

# The Effect of Testosterone Propionate on Mammary Tumors in Mice of the C3H Strain

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A relationship between estrogenic activity and the production of tumors of the mammary gland has been clearly demonstrated in various strains of mice. In view of the inhibitory action of testosterone on estrus, lactation, and the vaginal response to estrone in mice (4, 5), the possibility of affecting spontaneous tumor formation by inhibition of estrogenic activity seemed worthy of further investigation. In 1936, Lacassagne (2) reported that injections of testosterone had not altered the incidence of spontaneous mammary cancer in females of a high cancer strain, but the amount of hormone used was small.

This report presents the end results of long-continued administration of testosterone propionate on the development of spontaneous mammary carcinoma in female mice of a high cancer strain (a) when injections were begun by the 5th week of life, and (b) when injections were given to older females with a history of normal breeding. It also includes observations on the failure of testosterone to affect the growth rate of mammary carcinoma in mice when administered after the tumor is of palpable size.

The experiments were started in the spring of 1938 and extended over 2 years. They were well under way when the report of Nathanson and Andervont (3) was published, but since our experiments differed in some respects from theirs, it was decided to continue them.

Nathanson and Andervont (3) gave testosterone for a period of 4 months to C3H female mice, 4½ months of age or younger, which had previously borne one litter. Thirty per cent developed mammary carcinoma within 4 months after injections of the hormone were started, and died before the 11th month of age, with only a single tumor. Ten were still alive and without tumors at 16 months. The fate of the surviving mice has not been reported. All of the controls had developed at least one, and many of them multiple, tumors by the 11th month of life. The authors believe that the administration of testosterone prevented the development of tumors, "provided there were none present when the treatment became effective." They assume that those tumors which did develop were microscopic in size when treatment was started.

## MATERIALS AND METHODS

In the experiments reported here, female mice of the C3H strain<sup>1</sup> were likewise used. In the first experiment, 14 females received subcutaneous injections of testosterone<sup>2</sup> over a period of 45 weeks. Ages at the first injection ranged from less than one week to 5 weeks. The testosterone was diluted with olive oil so that each 0.05 cc. contained 0.5 mgm. of the hormone. For the first 4 weeks, 11 of the mice received 3 injections weekly, each containing 0.5 mgm. of the hormone. For the following 41 weeks, they received one injection containing 1.0 mgm. weekly. These mice were from 18 to 34 days of age at their first injection. The remaining 3 mice were given their first injection containing 0.1 mgm. of hormone at 2 days of age. After the first week, the amount was increased to 0.5 mgm. three times weekly for 3 weeks, and then reduced to 1.0 mgm. once a week for the remaining 41 weeks. These mice exhibited slight modifications of the external genitalia. None of the treated females became pregnant although a male of proven fertility was kept with them over long periods of time.

<sup>1</sup>The author is indebted to Dr. H. B. Andervont for the original C3H mice from which our strain has been developed. These consisted of two litters with 3 females and a sib male in each. The stock has been continued by brother-to-sister mating. Among the descendants of the two litters, 91 per cent of 300 bred females have developed mammary gland cancer, which is the same (91.37 per cent) as Andervont's (personal communication) for bred females of this strain in his laboratory. Virgin females, however, have not shown the expected high incidence of such tumors. In Andervont's stock (1), the cancer incidence is even higher (97.43 per cent) in virgin females. In our substrain, however, wide variation in tumor incidence in virgin females has been encountered among the offspring of the 6 original females. Unfortunately, the numbers are small, and as our present colony is composed of descendants from only one of the original females, we are unable to investigate the matter further.

<sup>2</sup>The crystalline propionic ester of testosterone in oil, Oreton, prepared by the Schering Corporation. We are indebted to Dr. Erwin Schwenk for the supply used in these experiments.

Injections were discontinued when the mice were 45 to 50 weeks old, the average age at which virgin females develop mammary tumors in this strain. They had thus received testosterone injections throughout the period during which tumors arise in the majority of such females.

*Controls.*—Thirty-eight virgin females from the same sublimes as the treated females have been used as controls on which to base expectancy of tumor incidence, age at appearance of tumors, and age at death of noncancerous individuals. Of these, 18, or 47 per cent, developed tumors of the mammary gland at an average age of 12.0 months. The average age at death of noncancerous mice was 19.7 months.

### RESULTS

Two treated mice died before reaching tumor age. Of the remaining 12, 3 developed adenocarcinoma of the mammary gland at 15, 22, and 24 months, 3, 10, and 12 months, respectively, after cessation of treatment. Injections of testosterone had been started in one of these at 18 days of age and in the other 2 at 34 days. In 2 of them, both ovaries were almost completely replaced by large cysts filled with clear fluid. Two mice developed tumors at other sites, one a sarcoma adjacent to an old oil abscess, at 27 months, and the other a primary liver cell tumor, discovered at autopsy at 27 months. Such tumors are known to occur in mice of this strain and have no bearing on the present problem. The remaining 7 mice died or were killed for autopsy at an average age of 21.7 months. Four females, in addition to the 2 with mammary tumors mentioned above, had cysts almost replacing one or both ovaries, leaving only a thin rim of ovarian tissue. Similar cysts were found in 3 of the control virgins, and have been found occasionally in bred females of this strain. In one treated mouse, a large cyst was found in the left adrenal gland.

Whole mounts of mammary glands were made from 8 treated females and from 10 control virgins of comparable age. The control glands are characteristically composed of extremely wide, cystic-appearing ducts, with no hyperplasia of the lining epithelium. Many of the ducts end in club-shaped or bulbous enlargements. In some glands, there are also a few narrow, branching ducts with grapelike clusters of alveoli and occasional isolated and localized areas of alveolar proliferation. Cellular infiltration is common around the alveoli, and rarely ducts and alveoli are surrounded by a cloud of lymphocytes.

Glands from testosterone-treated females are variable in appearance. Moderately wide, cystic ducts are present in all cases, but in none is the widening as exaggerated or extreme as in the controls. In almost all cases, in addition to the widened ducts, there are

also narrow, branching ducts with small clusters of alveoli. In 2 mice, the glands are predominately of the narrow, branching type. Small, localized areas of alveolar proliferation are present in several cases, and periductal infiltration is common although variable in amount.

In view of Nathanson and Andervont's report (3) that bred females (having had one litter which was removed at birth) injected with testosterone over a period of 4 months had a lower tumor incidence than control females, a study was undertaken of the effect of testosterone on a group of bred females which had suckled their young. Thirty-two females which had borne and nursed successfully 1 to 3 litters were divided into two groups with sister litter mates of similar breeding history in each group. Ages ranged from 4 to 8 months, with an average age of 5½ months at beginning of treatment. Eight females in each group had borne 1 litter, 7 had borne 2, and one had borne 3. Injections of 1.0 mgm. of testosterone were given once a week over a period of 4 months, or until the development of tumors. All females, both control and experimental, developed tumors, at an average age of 9 months for the controls and 9.6 months for the treated. Thus, in older females of normal breeding history, injections of this amount of testosterone (which was smaller by one-third than the amount given by Nathanson and Andervont) were ineffective in lowering tumor incidence and in delaying the appearance of tumors to any appreciable extent.

An attempt to influence the growth rate of tumors of palpable size was without effect, as previously reported by Nathanson and Andervont (3). Ten females were used in this study. Biopsy was done on each tumor and treatment started the second day following operation. Injections of 1.0 mgm. of testosterone were made subcutaneously on the side opposite the tumor, and were given three times weekly, or until the appearance of a second tumor. All tumors grew progressively and 3 of the mice developed a second tumor during the period of treatment.

### DISCUSSION

Testosterone was administered to C<sub>3</sub>H female mice from the 2nd to the 12th month of life, the period during which a majority of untreated females of this strain, virgin as well as bred, usually develop tumors. None developed tumors during the period of treatment. Three (25 per cent) of those living to cancer age developed tumors of the mammary gland at an average age of 20 months. Eighteen controls (47 per cent) developed tumors at an average age of 12.0 months. Thus, tumor incidence has been reduced by testosterone.

Because of the small number of mice involved, it is difficult to judge whether or not the age at macroscopic appearance of tumors has actually been increased. The 3 tumors among testosterone-treated individuals developed at 15, 22, and 24 months. Among the 18 control mice with tumors, 6 were seen for the first time after 12 months of age, at 13, 14 (2 individuals), 15, 16, and 24 months. Thus, the late age at which tumors developed in the treated mice may be a chance occurrence. The results, however, are interesting in view of the suggestion of Nathanson and Andervont that administration of testosterone propionate will prevent the development of tumors provided there was none present when treatment became effective. If this is the case, then tumors were in existence in the 3 individuals of the present experiment before 5 weeks of age. Since treatment with testosterone apparently does not influence the growth rate of tumors already established, the advanced age at macroscopic appearance of tumors can hardly be attributed to a retarding effect of the hormone. It seems reasonable to believe, therefore, that these tumors arose after cessation of treatment, when there may possibly have been renewed estrogenic activity stimulated by a pituitary reaction after the withdrawal of the male hormone.

## SUMMARY AND CONCLUSIONS

1. Injections of testosterone propionate to virgin female mice of the C<sub>3</sub>H strain from the 2nd to the 12th month of life lowers the incidence of spontaneous mammary gland tumors, and possibly increases the age at macroscopic appearance of tumors.
2. The incidence and age at appearance of tumors in females of normal breeding history are not influenced by testosterone in the amounts used in these experiments.
3. Injections of testosterone do not inhibit growth of mammary gland tumors once they have reached macroscopic size.

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