A Model for the Study of Experimental Bone Metastases.* (29708)

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This paper describes the technique of intra-medullary inoculation of fibrosarcoma cells into the femur of Sprague-Dawley rats. A tumor forms rapidly, expands and destroys the bone, providing an experimental model for the study of bone metastases.

Method and materials. The tumor used in the technique was originally a fibrosarcoma of the pituitary gland from a castrated male Sprague-Dawley rat bearing a pituitary graft. The rat received 10 doses of 10 mg of 3-methylcholanthrene a month after the pituitary transplantation. Fragments of this tumor have been successfully transplanted into the subcutaneous tissue of the abdomen of both female and male rats of the same strain for many generations in our laboratory. During this time the histology of the transplants has remained identical with that of the donor tumor, *i.e.*, a fibrosarcoma (Fig. 1).

Preparation of inoculum. The fibrosarcoma is transplanted into the subcutaneous tissues of the rat's abdomen. When the resulting growth has reached approximately 1.5 cm in diameter it is excised. Most of these tumors are cystic; contain clear yellow fluid protruding into which are festoons of tumor cells. These, to the naked eye, appear like aggregates of cells "beaded" along a capillary. This lining to the cyst is removed and pressed through stainless-steel wire cloth (mesh 100 × 90, Michigan Wire Cloth Co.). The sieved cells are suspended in synthetic medium 199 (Difco Laboratories) and subsequently introduced into the femoral shafts of female Sprague-Dawley rats.

Technique of inoculation. Under ether anesthesia the ligamentum patellae of the rat is exposed and transected at its upper end to leave sufficient ligament for later reconstruction of the joint.

Using a dental drill, No. 558, a hole is made in the intercondylar region of the articular surface of the lower end of the femur (Fig. 2). The condylar region admits the drill easily, and having overcome slight resistance at the metaphysis it can be removed. The drill is not passed down the diaphysis for it may damage the inner surface of the diaphyseal cortex directly or by over-heating.

Tumor suspension is drawn up into a ¼ ml tuberculin syringe using a size 20 needle. A 3 cm length of polyethylene tubing, internal diameter 0.7 mm and external diameter 1.2 mm, is then placed over the needle (Fig. 3) and filled to within 2 or 3 mm of its tip with the suspension. The tube, removed from the needle by clamping with a fine hemostat, can then be introduced into the medullary cavity of the femur to a depth of 2 cm. The

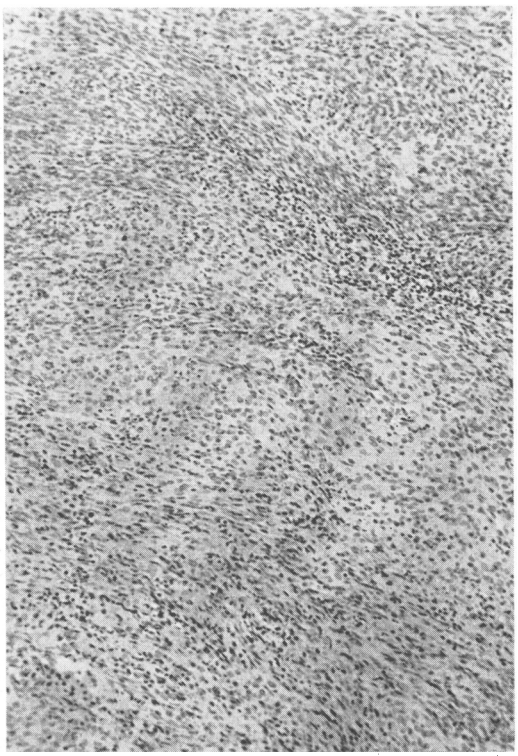

FIG. 1. Histology of original tumor.

* This study was supported in part by Grant CA-04632-05 from Nat. Cancer Inst., U.S.P.H.S.

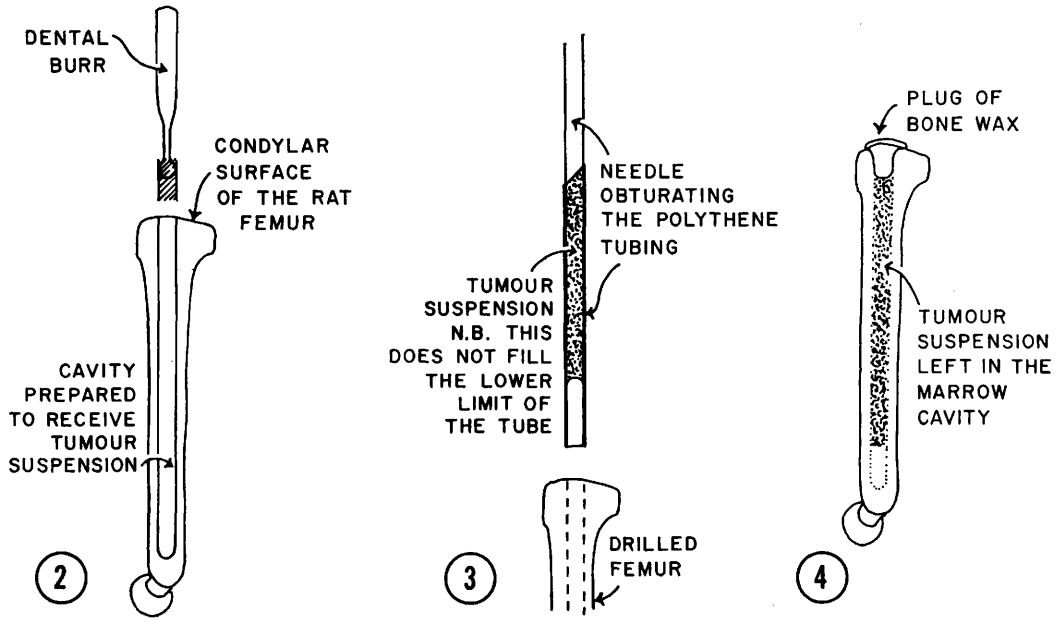

FIG. 2. Showing manner in which femur is drilled.
FIG. 3. Showing polyethylene tube, needle, and syringe containing suspension ready to insert into medulla of femur.
FIG. 4. Tumor sealed in the medullary cavity with bone wax.

hemostat removed from the section of tube projecting from the articular surface, the polyethylene can be withdrawn causing the contained suspension to be sucked into the prepared cavity in the femoral shaft (Fig. 4). By this means the tumor cells can be introduced without spillage or contamination of the joint and they are *not* put in under pressure. Bone wax is used to occlude the small hole in the articular surface of the femur; the operation site is washed with 1% Formalin and normal saline, the joint repaired with 40 braided black silk and the skin edges opposed with Michels 11 mm clips.

The method outlined has been studied in 3 phases:

Phase I. To demonstrate the possibility of transplanting tumor directly into bone; its viability and progression in this environment.

Phase II. To compare the effect of viable and non-viable tumor within bone.

Phase III. To study the effect of parathyroidectomy on the behavior of the intraosseous tumor.

Results. Phase I. Using the method described, fibrosarcoma cells were introduced into the medullary cavity of the left femur of eight female Sprague-Dawley rats. Within 19 days one of the animals (Rat 3) had a palpable tumor with a pathological fracture, well shown on X-ray. Films taken of the remaining animals showed evidence of bone erosion and deformity in all but one. Thus, 7 out of 8 femora X-rayed within 3 weeks of inoculation of the tumor had radiological evidence of successful invasion by the fibrosarcoma in its new environment (Fig. 5). The animal with no tumor involvement of the femur was the last to receive an inoculation and the bone was overheated in drilling. The latter was blamed for the sequestration of the bone during the ensuing 3 weeks.

The technique was considered satisfactory and repeated in a slightly modified form on a group of 10 animals all of which developed massive tumors of the femur within 3 weeks. The histology of the tumor was confirmed as identical to the primary growth after sacrifice of the animals at 6 weeks.

Phase II. Using 20 animals, the first 10 were inoculated in the manner described with viable tumor; the second group of 10 were inoculated with non-viable tumor cells. The suspension was heated for 20 minutes at

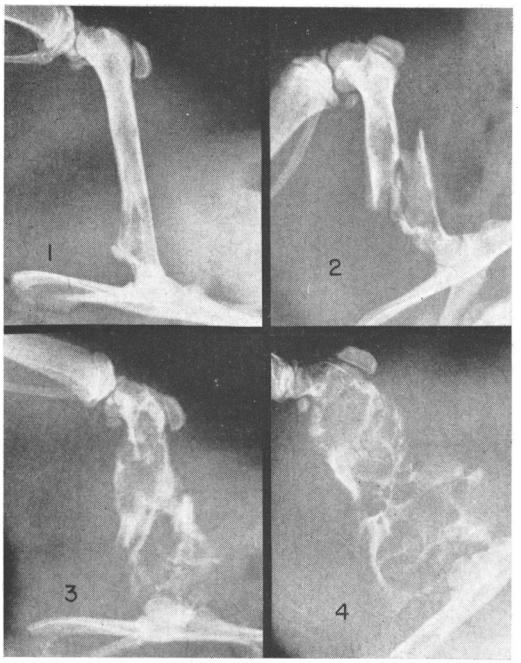

FIG. 5. Serial X-rays taken of femur in rat No. 38 showing rapid progression of tumor in the bone and apparent repair of a pathological fraction during invasion of tumor.

60°C to kill the cells but avoid changing the composition of their protein.

After injecting the first group with the suspension, a control animal was injected subcutaneously to insure that the suspension remained viable at the end of the inoculations in the femur. This animal developed a tumor 2.4 cm in diameter in the flank. Histological examination proved this to be identical to the donor tumor. Therefore, we could assume that the cells placed in the femora of the first 10 rats were viable.

All 10 animals receiving the non-viable tumor are alive 4 months after inoculation. Those with viable tumor were all dead within 55 days. The cause of death in this group was due to pneumonia or to the toxemia associated with the immense necrosing tumors that developed.

Phase III. Having established the possibility of growing the transplanted tumors within bone, it was decided to examine the effect of parathyroidectomy on their behavior. Three groups of 10 animals were used.

Group 1. These underwent parathyroidectomy before receiving intra-femoral tumor. Two died as an immediate result of the operation of parathyroidectomy and were thus excluded from the later statistical analysis. A group of 8 parathyroidectomized rats underwent later tumor inoculation.

Group 2. Ten animals in this group received tumor. Three died within 21 days from massive pulmonary metastases. The remainder underwent parathyroidectomy.

Group 3. Ten animals received tumor but 2 died of severe lung involvement prior to the date of the parathyroidectomy performed in Group 2. The death of these animals has been taken into account in the analysis of survival figures.

Four out of 8 animals undergoing parathyroidectomy prior to introduction of tumor survived appreciably longer (59 days) without lung metastases, whereas the animals in Groups 2 and 3 were all dead within 40 days.

Analysis of results, using the non-parametric test, gives a "t" value of 2.08 which is statistically significant, and would suggest that prior parathyroidectomy prolongs the life of animals receiving the intra-medullary tumor.

Comment. Toriyama *et al*(3) report the injection of a solid tumor from a trochar into the medullary cavity of the femur. This tumor was initiated by the intra-medullary implantation of 15 mg of 20-methylcholanthrene and 3,4-benzpyrene crystals in addition to the monthly injection of one microcurie of P^{32} in the form of $H_2P^{32}O_4$ per gram of body weight. The tumor that arose as a result of this was transplanted successfully and without histological change through many generations of Wistar rats. It was labelled an osteogenic sarcoma although only one animal showed osteoid formation in a subcutaneous transplant.

The fibrosarcomatous nature of the tumor arising from the pituitary region has been clear from the first and has remained histologically stable throughout.

The method outlined here is considered superior in that a uniform suspension is used, the introduction is not under pressure, and therefore, the possibility of cells being forced into veins is obviated. Furthermore, a measured number of cells can be used. In pre-

liminary experiments, 110 cells from a tissue culture of 67% viability have been introduced to grow very satisfactorily in the medulla of the femur.

From our results it would seem reasonable to suggest that in the absence of parathyroid glands the animals are more resistant to tumors invading bone than littermates with intact parathyroids. The place of parathyroid hormone in malignant invasion of bone remains to be clarified.

Summary. Fibrosarcoma induced in the pituitary by feeding 3-MC to a castrated male Sprague-Dawley rat, has been transplanted into the medullary cavity of the femur in female animals. Growth progress within the bone has been successfully demonstrated by X-rays. Parathyroidectomy prior to introduction of the tumor significantly prolongs the survival time of the animal.

The author wishes to thank Dr. T. L. Dao for his encouragement and advice in these studies. Technical assistance from Joan Glab and Daniel Gawlak is gratefully acknowledged.

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Received June 18, 1964. P.S.E.B.M., 1964, v117.

Effect of Two Lathyrogens on Cytochrome Oxidase in Cultures of the Strain L Fibroblast.* (29709)

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Osteolathyrism, a toxic syndrome involving the connective tissues of experimental animals, can be produced by a variety of chemical compounds. The pathogenesis of the disorder has been the subject of investigation for several years. One theory proposes that the disorder involves inhibition of cytochrome oxidase, an enzyme mediating transfer of electrons to molecular oxygen in the major chemical oxidative energy pathway of cell mitochondria(1). Aminoacetonitrile (AAN) and beta-aminopropionitrile (BAPN) are the 2 most widely used lathyrogenic compounds. Each has been shown, using different systems, to be capable of inhibiting cytochrome oxidase(1,2). The present experiment is an attempt to appraise the relative efficacy of these 2 substances as enzyme inhibitors in comparison with their relative efficacy as lathyrogens under the comparatively constant conditions of an *in vitro* tissue culture system.

* Supported by grants 5T1 GM-133 and GM06995 from U.S.P.H.S.

Material and methods. Cells of the L strain, originally derived from mouse fibroblasts, were used in this experiment. The growth medium consisted of Eagle Minimum Essential Medium(3) (Hyland Laboratories) to which was added 10% calf serum, 1% L-glutamine, and antibiotics (penicillin and streptomycin).

Di-BAPN fumarate, AAN hydrogen sulfate, and sodium fumarate solutions were each prepared in minimum essential medium and neutralized with one normal sodium hydroxide to pH 7. Sterilization was achieved by millipore filtration.

L cells from 4-day-old stock cultures were resuspended in fresh growth medium, to which BAPN, AAN or the fumarate control solution had been added. The suspended cells were pipetted in 4.0 ml aliquots into sterile one-ounce rectangular wide-mouth bottles containing glass cover slips. These were then sealed and incubated at 37°C for 72 hours.