Temporal Variation of Excess Mortality Rate from Solid Tumors in Mice Irradiated at Various Ages with Gamma Rays

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Age-dependence/Excess mortality rate/Temporal variation/Solid tumors/Gamma ray-irradiated mice.

The influence of age at the time of irradiation on the lifetime risk for excess mortality from solid tumors, and on the temporal pattern of variation in the excess mortality rate, was analyzed using data obtained from a study of female B6C3F1 mice, which was conducted at the National Institute of Radiological Sciences, Chiba, Japan. Mice were irradiated with 1.9-Gy gamma rays at day 17 in intra-uterine age, or day 0, 7, 35, 105 or 365 in postnatal age. Control and irradiated mice were allowed to live out their entire life span under a specific pathogen-free condition. The primary cause of death for each mouse was determined by macroscopic and microscopic examination. The lifetime excess mortality from solid tumors was apparently higher in the mice irradiated during the neonatal to puberty period than in the mice irradiated during the intra-uterine or adult period. The median of time for manifestation of lifetime excess mortality since irradiation was shortest among mice exposed at 365 days of age and longest among mice exposed at 17 days of intra-uterine age. The excess mortality rate at any attained age was not independent of the age at irradiation. The excess mortality rate increased with increasing age, and the excess relative risk decreased with increasing age. The temporal variations of the excess mortality rate and background mortality rate were analyzed using the additive multi-stage model, which includes the assumptions that radiation-related carcinogenesis superimposes on background carcinogenesis, and that both radiation-related and background carcinogenesis involve multiple stages. The results of the analysis strongly suggested that the number of stages for manifestation of radiation-related carcinogenesis was less than that in background carcinogenesis for various types of solid tumors, and that the majority of stages were common in both radiation-related and background carcinogenesis. The additive multi-stage model well described the observed findings on the length of the latent period and temporal variations of the excess mortality rate and excess relative risk. It should be stressed that the magnitude of the lifetime risk was not only determined by a decrease in the number of hits for carcinogenesis but was also determined by another parameter which decides the initial value of excess mortality rate. Furthermore, we estimated the rate of decrease in the number of remaining hits for carcinogenesis, and it was found that the rate of decrease in the number of remaining hits was higher in several irradiated groups than that in the background carcinogenesis. However, radiation-induced genomic instability and/or delayed mutation may be of secondary importance when radiation was delivered promptly, because the present analysis revealed that the major action of radiation took place soon after irradiation, as one or more hits for transitions of stages for carcinogenesis.

INTRODUCTION

In previous reports we showed that mice of the ages from the neonatal to puberty period are most susceptible to the life-shortening effect of gamma rays.1–2) The age-depen-

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The influence of age at the time of irradiation with ionizing radiation on the temporal pattern of the age-specific mortality from solid cancer is an important problem with regard to the estimation of lifetime cancer risk. In particular, the lifetime risk for solid cancer after irradiation during the childhood period has not been estimated precisely, because a decrease in the relative risk with increasing age was found in several populations exposed as children, and the follow-up time is still not sufficient. Various risk models have been proposed to project the lifetime cancer risk from results of epidemiological investigations. Kellner and Barley proposed the age-attained model, in which the excess rate of solid cancer at any specific age does not depend on age at irradiation but depends only on the dose of radiation. Pierce and Mendelsohn also reported that the excess incidence rate of solid cancer except endocrine gland cancer seemed to be independent of age at exposure among atomic bomb survivors in Hiroshima and Nagasaki. Little et al. analyzed the temporal pattern of variation in the mortality rate from solid cancer using various risk models and noticed that the mortality rate was not independent of the age at exposure among atomic bomb survivors and other cohorts. Heidenreich et al. mentioned that the fitness of various risk models could not be discriminated even by data of atomic bomb survivors, which are the largest cohort of epidemiological study on the late effects of ionizing radiation. The first aim of the present study was to analyze whether the excess mortality rate attributable to solid tumors is independent of age at irradiation in a life span experiment using mice.

The persistent increase in the mortality rate from solid tumors after irradiation is a biologically interesting phenomenon. Armitage and Doll proposed the multi-stage model of carcinogenesis based on findings that the mortality rate from solid cancer increases as a power function of age. Mendelsohn and Pierce found that the excess incidence rate for solid cancer also increased as a power function of time after exposure in atomic bomb survivors. They proposed a multi-mutation model of carcinogenesis, in which radiation induces only one mutation within the multiple mutations necessary for manifestation of carcinogenesis. The second aim of the present study was to analyze the temporal variation of the excess mortality rate from solid tumors in mice irradiated at various ages.

In the first part of the present report, basic data on mean life span, lifetime excess relative risk for all causes of death, number of solid tumors per mouse at the time of death, incidence of each type of neoplasm, and incidence of each type of solid tumor as a primary cause of death are described in detail. In the second part, the influence of age at irradiation on the lifetime risk for mortality from solid tumors and on the temporal pattern of variation of the mortality rate attributable to solid tumors are analyzed. Finally, we consider the implications of the results of this study.

**MATERIALS AND METHODS**

**Methods for obtaining basic data for analysis**

For the present analysis we used data from a study of female B6C3F1 mice which was conducted at the National Institute of Radiological Sciences, Chiba, Japan. In a series of experiments, mice were irradiated with gamma rays from 137 Cs at day 17 of the intra-uterine period, or day 0, 7, 35, 105 or 365 of the postnatal period. All of the mice were allowed to live out their entire life span under a specific pathogen-free condition. The methods of the experiments have been described in previous reports. The observed incidences of neoplastic diseases including lethal and incidental diseases were compared among mice irradiated at different ages with 3.8-Gy gamma rays in a previous paper.

The main purpose of the present study was to analyze the influence of the age at irradiation on the lifetime excess mortality from solid tumors and on the temporal variation of the mortality rate from solid tumors. For this purpose, data of the dose level of 1.9 Gy were selected, because the incidences of thymic lymphoma and myeloid leukemia were low at the dose level of 1.9 Gy. The number of mice and the mean life span in the control and each irradiated group are summarized in Table 1. In order to obtain the mortality rate from solid tumors, the primary cause of death of each mouse was determined using gross and histopathological findings, which were maintained as a card system, photographs and/or computer files. In the first part of this report, the incidences of neoplastic diseases as the primary cause of death are described in detail. The mortality rate from solid tumors was represented as the number of deaths per 10,000 mouse-day at risk.

The mean and distribution of the number of types of solid tumors per mouse at death were also compared among groups irradiated at various ages, since an increase in the number of types of solid tumors at death has been shown to be a sensitive measure of radiation carcinogenesis. Differences in the number of types of solid tumors per mouse were examined with Student’s t-test, and the difference in tumor incidence was analyzed with C2 test or Fisher’s direct probability method.

**Methods of analysis**

A. Lifetime excess mortality from solid tumors

The lifetime excess mortality from solid tumors was defined as the fraction of deaths from solid tumors attributable to radiation exposure. Lifetime observed mortality from solid tumors in the irradiated group, Mobs(X), includes not only lifetime excess mortality from solid tumors, Mexc(X), but also lifetime background mortality from solid tumors, Mbg(X). Lifetime background mortality in the irradiated group was estimated by following equation:

\[ M_{bg}(X) = M_{obs}(X) - M_{exc}(X) \]
The temporal variation of the excess relative risk, $\text{ERR}(t)$, can be described by the following equation:

$$
\text{ERR}(t) = \text{ERR}(t_1)^{k(\text{exc})-k(\text{bg})},
$$

where $\text{ERR}(t_1)$ is excess relative risk at unit time $t_1$, and is given by $\text{ERR}(t_1) = \frac{a(\text{exc})}{a(\text{bg})}$.

The interval of age for examination can be set in any age interval, and in the present analysis the interval was set as the following: (1) The interval from the age at irradiation to the longest life span. (2) The interval from 300 days of age to the longest life span. Parameters $a(\text{exc})$, $a(\text{bg})$, $k(\text{exc})$ and $k(\text{bg})$ were estimated by regression analysis.

The following methods were used in order to analyze whether the rate of decrease in the number of remaining stages for manifestation of carcinogenesis was more rapid in the irradiated mice than in the control group. As described above, the value of parameter $k$ represents the number of remaining transitions to another stage for carcinogenesis at the start point of the interval for examination. By specifying the start point of the interval for examination, we obtained the temporal variation of parameter $k$ after irradiation. The end point of the duration for examination was always set as the age of the longest life span. A similar analysis was performed for the corresponding background mortality rate. The temporal variation was represented as a function of time after irradiation. Two functions were applied. One was the linear function: $k(T) = k(0) (1 – bT)$, where $k(T)$ and $k(0)$ represent the $k$ value at time $T$ after irradiation and that at time 0 after irradiation. The other was the exponential function: $k(T) = k(0) \text{exp}(-cT)$. Parameters $b$ and $c$ are rate coefficients of the decrease in the number of remaining hits for transitions for carcinogenesis. Fitness to the function was tested with Akaike’s information criterion.\textsuperscript{21)

**RESULTS**

1. Description of experimental data

1-1. Life-shortening and excess mortality from all causes

The sample size, mean life span, lifetime risk from all causes of death and temporal variation of the cumulative excess mortality are summarized in Table 1. The lifetime risk from all causes of death is an excellent comprehensive measure of the late effects of radiation, as shown in a previous report,\textsuperscript{5} and is expressed as the lifetime excess mortality and lifetime excess relative risk. The lifetime risk from all causes of death was calculated from the increase in the mortality rate, as shown in a recent paper.\textsuperscript{10} The lifetime excess mortality in a group irradiated at day 0 was 0.568, which implies that 56.8% of the mice died from causes attributable to radiation exposure. The lifetime risk of neonatal irradiation can be expressed by an alternative method so that the excess relative risk was 1.315. The lifetime excess mortality from all causes after irradiation at day 7 or 35 was not sta-
tistically different from that in mice irradiated at day 0. However, it was evident that irradiation at day 17 of intra-uterine age, or day 105 or 365 of postnatal age, was less effective in inducing the lifetime excess mortality from all causes than that at day 0. In Fig. 1, the lifetime excess relative risks for mortality rate from all causes are plotted against age at irradiation. These results show clearly that the magnitude of the lifetime excess mortality was not independent of age at irradiation.

1-2. Number of types of solid tumors at death

The mean number of types of solid tumors observed at autopsy in each group is summarized in Table 2. For example, when three hepatocellular tumors and two Harderian gland tumors were found in a mouse, the number of types of solid tumors was counted as two. The mean number of types of solid tumors in the control group was 0.540 and the standard error was 0.021. The mean numbers of types of solid tumors in all irradiated groups were significantly larger than that in the control group (P<0.05). In mice irradiated at day 0 of postnatal age, the mean number reached 1.468 with a standard error of 0.048. The number of types of solid tumors also increased remarkably after irradiation at day 7 and 35 of postnatal age. Mice of day 17 of intra-uterine age were found to be less susceptible in increasing number of solid tumors at death, but the mean was sufficiently larger than that in the control group. After irradiation at day 105 or 365, the number of types of solid tumors increased slight-
ly, with statistically significant difference from that in the control group.

The distributions of the numbers of types of solid tumors at death are shown in Table 2. We tested whether the number of types of solid tumors per mouse fit the Poisson distribution and found a significant lack of fit in groups irradiated at day 0, 7 and 35 (P < 0.01). This result indicated that there was not a homogeneous probability of multiple types of solid tumors, which suggests that there is a difference in susceptibility for induction among solid tumors. In Table 2, the numbers of types of solid tumors in mice that died at different ages are also summarized. An increasing trend in the number of types of solid tumors with increasing age at death was observed in the control and all irradiated groups. In a group irradiated at day 0, an increase in the number of types of solid tumors was detected in the mice that died between 51–450 days of age or later. After irradiation at day 7, 35 or 105, an increase in the number of types of solid tumors was first detectable in mice that died between 451–550 days of age. When mice were irradiated at day 17 of intrauterine age, a significant increase in number was found in the animals that died between 651–750 of age or later, which indicated that the latent period for excess occurrence of solid tumors after irradiation in utero was longer than those after irradiation during the neonatal, puberty or young adult periods. In a group irradiated at day 365, a tendency of increase in the number of types of solid tumors was observed in mice that died between 651–750 days of age and later.

These results showed that the mean number of types of solid tumors at the time of death is a sensitive measure of tumorigenic effect, and that the magnitude of the tumorigenic effect of radiation and the temporal pattern of manifestation are apparently dependent on the age at exposure.

### Table 2. Number of types of solid tumors per mouse at autopsy.

| Age at irradiation | Control | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
|--------------------|---------|----------------|-------|-------|--------|---------|---------|
| A. Mean number of tumors, ±SE* | 0.540±0.021 | 0.989±0.083 | 1.468±0.048 | 1.352±0.085 | 1.325±0.112 | 0.864±0.087 | 0.709±0.061 |
| B. Distribution of number of tumors per mouse | | | | | | | |
| n=0 | 0.531 | 0.269 | 0.113 | 0.132 | 0.200 | 0.358 | 0.433 |
| n=1 | 0.403 | 0.516 | 0.444 | 0.495 | 0.438 | 0.457 | 0.440 |
| n=2 | 0.060 | 0.183 | 0.323 | 0.330 | 0.225 | 0.148 | 0.113 |
| n=3 | 0.006 | 0.022 | 0.105 | 0.077 | 0.113 | 0.037 | 0.014 |
| n=4 | 0.000 | 0.011 | 0.016 | 0.000 | 0.025 | 0.000 | 0.000 |
| Variance | 0.402 | 0.634 | 0.781 | 0.651 | 0.994 | 0.636 | 0.518 |
| C. Number of tumors in mice died during age (days): | | | | | | | |
| 51–450 | 0.667±0.157 | | | | | | |
| 451–550 | 0.278±0.106 | 1.000±0.283 | 0.692±0.200 | 0.714±0.171 | 0.750±0.217 |
| 551–650 | 0.253±0.073 | 0.400±0.219 | 1.129±0.127 | 1.143±0.223 | 1.200±0.438 | 1.000±0.286 | 0.333±0.111 |
| 651–750 | 0.304±0.046 | 1.000±0.163 | 1.464±0.121 | 1.350±0.177 | 1.091±0.169 | 0.813±0.182 | 0.514±0.102 |
| 751–850 | 0.490±0.038 | 1.069±0.161 | 1.659±0.096 | 1.360±0.148 | 1.261±0.176 | 0.858±0.131 | 0.694±0.103 |
| 851–950 | 0.603±0.041 | 1.039±0.139 | 1.651±0.118 | 1.824±0.190 | 1.923±0.288 | 0.938±0.125 | 0.968±0.148 |
| 951–1050 | 0.689±0.056 | 1.143±0.245 | 1.833±0.285 | 1.000±0.236 | 2.167±0.281 | 1.400±0.253 | 0.944±0.161 |
| >1050 | 0.693±0.079 | | | | | | |

*SE: standard error
after irradiation at day 0, 7 and 35. In particular, the increase in incidence was remarkable in mice irradiated at day 0 and 7. Liver tumors were the most frequent cause of death in these groups. The increase in the incidence of liver tumors was not detected in mice irradiated in adulthood. The incidence of liver tumors was not statistically different from that in the control in a group irradiated at day 17 of intra-uterine age with 1.9 Gy in the present experiment, but liver tumors developed in excess after irradiation with higher doses at the same age in the B6C3F1 and B6WF1 strains.

Development of pituitary tumors seemed to be lethal in mice. The evagination of the skull and a remarkably emaciated body were characteristic features of the occurrence of this tumor. The highest incidence of pituitary tumors was found in mice irradiated at day 17 of intra-uterine age. Statistically significant increase in incidence was also detected.

### Table 3. Incidences of neoplasms (%) in mice irradiated with 1.9-Gy gamma rays at various ages.

| Age at irradiation | Control | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
|--------------------|---------|-----------------|------|-------|-------|--------|--------|
| A. Solid tumors    |         |                 |      |       |       |        |        |
| Liver tumor        | 19.7±1.2| 22.6±4.3        | 53.8±3.1 | 54.9±5.2 | 31.3±5.2 | 17.3±4.2 | 22.7±3.5 |
| Pituitary tumor    | 8.0±1.0 | 31.2±4.8        | 25.0±2.4 | 22.0±4.3 | 8.8±3.2 | 7.4±2.9 | 4.3±1.7 |
| Ovarian tumor      | 1.8±0.5 | 7.5±2.7         | 33.7±3.0 | 34.1±5.0 | 38.8±5.4 | 24.7±4.8 | 7.1±2.2 |
| Harderian gland tumor | 1.1±0.4 | 1.1±1.0         | 0.3±0.3 | 5.5±2.4 | 17.5±4.2 | 12.3±3.6 | 1.4±1.0 |
| Bone tumor         | 0.8±0.3 | 2.2±1.5         | 3.0±0.9 | 2.2±1.5 | 2.5±1.7 | 0.0±0.0 | 0.0±0.0 |
| Lung tumor         | 5.9±0.8 | 11.8±3.3        | 16.2±2.3 | 12.1±3.4 | 8.8±3.2 | 4.9±2.4 | 9.2±2.4 |
| Gastro-intestinal tumor | 0.6±0.3 | 2.2±1.5         | 1.8±0.7 | 0.0±0.0 | 1.3±1.3 | 1.2±1.2 | 3.5±1.5 |
| Renal tumor        | 0.0±0.0 | 0.0±0.0         | 0.6±0.4 | 1.1±1.1 | 1.3±1.3 | 0.0±0.0 | 0.0±0.0 |
| Adrenal tumor      | 0.1±0.1 | 1.1±1.1         | 1.5±0.7 | 2.2±1.5 | 1.3±1.3 | 1.2±1.2 | 0.0±0.0 |
| Splenic hemangioma | 0.3±0.2 | 1.1±1.1         | 1.5±0.7 | 0.0±0.0 | 1.3±1.3 | 0.0±0.0 | 0.7±0.7 |
| Other solid tumors | 16.8±1.3| 17.2±3.9        | 6.1±1.3 | 14.3±3.7 | 22.6±4.7 | 19.7±4.4 | 14.2±2.9 |
| B. Lympho-hematopoietic tissue neoplasms |         |                 |      |       |       |        |        |
| Myeloid leukemia   | 0.0±0.0 | 0.0±0.0         | 0.0±0.0 | 0.0±0.0 | 2.5±1.7 | 4.9±2.4 | 1.4±1.0 |
| Malignant lymphoma, lymphocytic | 0.3±0.2 | 0.0±0.0         | 0.3±0.3 | 2.2±1.5 | 3.8±2.1 | 1.2±1.2 | 0.7±0.7 |
| Malignant lymphoma, histiocytic | 53.8±1.7| 33.3±4.9        | 23.4±2.3 | 31.1±4.9 | 32.3±5.0 | 56.8±5.5 | 45.4±2.4 |
|                    | (52.8±1.7) | (28.0±4.7) | (18.5±2.1) | (19.7±4.3) | (22.0±4.2) | (47.4±5.4) | (42.6±4.2) |
in groups irradiated at day 0 and 7. Irradiation at day 35 or later was not effective in increasing the incidence of pituitary tumors.

Ovarian tumors developed in excess, with statistically significant difference in all irradiated groups examined here. The highest incidence of ovarian tumors was found in mice irradiated at day 35. Mice of day 17 of intra-uterine age and day 365 of postnatal age were apparently less susceptible to increase the incidence of ovarian tumors by radiation exposure. A majority of the ovarian tumors seemed to be non-lethal, but a fraction of them were recognized as the primary cause of death.

The incidence of Harderian gland tumors did not increase after irradiation at day 17 of intra-uterine age or day 0 of postnatal age. A distinct increase in the incidence of Harderian gland tumors was observed in mice irradiated at day 35 and 105. A slight but significant increase in the incidence of Harderian gland tumors was detected in a group exposed at day 7 of postnatal age. When mice were irradiated at day 365 with 1.9-Gy gamma rays, Harderian gland tumors did not occur in excess. As shown in previous papers,2–3) the incidence of Harderian gland tumors increased significantly after irradiation with 3.8-Gy gamma rays at day 365 in the same hybrid mice. A fraction of the Harderian gland tumors were recognized as the primary cause of death, as multiple metastatic nodules were observed in the lung in several cases.

Bone tumors developed at low incidences in the control and irradiated groups. The majority of the bone tumors were histologically shown to be osteosarcoma, and massive metastasis was frequently observed. A tendency of increase in the incidence of bone tumors was found in mice irradiated at day 17 of intra-uterine age, or day 0, 7 or 35 of postnatal age, but statistically significant increase was detected in only one group irradiated at day 0.

A significant increase in the incidence of lung tumors was observed in mice irradiated at day 0 of the neonatal period. Although a tendency of increase in the incidence of lung tumors was found after irradiation at day 17 of intra-uterine age, or day 7 or 35 of postnatal age, the increase was not statistically significant.

In a group irradiated at day 0, the incidences of adrenal

| Table 4. Temporal variation of the cumulative observed mortality and the mortality rate from solid tumors. |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age at irradiation                               | Control         | Day 17 IU       | Day 0           | Day 7           | Day 35          | Day 105         |
| Age (days)                                       |                 |                 |                 |                 |                 |                 |
| A. Cumulative observed mortality                 |                 |                 |                 |                 |                 |                 |
| 450                                             | 0.002±0.002     | 0.024±0.008     | 0.033±0.019     | 0.050±0.024     | 0.012±0.012     |                 |
| 550                                             | 0.007±0.003     | 0.049±0.011     | 0.077±0.029     | 0.088±0.032     | 0.049±0.024     |                 |
| 650                                             | 0.016±0.004     | 0.022±0.015     | 0.142±0.019     | 0.198±0.044     | 0.212±0.049     | 0.099±0.034     |
| 750                                             | 0.045±0.007     | 0.150±0.038     | 0.327±0.028     | 0.374±0.059     | 0.337±0.061     | 0.185±0.046     |
| 850                                             | 0.145±0.012     | 0.366±0.056     | 0.597±0.036     | 0.560±0.070     | 0.512±0.074     | 0.247±0.053     |
| 950                                             | 0.256±0.016     | 0.559±0.067     | 0.751±0.040     | 0.726±0.077     | 0.637±0.079     | 0.366±0.062     |
| 1050                                            | 0.344±0.018     | 0.656±0.072     | 0.791±0.041     | 0.781±0.079     | 0.700±0.083     | 0.432±0.067     |
| 1150                                            | 0.368±0.019     | 0.666±0.072     | 0.799±0.042     | 0.712±0.083     | 0.456±0.068     | 0.440±0.050     |
| 1250                                            | 0.372±0.019     |                 |                 |                 |                 |                 |
| B. Mortality rate*                               | 0.11±0.08       | 0.00±0.00       | 1.23±0.40       | 1.68±0.95       | 2.55±1.24       | 0.63±0.62       | 0.00±0.00       |
| 451–550                                         | 0.46±0.23       | 0.00±0.00       | 2.56±0.83       | 4.79±2.34       | 4.05±2.29       | 3.92±2.22       | 0.00±0.00       |
| 551–650                                         | 0.94±0.33       | 2.25±1.57       | 10.7±1.7        | 14.4±4.1        | 14.6±3.3        | 5.48±2.67       | 4.46±1.78       |
| 651–750                                         | 3.31±0.64       | 15.2±4.1        | 26.5±2.8        | 27.2±6.0        | 18.0±5.3        | 11.2±4.0        | 11.5±3.2        |
| 751–850                                         | 14.3±1.4        | 33.5±6.4        | 69.1±5.6        | 48.0±9.8        | 47.1±11.1       | 22.6±5.4        | 21.2±5.1        |
| 851–950                                         | 27.3±2.5        | 59.7±11.1       | 125±13          | 111±22          | 66.7±16.0       | 46.2±14.8       | 45.7±9.0        |
| 951–1050                                        | 51.4±5.1        | 121±33          | 180±42          | 278±88          | 187±73          | 101±30          | 105±29          |
| 1051–1150                                       | 57.7±11.9       | 143±66          |                 |                 |                 |                 |                 |

* Number of deaths per 10,000 mouse-day at risk
tumors, gastro-intestinal tumors and splenic hemangioma increased with statistically significant difference. A slight but significant increase in the incidence of gastro-intestinal tumors was detected in mice irradiated at day 365.

In the category of “other solid tumors”, soft part tumors, mammary tumors, uterine tumors, thyroid tumors and tumors of the nervous system were included. Significant increase in the incidences of these tumors was not found after irradiation in the present study.

Female B6C3F1 mice seemed to have low susceptibility for induction of lympho-hematopoietic tissue neoplasms. The incidences of myeloid leukemia increased significantly after irradiation at day 35, 105 or 365 of postnatal age. On the other hand, irradiation at day 17 of intra-uterine age, or day 0 or 7 of postnatal age was not effective in inducing myeloid leukemia. Statistically significant increase in the incidence of malignant lymphoma of the lymphocytic type was found only in a group irradiated at day 35 in the present study. As shown in a previous report, malignant lymphoma of the lymphocytic type developed at high incidences after irradiation at day 35, 105 or 365 of postnatal age. On the other hand, irradiation at day 17 of intra-uterine age, a distinct increase in the lifetime cumulative mortality from solid tumors was detected. The lifetime cumulative mortality from solid tumors also increased after irradiation during the adulthood period, but was apparently less than that in groups exposed at earlier ages. It was evident that deaths from solid tumors occurred earlier in mice irradiated during the neonatal, puberty or young adult periods than in the control group.

Female B6C3F1 mice seemed to have low susceptibility for induction of lympho-hematopoietic tissue neoplasms. The primary cause of death for each mouse was determined, as described above, and we obtained the cumulative mortality and mortality rate from solid tumors in the control and irradiated groups (Table 4). It is notable that the lifetime cumulative mortality from solid tumors was remarkably high in mice irradiated at day 0, 7 or 35 of postnatal age. After irradiation at day 17 of intra-uterine age, a distinct increase in the lifetime cumulative mortality from solid tumors was detected. The lifetime cumulative mortality from solid tumors also increased after irradiation during the adulthood period, but was apparently less than that in groups exposed at earlier ages. It was evident that deaths from solid tumors occurred earlier in mice irradiated during the neonatal, puberty or young adult periods than in the control group. The cumulative mortality from solid tumors in the irradiated group includes the spontaneous mortality from solid tumors, as described in the section on the analysis of the experimental data.

The mortality rates from solid tumors in the control and irradiated groups are summarized in Table 4. The mortality rate is expressed as the number of deaths per 10,000 mouse-day at risk for each age interval. The mortality rate is the most reliable measure of the late effects of radiation, because the mortality rate is not confounded by competing risks. The mortality rate and its derivatives, therefore, were.

### Table 5. Temporal variation of the excess mortality rate and excess relative risk.

| Age at irradiation | Age (days) | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
|--------------------|-----------|----------------|-------|-------|--------|---------|---------|
| A. Excess mortality rate* |
| 251–450           | 2.10±0.86 | 1.56±0.96      | 3.59±2.30 | 4.33±2.35 | 4.54±2.65 | 3.46±2.21 |
| 451–550           | 1.31±1.61 | 9.73±1.73      | 13.5±4.1 | 13.7±4.3 | 4.54±2.65 | 3.52±1.81 |
| 551–650           | 11.9±4.1  | 23.2±2.8       | 23.9±6.1 | 14.7±5.3 | 7.89±3.97 | 8.17±3.23 |
| 651–750           | 19.2±6.6  | 54.8±5.7       | 33.7±9.9 | 32.8±11.0 | 7.90±4.05 | 6.95±5.25 |
| 751–850           | 32.5±11.4 | 97.8±13.5      | 83.5±21.8 | 39.4±16.2 | 18.9±14.6 | 18.4±9.3 |
| 851–950           | 69.8±33.7 | 129±42         | 226±88 | 136±74 | 49.9±29.5 | 53.9±29.3 |
| 951–1050          | 10.83±7.52 | 14.79±13.41   | 22.54±19.34 | 8.82±6.66 | 8.55±6.45 |
| B. Excess relative risk |
| 251–450           | 5.58±3.01 | 10.44±7.29     | 8.82±6.66 | 3.39±1.38 | 3.37±1.17 |
| 451–550           | 2.40±1.88 | 11.39±3.91     | 15.40±6.94 | 15.63±7.16 | 5.85±3.52 | 4.76±2.53 |
| 551–650           | 4.61±1.52 | 8.00±1.63      | 8.22±2.42 | 5.44±1.91 | 3.39±1.38 | 3.47±1.17 |
| 651–750           | 2.34±0.51 | 4.84±0.59      | 3.65±0.77 | 3.30±0.84 | 3.38±1.37 | 3.47±1.18 |
| 751–850           | 2.19±0.46 | 4.59±0.63      | 4.06±0.88 | 2.45±0.63 | 1.69±0.56 | 1.68±0.36 |
| 851–950           | 2.36±0.69 | 3.51±0.90      | 5.40±1.79 | 3.65±1.47 | 1.97±0.61 | 2.05±0.60 |
| 951–1050          | 1.47±1.97 | 3.47±1.17      | 3.65±1.47 | 1.97±0.61 | 2.05±0.60 |

* Number of deaths per 10,000 mouse-day at risk.
used in the analysis of experimental data, which is described below. The excess mortality rates from solid tumors in the irradiated groups are given in Table 5. The excess mortality rate increased with increasing age, as was the case with the mortality rate from solid tumors. The following three characteristics in these results should be noticed. (1) The excess mortality rate from solid tumors at any specific age was not independent of the age at irradiation. (2) The excess mortality rates in mice irradiated at day 17 of intra-uterine age were lower than those after irradiation at day 0, 7 or 35 of postnatal age. (3) The excess mortality rate at each specific attained age in mice irradiated at day 105 was virtually similar to that in a group irradiated at day 365. The temporal variation in excess relative risk is summarized in Table 5. A tendency of decrease in the excess relative risk with increasing attained age was recognized in all irradiated groups examined here.

2. Analysis of experimental data

2-1 Lifetime excess mortality, excess relative risk and attributable risk

The expected mortality in each irradiated group was calculated using mouse-day in the irradiated group and the mortality rate in the control group, and we obtained the lifetime excess mortality, excess relative risk and attributable risk (Table 6). In epidemiological studies, the lifetime risks are usually estimated with projection models. However, the lifetime risks can be obtained directly from experimental data in life-span animal studies, as shown here. In a group irradiated at day 0 of postnatal age, the lifetime excess mortality from solid tumors was a very high value of 0.659. The lifetime excess relative risk, which is an alternative measure for the expression of the lifetime risk, was 4.72. The attributable risk was 82.5% in this group. These three measures were used to express the lifetime risk of radiation. This result clearly showed that mice of the neonatal period are highly susceptible for the increase in mortality rate by gamma irradiation. The lifetime risks for mortality from solid tumors after irradiation at day 7 or 35 were also high values, and were not significantly different from that in the neonatally irradiated group. The lifetime risk for mortality from solid tumors in mice irradiated at day 17 of prenatal period was a considerably high value, but was apparently lower than that in mice irradiated in the neonatal period. The lifetime risks in mice irradiated in adulthood were lower that in the neonatally irradiated group, but it should be noted that the attributable risk reached about 50% in these groups, as shown in Table 6.

In Fig. 2, the excess relative risks from solid tumors are plotted against age at irradiation. The age-dependence of susceptibility for the lifetime risk from radiation-related solid tumors was nearly similar to that for the lifetime risk from radiation-related all causes of death shown in Fig. 1. However, the nominal values of the excess relative risks for solid tumors were higher than those for all causes of death. The high susceptibility of mice during the neonatal to puberty period can be seen in this figure. The excess relative risk for solid tumors after irradiation at day 17 of intra-uterine period was higher than that in mice irradiated during adulthood period. On the other hand, the excess relative risk for all causes of death was lower in mice irradiated during intra-uterine period, as shown in Fig. 1.

2-2 Mean age at death and median time for manifestation of excess mortality from solid tumors

The mean ages at death from solid tumors in the control...
and irradiated groups are summarized, together with their standard errors, in Table 7. In all groups, the mean ages at death of the mice that died from solid tumors were slightly longer than those that died from all causes, as shown in Table 1. The mean life spans of the mice that died from solid tumors in all irradiated groups were significantly shorter than that of the control group. The mean life span was shortest in a group irradiated at day 7 and longest in mice irradiated at day 17 of intra-uterine period. The mean life span of the mice that died from solid tumors after irradiation at day 365 was fairly long.

The excess mortality from solid tumors was calculated using equation 1 as described in Materials and Methods. The cumulative distributions of the excess mortality from solid tumors in all irradiated groups are shown in Table 8. The medians of the time for manifestation of the excess mortality were estimated using equation 2 and are shown in Table 7. Interestingly, the median of the time for manifestation of the excess mortality was shortest in the group irradiated at day 365 (Tmed = 445.2 days) and longest after irradiation at day 17 of intra-uterine period (Tmed = 832.9 days). There was a tendency for the median of the time for manifestation of the excess mortality to decrease with increasing age at the time of irradiation. This tendency is clearly shown in Fig. 3, in which the cumulative excess mortalities are plotted against time after irradiation. The attained age at the median time for manifestation of the excess mortality in each irradiated group is also shown in Table 7. Ages at the median of the time for manifestation among groups irradiated at different ages was apparently narrower than that of the medians of the time for manifestation since irradiation, as shown in Fig. 4 in which the cumulative excess mortalities are plotted against attained age.

### 2-3 Fitting the additive multi-stage model to temporal variation of the excess mortality rate

Equations 3 and 4 were fitted to the temporal variation of the excess mortality rate and background mortality rate, and values of the parameters were estimated by regression analysis. First, we will describe the results of analysis when the interval of age for examination was set as a whole interval of the life span after irradiation. The slope of the regression line in log-log plotting (Fig. 5) implies the parameter $k$ value. In a group irradiated at day 0, for example, parameter $k$ was estimated to be 5.01. As shown in Fig. 5-A, the slope

### Table 7. Mean age at death from solid tumors and median of time for manifestation of excess mortality from solid tumors.

| Age at irradiation | Control | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
|--------------------|---------|-----------------|------|------|-------|--------|--------|
| A. Mean age at death, days | 882.4±7.1 | 849.9±13.3 | 773.8±8.2 | 744.9±18.2 | 786.8±20.5 | 796.7±31.0 | 838.8±15.2 |
| B. Median time for excess mortality manifestation | | | | | | |
| Days after irradiation | 832.9 | 762.9 | 732.5 | 688.5 | 648.4 | 445.2 |
| Attained age, days | 830.9 | 762.9 | 739.5 | 723.5 | 753.4 | 810.2 |
| B. Parameters of regression curve | | | | | | |
| Parameter $p$ | 12.362 | 10.332 | 7.914 | 6.490 | 4.631 | 4.949 |
| Parameter $q$ | 1.488 | 1.354 | 1.080 | 0.943 | 0.714 | 1.112 |

### Table 8. Temporal variation of the cumulative excess mortality from solid tumors.

| Age | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
|-----|-----------------|------|------|-------|--------|--------|
| 450 | 0.022±0.008 | 0.031±0.019 | 0.048±0.024 | 0.010±0.012 | |
| 550 | 0.042±0.011 | 0.071±0.029 | 0.081±0.032 | 0.043±0.024 | |
| 650 | 0.006±0.016 | 0.127±0.019 | 0.183±0.045 | 0.198±0.049 | 0.084±0.034 | 0.027±0.018 |
| 750 | 0.106±0.038 | 0.289±0.028 | 0.338±0.059 | 0.300±0.061 | 0.145±0.047 | 0.088±0.030 |
| 850 | 0.230±0.057 | 0.504±0.037 | 0.469±0.071 | 0.422±0.074 | 0.136±0.054 | 0.122±0.040 |
| 950 | 0.335±0.068 | 0.623±0.041 | 0.594±0.078 | 0.495±0.080 | 0.177±0.063 | 0.174±0.047 |
| 1050 | 0.390±0.073 | 0.652±0.042 | 0.638±0.080 | 0.541±0.084 | 0.219±0.068 | 0.210±0.051 |
| 1150 | 0.399±0.073 | 0.659±0.042 | 0.553±0.084 | 0.239±0.069 | 0.216±0.052 |
of the regression line for the group irradiated at day 0 was less than that for the corresponding background mortality rate. The estimate of the k value in the control group during the age from day 0 to the longest life span was 6.11 (Table 9). The difference between k(bg) and k(exc) was 1.10. The value of a(exc) in the group irradiated at day 0 was 13.5 deaths per 10^6 mouse-day at risk, which implies an estimate of excess mortality rate at 100 days since irradiation. The value of a(bg) for the corresponding background mortality rate was 0.32 deaths per 10^6 mouse-day at risk, which indicates an estimate of the mortality rate at 100 days of age in the control group. The ratio of a(exc) to a(bg) was 42.2, which is the estimate of the excess relative risk at 100 days after irradiation at day 0, although an increase in the mortality rate from solid tumors is usually not detectable in experimental studies using mice. Thus, the temporal variation of the excess mortality rate after irradiation at day 0 was estimated to be described as 13.5T^{0.01}, and that of the corresponding background mortality rate as 0.32T^{0.11}. The temporal variation of the excess relative risk was estimated to be 42.2T^{-1.10}, which is linear in log-log plotting. The temporal variations of the excess mortality rate and corresponding background mortality rate in groups irradiated at day 0, 35, 105 and 365 are shown in Fig. 5. The estimated values of parameters in all groups are summarized in Table 9. The value of parameter k decreased with increasing age at irradiation for both the excess and background mortality rate. In contrast, the values of parameters a(exc) and a(bg) increased with increasing age at irradiation. Parameter k(exc) was less than parameter k(bg) in 5 out of the 6 irradiated groups examined here. The difference between k(bg) and k(exc) was not independent of the age at irradiation. This difference was largest in a group irradiated at day 35 (2.53). These results of analysis imply that the number of stages for manifestation of radiation-related carcinogenesis was less than that of background carcinogenesis in 5 of the irradiated groups. As an exception, in a group irradiated at day 17 of intra-uterine age, k(exc) was larger than corresponding k(bg). This result implies that the number of hits for transitions of stage for manifestation of radiation-related carcinogenesis in this group was larger than that for spontaneous carcinogenesis. Because this result seemed to be unreasonable at first appearance, further analysis was performed. As shown in Table 3, the increase in the mortality rate after irradiation at day 17 of intra-uterine age was largely attributable to the occurrence of pituitary tumors. The estimate of k(exc) in the excess pituitary tumorigenesis in the fetally irradiated group was 7.09, and was less than that in the background
pituitary tumorigenesis (k(bg) = 9.37). Therefore, result of the analysis mentioned above was not unreasonable. It should be noted that the radiation-related carcinogenesis was qualitatively and quantitatively different from the background carcinogenesis and that there were same differences in the radiation-related carcinogenesis among the irradiated groups. Therefore, the estimated values of k(exc) and k(bg) were averages of values for various types of solid tumors involved in radiation-related and background carcinogenesis. In spite of this limitation, results of the analysis strongly suggested that the number of hits for transitions of stages for manifestation of radiation-related carcinogenesis of specific neoplasm was generally less than that of spontaneous carcinogenesis. In order to verify this conclusion, we must analyze the data on the various types of solid tumors. These results will be shown in consecutive reports. However, we show here another result of the analysis on the specific neoplasm. The estimate of the number of hits for transitions of stages for manifestation of the radiation-related liver tumorigenesis after irradiation at day 0 was 6.12, and that for the background liver tumorigenesis was estimated to be 7.61.

The ratio of parameter a(exc) to a(bg) was apparently not independent of the age at irradiation. The ratio was a high value, 496, in mice irradiated at day 35, whereas the ratio was 0.60 after irradiation at day 17 of intra-uterine age. Generally speaking, a large value of the ratio of parameter a(exc) to a(bg) shows that the excess relative risk is high during the early time after irradiation, and a high value of difference between parameter k(bg) and k(exc) shows that the excess relative risk decreases rapidly with increasing time after irradiation. In a group irradiated at day 35, the excess relative risk was indeed high during the early ages and decreased rapidly with increasing age, as shown previously in Table 5. The lifetime excess relative risk in the mice irradiated at day 35 was not larger than that in mice irradiated at day 0, in which the ratio of a(exc) to a(bg) was fairly large and the difference between k(bg) and k(exc) was relatively small. In contrast, the mortality rate increased after a very long latent period in mice irradiated at day 17 of intra-uterine age, but the lifetime risk in this group was larger than that in groups irradiated during adulthood period. These results showed that the overall susceptibility for radiation-related carcino-

![Fig. 5. Temporal variation of excess mortality rate (EX) and background mortality rate (BG) from solid tumors after irradiation with 1.9-Gy gamma rays at day 0, 35, 105 or 365 of postnatal age.](https://academic.oup.com/jrr/article-abstract/46/1/1/927081)
genesis was determined by both parameters of the multi-stage model that were dependent on the age at the time of irradiation.

Table 10 summarizes the parameters of the multi-stage model applied to the temporal variation of the excess and background mortality rates during the interval from 500 days of age to the longest life span. The value of parameter $k(bg)$ was 2.55, which is an estimate of the number of remaining hits for transitions of stage for manifestation of carcinogenesis at 500 days of age in the control group. Parameters $a(exc)$ and $a(bg)$ are estimates of the excess and background mortality rates at 600 days of age (100 days after start point of the interval of the age for examination). The estimates of the excess relative risk at 600 days of age consisted well with the observed values (Table 5). The least value of parameter $k(exc)$ was also found in a group irradiated at day 35 ($k(exc) = 1.27$), and the largest value of $k(exc)$ was 2.33 in mice irradiated at day 17 of intra-uterine age.

The present analysis showed that the influence of age at the time of irradiation on the magnitude of increase in mortality rate and on the temporal variation of the mortality rate during every optional interval of age can be described using parameters of the multi-stage model of carcinogenesis. It should be stressed that the difference between the number of hits for transitions of stage for manifestation of radiation-related carcinogenesis and that for spontaneous carcinogenesis did not correspond to the magnitude of the lifetime risk of radiation.

The rate of decrease in the remaining number of hits for transitions of stages for manifestation of radiation-related carcinogenesis was estimated and compared with that for the corresponding background carcinogenesis. The number of remaining hits for manifestation was estimated by specifying the start point of the age interval for examination, in which the end point was always set as the age of the longest life span.

First, we will show results of the analysis on the background carcinogenesis. When start of the interval was set as the time of fertilization, parameter $k(bg)$ was estimated to be 6.29, which implies that the mean number of hits for the manifestation of solid tumors in the control group was 6.29. The remaining number of hits for manifestation at day 0,

| Age at irradiation | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
|--------------------|-----------------|-------|-------|--------|---------|---------|
| Parameter $k(exc)$ | 7.15            | 5.01  | 4.57  | 3.43   | 3.15    | 2.43    |
| Parameter $a(exc)^*$| 0.18            | 13.5  | 37.3  | 311    | 352     | 3698    |
| Parameter $k(bg)$  | 6.13            | 6.11  | 6.04  | 5.97   | 5.04    | 3.29    |
| Parameter $a(bg)^*$| 0.30            | 0.32  | 0.38  | 0.63   | 6.31    | 993     |
| $k(exc) - k(bg)$   | 1.02            | -1.10 | -1.47 | -2.54  | -1.89   | -0.86   |
| $a(exc) / a(bg)$   | 0.60            | 42.2  | 98.2  | 494    | 55.8    | 3.7     |

* Number of deaths per 1,000,000 mouse-day at risk

Table 10. Parameters of the additive multi-stage model fitted to temporal variation of excess and background mortality rates during interval from 500 days of age to the longest life span.

| Age at irradiation |
|--------------------|-----------------|-------|-------|--------|---------|---------|
| Background         | Day 17 in utero | Day 0 | Day 7 | Day 35 | Day 105 | Day 365 |
| Parameter $k$      | 2.55            | 2.33  | 1.66  | 1.61   | 1.27    | 1.37    | 1.46    |
| Parameter $a^*$    | 0.79            | 1.58  | 8.84  | 9.87   | 9.57    | 3.83    | 2.83    |
| $k(exc) - k(bg)$   | -0.22           | -0.89 | -0.94 | -1.28  | -1.18   | -1.09   |
| $a(exc) / a(bg)$   | 2.00            | 11.19 | 12.49 | 12.11  | 4.85    | 3.58    |

* Number of deaths per 10,000 mouse-day at risk

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365, 500 and 600 was estimated to be 6.11, 3.29, 2.55 and 1.96, respectively (Tables 9, 10). The linear and exponential functions were fitted to the decrease in the remaining number of hits with increasing the age, and regression coefficients (parameters b and c) were estimated. Results of the analysis (Table 11) showed that the remaining number of hits decreased exponentially with age rather than linearly, although the difference in goodness of fit was not so large.

The remaining number of hits for manifestation of radiation-related carcinogenesis decreased exponentially with age after irradiation at day 7, 35, 105 or 365, but decreased linearly with age after irradiation at day 17 of intra-uterine period or day 0 of postnatal period (Table 11). A remarkable enhancement of the rate of the exponential decrease in the number of remaining hits was found in a group irradiated at day 365. The significant increase in rate of exponential decrease was also detected in groups irradiated at day 7 and day 105 of postnatal age. The rate coefficient of exponential decrease in the number of remaining hits for manifestation of radiation-related carcinogenesis after irradiation at day 35 was not different from that in background carcinogenesis. The rate of decrease in the fatally irradiated group was less than that in the background carcinogenesis, but it should be noted that the radiation-related carcinogenesis in this group was largely attributable to pituitary tumors, as mentioned above.

**DISCUSSION**

In a previous report we described a method of analysis of the lifetime risk for all causes of death after irradiation.\(^5\) Method in the present study of the lifetime risk for mortality from solid tumors is essentially same. Measures of the lifetime risk are the cumulative excess relative risk, cumulative mortality and attributable risk (Table 6). These measures of the lifetime risk are not confounded by competing risks. This plain method may be widely applicable for analysis of data obtained from the animal experiments, and facilitates comparison with results of the epidemiological investigations. In the analysis of results of a large-scale experiment using RFM strain of mice which was conducted at Oak Ridge National Laboratory, Ullrich and Storer (1979) used direct age-adjustment procedures to adjust the incidence values to those that presumably would have been observed had all the groups shown the same distribution of ages at death or similar.
ilar days at risk as a common reference population.\textsuperscript{23–24)} Age-adjusted incidences of the lethal neoplasms were estimated using mortality rates and those of the incidental neoplasms were obtained using prevalence rates on an assumption mentioned above. Myeloid leukemia and thymic lymphoma were classified as lethal neoplasms, and all solid tumors and reticulum cell sarcoma were classified as incidental neoplasms. It seemed to be uncertain whether their assumption and classification were reasonable. In the procedure of present analysis, number of deaths attributable to irradiation was estimated using mortality rates among irradiated and control mice. Substantial fraction of the solid tumors was recognized to be lethal in B6C3F\textsubscript{1} mice (Table 3). Furthermore, we analyzed the temporal variation of mortality rates which offered valuable informations.

The present study showed that the excess mortality rate from solid tumors increased with age and that the excess relative risk decreased with age. These temporal variations are not consistent with the constant relative risk model or the constant absolute risk model. The additive multi-stage model fitted well to the observed temporal variation of the excess mortality rate and of the excess relative risk. This model can be applied to any age interval after irradiation, as shown here. The additive multi-stage model hypothesizes that radiation-related carcinogenesis superimposes on background carcinogenesis, and that multiple hits are necessary for the transitions of stages for manifestation of radiation-related and background carcinogenesis. Another important hypothesis is whether the magnitude of the excess mortality rate depends on the background mortality rate is inherent to each type of neoplasm.

The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) reviewed the mathematical models for radiation-related carcinogenesis in a year 2000 report and concluded that firm statements on the relative validity of different biologically based models of radiation tumorigenesis must await further developments.\textsuperscript{25)} However, the multi-stage nature of carcinogenesis has become evident by a large body of work. Hanahan and Weinberg (2000)\textsuperscript{26)} suggested that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: 1) self-sufficiency in growth signals, 2) insensitivity to growth-inhibitory signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained angiogenesis, and 6) tissue invasion and metastasis. These alterations have been revealed to be attributable to mutations that produce oncogenes with a dominant gain of function and tumor suppressor genes with a recessive loss of function, chromosomal aberrations, and alterations of the epigenetic regulatory mechanisms. Cox (1998) reviewed and discussed the possible actions of radiation in the multi-stage nature of carcinogenesis.\textsuperscript{27} He listed a set of questions that are judged to be of particular importance to the future development of biological models. The two-mutation model is another biologically based model which has been frequently used for the analysis of carcinogenesis. Moolgavkar and Knudson\textsuperscript{28)} showed that the temporal distribution of incidence of retinoblastoma was well fitted by the two-mutation model. Although the two-mutation model was used to analyze the temporal distribution of risk of various types of neoplasms,\textsuperscript{29–32)} it seems to be rather doubtful whether application of this model to neoplasms other than retinoblastoma is adequate.

Hall (1998)\textsuperscript{33)} mentioned the possibility that radiation causes a mutation in one of the genes responsible for the stability of the genome and/or the fidelity of replication, leading to what has been called a mutator phenotype. Ullrich (1998)\textsuperscript{34)} proposed a model that identifies radiation-induced genetic instability as the earliest cellular event in the multi-step sequence leading to radiation-induced cancer. He showed that mice resistant to transformation and mammary development are also resistant to the development of chromosomal instability after irradiation. Little (1998),\textsuperscript{35)} Huang et al. (2003)\textsuperscript{36)} and Ohtaki and Niwa (2001)\textsuperscript{37)} also mentioned the important role of genomic instability in radiation carcinogenesis. The results of the present study were partially consistent with these theories. However, the analysis indicated that radiation produced one or more hits for transitions of stages of carcinogenesis soon after exposure, and that the rate of decrease in the remaining number of hits for carcinogenesis was enhanced slightly. Therefore, the role of genomic instability and/or delayed mutation may be second-drawer in the condition of prompt irradiation.

Mendelsohn and Pierce proposed the multi-mutation model,\textsuperscript{15–18)} in which it was assumed that cancer is caused by mutations that accumulate in a stem cell throughout life, and that radiation can cause only one of these mutations. Our additive multi-stage model is not identical to the multi-mutation model of Mendelsohn and Pierce. We can agree with the concept that radiation exposure does not in itself cause cancers, but contributes to their cause. Surely, the present study indicated that radiation-related and background carcinogenesis pursued the common multi-stage pathway. We will point out several problems for the application of the multi-stage model to radiation carcinogenesis, as follows.

[1] Is the number of hits necessary for radiation-related carcinogenesis always only one?

Mendelsohn and Pierce stated emphatically that the linear dose-response relationship for solid cancer among atomic bomb survivors is due to the necessity of only one mutation adding to the spontaneous mutations for manifestation of carcinogenesis. However, many epidemiological and experimental studies have reported non-linear dose-response relationship for solid tumorigenesis. Little and Charles (1997)\textsuperscript{18)} analyzed the atomic bomb survivor non-melanoma skin cancer incidence data and indicated substantial curvilinearity in
the dose-response relationship, consistent with a possible dose threshold of about 1 Sv, or with a dose-response in which the excess relative risk is proportional to the fourth power of dose. Hulse et al. (1973)\(^{49}\) analyzed the dose-response relationship for induction of skin tumors by beta rays in mice and showed that the dose-squared-exponential equation fitted well to the dose response. Epidemiological investigations on the long-term effects among radium dial painters demonstrated that the incidence of osteosarcoma was proportional to the square of absorbed dose 40–42. We showed in a previous study using newborn mice that the incidence of bone tumors was also proportional to the square of dose of gamma rays.\(^{51}\) A threshold-like dose was found in the dose-response relationship for induction of renal tumors by localized X-irradiation of Wistar strain rats, and high doses were required to induce renal tumor (Maldague, 1969).\(^{43}\)

It is worthy of remark that the spontaneous incidences of these neoplasms are very low. Therefore, The probability of spontaneous hits in target cells leading to malignant neoplasm may be low. Multiple hits by radiation may be necessary for carcinogenesis when the probability of spontaneous hits is low.

[2] Does magnitude of the excess mortality rate depend on the background mortality rate?

Fry and Carnes (1989)\(^{44}\) examined the correlation of the incidence in unirradiated mice and the susceptibility for cancer induction by radiation and it became clear that the ratios of the slopes of the dose responses of radiation-induced cancer to the natural incidence were similar for specific solid cancers (breast cancer and lung cancer) in different strains. They also pointed out that both the natural incidence and the radiation-induced incidence of myeloid leukaemia in RFM strain mice were higher in males than in females, whereas, in females of the same strain, both the incidence and the susceptibility for induction by radiation of lymphoma were greater than those in males. Storer et al. (1988)\(^{45}\) demonstrated the positive relationship between the natural incidence and susceptibility for induction of solid cancers (lung, breast, liver, ovary, and adrenal tumors) among four different strains of mice. These findings strongly suggested that radiation-related and background carcinogenesis pursue the common pathway in the development of several neoplasms. On the other hand, the incidence of malignant lymphoma of the histiocytic type did not increase after irradiation (Table 3). Similar result has been obtained in many experiments using various strains of mice\(^{23,46–47}\). This neoplasm developed spontaneously at high incidences, but radiation exposure was not effective in increasing the incidence. Similarly, X-irradiation did not increase the incidence of the uterine tumors (leiomyoma and leiomyosarcoma) that developed spontaneously at a substantial incidence in B6WF\(_1\) mice (Sasaki and Kasuga, 1981).\(^{48}\) The ICRP estimated the risk of solid cancer in the 1990 recommendation using the multiplicative model.\(^{49}\) However, Pierce et al. (1996)\(^{50}\) showed that the excess relative risk at a given age depends substantially on sex, but the age-specific excess absolute risk depends little on sex factors. Preston et al. mentioned in a recent report\(^{51}\) that the excess absolute risk (EAR) in LSS might best be used to estimate risks of breast cancer in other populations, and that for stomach and thyroid cancer the excess relative risk (ERR) might be the most appropriate for this purpose. Because the mechanism of radiation carcinogenesis is not yet clear, we must conclude whether the magnitude of the excess mortality rate depends on the background mortality rate is inherent to each type of neoplasm.

[3] Is the excess mortality rate independent of age at exposure?

Kellerer and Barclay (1992)\(^{10}\) proposed the age-attained model, in which it was assumed that the excess mortality rate at any attained age does not depend on the age at exposure. Mendelsohn and Pierce\(^{11,15–18}\) reported that, for most solid cancers, the excess incidence rates among atomic bomb survivors depend very little on age at exposure or time since exposure, but mainly on attained age. The findings obtained from investigations of atomic bomb survivors are yet interim data, because the majority of individuals exposed during childhood and young adult period are still alive. It may not be appropriate that tumors of endocrine organs and endocrine-related organs and individuals who died during young age were omitted from the analysis of ‘solid cancer’, because the higher susceptibility of young individuals for induction by radiation of thyroid cancer,\(^{25,50–51}\) breast cancer\(^{52–53}\) and other cancers\(^{54–56}\) has been reported. Pierce noted that the age-at-exposure effect is confounded by the birth cohort trends in background cancer rates.\(^{18}\) They challenged the interpretation of the age-time patterns of risk which they obtained in the LSS of atomic bomb survivors with their multi-mutation model. The present study showed, however, that the excess mortality rate at any age was not independent of age at irradiation. All of the mice in our experiment were allowed to live out their entire life span, and the age-at-exposure effect was not confounded by birth cohort trends in background mortality rate. It has been clearly demonstrated by application of the additive multi-stage model that the lifetime risk is determined by both parameters K(exc) and a(exc) of the equations in the model. We have shown in previous reports that the age-dependence of susceptibility for induction of neoplasm is inherent to the specific type of neoplasm.\(^{2–3,20}\) The age-dependence of susceptibility for induction of each type of neoplasm may be determined by the number of target cells, the state of cell proliferation at and/or after exposure, and the state of gene expression in target cells. It should be noted that the target cells for radiation carcinogenesis during the infant period are the expanding cell population, in which the cell kinetics is different from that of stem cells during adult period. The suggestive results were obtained by studies using cultured cells in which a marked increase in frequency of malignant
transformation was detected by serial passages after irradiation of C3H10T1/2 cells,53 human bronchial epithelial cells54) and rat fetal glial cells.59)

We proposed a hypothesis that the age-dependence of susceptibility for induction of specific type of neoplasm is common beyond animal species of mammals.2–3) This hypothesis seems to be strengthened by the results of recent epidemiological investigations that showed the increase in incidences of pituitary tumors by radiation in cohorts of atomic bomb survivors,60) patients who had been irradiated in childhood for tinea capitis in Israel,61) and individuals irradiated during infancy for treatment of hemangioma in Sweden.62) The age-dependence of susceptibility for induction of specific type of neoplasm, which has become evident in the present study (Table 3), is well consistent with the results of previous experiments.2–3) It should be noted that the developmental stage at the time of birth in mice is much earlier than that in humans.63–65) The status of development of the late fetal period in mice corresponds to that of the second trimester of gestation in humans, and the early postnatal period in mice to the third trimester of gestation in humans. Therefore, it may be reasonable to expect the substantial susceptibility of fetuses during the second trimester of gestation for induction of several types of neoplasms, the high susceptibility of fetuses during the third trimester of gestation for induction of various types of neoplasms and the higher susceptibility of children compared to that of adults for enhancement of the mortality rate from solid cancers by radiation in humans.

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