Effect of Testosterone Propionate on the Adrenals and on the Incidence of Mammary Cancer in the RIII Strain of Mice

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INTRODUCTION

The hormonal relationship that Cramer and Horning (6, 8, 9) believe to exist between brown degeneration of the adrenals and carcinoma of the mammae in high tumor strain mice has been questioned by other observers, but not definitely disproved. Details may be found in the papers of Dobrovolskaïa-Zavadskaïa and her group (15-17); Lacassagne and Raynaud (25, 26); Blaisdell, Gardner, and Strong (1); Bonser (3); and Kreyberg (23). This degeneration occurs frequently at an early age in female mice of a high tumor strain, according to Cramer (8) and Dobrovolskaïa-Zavadskaïa (17), but is seen also in the tumor-free males of the same strain at a later age. It has been described by Whitehead (31), Martin (28), Howard-Miller (21), Burrows (5), Daughady (11), and Deanesly (12) as seen at the cortico-medullary junction, in the periphery of the medulla, or in the medial part of the zona reticularis. The change significantly begins in the x-zone, which, according to Howard-Miller, is "A transitory zone showing age and sex relationships in mice."

The appearance of brown degeneration around the x-zone in various strains of mice after estrinization as described by Burrows (4, 5), Cramer (6), and Martin (28), and the induction of mammary tumors in similar strains also by estrinization reported by Cramer (10), Bonser and her associates (2), Lacassagne (24), Twombly (30), and Gardner (18) warranted further studies on this apparent relationship.

Burrows (5) states that the effect of castration, pregnancy, and estrogen on the presence or absence of the x-zone suggests that it may be influenced by testosterone or progesterone, and notes that these hormones seem to hasten the involution or absorption that normally occurs in the x-zone of adrenals in female mice. Deanesly points out that castration of the male causes reappearance of the x-zone, converting the male adrenal to the female type. Woolley, Fekete, and Little (32) have observed nodular hyperplasia and carcinoma of the adrenals in ovariectomized mice that show a return of estrogen effect. Martin (28) noted the disappearance of the x-zone in females after testosterone. Thus a definite relationship has been shown to exist between sex hormones and adrenals on the one hand, and the same hormones and certain mammary tumors on the other.

The problem that presented itself, therefore, dealt with the administration of certain hormones to mice with a high incidence of both mammary tumors and adrenal degeneration.

MATERIALS AND METHOD

The experimental data reported here deal with brown degeneration of the adrenals, in relation to mammary carcinoma in female mice of the RIII strain, after the administration of testosterone propionate.[1] These mice were inbred from animals procured from Dr. N. Dobrovolskaïa-Zavadskaïa (13) by Dr. F. C. Wood in May, 1935.

In order to determine approximately the incidence of spontaneous mammary carcinoma in RIII breeding female mice in this laboratory, 486 were observed from the fourth to the eighteenth month of life. For several reasons, including the diminishing incidence after 18 months (14), all animals without tumors were eliminated at that age and classed with those dying before that age as negative. Figures for comparison between untreated and treated animals were sought because various observers have reported incidences ranging between 56 and 79 per cent in untreated mice (15, 24). Of the 486 stock mice observed, 254 developed mammary adenocarcinoma (52.2%). A small number had 2 or more tumors. The earliest tumor to be noted arose at the age of 180 days, the remainder appearing between 200 and 326 days of age. Dobrovolskaïa-Zavadskaïa and Kobozieff (14) reported that tumors appeared in 37.3 per cent of RIII mice 18 months old.

[1] Testosterone propionate for these experiments was generously furnished by Dr. Erwin Schwenk, of the Schering Corporation; Dr. R. J. Floody, of Roche-Organon, Incorporated; and Dr. E. Oppenheimer, of Ciba Pharmaceutical Products, Incorporated.

Observations on the Growth of Tumors in RIII Mice after Injection of Testosterone

Between May 2, 1941 and August 11, 1942, 96 breeding female mice, 5 to 7 months old, were injected subcutaneously every 2 weeks with 0.5 to 1.0 mgm. of testosterone propionate in peanut oil (total of 8 to 9 mgm.). Animals between these ages were chosen because the mammary gland was fully developed. As Lacassagne (24) has shown that mammary tissue atrophies when androgens are administered to female mice soon after birth, it would have been impossible to evaluate the pharmacologic effect of testosterone on a mature precancerous gland unless treatment were begun at maturity. Twelve mice, 6 months old, received cutaneous inunctions over epilated areas every 2 weeks (total 18 mgm.). As the animals aged the injections and inunctions were given every third and finally every fourth week. All were observed until death, which occurred between 8 and 20 months of age.

Table I: Tumors in Testosteronized Female Mice of the RIII Strain

Series	Number of mice	Age at beginning of treatment	Amount of testosterone	Duration of treatment	Number of tumors	Per cent
I Box 1	24	7 mos.	8.7 mgm.			
Box 2			16 injections	14 mos.	6	25.0
II Box 3	24	5½ "	9.7 mgm.	15.6 "	5	20.8
Box 4			18 injections			
III Box 1	12	6 "	18 mgm.			
			18 inunctions	15 "	2	16.6
IV Box 1	48	5½-6 "	9 mgm.	8 "	8	16.6
Box 2			9 injections			
Box 3						
Box 4						

In 5 mice tumors appeared within 28 days after first treatment.
In 1 mouse tumor " " 42 " " " "
" " " " " " 56 " " termination of "
" " " " " " 372 " " first "
24 mice without tumors died between the 8th and 12th months.

Table I gives details of the experiment. Spontaneous mammary tumors appeared in 19.4 per cent (21 of 108) of androgen treated female mice; when 24 tumor-free mice that died before the age of 1 year were eliminated the tumor-incidence was 25 per cent (21 tumors in 84 mice). The incidence in untreated animals, as previously mentioned, was 52.2 per cent. In the different series of treated mice the percentage of tumors arising varied between 16.6 and 25.0 per cent depending on dosage, time, and method of administration, and age of animals at death. Mice receiving a larger dose (18 mgm.) in series III, and those treated for a shorter time in series IV (8 months), gave a lower percentage of growths. Nathanson and Andervont (29) reported an incidence reduced to 30 per cent after testosterone injections. Similar results were reported by E. E. Jones (22) and also by A. A. Loeser (27).

None of the mice subjected to testosterone treatment bore any litters.

Tumors arising in injected mice were not influenced or inhibited by further treatment with testosterone. Multiple tumors did not appear in treated mice, but occurred frequently in the untreated controls. The time elapsing between the first treatment and the discovery of tumors varied between 28 and 372 days. It is probable that in 5 mice minute tumors were already present when treatment was started, since they became manifest within 4 weeks after the first injection of 0.5 mgm. of testosterone propionate. It might have been better, therefore, if treatment had been started at 4 months of age instead of 6. In addition to these early tumors 4 appeared after treatment had been discontinued for 56 days or more.

The various types of mammary carcinoma arising in androgen treated mice differed in no way from those in the untreated. The tumors ranged between adenocarcinoma of the simple or medullary forms, cystic degenerative types, and tumors with extensive hemorrhagic areas.

Observations on Brown Degeneration in the Adrenals of Normal and Testosteronized RIII Mice

Histologic studies of both adrenals of 135 mice were made. Some of the glands were stained with osmic acid vapor as described by Cramer (7), others with Sudan IV and hematoxylin or eosin and hematoxylin. The amount of brown degeneration found was classi-

fied approximately between 0 and 4+ (Table II). The greatest number of adrenals with degeneration appeared in untreated mice (92.8%) with tumors; in treated mice with tumors the number was somewhat smaller (85.7%). In certain treated mice tumors continued to grow and brown degeneration occurred despite the injections. On the other hand, a still smaller proportion (80.9%) of degenerated adrenals in untreated tumor-free mice, and considerably fewer (56.6%) in tumor-free androgen-treated female mice according to Burrows (5), Martin (28), and others seems to effect a rapid involution of the x-zone, and in our series of females the same hormone produced a significant reduction of adrenal degeneration in the same region. Jones (22) and others, as previously mentioned, reported a reduced incidence of tumors in mice after testosterone injections. Since both brown degeneration and tumor incidence are reduced by testosterone, it seems not unreasonable to assume that more than a casual relationship exists between the two.

TABLE II: BROWN DEGENERATION IN THE ADRENALS OF FEMALE MICE OF THE RIII STRAIN

	Number of females	Brown degeneration 0	1+	2+	3+	4+	Per cent
Untreated controls; no tumors	36	4	6	14	8	4	80.9
Treated: Testosterone propionate; no tumors	50	22	13	10	3	2	56.6
Untreated controls; tumors	28	2	8	7	2	9	92.8
Treated: Testosterone propionate; tumors	21	3	12	2	1	3	85.7

supplements the observation that in testosterone-sensitive mice a parallel reduction in tumor growth and brown degeneration occurs (Table III).

In other words, animals developing tumors in spite of the treatment had almost as much adrenal degeneration as untreated tumor-bearing mice. Those subjected to treatment and remaining free of tumors exhibited much less brown degeneration. A parallel reduction of tumor incidence and adrenal degeneration after testosterone, therefore, appears in this strain.

TABLE III: BROWN DEGENERATION AND TUMOR INCIDENCE IN RIII MICE

	Tumors, per cent	Brown degeneration, per cent
Untreated (36 mice)	0	80.9
Treated (50 ")	0	56.6
Untreated (28 ")	52.2	92.8
Treated (21 ")	19.4	85.7

Corresponding drop in tumor incidence and brown degeneration in treated mice.

In androgen-treated mice with tumors the adrenals, as well as the ovaries and adnexa, were atrophied, while in a similarly treated group of mice without tumors the adrenals were notably enlarged, but the ovaries and uterine horns were atrophied.

DISCUSSION

Since the hormones of the adrenal cortex are not sex-specific the possibility of assigning androgenicity or estrogenicity to special cells or layers is problematic. The x-zone has been described as a transitory structure, whose morphologic status is influenced by the gonadal or hormonal status of the host. Testosterone,

Apart from this relationship the effect of testosterone as a possible inhibitor of mammary gland proliferation may be considered. In 1940 reports were published (19) describing the inhibiting effect of androgens on the epithelium of benign fibroadenomas of the rat, and (20) the considerably reduced occurrence of the same tumors in male castrates after testosterone was administered. On the basis of these observations and of those recorded in the present paper the suggestion is advanced that testosterone in controlled dosage be used under careful supervision in women with a family history of mammary cancer as a prophylactic measure shortly before the cancer age. The amount of testosterone given orally or by injection may be about the same as that now used for the menopausal syndrome, or other gynecological disturbances, or in certain conditions of the mammary gland such as mastodynia.

CONCLUSION

1. In testosteronized female mice of the RIII strain the incidence of mammary carcinoma was reduced from 52.2 to 19.4 per cent.

2. In the same series brown degeneration of the adrenals was reduced from 80.9 per cent in controls to 56.6 per cent in tumor-free treated mice.

3. In testosteronized mice exhibiting tumors brown degeneration of the adrenals was not diminished (85.7%).

4. A lowered tumor incidence and brown degeneration occurred in testosteronized female mice of the RIII strain. The reduction of a stimulating factor in the mammary gland, and of an inhibiting factor in the adrenal gland, appears to represent more than a casual

relationship, established, perhaps, by the effects of testosterone.

5. The lowering of tumor frequency may presumably be due to reduction of the growth activity of the mammary gland epithelium, when associated in testosteronized mice with adrenal changes.

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