THE EFFECT OF ANDROGENS AND ESTROGENS ON SPONTANEOUS BENIGN MAMMARY TUMORS IN THE RAT

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The effect of exogenous hormones on transplanted mammary fibroadenomata in the rat has previously been described (1, 2). The present report is of a similar study of spontaneous benign mammary tumors.

Fig. 1. Spontaneous Mammary Adenofibroma in White Rat. Magnified 300 diameters. All other cuts are the same magnification.

For this study 97 rats were used, 94 females and 3 males. All the animals were observed until death, the period of observation extending from one to two years. In every instance the tumors were examined and measured, and the weight of the animal and of the tumor was determined at autopsy. The ovaries, adrenals, mammary glands, and pituitary glands were studied histologically.

Spontaneous fibroadenomata of the rat's breast vary widely in shape, size, and rate of growth. Detailed studies on the morphology of these tumors have been published by Bullock and Curtis (3), Curtis, Bullock and Dunning (4) Heiman (5), Wright, Klinck, and Wolfe (6).

In the present series all the tumors, with one exception, occurred in either the right or left axillary or inguinal mammary glands. Eighty-four rats had
Fig. 2. Spontaneous Mammary Fibroadenoma (Rat 2418) Before Implantation of Crystalline Estrogen

Fig. 3. Third Autotransplant (2418/0-3rd) After Administration to Rat of 1.5 mg. Estrogen
Spontaneous fibroadenoma becomes adenoma.
one primary tumor each; 13 had two each. None of the tumors seemed to originate in the thoracic glands and only one in the lower abdominal region. The breasts rarely suckled by the young are the ones in which the tumors are most frequent and the absence of nipple stimulation, followed by duct occlusion and hormonal action, is probably a factor in initiating changes leading to neoplastic growth. The morphologic types were as follows: adenofibroma 70 per cent, adenoma 16 per cent, cystadenoma 6 per cent, fibroma 8 per cent.

Two male rats, under one year of age, had one fibroma each, while one young male, six months of age, with cystic degeneration and atrophy of the testicles, bore a subcutaneous fibroadenoma. Ninety per cent of the females with fibro-epithelial or epithelial tumors had enlarged, cystic, or abscessed ovaries (7). No morphologic correlation could be established between the size of the adrenals or pituitaries and the mammary tumors (8).

Series I: In the first series of experiments autotransplantations and homotransplantations were done in 48 female white rats. The original tumors were removed with their capsules, and fragments weighing 0.3 gm. were introduced subcutaneously in both axillae and groins of the same animal. One to four of these implants grew in all rats after a latent period of six to twelve weeks. The most rapid growth occurred usually at the primary tumor site. The variations which occurred were probably due to the increased availability of nutrition to certain tumor fragments, through greater surface exposure to circulatory influences, such as food and hormones. The more rapidly growing tumors were more cellular and vascular, and the acini appeared less compressed. When the autotransplants reached a certain size, they were removed and small fragments were implanted in the same animals. Such serial autotrans-
Fig. 5. Autotransplant (2236/0) Sixty Days Old, Before Implantation of Estrogen Crystals

Fig. 6. Second Autotransplant (2236/0) Twenty-one Days Old with Synchronous Implantation of Crystalline Estrogen
plantations were repeated from two to five times, until the animal died. No pronounced morphologic variations were observed in the transplants as compared with the primary tumor. (Fig. 1.)

Fragments of spontaneous adenofibroma were also implanted in other rats from which spontaneous tumors were first removed. These homotransplants retained the morphologic characteristics of the primary tumor only if the inoculated host was originally the bearer of a spontaneous fibroadenoma. In animals from whom spontaneous fibromata had been previously excised, the adenofibroma transplants lost their glands and were transformed into richly cellular fibroma similar to the spontaneous tumor originally removed. On the contrary, with certain exceptions, to be explained later, homotransplants from spontaneous fibromata remained fibromata in hosts from which spontaneous adenofibromata had been removed.

Series 2: The second series comprised 18 female rats treated with estrogen. Animals bearing spontaneous benign tumors, or with auto- or homotransplants after removal of the original tumors, were injected subcutaneously with estrogens in oil or had the crystalline hormone introduced into subcutaneous pockets (9). The hormone used was an estradiol benzoate and the dosage 0.1 to 2.5 mg.

After treatment with the estrogen, large spontaneous tumors showed no change in morphology. Autotransplants and homotransplants, however, grew more rapidly. The latent period was reduced to twenty-one days or less, and morphologically the tumor changed to a soft adenoma, cystadenoma, or papillary cystadenoma (Figs. 2 to 7). The proliferative capacity of the epithelial fraction of the tumor was markedly enhanced, and a secretory phase
with desquamation in and dilatation of alveoli and ducts was observed. An irregular epithelial over-growth appeared in many alveoli. In one section there was seen epithelial invasion between strands of muscle fibers.

Autotransplanted fibromata in rats injected with estrogens remained fibromata through four inoculations. All animals otherwise showed marked general effects of estrinization. Homotransplants of fibroma in injected rats from which spontaneous epithelial tumors were first entirely removed showed a moderate growth of ducts and glands. (Without injections this did not occur, as noted above.) The possibility of latent epithelial elements in mammary fibroma must be assumed to explain this appearance of glands and ducts in the estrogen-stimulated fibroma growing in another host. It would seem, however, that another growth factor must be present, since estrogens alone did not produce epithelial elements in an autotransplanted fibroma.

Homotransplants of epithelial tumors in injected rats from which spontaneous fibromas were first removed developed into actively growing adenofibromata or adenomata, depending on the amount of estrogen administered. If the amount was small, with eventual elimination, the fibroadenoma gradually lost its epithelial components through necrosis, pressure, and connective-tissue over-growth, and became a fibroma similar in morphology to the spontaneous fibroma originally removed. It is thus evident that the host lacks hormonal or other growth factors for abnormal epithelial stimulation after the exogenous hormonal effect is lost.

It appears that growth-stimulating factors necessary for abnormal connective-tissue growth are not identical with those necessary for abnormal epithelial proliferation. Estrogen is evidently not a factor in promoting growth

Fig. 8. Small Spontaneous Fibroadenoma in a Female Rat; No Androgens Administered
Fig. 9. Small spontaneous fibroadenoma after subcutaneous implantation of 10 mg. of androgen, showing increase in stroma with few glands. Biopsy six weeks after implantation of hormone.

Fig. 10. Autotransplant (2515/0-1A) four months after subcutaneous implantation of 10 mg. of androgen.
of mesodermal tissue, but is an accelerator of the growth of specific normal and abnormal glandular epithelium.

**Series 3:** A third series of experiments was carried out with 16 female white rats treated with androgen. Animals from which the original tumors—spontaneous benign tumors or auto- or homotransplants—had been removed were treated with androgens, injected subcutaneously in oil or introduced in crystalline form in subcutaneous pockets. The total dose administered was 5 to 15 mg. of testosterone propionate. A large spontaneous tumor showed no histologic change. Autotransplanted or homotransplanted adenofibroma did not grow in 13 (80 per cent) of the treated rats. In a few animals, after a prolonged latent period of sixteen to twenty-four weeks, small, hard, white, fibrous tumors appeared, growing very slowly (Figs. 8–13). The possibility

![Image](image_url)

**Fig. 11. Autotransplant Six Months After Subcutaneous Implantation of 10 mg. of Androgen**

of elimination of the injected androgen or the production of specific anti-hormones may be considered as factors in the release of inhibition and recurrence of tumor growth. Murlin and his co-workers (10) have reported a reduction in mortality and metastases from Brown-Pearce carcinoma in rabbits treated with androgen. Their explanation of this phenomenon is that androgens and estrogens act in opposite ways as oxidizing and reducing agents. Whereas, according to their view, the estrogens deprive the cells of oxygen, compelling anaerobic glycolysis, androgens prevent this action. As previously reported by us, the growth inhibition exerted by androgens is directed mainly to the epithelial elements of the adenofibroma (1). The growth of spontaneous fibroma, however, is moderately inhibited but the growth of auto- or homotransplants of fibroma is not prevented.
FIG. 13. Spontaneous Adenofibroma (2537/0) After Subcutaneous Implantation of 10 mg. of Male Sex Hormone, now a Fibroma
No epithelial elements are present in the section.
In androgen-treated rats in which tumor growth was inhibited, the ovaries were atrophic and fibrotic.

Series 4: Twelve female rats with spontaneous benign tumors or bearing auto- or homotransplants were injected subcutaneously with estrogens and androgens, together or in sequence. The total dosage of estrogen was 1 to 2.5 mg., that of androgen 10 to 15 mg. Large spontaneous tumors were not affected. Very early small spontaneous tumors, homotransplants, and autotransplants, all grew. The latent period was prolonged to six weeks (three weeks in estrinized and twenty weeks in androgenized rats) and adenofibroma became fibroadenoma. It seems that in the ratio given—estrogens 1 to androgens 10—the estrogens have a stronger stimulating effect, overcoming the inhibiting action of the androgens (Figs. 14 and 15). This observation is borne out by Mark and Biskind (11), who noted that when estrogens and androgens are given together to normal rats, the effect of the former is predominant. We have also reported that androgens in 5 to 10 mg. doses administered to tumor-bearing pregnant rats do not alter the morphology of transplanted fibroadenoma (2).

Series 5: Spontaneous tumors in 10 female rats were injected with various chemicals, such as Sudan III, scarlet red, methylene blue, tar, and benzpyrene. The animals were observed for the duration of life. No morphologic change toward malignancy appeared. The tumors all became gradually more fibrotic. The details of these experiments have been published previously (5, 8).

Discussion

Rats with spontaneous tumors were chosen for auto- and homotransplantation experiments because thus many complicating genetic factors are eliminated, and the margin of error in interpretation is possibly lessened. A simpler basis of comparison is established between original tumors and autotransplants for observing experimental variations. Also, as the tumors are benign, a longer observation period is possible.

The results obtained, however, compare closely with those obtained in normal animals inoculated with tumors from other rats and then injected with androgens and estrogens. The experiments do not explain the initiation of benign spontaneous growths, the stimulating effect of estrogens on specific epithelium, or the inhibiting effect of androgens on epithelium and connective tissue.

Although estrogen-stimulated animals showed an activation of tumor growth (see, for example, Fig. 15), in no instance did a true carcinoma develop. Other growth factors are evidently necessary to initiate malignant transformation.

Homotransplants of adenofibroma in rats with spontaneous fibroma became cellular fibromata unless the animals were treated with estrogen. As long as the hormonal stimulus was present, the epithelial elements grew. Homotransplants in normal animals showed the same behavior.
Fig. 14. Second autotransplant of spontaneous benign fibroadenoma six months after implantation of 1 mg. crystalline estrogen and three months after implantation of 10 mg. crystalline androgen.

Fig. 15. Autotransplant after administration of 2.5 mg. of estrogen and 10 mg. of androgen.
Conclusions

1. With minor variations, autotransplants, for two to five tumor generations, retain the structural identity of the original tumor.

2. Homotransplants of adenofibromata in rats freed from spontaneous fibromata become cellular fibromata, in some instances resembling sarcoma but never undergoing malignant change.

3. Homotransplants of fibromata in rats freed from adenofibromata remain fibromata unless the host is estrinized.

4. Estrogens stimulate the epithelial components of early spontaneous, autotransplanted, or homotransplanted adenofibroma, producing adenoma or cystadenoma.

5. Androgens, on the other hand, inhibit the epithelial components of these tumors.

6. Estrogens administered with ten times the dose of androgens overcome the inhibitory action of the androgens and exert a stimulating effect on growing specific epithelium.

7. Connective-tissue elements of the tumor are not directly affected by estrogens and are moderately inhibited by androgens.

8. The morphological changes are clearly shown in the accompanying photomicrographs, all taken with exactly the same magnification.

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Bibliography