Effect of Chronic Exposure to Fast Neutrons on the Development of Mammary Tumors in the Rat*

QUENTIN L. HARTWIG†, SIDNEY P. KENT,‡ AND JAMES A. SPROUL, JR.§

(Department of Radiobiology, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas)

Neoplastic changes resulting from radiations have interested investigators since the discovery of the x-ray. The initial observations were made on humans. Later, the neoplastic effect of various types of ionizing radiations was studied in animals. With the advent of nuclear stockpiles, it became evident that personnel working about radioactive materials, even though exposed at a very low dose rate, might, over a period of years, accumulate a substantial total dose. An evaluation of the carcinogenic effect of this type of exposure is of interest. Experiments designed to evaluate the problem suggest that at least two end-points should be observed: the incidence of tumors in the irradiated animals versus their controls and the effect on longevity.

This paper is a report on tumor incidence and longevity of a group of rats chronically exposed for 6 months to fast neutrons.

MATERIALS AND METHODS

One hundred female Sprague-Dawley rats were used in this experiment; 50 were irradiated, and 50 served as non-irradiated controls. At the beginning of the irradiation, the animals were 3 months old and averaged 220 gm. in weight. The colony was maintained on Wayne Lab Blox “R” rat pellets and water ad libitum throughout the experiment, with the animals. In addition to a gross examination, sections of the tumors were subcutaneous, they were easy to find appearing rats found to be tumorous at autopsy. Since most of the tumors were subcutaneous, they were easy to find in the living animal, and in only two instances were normal-appearing rats found to be tumorous at autopsy.

No animals were sacrificed; autopsies were performed at time of spontaneous deaths of both irradiated and control animals. In addition to a gross examination, sections of the kidneys, spleen, lungs, liver, bone marrow, heart, and any tumors present were examined microscopically. The tissues were stained by the hematoxylin-eosin technic, and calcium deposits were demonstrated by the Alizarin Red S and Von Kossa methods (11).

RESULTS

Tumors began to appear in the irradiated group 248 days after the beginning of the 6-month chronic exposure and 259 days in the controls as shown in Chart 1. The incidence of visible tumors steadily increased in the irradiated group, and by day 468 after commencement of the exposure all living irradiated rats bore tumors. At that time only 8 per cent of the controls exhibited visible palpable tumors. Table 1 gives the percentage of tumors in both irradiated and control rats at the time all animals were dead. At the termination of the experiment, 68 per cent of the irradiated rats possessed one or more tumors, as compared with 14 per cent of the controls. Table 2 presents the tumor data in reference to 4-month periods throughout the span of the experiment. Both irradiated and control rats began to exhibit tumors in the third period, the greatest per cent being in the irradiated animals. During the fourth period, all remaining irradiated rats...
developed tumors and consequently showed no additional tumor incidence in Groups V and VI. Since the controls did not exhibit a similar high percentage of tumors in the early periods, some tumors appeared in Group III and in subsequent groups of the controls.

The other nonmammary tumor was a carcinoma of the ovary. The remaining malignant and benign tumors of both the irradiated and control animals were of the mammary gland. In the irradiated rats, four of the adenocarcinomas metastasized to the lungs. No metastasis occurred in the controls.

As noted in Table 1, 22 per cent of the tumor-bearing irradiated rats bore a malignant tumor at autopsy, as compared with 50 per cent with benign tumors. Two irradiated animals carried both benign and malignant tumors. Each was scored in both groups. Chart 2 is a plot showing the date at which each rat with a malignant or benign tumor (as determined at autopsy) first exhibited a visible tumor. It would appear that tumors which developed after about 340 days following the first exposure were benign.

Only a small percentage of the tumors in the irradiated animals (Table 3) were nonmammary in origin. The hemangioma was located in a fallopian tube. In addition, the same rat had two breast fibroadenomas and one breast carcinoma.
of 34) had four. No control rats had more than one tumor at autopsy.

In the latter period of the 6-month exposure, the rat pellet supply ran short, and it was necessary to place the rats on monkey food for 2.5 weeks until a new shipment arrived. Owing to an error by the manufacturer of the monkey food, it contained 1,430 USP units of Vitamin D per gram of food rather than the stipulated 37.3 USP units per gram. Based on the average consumption of food per day by the rat, the animals were receiving about 29,000 USP units of Vitamin D per day for the 2.5-week period. The first indication of Vitamin D toxicity was weight loss in both irradiated and control animals.

Extensive deposits of calcium were noted in the lungs, coronary vessels, and kidneys of all irradiated and control animals autopsied after day 271 following the first exposure. The pathological picture closely resembled that of hypervitaminosis D and probably explains the early loss of weight and the deaths which occurred in both groups during this period.

The average life span was 463 days for the irradiated rats and 511 days for the controls. The life span for both groups was considerably shorter than that reported by Davis et al. (4) and probably can be attributed to the hypervitaminosis D episode.

Brochiectatic abscesses were found in over half of the irradiated and control animals autopsied. Purulent bronchiectasis is frequently found in the rat at autopsy (12).

DISCUSSION

It is evident from the results that neutrons delivered at a low dose rate over a period of time can accelerate the onset of tumors in the female Sprague-Dawley rat. In this experiment, the advent of the first visible tumor in the irradiated group was followed by a steady increase in incidence until, at the termination of the experiment, 68 per cent of the irradiated group had a tumor in comparison with 12 per cent in the controls. Thus, a difference of 56 per cent in tumor incidence was noted in the group receiving 154 rep fast neutrons, as compared with their nonirradiated controls. Barnett (2), over a period of a year, delivered approximately 72 rep to a group of rats. This resulted in a difference of 29 per cent in tumor incidence between the irradiated and control groups. Ely et al. (5) exposed rats daily to a beam of cyclotron neutrons at a dose rate of 0.11 n per day to a total dose of about 58 rep. In the irradiated group, tumors developed in 42 per cent of the animals, whereas tumors occurred in only 10 per cent of the controls.

| TABLE 3 |

| INCIDENCE OF EACH TYPE OF TUMOR FOUND |

| Ratio is Number per Total of Number of Rats Possessing One or More Tumors |

<table>
<thead>
<tr>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Fibroadenoma</td>
</tr>
<tr>
<td>Adenofibroma</td>
</tr>
<tr>
<td>Fibroma</td>
</tr>
<tr>
<td>Hemangioma</td>
</tr>
<tr>
<td>Carcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
</tr>
</tbody>
</table>

| Davis et al. (4) found that 87 per cent of all tumors normally develop in female Sprague-Dawley rats after the rats reach the age of 540 days, the average life span of their rats being about 780 days. In our study, 100 per cent of the tumors which occurred in the irradiated group developed before the animals were 540 days old. There is little information in the literature concerning the incidence of tumors during the life span of the Sprague-Dawley rat. However, in comparing the incidence of tumors in our irradiated rats with their controls and with those of Davis et al. (4), it would appear that a chronic exposure to 154 rep fast neutrons significantly accelerated the appearance of the tumors. In our study, only 6 per cent of the tumors occurring in the chronically irradiated rats developed from nonmammary tissue. Brecher et al.
(3) exposed Sprague-Dawley rats to acute doses of 700–1,000 r of x-rays. The authors state that fibroadenomas of the breast were common in both irradiated and untreated litter-mates, although exact numbers are not given. However, they also reported a number of tumors arising in other tissues such as an adamantinoma, a glioma, a sarcoma of the uterus, and various malignant tumors of the digestive tract. Finerty et al. (7) exposed female Holtzman litter-mate rats to an acute dose of 700 r x-rays. Within 6 months after the irradiation, tumors developed in muscle, the clitoris, and cheek. 

There have been no previous data published on the chronological appearance of the benign or malignant tumor over the life span of irradiated rats. Our data covering the life span of both irradiated and control animals suggest that benign tumors develop randomly during the postexposure life span, whereas malignant tumors are more apt to appear soon after the exposure period. 

This experiment suggests that a 6-month chronic exposure to 154 rep of fast neutrons can accelerate the onset of mammary gland tumors in the Sprague-Dawley rat and increase their frequency.

ACKNOWLEDGMENTS

The authors express their appreciation to Major Richard E. Benson, Veterinary Research Group, and Captain Loren C. Logie, Physics and Engineering, for their generous aid in the progress of this study.

REFERENCES

13. SHELLABARGER, D. J.; CRONKITE, E. P.; BOND, V. P.; and LIPPINCOCK, S. W. The Occurrence of Mammary Tumors in the Rat after Sublethal Whole Body Irradiation. Radiation Research, 5:501-12, 1957.
Effect of Chronic Exposure to Fast Neutrons on the Development of Mammary Tumors in the Rat

Quentin L. Hartwig, Sidney P. Kent and James A. Sproul, Jr.