The Experimental Production of Fibrosarcomas of Bone*

Clifford C. Franseen, M.D., Joseph C. Aub, M.D., and Carol L. Simpson

(From the Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Boston, Massachusetts)

(Received for publication March 6, 1941)

From a desire to obtain experimental material for a continuation of studies on phosphatase in bone tumors (5, 6) an attempt has been made for over four years to produce sarcomas of bone in rats and mice.

The spontaneous occurrence of bone tumors in animals is so rare that it is a very uncertain source of experimental material. Pybus and Miller (12) have succeeded, however, in developing a strain of mice with a high incidence of spontaneous bone tumors which may prove to be of value in future experimental studies.

Martland's observation (11) on the development of osteogenic sarcomas in radium dial painters stimulated many attempts to produce experimental bone tumors with radioactive substances. Production of bone tumors has subsequently been reported by Sabin, Doan, and Forkner (15) to follow intravenous administration of radium and of mesothorium. Bone tumors were produced by implantation of radium sulfate, by Duels and Biltris (4); by intramedullary implantation of mesothorium, by Schürch and Uehlinger (16-18); and by subcutaneous injection of thorotrast, by Selbie (19). In all these instances the radiation from the radioactive material was unfiltered. However, bone tumors have also been reported by Lacassagne (8) after interstitial implantation of filtered radon, and were observed by Ross (13) to occur after the implantation of unfiltered radium.

Bone tumors in animals have also followed heavy application of filtered radium applied externally (7) and also following exposure to x-rays, as reported by Lüdin (9). A spindle cell sarcoma following x-ray treatment was experimentally produced in a rabbit as early as 1910 by Marie, Clunet, and Rault-Lapinte (10). Bone tumors have therefore been found to result from prolonged exposure to both filtered and unfiltered rays from radium, unfiltered radiation from thorium, and roentgen radiation.

Brunschwig (2) has shown that transplantation of epidermoid carcinoma beneath the periosteum or into the bone marrow cavity produces spicules of new bone as in osteogenic sarcomas but, as Wephwadze (21) suggests, these spicules are probably formed by the osteogenic elements of the exfoliated periosteum and are not true tumors of bone.

The first tumor containing bone elements which was produced by carcinogenic chemicals was reported by Anardi (1) in 1934. He produced an osteochondroma in a rabbit by painting with tar. During the course of the present experiments, Brunschwig and Bissell (3) have reported the production of an ossifying sarcoma in a mouse following implantation of benzpyrene and cholesterol and a parosteal or central fibrosarcoma by intramedullary injection of methylcholanthrene. Council.

* This investigation was aided in part by a grant from the Committee on Research in Endocrinology, National Research Council.
14 rats less than 15 days old. Although the last animal died 635 days after implantation, no tumor had developed. In 2 rats in which a radon seed had been implanted, fractures occurred at the site in both animals about 2 months postoperatively. These fractures appeared to unite firmly again, but the animals died subsequently of intercurrent disease without tumors. Unfortunately, a disease which produced a large inflammatory mass about the cecum with accompanying diarrhea attacked the rat colony and many animals died prematurely from this cause.

Unfortunately, a disease which produced a large inflammatory mass about the cecum with accompanying diarrhea attacked the rat colony and many animals died prematurely from this cause.

Of the 13 mice which developed fibrosarcomas at the site of implantation in the upper tibial epiphysis, 3 developed bilateral tumors almost simultaneously. All the tumors were fibrosarcomas, usually of spindle cell type, but with some undifferentiation (Figs. 1 and 2). Two of these tumors are shown in Figs. 3 and 4. None, however, showed new bone formation except in immediate relation to the periosteum where, in the roentgenograms, small areas with radiating spicules of bone could be seen.

The application of methylcholanthrene against traumatized periosteum was no more successful in producing true bone sarcomas, as only 1 tumor was produced by this method, a spindle cell sarcoma without osteogenic characteristics.

Because of the reported successes in the production of osteogenic sarcoma by the use of radioactive substances, an attempt was made to produce similar tumors with the crystals resulting from the evaporation of thorotrast. However, these crystals were readily soluble in the tissue fluids and the solution appeared to be transported quickly from the site of implantation. No tumors have developed although 11 of the 12 mice lived for more than 1 year after implantation. It is possible that if the removal of the thorotrast from the site of implantation is prevented by mixing with vaseline, as Schürch and Uehlinger (16-18) have done, the likelihood of the development of tumors at the site will be increased.

We have presumed that all these fibrosarcomas were of parosteal origin, for by following the process at the site of implantation, it was seen that a small zone of necrosis developed about the agent and was followed by repair by fibroblasts which appeared to enter the area from the extra-osseous connective tissue through the small drill hole in the bone. The development of malignant tumors subsequently occurred, probably from this repair tissue.

In three instances where methylcholanthrene had been implanted into both tibiae, tumors were discovered on both sides when microscopic sections were examined, although a tumor had been palpable on only one side (Figs. 3 and 4). This would seem to indicate that similar tissues in an individual mouse respond equally to similar stimuli and may develop tumors almost simultaneously.

It is interesting to note in this connection that most of the mice of the strain of Pybus and Miller (12) in which a high incidence of spontaneous bone tumors occurred had multiple tumors.

### Table I: Experiments on Bone

<table>
<thead>
<tr>
<th>Animals</th>
<th>No.</th>
<th>Substance used</th>
<th>Site</th>
<th>Tumors produced</th>
<th>First observation of palpable tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>11</td>
<td>10% methylcholanthrene in cholesterol</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>2</td>
<td>Cholesterol</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>4</td>
<td>100% methylcholanthrene</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats (15 days old)</td>
<td>14</td>
<td>100% methylcholanthrene</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>2</td>
<td>Radon seeds (0.16 mc each)</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>9</td>
<td>Thorotrast crystals</td>
<td>Upper tibial epiphysis</td>
<td>1 fibrosarcoma</td>
<td>91 to 225 days in 13 animals</td>
</tr>
<tr>
<td>Mice (C3H)</td>
<td>20</td>
<td>100% methylcholanthrene</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Mice (DBA)</td>
<td>2</td>
<td>100% methylcholanthrene</td>
<td>Upper tibial epiphysis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Mice (A)</td>
<td>12</td>
<td>Thorotrast crystals</td>
<td>Tibia subperiosteal</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Mice (C3H)</td>
<td>5</td>
<td>100% methylcholanthrene</td>
<td>Tibia subperiosteal</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Mice (A)</td>
<td>5</td>
<td>100% methylcholanthrene</td>
<td>Tibia subperiosteal</td>
<td>1 fibrosarcoma</td>
<td>132 days</td>
</tr>
</tbody>
</table>

Of the 13 mice which developed fibrosarcomas at the site of implantation in the upper tibial epiphysis, 3 developed bilateral tumors almost simultaneously. All the tumors were fibrosarcomas, usually of spindle cell type, but with some undifferentiation (Figs. 1 and 2). Two of these tumors are shown in Figs. 3 and 4. None, however, showed new bone formation except in immediate relation to the periosteum where, in the roentgenograms, small areas with radiating spicules of bone could be seen.

The application of methylcholanthrene against traumatized periosteum was no more successful in producing true bone sarcomas, as only 1 tumor was produced by this method, a spindle cell sarcoma without osteogenic characteristics.

Because of the reported successes in the production of osteogenic sarcoma by the use of radioactive substances, an attempt was made to produce similar tumors with the crystals resulting from the evaporation of thorotrast. However, these crystals were readily soluble in the tissue fluids and the solution appeared to be transported quickly from the site of implantation. No tumors have developed although 11 of the 12 mice lived for more than 1 year after implantation. It is possible that if the removal of the thorotrast from the site of implantation is prevented by mixing with vaseline, as Schürch and Uehlinger (16-18) have done, the likelihood of the development of tumors at the site will be increased.

We have presumed that all these fibrosarcomas were of parosteal origin, for by following the process at the site of implantation, it was seen that a small zone of necrosis developed about the agent and was followed by repair by fibroblasts which appeared to enter the area from the extra-osseous connective tissue through the small drill hole in the bone. The development of malignant tumors subsequently occurred, probably from this repair tissue.

In three instances where methylcholanthrene had been implanted into both tibiae, tumors were discovered on both sides when microscopic sections were examined, although a tumor had been palpable on only one side (Figs. 3 and 4). This would seem to indicate that similar tissues in an individual mouse respond equally to similar stimuli and may develop tumors almost simultaneously.

It is interesting to note in this connection that most of the mice of the strain of Pybus and Miller (12) in which a high incidence of spontaneous bone tumors occurred had multiple tumors.

### Description of Figures 1 to 4

**Fig. 1.**—Fibrosarcoma showing considerable undifferentiation appearing 211 days after introduction of methylcholanthrene into the head of the right tibia of a mouse of C3H strain.

**Fig. 2.**—Fibrosarcoma with giant cells which appeared 176 days after a crystal of methylcholanthrene had been placed in the head of the right tibia of a mouse of C3H strain.

**Fig. 3.**—Fibrosarcoma produced by methylcholanthrene in head of left tibia after 178 days, in a mouse of C3H strain. A similar tumor was found in the opposite leg. (See Fig. 4.)

**Fig. 4.**—Fibrosarcoma produced by methylcholanthrene in head of the right tibia after 178 days, appearing simultaneously with the tumor in the opposite tibia shown in Fig. 3. The epiphyseal line may be seen in the right upper corner.
Branschwig has suggested that fibrosarcomas develop from carcinogenic agents in bone because "the number of fibroblasts in bone which cannot function as osteoblasts is much in excess of active and potential osteoblasts and . . . the carcinogenic agent therefore is in contact with relatively greater numbers of the former." However, it is possible that the more highly differentiated tissues are more resistant to the carcinogenic agents that are young fibroblasts which engage in the repair process. This repair process is so active that the agent quickly becomes surrounded by young fibroblasts and contact with the more differentiated cells cannot be maintained.

As many investigators have shown, when radium or mesothorium is injected intravenously or ingested, the deposition occurs preferentially in bone. In these cases the action of the rays from the radioactive substance must in many cases act upon fibroblasts as well as upon osteoblasts, yet the predominance of tumors of osteoblastic origin resulting from these substances is well known (11). An alternate hypothesis is, of course, that a certain selectivity exists so that the fibroblasts are more susceptible to the carcinogenic action of chemical agents and the osteoblasts more susceptible to the carcinogenic action of radioactive substances. In the experiments of Selbie (19), a thorotrast deposit lay in contact with a rib, presumably in contact with both fibroblasts and osteoblasts, and an osteosarcoma developed. In experiments of Schürch and Uehlinger (16-18) in which vaseline containing radium or mesothorium was inserted into the medullary cavity of the femur of rabbits, 4 osteogenic sarcomas and 1 Ewing's sarcoma were found among the 9 tumors which resulted. The remaining tumors were fibrosarcomas, suggesting in this case that, with the application of these substances in contact with both fibroblasts and osteoblasts, an approximately equal susceptibility to the development of malignancy exists.

Phosphatase determinations on 3 of the fibrosarcomas reported here showed only the faintest traces of phosphatase, a characteristic of sarcomas of fibroblastic origin in both animals and humans in contrast with a high phosphatase activity in the tissues of osteogenic sarcoma. This suggests that the tumors produced in these experiments were not true osteogenic sarcomas but were sarcomas of extra-osseous origin; since the experiments of Fransen and McLean (5) have shown that in tumors of osteoblastic origin, even when osteoid tissue is absent and a histological picture characteristic of fibrosarcoma obtains, phosphatase is produced in the tumor tissue.

Summary

Attempts have been made over a period of 4 years to produce osteogenic sarcomas in rats and mice by implanting methylcholanthrene, thorotrast crystals, and radon seeds near the upper tibial epiphysis and also methylcholanthrene subperiosteally. Seventeen tumors were produced in mice but all proved histologically to be fibrosarcomas of probable extra-osseous origin.

The authors are indebted to Dr. Shields Warren and Dr. Olive Gates for assistance in interpreting the microscopic sections of the tumors produced, and to Miss Kathryn Hooper for preparation of the photomicrographs.

REFERENCES

The Experimental Production of Fibrosarcomas of Bone

Clifford C. Franseen, Joseph C. Aub and Carol L. Simpson

Cancer Res 1941;1:393-396.

Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/1/5/393.citation

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.