M., Tomatis, L. and Yamasaki, H. (ed.) "Perinatal and Multigeneration Carcinogenesis," IARC Scientific Publications, No. 96 (1989). IARC, Lyon.
2) Tomatis, L., Narod, S. and Yamasaki, H. Transgeneration transmission of carcinogenic risk. *Carcinogenesis,* **13,** 145–151 (1992).
3) Tomatis, L. Transgeneration carcinogenesis: a review of the experimental and epidemiological evidence. *Jpn. J. Cancer Res.,* **85,** 443–454 (1994).
4) Gardner, M. J., Snee, M. P., Hall, A. J., Powell, C. A., Downes, S. and Terrell, J. D. Results of the case-control study of leukemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *Br. Med. J.,* **300,** 423–429 (1990).
5) Gardner, M. J., Hall, A. J., Snee, M. P., Downes, S., Powell, C. A. and Terrell, J. D. Methods and basic data of case-control study of leukemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *Br. Med. J.,* **300,** 429–434 (1990).
6) Roman, E., Watson, A., Beral, V., Buckle, S., Bull, D., Baker, K., Ryder, H. and Barton, C. Case-control study of leukemia and non-Hodgkin's lymphoma among children aged 0–4 years living in West Berkshire and North Hampshire health districts. *Br. Med. J.,* **306,** 615–621 (1993).
7) Tomatis, L., Cabral, J. R. P., Likhachev, A. J. and Ponomarkov, V. Increased cancer incidence in the progeny of male rats exposed to ethylnitrosourea before mating. *Int. J. Cancer,* **28,** 475–478 (1981).
8) Nomura, T. Parental exposure to X rays and chemicals induces heritable tumors and anomalies in mice. *Nature,* **296,** 575–577 (1982).
9) Takahashi, T., Watanabe, H., Dohi, K. and Ito, A. ^{252}Cf relative biological effectiveness and inheritable effect of fission neutrons in mouse liver tumorigenesis. *Cancer Res.,* **52,** 1948–1953 (1992).
10) Goud, S. N., Feola, J. M. and Maruyama, Y. Sperm shape abnormalities in mice exposed to californium-252 radiation. *Int. J. Radiat. Biol.,* **52,** 755–760 (1987).
11) Hugenholtz, A. P. and Bruce, W. P. Radiation induction of mutations affecting sperm morphology in mice. *Mutat. Res.,* **107,** 177–185 (1987).
12) Nomura, T. Congenital malformation as a consequence of parental exposure to radiation and chemicals in mice. *J. UOEH.,* **11** (Suppl.), 406–415 (1985).
13) Kirk, K. M. and Lyon, M. F. Induction of congenital anomalies in offspring of female mice exposed to varying doses of X-rays. *Mutat. Res.,* **106,** 73–83 (1982).
14) Kirk, K. M. and Lyon, M. F. Induction of congenital malformations in the offspring of male mice treated with X-rays at pre-meiotic and post-meiotic stages. *Mutat. Res.,* **125,** 75–85 (1984).
15) West, J. D., Kirk, K. M., Goyder, Y. and Lyon, M. F. Discrimination between the effects of X-ray irradiation of the mouse oocyte and uterus on the induction of dominant lethals and congenital anomalies. I. Embryo-transfer experiments. *Mutat. Res.,* **149,** 221–230 (1985).
16) West, J. D., Kirk, K. M., Goyder, Y. and Lyon, M. F. Discrimination between the effects of X-ray irradiation of the mouse oocyte and uterus on the induction of dominant lethals and congenital anomalies. II. Localized irradiation experiments. *Mutat. Res.,* **149,** 231–238 (1985).
17) Lyon, M. F. and Renshaw, R. Induction of congenital malformations in mice by parental irradiation: transmission to later generations. *Mutat. Res.,* **198,** 277–283 (1988).
18) Kurishita, A., Ono, T., Okada, S., Mori, Y. and Sawada, S. Induction of external abnormalities in offspring of male mice irradiated with ^{252}Cf neutron. *Mutat. Res.,* **268,** 323–328 (1992).
19) Nomura, T. X-ray-induced germ-line mutation leading to tumors. Its manifestation in mice given urethane postnatally. *Mutat. Res.,* **121,** 59–65 (1983).
20) Vorobtsova, I. E. and Kitaev, E. M. Urethane-induced lung adenomas in the first-generation progeny of irradiated male mice. *Carcinogenesis,* **9,** 1931–1934 (1988).
21) Mewissen, J., Ugarte, A. S. and Rust, J. H. Tumeur intestinale l'hereditaire observee apres irradiation de generations multiples d'une lignee germinale male de la Souris C57BL/6. *C. R. Soc. Biol.,* **178,** 230–235 (1984).
22) Strong, L. C. A genetic analysis of the induction of tumors by methylcholanthrene. *Am. J. Cancer Inst.,* **39,** 347–349

(1940).
23) Strong, L. C. Genetic analysis of the induction of tumors by methylcholanthrene. IX. Induced and spontaneous adenocarcinomas of the stomach in mice. *J. Natl. Cancer Inst.*, **5**, 339–362 (1945).
24) Boutwell, R. K. Some biological aspects of skin carcinogenesis. *Progr. Exp. Tumor Res.*, **4**, 207–250 (1964).
25) Shay, H., Gruenstein, M. and Weinberger, M. Tumor incidence in F_1 and F_2 generations derived from female rats fed methylcholanthrene by stomach tube prior to conception. *Cancer Res.*, **12**, 296 (1952).
26) Nomura, T. Role of radiation-induced mutations in multigeneration carcinogenesis. *In* "Perinatal and Multigeneration Carcinogenesis," ed. N. P. Napalkov, J. M. Rice, L. Tomatis and H. Yamasaki, IARC Scientific Publications, No. 96, pp. 375–387 (1989). IARC, Lyon.
27) Ward, J. M., Goodman, D. G., Squire, R. A., Chu, K. C. and Linhart, M. S. Neoplastic and nonneoplastic lesions in aging (C57BL/6N×C3H/HeN)F_1 (B6C3F_1) mice. *J. Natl. Cancer Inst.*, **63**, 849–854 (1979).
28) Taron, R. E., Chu, K. C. and Ward, J. M. Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N×C3H/HeN)F_1 (B6C3F_1) mice. *J. Natl. Cancer Inst.*, **66**, 1175–1181 (1981).
29) Ito, A., Takahashi, T., Watanabe, H., Ogundigie, P. O. and Okamoto, T. Significance of strain and sex differences in the development of ^{252}Cf neutron-induced liver tumors in mice. *Jpn. J. Cancer Res.*, **83**, 1052–1056 (1992).
30) Ito, A. Host factors influencing the development of radiation induced hepatic tumors in mice. *In* "Induction of Radiation Resistance," ed. M. Shikita and T. Yamada, pp. 204–211 (1992). Jitsugyokouhousha, Tokyo (in Japanese).
31) Drinkwater, N. R., Hanigan, M. H. and Kemp, C. Genetic determinants of hepatocarcinogenesis in the B6C3F_1 mouse. *Toxicol. Lett.*, **49**, 255–265 (1989).
32) Lee, G.-H., Melino, G. and Fausto, N. Development of liver tumors in transforming growth factor α transgenic mice. *Cancer Res.*, **52**, 5162–5170 (1992).
33) Murakami, H., Sanderson, N. C., Nagy, P., Marino, P. A., Merlino, G. and Thorgeirsson, S. S. Transgenic mouse model for synergistic effects of nuclear oncogenes and growth factors in tumorigenesis: interaction of c-*myc* and transforming growth factor α in hepatic oncogenesis. *Cancer Res.*, **53**, 1719–1723 (1993).
34) Dunsford, H., Sell, S. and Chisari, F. V. Hepatocarcinogenesis due to chronic liver cell injury in hepatitis B virus transgenic mice. *Cancer Res.*, **50**, 3400–3407 (1990).
35) Kim, C.-M., Koike, K., Saito, I., Miyamura, T. and Jay, G. *HBx* gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature*, **351**, 317–320 (1991).