

## **Influence of the Age of Mice at Exposure to Radiation on Life-shortening and Carcinogenesis**

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### **Age dependence/carcinogenesis/life-shortening/ $\gamma$ rays/B6C3F<sub>1</sub> mice**

Female B6C3F<sub>1</sub> mice were irradiated on day 17 prenatal age, or day 0, 7, 35, 105, 240 or 365 postnatal age with 0.95, 1.9, 2.85, 3.8 or 5.7 Gy of  $\gamma$ -rays from <sup>137</sup>Cs. They were allowed to live out their entire life spans under specific pathogen free conditions. All the mice were given autopsies at death and were examined histologically for neoplastic and non-neoplastic diseases. The mice in the early postnatal period were most sensitive to the life-shortening effect of radiation. The shortening effect of irradiation given during the late fetal period was almost the same as that given during the young adult period. Incidences of lung, liver, pituitary, ovarian and bone tumors and malignant lymphoma of the lymphocytic type increased after irradiation of mice in the late fetal period. Mice in the early postnatal period are more susceptible to the induction of liver and ovarian tumors and malignant lymphoma of the lymphocytic type than are fetal mice. Myeloid leukemia and Harderian gland tumor did not develop in excess when mice were irradiated in fetal or in neonatal period; whereas, these neoplasms were induced by irradiation during the adult period.

## INTRODUCTION

The most significant risk among individuals exposed to radiation may be the development of cancer, as reflected in the increase in age-specific mortality and the shortening of life expectancy. Age at the time of irradiation is one of the biological variables which determine susceptibility to cancer induction and age at death from cancer. As development and aging in mammals are generally similar, the age dependence of susceptibility for each type of neoplasm may be similar in mammals. A series of experiments using mice was designed to investigate the age-dependence of susceptibility to the induction of various types of neoplasms, with emphasis on carcinogenesis after irradiation during the prenatal, neonatal or juvenile period. Because cancers are life-limiting diseases that are manifested after a long latent period, our experiments were planned as life-span studies. Previous experiments showed that exposure of B6WF<sub>1</sub> mice to X-rays between day 16 and 18 prenatal age led to increases in the incidence of such neoplasms as pituitary, lung, ovarian and liver tumors (Sasaki *et al.*

(1978a), Sasaki *et al.* (1978b)). In contrast, radiation exposure on day 12 prenatal age did not increase the incidence of any type of neoplasm (Sasaki *et al.* (1978a)). A subsequent study using female B6C3F<sub>1</sub> mice also showed development in excess of neoplasms after irradiation on day 17 prenatal age (Sasaki and Kasuga (1986)). Dose-related shortening of the mean life span and induction of various types of neoplasms have been investigated in both sexes of B6WF<sub>1</sub> mice irradiated on day 0 of the neonatal period with X-rays (Sasaki and Kasuga (1981)). Neonatal mice have been found to be more susceptible than adults to the induction of malignant lymphomas of the lymphocytic type, as well as to liver, lung, pituitary and bone tumors. Myeloid leukemias and Harderian gland tumors were not induced by irradiation given during neonatal period of mice, whereas these neoplasms developed in excess after irradiation during young adult period (Sasaki and Kasuga (1986), Sasaki (1987)).

The experimental studies have been taken place on the age dependence of susceptibility to radiation-induced late effects. Lindop and Rotblat (1962) showed that mice in the early postnatal period were highly sensitive to life-shortening by radiation. Upton *et al.* (1960) investigated the influence of age at irradiation on the induction of thymic lymphomas, myeloid leukemias and ovarian tumors for the period of 1–3 days before birth up to 180 days of age. The respective peak incidences for thymic lymphomas and myeloid leukemias were during the early postnatal and young adult periods. They reported that increase in the incidence of neoplasms was not detectable in mice irradiated during the prenatal period. Vesselinovitch *et al.* (1971) examined age-associated change in susceptibility to radiation carcinogenesis from day 1 to 42 after birth and showed that susceptibility to the induction of liver tumors decreased with age and that the incidence of Harderian gland tumors was lower when mice were irradiated at a young age. They also showed that the incidences of malignant lymphomas, ovarian and pituitary tumors increased in mice irradiated during the early postnatal period. Benjamin *et al.* (1986) studied the age-dependence of susceptibility to radiation carcinogenesis in beagle dogs over a wide age range from the prenatal period to adulthood. An intermediate report of their study showed there is a tendency for incidences of thyroid tumors and malignant lymphomas to increase after irradiation during the perinatal period. Schmahl (1984) reported that the incidences of ovarian, lung and liver tumors increased in mice irradiated during the late fetal period. Walinder and Sjöden (1973) presented important experimental results showing that thyroid tumors developed in mice after transplacental incorporation of <sup>131</sup>I, and that the susceptibility of fetal mice was higher than that of adult mice. In contrast, mammary tumors did not develop in excess in rats exposed to tritiated water throughout intrauterine period; whereas, incidence of mammary tumors increased among mother rats exposed to tritiated water during pregnancy (Cahill (1975)). Age-associated change in the susceptibility of rats to the induction of mammary tumors was shown by Huggins and Fukunishi (1963). They reported that during the early postnatal period rats are susceptible to induction of mammary tumors and that the most susceptible time may be during the period of sexual maturation.

Recent epidemiological findings about humans exposed to ionizing radiation during juvenile or intrauterine period are important. Modan *et al.* (1989) reported that children

have an extremely high susceptibility to the induction of breast cancers, based on investigation of a population in Israel given X-ray treatment for skin diseases of head. Life span studies on cancer mortality among A-bomb survivors have indicated that the relative risk among individuals exposed under 10 years of age is higher than that for population exposed as adult (Preston *et al.* (1987), Shimizu *et al.* (1988)). Yoshimoto *et al.* (1988) have showed that mortality from adult-type cancer was higher among individuals exposed *in utero* to atomic bomb radiation than in unexposed population, but no increase in mortality from childhood cancers was detected.

The study presented here was designed to confirm and to amplify the previous findings on the age dependence of susceptibility to the shortening of life expectancy and cancer induction.

## MATERIALS AND METHODS

First generation female hybrid mice between C57BL/6JNrs and C3H/HeNrs (B6C3F<sub>1</sub>) were used. Mice were given whole-body irradiation with  $\gamma$ -rays from <sup>137</sup>Cs at a dose rate of 0.98 Gy per minute at day 17 post-coitus (dpc) prenatal age or at day 0, 7, 35, 105, 240 or 365 post-partum (dpp) postnatal age. Doses of  $\gamma$ -rays were 1.9, 3.8 and 5.7 Gy for irradiation at 17 dpc, 0 dpp, 35 dpp and 105 dpp. Mice at 7 dpp, 240 dpp and 365 dpp were irradiated with a dose of 3.8 Gy. The number of mice in each group is given in Table 1. All the mice were allowed to live out their entire life spans in a specific pathogen-free environment. Mice were lived on a pellet diet (MB1, Funabashi Farm Co.) which had been disinfected by autoclaving it. Chlorinated water (pH 2.8–3.0) were available *ad libitum*. Mouse cages were checked for dead animals once a day, 6 days a week. Upon death the mice were given autopsies, the gross findings being recorded on photographs. Suspicious neoplastic and non-neoplastic diseases were examined histologically. Mice that died before 100 days of age were not included in the calculation of the mean life span and incidence of neoplasm. Effects of radiation exposure on mortality and the development of neoplasms were analyzed statistically. Age-specific mortality was presented as the number of deaths per 10<sup>5</sup> mouse-day (MD), and the age-specific and cumulative tumor rates were expressed as the number of cases per 10<sup>5</sup> MD. The observed incidence was used as a simple indicator of cancer development.

## RESULTS

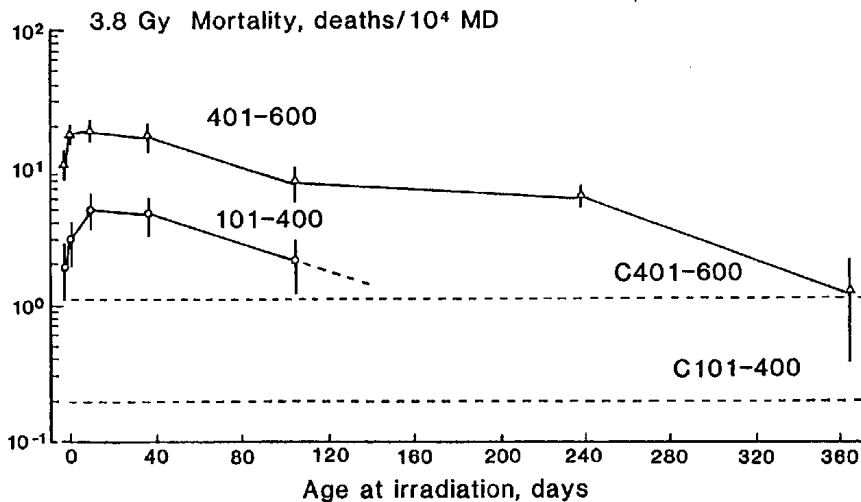
### *Mean life span and age-specific mortality*

The mean life span and the percent of shortening are given in Table 1. The means of the life spans for all the irradiated groups were significantly shorter than the mean for the unirradiated group ( $P < 0.05$ ). The life-shortening effect of exposure to radiation was appeared to be dependent on the dose and age at exposure. The life-shortening effect was remarkably high for irradiation given on day 0, 7 and 35 of the postnatal period. Exposure

**Table 1.** Influence of age at irradiation on shortening of the mean life span

Age at irradiation	Dose, Gy	No. of mice	Mean life span, days, (se)	Shortening, %, (se)
Control	0	332	871.1 ( 7.5)	
17 dpc	1.9	93	831.3 (13.8)	4.6 (1.8)
	3.8	87	709.9 (15.5)	18.6 (2.0)
	5.7	65	583.8 (20.8)	33.0 (2.6)
0 dpp	1.9	85	784.4 (14.2)	10.0 (1.8)
	3.8	81	641.8 (16.2)	26.3 (2.1)
	5.7	91	453.2 (16.0)	48.0 (2.1)
7 dpp	3.8	87	623.8 (23.3)	28.4 (2.8)
35 dpp	1.9	80	752.0 (17.4)	13.7 (2.2)
	3.8	84	622.0 (20.2)	28.6 (2.5)
	5.7	84	477.1 (21.1)	45.2 (2.6)
105 dpp	1.9	81	788.5 (16.9)	9.5 (2.1)
	3.8	80	723.9 (18.4)	16.9 (2.8)
	5.7	83	585.7 (21.1)	32.8 (2.6)
240 dpp	3.8	85	789.4 (13.8)	9.4 (1.8)
365 dpp	3.8	82	826.0 (12.1)	5.5 (1.6)

dpc: days post-coitus; dpp: days post-partum;  
se: standard error



**Fig. 1A.** Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on age-specific mortality for 100–400, 401–600 days of age. Mortalities in the control group are shown as horizontal broken lines marked C101–400 and C401–600. Vertical bars indicate standard errors.

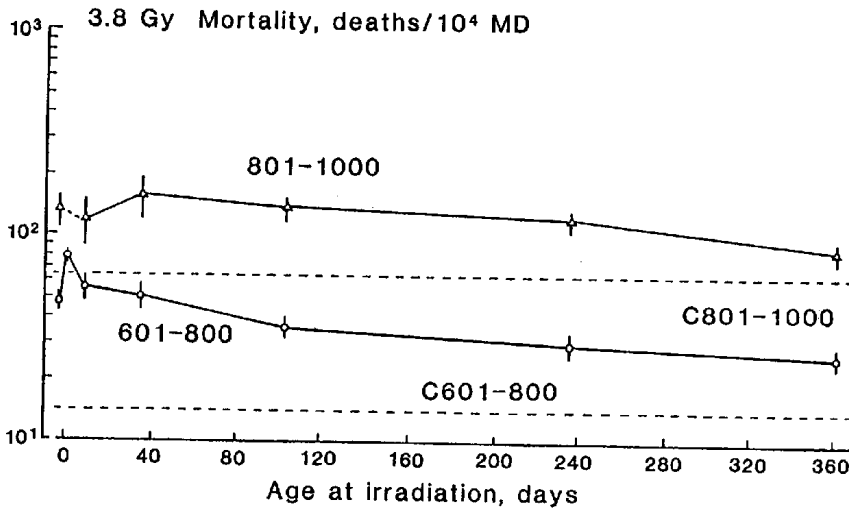


Fig. 1B. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on age-specific mortality for 601-800 and 801-1000 days of age.

on day 17 of the prenatal period was less effective than exposure during the early postnatal period. During adulthood sensitivity to the life-shortening effect of radiation decreased with age. Age-specific mortalities between 101-400, 401-600, 601-800 and 801-1000 days of age are plotted against the age at irradiation with 3.8 Gy in Fig. 1. It is clear that the age-specific mortalities of mice irradiated during neonatal or juvenile period were higher throughout the entire lifespan than were those of mice irradiated at other ages. These results indicate that during the neonatal and juvenile period mice are susceptible to both early- and late-occurring lethal diseases.

### Carcinogenesis

The relation of the incidence of malignant lymphomas of the lymphocytic type to the age at exposure to 3.8 Gy of  $\gamma$ -rays is shown in Fig. 2. The highest incidence was in the group irradiated on day 35 during the juvenile period. An increase in incidence also was detected when mice were irradiated during the neonatal and young adult periods. Development in excess of malignant lymphomas of the lymphocytic type was not found after irradiation with 3.8 Gy during the fetal period. But, irradiation with 5.7 Gy during the late fetal period did result in an increase in incidence (Fig. 3).

The incidence of malignant lymphomas of the histiocytic type, which developed spontaneously with high frequency was decreased by exposure to radiation, which effect was dependent on the age at exposure. A marked decrease in incidence was found for irradiation on day 17 prenatal age and days 0 and 7 postnatal age (Fig. 4).

Myeloid leukemias were not induced by irradiation on day 17 prenatal age or on days 0 or 7 postnatal age; whereas, myeloid leukemias developed in excess when mice were irradiated on day 35 or later (Fig. 5).

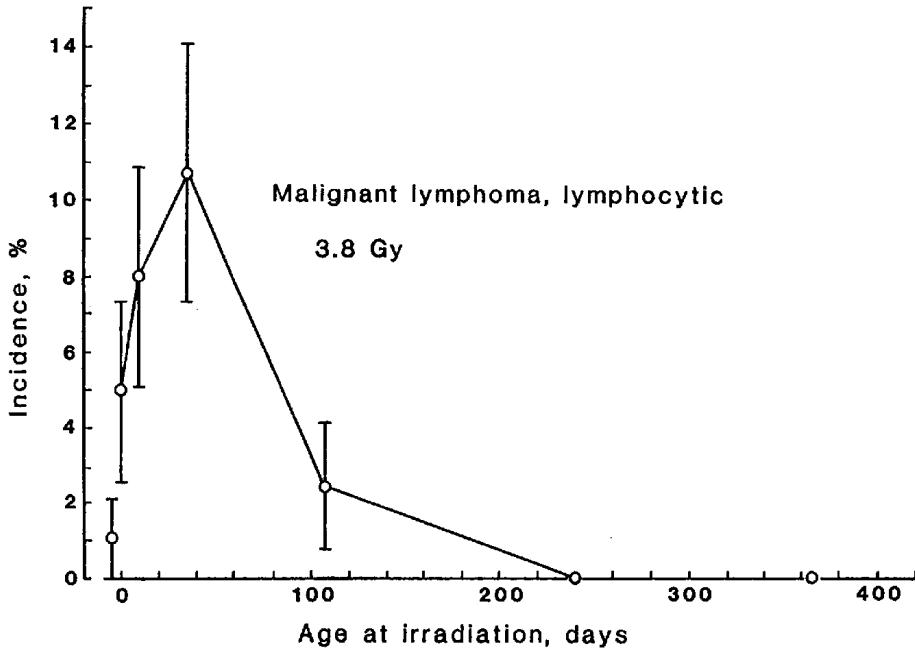


Fig. 2. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of malignant lymphomas of the lymphocytic type. Incidence in the control group: 0.3% (1/332).

Mice in the early postnatal period were remarkably susceptible to the induction of liver tumors (Fig. 6). Radiation exposure during the late fetal or juvenile period also induced liver tumors, but the incidence of liver tumors did not increase when mice were irradiated during adulthood.

Harderian gland tumors were induced by irradiation during the juvenile or young adult period. Middle-aged adults, 240 and 365 days of age, were susceptible to induction of this type of tumor. Incidences in mice irradiated at 17 dpc or 0 dpp did not differ from the value for the controls. It is noteworthy that the increase in the incidence of tumor was statistically significant for irradiation at 7 dpp (Fig. 7).

The incidence of lung tumors increased when  $\gamma$ -rays were given during the late fetal, neonatal or juvenile period. The incidence of lung tumors decreased with age at the time of irradiation as shown in Fig. 8.

Irradiation during the late fetal or neonatal period resulted in high incidences of pituitary tumors; but, the incidences did not differ from the incidence of the control for irradiation on day 35 or later (Fig. 9). Susceptibility appeared to decrease rapidly during the early postnatal period.

Ovarian tumors developed in excess in all irradiated groups with statistically significant difference. As shown in Fig. 10, during the late fetal period mice were less susceptible than during the neonatal period. During adulthood the susceptibility to the induction of ovarian

Malignant lymphoma, lymphocytic  
Dose: 5.7 Gy

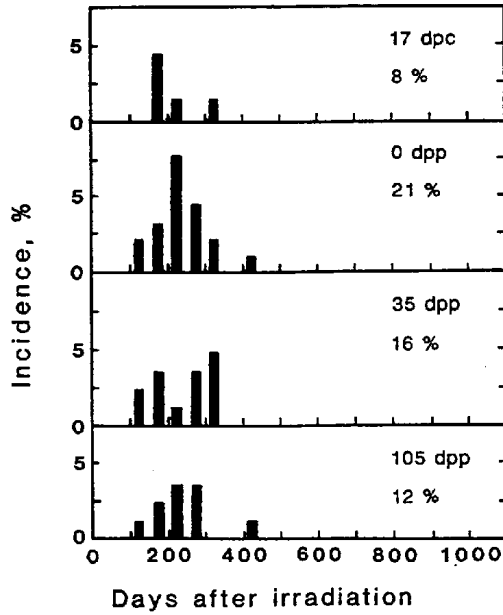


Fig. 3. Influence of the age of mice at irradiation with 5.7 Gy  $\gamma$ -rays on the incidence of malignant lymphomas of the lymphocytic type.

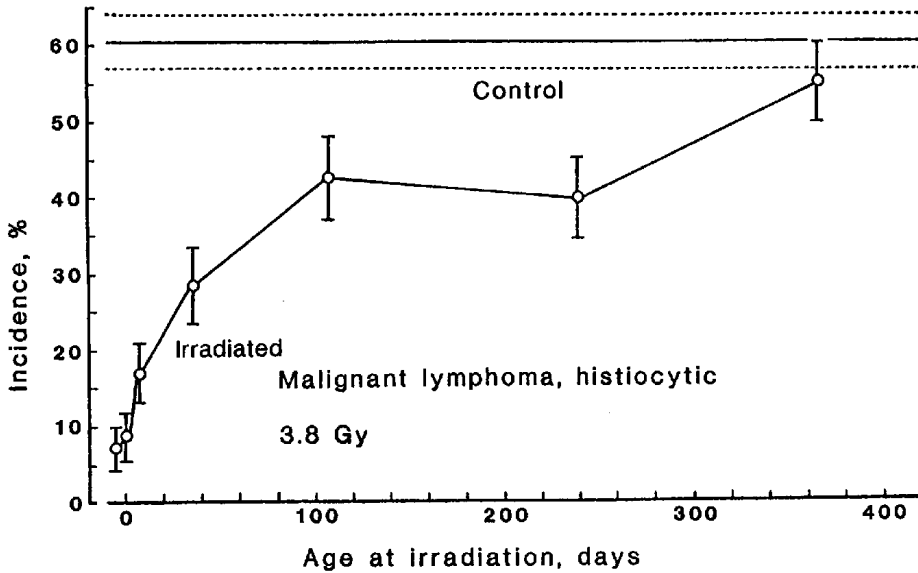


Fig. 4. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of malignant lymphomas of the histiocytic type.

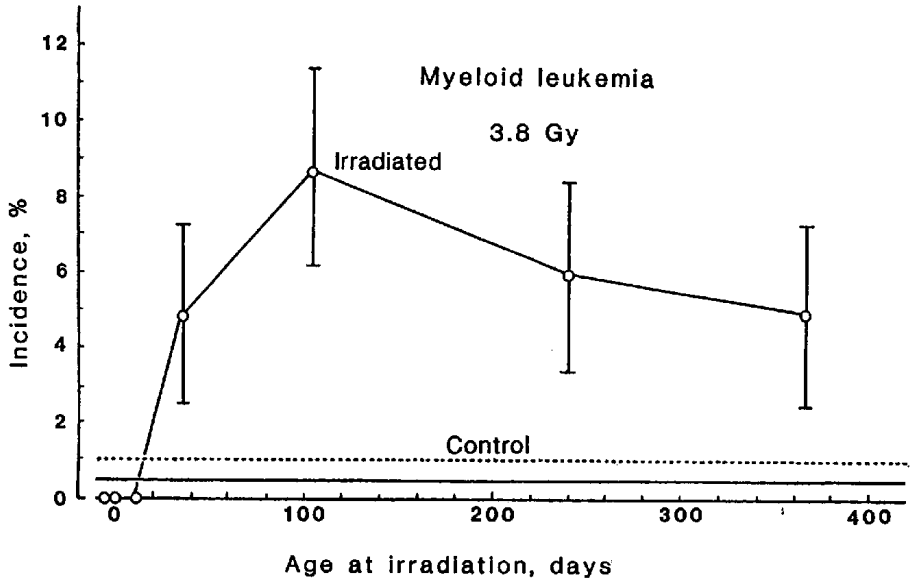


Fig. 5. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of myeloid leukemias.

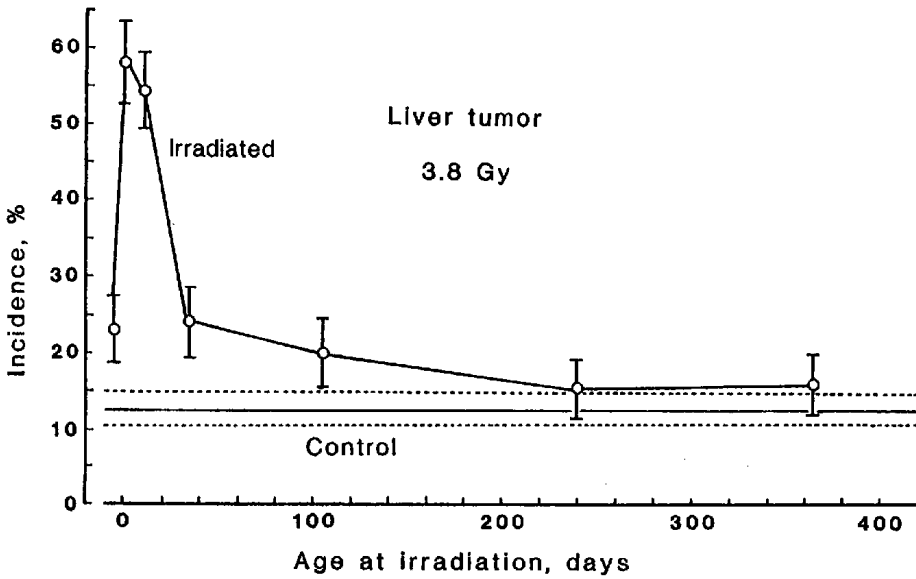


Fig. 6. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of liver tumors.



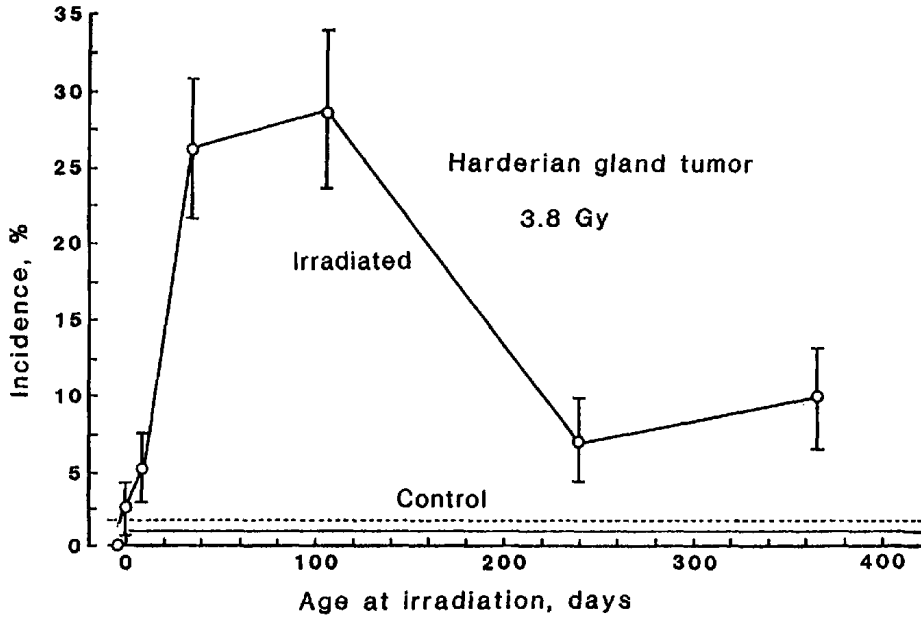


Fig. 7. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of Harderian gland tumors.

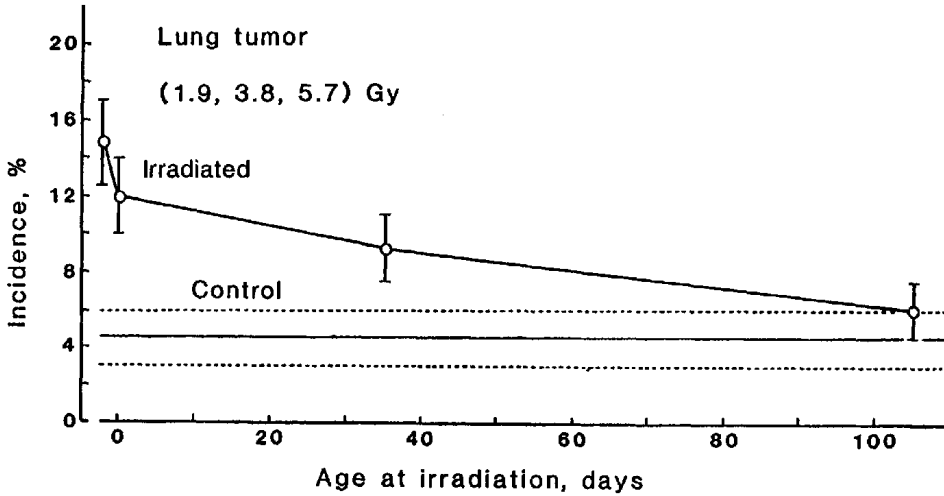


Fig. 8. Influence of the age of mice at irradiation with  $\gamma$ -rays on the incidence of lung tumors. Data for the different dose groups were pooled.

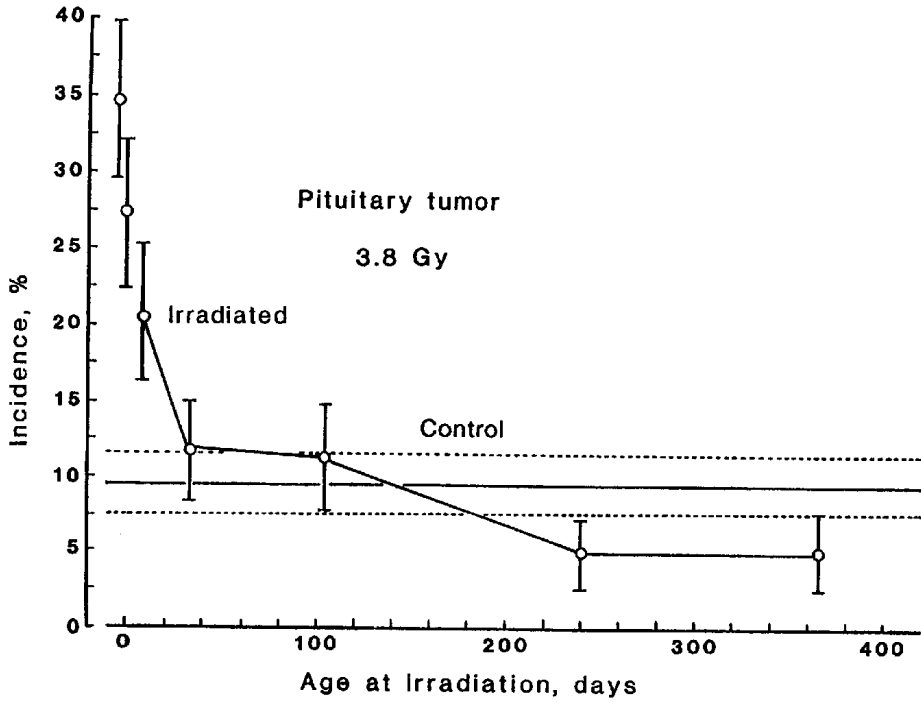


Fig. 9. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of pituitary tumors.

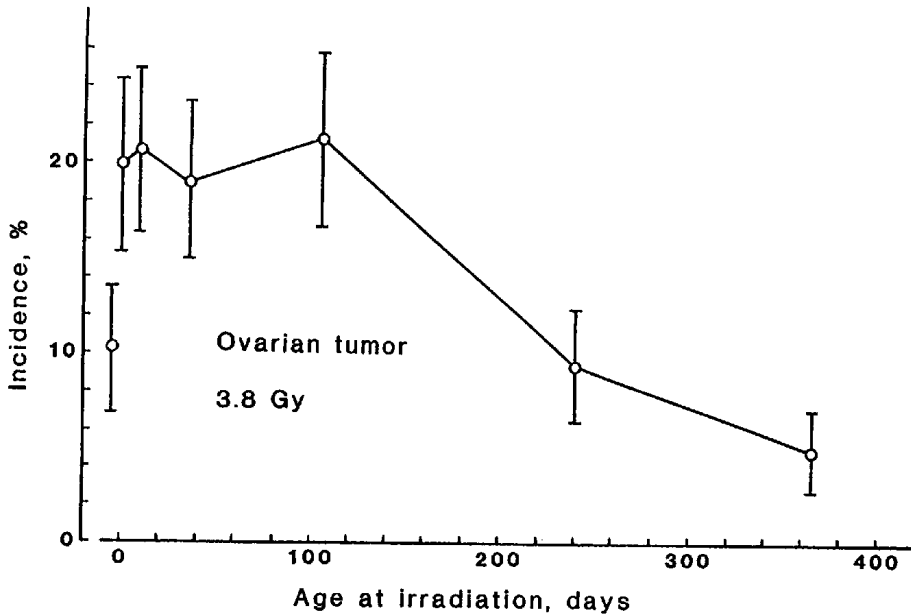


Fig. 10. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of ovarian tumors. The control incidence: 0.6% (2/332).

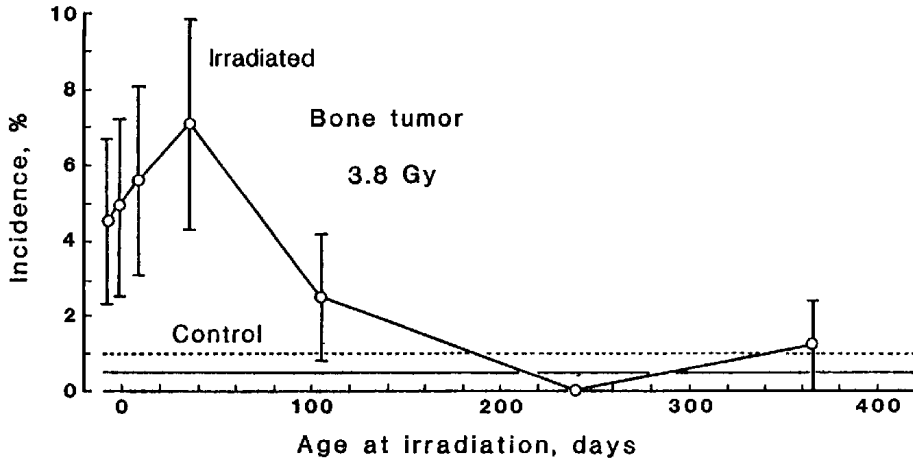


Fig. 11. Influence of the age of mice at irradiation with 3.8 Gy  $\gamma$ -rays on the incidence of bone tumors.

tumors appeared to decrease with age.

The incidences of bone tumors are plotted against the age at irradiation with 3.8 Gy Fig. 11. There is a tendency for an increase in incidence for irradiation during the late fetal, neonatal or juvenile period. The incidence of bone tumors did not increase after irradiation during adulthood.

## DISCUSSION

The age-specific mortalities of mice irradiated during the neonatal or juvenile period were higher throughout the entire lifespan than were those of mice irradiated during the fetal or adult period. This may be because of higher susceptibility to the induction of both the early-occurring neoplasms and the majority of the late-occurring neoplasms.

Results of the study reported here have confirmed and added to our previous findings that radiation exposure during the late fetal period resulted in the increase in incidences of various types of neoplasms (Sasaki *et al.* (1978)). It is now clear that mice in the late fetal period are susceptible to the induction of pituitary, liver, lung, ovarian and bone tumors and malignant lymphomas of the lymphocytic type.

The age-dependence of the susceptibility to tumor induction is specific for each type of neoplasm. This age-associated change in susceptibility is attributable to two major factors: (1) the proliferative activity of the target cells during or after (or both) exposure to radiation, and (2) the number of cells at risk. The active proliferation of hepatocytes (Sell *et al.* (1974)) and lung cells (Crocker *et al.* (1970)) during the early postnatal period supports this hypothesis. The susceptible ages for the induction of malignant lymphomas of

the lymphocytic type and bone tumors agree respectively with the rapid development of the immune and skeletal systems. The age-dependence of susceptibility to the induction of Harderian gland tumors may be related to proliferative activity and the number of cells at risk, but the proliferation kinetics of the cells in the Harderian gland has yet to be clarified. Why no myeloid leukemias were induced by irradiation during the late fetal or neonatal period is not known, but experimental results suggest that the hematopoietic cells in the liver of fetal and neonatal mice were not at risk for the induction of leukemias. The marked decrease in the incidence of malignant lymphomas of the histiocytic type owing to exposure to radiation during the fetal or neonatal period is interesting; this phenomenon also was found by Schmahl (1984). At this time, however, we are not able to speculate about the mechanism(s) that produces this phenomenon.

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