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# Fast-Neutron Irradiation of Mouse Embryos in the Pronuclear Zygote Stage: Mortality Curves and Neoplastic Diseases in 30-Day Postnatal Survivors (39312)

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This investigation was undertaken because of concern regarding the risk of injury to air travelers from exposure to the elevated levels of neutrons that occur in the earth's atmosphere during solar radiation events. Information on late effects of neutron irradiation in mammals, particularly the consequences of prenatal exposure, is inadequate. Interest in the biological actions of neutrons was also stimulated by the prospect of large-scale use of nuclear power and the increasing use of neutrons in industry and medicine.

Mice in the pronuclear zygote stage of embryonic development are highly vulnerable to radiation injury leading to prenatal death (1, 2). Survivors have been found to be free of radiation-induced congenital anomalies by almost every criteria used (1, 3, 4). Two exceptions reported are XO aneuploidy (5, 6) and exencephalia (7, 8; see comments by Brent, 4).

Rugh *et al.* (9) investigated possible delayed effects of irradiation in mice that were exposed in utero to 275 R of X rays during the pronuclear zygote stage and lived at least 30 days after birth. Life span tended to be shorter in the irradiated mice of both sexes than in unirradiated control animals; however, the differences were not statistically significant. With respect to other indices measured (body weight, hemoglobin concentration, and blood cell counts), differences between control and irradiated mice were small and probably not statistically significant, although statistical analyses were not reported. Leukemia was not found in any of the mice examined. Rugh *et al.* (10) also reported that exposure of mouse pronuclear zygotes to 100 R of X rays did not change the percentage of 30-day postnatal survivors that developed externally palpable tumors. Russell *et al.* (11, personal communication of final results) exposed mouse pronuclear zygotes to 200 R of X rays and found no statistically significant difference in postnatal survival between irradiated and unirradiated animals. An increase in cataracts was reported (12) for adult mice that had been X-irradiated during the pronuclear zygote stage; however, inconsistencies between results of the different experiments suggest that the increase may be a chance occurrence.

We report here long-term observations on mice that were irradiated with fast neutrons during the pronuclear zygote stage of embryonic development. Cumulative mortality distributions were investigated as were the percentage incidence and mean age at death for each of the principal neoplastic diseases seen on postmortem examination.

*Methods.* Randomly bred albino mice (CD-1 strain, Charles River Breeding Laboratories, Wilmington, Mass.) were received in Oak Ridge at 3 weeks of age. At Charles River and in Oak Ridge they were kept in rooms that were lighted from 0600 to 1800. The mice were mated and irradiated at 7 weeks of age. Mating opportunity was for a single 12- to 13-hr period beginning at 1900. At the termination of the mating opportunity, those females that appeared to

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have a copulation plug were segregated, and the same day, between 1400 and 1500, they were either irradiated or sham irradiated. At the time of irradiation the conceptuses were in the pronuclear stage (2).

The Oak Ridge National Laboratory Health Physics Research Reactor was the source of fast neutrons, providing a neutron absorbed dose of 15 rad at a dose rate of 2 rad/min. From a previous study we estimate that as a result of the radiation, approximately 54% of the embryos died *in utero* (2). During irradiation the mice were confined individually in rotating nylon tubes, lateral to the reactor core. Sham irradiation was performed by using the same physical arrangement without radiation. Other details of the irradiation have been described previously (2).

On the day of birth both the irradiated and the sham-irradiated litters were transferred to sham-irradiated foster mothers (usually 10 pups per mother). The pups were weaned at 3 weeks of age and flown to Oklahoma City the following week. There they were observed until natural death.

When an animal died, the abdomen, thorax, and cranium were opened and the carcass was preserved in 10% buffered formalin for later examination. Gross pathologic examination was performed on all animals that survived at least 30 days after birth except those that had been eaten or were badly decomposed. To verify gross diagnoses, tissue samples from 19 to 23% of the mice in each of the four groups were embedded in paraffin, sectioned at 5 $\mu$m, stained with hematoxylin and eosin, and examined microscopically.

In the statistical analyses of the results probability levels $\leq 0.05$, when adjusted for multiple comparisons, are considered significant.

*Results and discussion.* Cumulative mortality distributions of the irradiated and sham-irradiated mice that survived at least 30 days after birth are shown in Fig. 1. Between 500 and 650 days of age, 35% of the sham-irradiated females and 12% of the irradiated females died. Despite this difference—which accounts for most of the dissimilarity in the curves—the cumulative mortality distributions are not significantly different (see below). Differences in the distributions at 1-day intervals were tested for significance by use of the Kolmogorov-Smirov two-sample test (13). The test is sensitive to any kind of difference in distribution between two populations, e.g., central tendency, dispersion, and skewness. The probability level corresponding to an overall type I error of 0.05 is 0.025 when adjustment is made for two comparisons (14). At the adjusted probability (0.025), the difference in mortality distribution between irradiated and sham-irradiated mice is not significant for either the females ($P = 0.029$) or the males ($P = 0.54$).

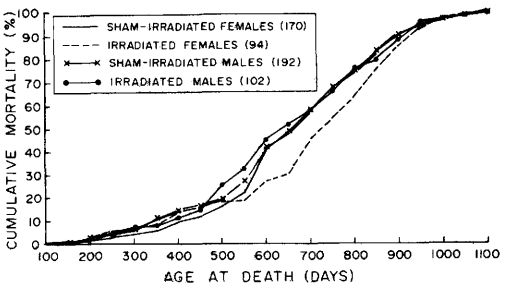


FIG. 1. Cumulative mortality distributions (50-day intervals) of 30-day postnatal survivors.

Table I shows the percentage incidence and mean age at death for each of the principal neoplastic diseases seen on postmortem examination. Differences between irradiated and sham-irradiated mice were tested for significance by use of two-by-two chi-square tests for disease incidence and *t* tests for mean age at death. The analyses performed do not take into account possible dependencies suggested by the fact that some of the mice had tumors of more than one type. Because four diseases were considered in the males and six in the females, the probability corresponding to an overall type I error of 0.05 is approximately 0.01. At the adjusted probability, the difference between irradiated and sham-irradiated mice of the same sex with regard to either mean age at death or incidence of neoplastic disease is not significant for any of the neoplasms observed.

Thus, for two of the major delayed effects of radiation, induction of neoplastic disease and shortened life span, no significant increase in risk resulted from irradiating the pronuclear zygotes with fast neutrons.

*Summary.* Mouse embryos in the pronu-

TABLE I. PERCENTAGE INCIDENCE AND MEAN AGE AT DEATH FOR THE PRINCIPAL NEOPLASTIC DISEASES SEEN ON POSTMORTEM EXAMINATION.[a]

| Disease | Percentage incidence[b] | | | Mean age at death (SE)[c] | | |
|---|---|---|---|---|---|---|
| | Sham-irradiated | Irradiated | $P^d$ | Sham-irradiated | Irradiated | $P^d$ |
| | | | Females[e] | | | |
| Reticulum cell sarcoma | 33 | 22 | 0.084 | 665 (20) | 752 (34) | 0.026 |
| Thymic lymphoma | 5 | 7 | 0.56 | 378 (43) | 323 (45) | 0.40 |
| Other tumors | | | | | | |
| Lung | 21 | 26 | 0.39 | 705 (23) | 734 (35) | 0.47 |
| Liver | 4 | 11 | 0.038 | 839 (22) | 882 (26) | 0.26 |
| Ovary | 20 | 22 | 0.63 | 740 (22) | 778 (30) | 0.30 |
| Breast | 9 | 9 | 0.93 | 664 (42) | 729 (60) | 0.38 |
| | | | Males[f] | | | |
| Reticulum cell sarcoma | 24 | 17 | 0.22 | 671 (25) | 613 (27) | 0.19 |
| Thymic lymphoma | 5 | 7 | 0.42 | 389 (35) | 347 (37) | 0.43 |
| Other tumors | | | | | | |
| Lung | 26 | 23 | 0.60 | 759 (24) | 709 (37) | 0.25 |
| Liver | 19 | 12 | 0.13 | 788 (23) | 712 (54) | 0.14 |

[a] Only mice that survived 30 days or longer after birth were included in the study.
[b] Percentage of mice that had one or more neoplasms of the type indicated. Results are given only for neoplastic diseases that affected at least 2% of the animals in one or both of the groups compared.
[c] Standard error of mean is shown in parentheses.
[d] Probability level for difference between sham-irradiated and irradiated groups.
[e] Sham irradiated, 163; irradiated, 90.
[f] Sham irradiated, 186; irradiated, 98.

clear zygote stage were either irradiated *in utero* with a dose of 15 rad of fast neutrons or were sham-irradiated. Those animals that survived at least 30 days after birth were observed until their natural death. We investigated the percentage incidence and mean age at death for each of the principal neoplastic diseases seen on postmortem examination and also cumulative mortality distributions. No statistically significant differences were found between irradiated and sham-irradiated mice of the same sex.

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