Development of a Somatotropic Variant of the Mammosomatotropic Tumor MtT/W5[1] (35746)

NINA HOLLANDER[2] AND VINCENT P. HOLLANDER

Research Institute of the Hospital for Joint Diseases, Mount Sinai School of Medicine, 1919 Madison Avenue, New York, New York 10035

The rat pituitary tumor MtT/W5 arose originally by X-ray induction in a W/Fu rat (1). It secretes growth hormone and prolactin but no ACTH. Its secretory characteristics have been amply documented (1–3) and the present experiments confirm these findings. The tumor was obtained from Dr. Furth of Columbia University, New York, and has been stable for approximately 30 generations in this laboratory. With the methods used here routinely, the usual time of survival of the rat after implantation of the tumor is 8–12 weeks. By the expedient of resection and reimplantation, the length of time that the tumor is present in a single animal has been approximately doubled. During this increased length of time the effects of growth hormone and prolactin continue to be demonstrable in the animal. In addition, metastases appear primarily in ovaries and bone. Some of these metastases are functionally different from the parent tumor; that is, when subtransplanted, they secrete only growth hormone. The present report documents these findings. It is possible that during the metastatic spread a selective cloning occurs for this tumor.

Materials and Methods. Wistar-Furth female rats were obtained from A. R. Schmidt Company, Madison, Wis., fed Wayne Lab Blox and water *ad libitum*. When they weighed approximately 150 g, they were implanted subcutaneously via number 13 trochar with a 2 × 4-mm fragment of MtT/W5 tumor near the scapular region. The tumor is maintained in this laboratory in W/Fu female rats and transplanted every 2 or 3 months. Each generation of tumor is tested for hormonal function by comparison of body weight with intact littermate controls and by comparing the growth of a prolactin-responsive mammary tumor MT/W9 in non-tumor-bearing animals and animals with the pituitary tumor. The MT/W9 tumor does not grow unless prolactin or a prolactin-secreting tumor is also present.

When the MtT/W5 tumor had grown to approximately 2 × 2 cm, an additional tumor fragment was implanted in the opposite flank. Then, when the first tumor had grown to approximately 3 × 5 cm, it was resected totally under light ether anesthesia, and the skin was closed with metal clips. This procedure of implantation in a different site and resection of large tumors was continued so that the animal had an MtT/W5 tumor present at all times. After the initial implantation, and after each surgical procedure, the animals were given Veterinary Terramycin (Pfizer and Company) in their drinking water (30 g/liter) for 1 week, after which they were returned to tap water.

Animals were sacrificed periodically up to 21 weeks after initial tumor implantation. The day before sacrifice, an X-ray was taken of each rat. At autopsy, weights were obtained of the animal, the subcutaneous tumor, spleen, kidneys, adrenals, ovaries, and thymus. Fragments of mammary tissue, spine, femur, and tibia were removed for histologic examination.

A fragment of the subcutaneous tumor present at the time of sacrifice was implanted into a new W/Fu female rat (test animal). If metastases were present in the ovary, or if the X-ray picture indicated the presence of

[1] This investigation was supported by Contract PH 43-64-46, Endocrine Evaluation Branch, General Laboratories and Clinics, National Cancer Institute.

[2] Present address: Rockefeller University, New York, New York.

bone metastasis, a fragment of the metastatic tumor was obtained and subtransplanted into a test animal. The test animals, that is, those with secondary subcutaneous implants from parent tumor or from metastases, were kept until the tumor grew to 2 × 3 cm or larger. The test animal was then sacrificed, weighed, and fragments of mammary tissue were taken for histologic examination.

Histologic sections were stained with hematoxylin–eosin. The bones were decalcified with nitric acid or EDTA before sectioning and staining.

X-Rays were obtained with a Waite machine at a distance of 100 cm for 4 sec, using Agfa-Gevaert Curix Rapid DW base film. They were developed in a Kodak M-4 X-omat machine.

Results. The autopsy results of the experimental animals are summarized in Table I. Growth hormone activity was indicated in tumor-bearing rats as expected by increased body and organ weights. These are contrasted with the weights in littermates without tumor. Evidence of prolactin secretion was present in all the animals as seen in sections of mammary gland, which showed considerable lobular stimulation and secretion. ACTH secretion is not usually expected with the MtT/W5 tumor although it is prominent in some of the other rat pituitary tumors. However, the increased adrenal weights after the 17th week as well as decrease of thymus weight in some of the animals suggests an increase in circulating ACTH.

Metastatic tumors were found first in the ovaries after 15 weeks. These masses varied in size up to 2 cm in diameter and involved either left or right or both ovaries with no consistent preference. Although in some instances the remaining ovarian tissue consisted of a thin layer at the periphery of the tumor, its functional capacity continued as shown by the uterine weights. X-Ray evidence of bone metastasis appeared after 21 weeks. An X-ray picture of bone metastasis is shown in Fig. 1 along with the microscopic appearance of one of the areas of metastasis. The site of preference appeared to be the distal end of the femur, although in one rat tumor was present in the proximal end of the humerus, as well as in the femur.

After the appearance of metastases any additional hormonal secretion by the metastatic tumor could not be assessed. In fact, there was no indication that the metastatic tumors were in any way different from the primary tumors until the subtransplants grew out and were found in some instances to be functionally different.

Proliferation of mammary tissue and appearance of mammary secretion on microscopic section were considered evidence for prolactin secretion by the subtransplanted tumor. In every instance the parent tumor was positive, as was expected. Three out of nine tumors from ovarian metastases were negative, that is, the mammary tissue from animals with these subtransplants was not stimulated.

In tumor subtransplants from four bone metastases, one showed evidence of prolactin secretion, one showed none. In two instances, tumor was taken from two different sites of the same metastasis and implanted into different rats. One rat in each instance showed prolactin secretion while the other did not.

Growth hormone secretion by the tumors in the test animals was shown by the increase in body weight and length of these animals over those of nontumor-bearing littermate controls.

Two of the subtransplanted ovarian metastases which showed growth hormone secretion had no prolactin secretion have retained these characteristics over at least eight transplant generations in this laboratory. Immunoassay of the blood of these animals, done by Dr. Jacob Furth, showed a high level of growth hormone and a normal level of prolactin.

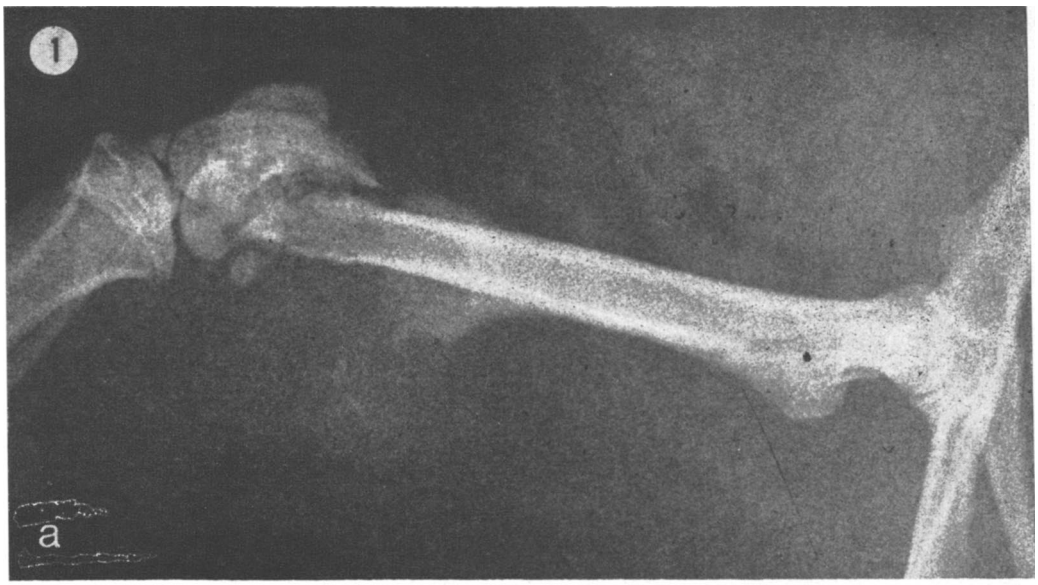
It is possible that there is a generally decreasing hormonal output with disappearance of prolactin before the eventual disappearance of growth hormone. However the marked growth response in the animal shown in Fig. 2 suggests that the new line of tumors probably secretes even higher amounts of growth hormone than the MtT/W5 parent tumor.

Discussion. The rat pituitary tumor MtT/W5 is a stable tumor which over many

TABLE I. Body and Organ Weights of Experimental Animals.

Duration of experiment (weeks)	No. of animals	Body weight without tumor (g)	Liver (g)	Spleen (g)	Two kidneys (g)	Two adrenals (mg)	Two ovaries (mg)	Uterus (mg)	Thymus (mg)
11	5	205[a]	10.9	0.94	1.66	67.0	109.0	301.1	226.8
		±6	+0.9	±0.14	±0.07	±3.3	±5.2	±18.4	±18.6
15	4	239	13.4	1.30	1.99	75.5	—	291.2	212.0
		±8	±0.9	±0.39	±0.16	±5.2	—	±33.6	±17.5
17–18	7	206	12.9	1.32	2.03	142.7	—	478.3	115.5
		±14	±0.9	±0.25	±0.39	±13.9	—	±30.9	±24.1
21	4	245	15.5	2.20	2.38	115.8	—	336.0	213.0
		±11	±0.9	±0.62	±0.14	±13.8	—	±37.2	±31.0
Littermates with no tumor:									
11		157	5.0	0.33	1.16	48.0	53.0	132.0	137.0
15		185	6.5	0.43	1.40	57.0	87.0	339.0	165.0

[a] Values represent mean ± SE.



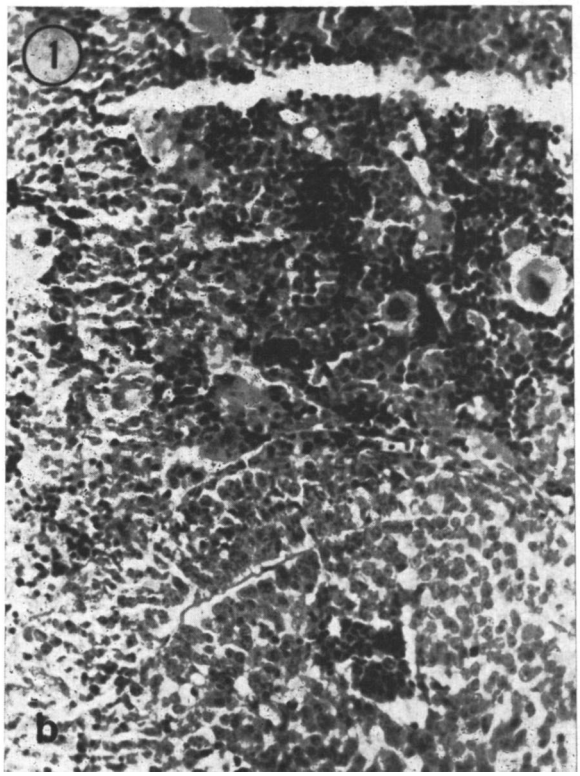


FIG. 1. a. X-Ray picture of MtT/W5 metastatic to rat femur. There is a pathologic fracture at the tumor site with a collar of callus formation. This tumor, and occasionally the subcutaneous tumors also, shows small areas of extraosseous calcification. b. Microscopic section of tumor metastatic to bone, showing sheets of tumor cells (lower portion) invading and replacing the bone marrow.

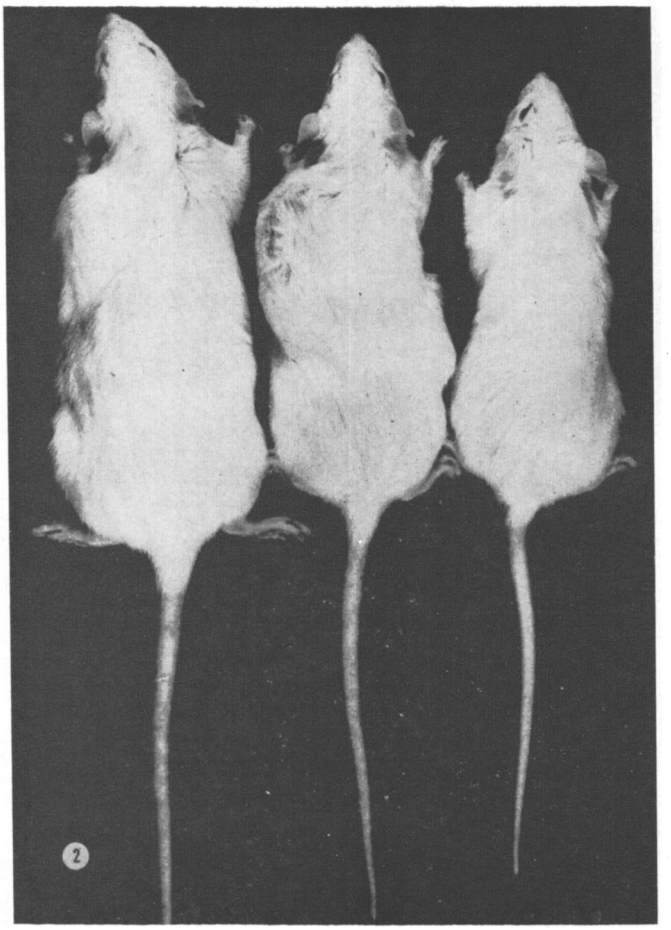


FIG. 2. Littermate rats of identical size were implanted with tumor in the left scapular region 8 weeks prior to this picture. The rat on the left has a subcutaneous implant of the exclusively growth-hormone-secreting metastatic line of tumor. The rat in the center has a subcutaneous implant of MtT/W5. The rat on the right has no tumor. Note that the tumors are of comparable size.

transplant generations (30 in our laboratory) has shown evidence of growth hormone and prolactin secretion. MacLeod reported the spontaneous appearance of a variant of this tumor which secreted only growth hormone (4). Welsch (personal communication) found in a fast-growing variety of MtT/W5 the appearance of ovarian metastases 3 months after subcutaneous implantation, but did not test the hormone-secreting capacity of the metastatic tumors. The predilection of metastasis for the ovary suggests that either estrogens or local unknown factors provide a favorable soil for the growth of the tumor.

The possibility of *in vivo* cloning through metastatic spread of at least two cell types should be considered. In routine methods of transplanting this tumor, because it is remarkably necrotic and bloody, a selection is usually made of a solid, healthy-looking area for transplantation. This selection may be the means by which, if there are two cell types (growth hormone and prolactin-secreting) their continued coexistence is assured. Tashjian *et al.* (5) demonstrated growth hormone secretion by three strains of cells from MtT/W5 cloned by means of tissue culture, but prolactin secretion or its absence is not mentioned.

In the metastatic process, single cells may

spread and suggest evidence of cloning. If this hypothesis is true, it should be possible by this means to obtain tumors which secrete only prolactin.

Summary. Repeated resection and reimplantation of the pituitary tumor MtT/W5 in W/Fu rats leads to the appearance of metastatic tumor chiefly in the ovaries and long bones. Metastatic tumor begins to appear about 15 weeks after initial subcutaneous implantation. Subtransplantation of some of the metastatic tumors results in a number of stable somatotropic tumors devoid of mammotropic activity. Whether this change in secretory activity is due to cloning or transformation cannot be determined at this time.

1. Yokoro, K, Furth, J., and Haran-Ghera, N., Cancer Res. **21**, 178 (1961).

2. Takemoto, H., Yokoro, H., Yokoro, K., Furth, J., and Cohen, A. I., Cancer Res. **22**, 917 (1962).

3. Cohen, A. I., and Kim, U., Cancer Res. **23**, 93 (1963).

4. MacLeod, R. M., Smith, M. C., and DeWitt, G. W., Endocrinology **79**, 1149 (1966).

5. Tashjian, A. H., Jr., Yasumura, Y., Levine, L., Sato, G. H., and Parker, M. L,. Endocrinology **82**, 342 (1968).

Received Feb. 19, 1971. P.S.E.B.M., 1971, Vol. 137.