The Effect of Progesterone and Testosterone Propionate on the Incidence of Mammary Cancer in Mice*

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It has been variously reported (1, 2, 8, 11) that the incidence of spontaneous mammary adenocarcinoma in breeders of the RIII strain from 6 to 12 months old ranges between 54 and 90 per cent, and is about 37.3 per cent after the first 18 months. Haagensen and Randall (6) found that 52.1 per cent of virgin females and 71.5 per cent of bred females of the same strain developed mammary cancer. Twombly (17) states that mammary tumors occur in 74.4 per cent of virgin females of the RIII strain.

It has been found in this laboratory (8) that the incidence can be reduced to 19 per cent when young females of this strain are injected subcutaneously with testosterone propionate as a prophylactic measure. Similar results have been reported by Gardner (5), Jones (9), Nathanson and Andervont (15), and by Loeser (13).

Lipschütz (12) has reported that testosterone with progesterone inhibited the growth of fibromas of the guinea pig uterus; and Heiman (7), that this combination reduced the glandular components of benign mammary adenofibroma of the rat and lowered the percentage of takes from 66.6 to 8.3.

MATERIAL AND METHODS

It has been suggested that progesterone be given alone or with testosterone to females of the RIII strain under conditions similar to those obtaining when only testosterone was administered (8). Accordingly 96 RIII mice of a brother-to-sister bred strain were treated. Inasmuch as the incidence of mammary cancer in virgin or bred mice of this strain varied in different laboratories, the reproductive history was not ascertained. The animals were fed on one part Purina dog chow checkers and two parts Rockland rat diet, given water *ad libitum*, and observed until death. The females littered in the early months of treatment.

The experiments were begun in February, 1942, and continued to December, 1944. The accompanying tables set forth in detail the procedure and observations.

Twenty-four female mice (Table I, Boxes I and II) 6½ months old were given subcutaneous injections of progesterone between February 11, 1942 and June 9, 1942. The injections, each of 0.2 mgm. dissolved in 0.1 cc. of sterile peanut oil, were given alternately on the right and left sides. One animal, which had received a total of 0.6 mgm. of progesterone, developed a mammary carcinoma 3 weeks after injections were started. A second mouse developed a similar growth after 8 weeks, following 1.6 mgm. progesterone; a third, 11 weeks after the first injection and after 2.2

TABLE I: PROGESTERONE IN FEMALE RIII MICE

Box I	Age at beginning of treatment, days	Dose, mgm.	Survival, days	Tumor
TREATMENT EVERY 6 DAYS: 4 TO 18 INJECTIONS, EACH 0.2 MGM.				
1	195	0.8	215	+
2	195	2.0	257	0
3	195	2.0	257	0
4	195	2.0	257	0
5	195	2.2	265	0
6	195	2.4	271	+
7	195	2.6	277	0
8	195	3.4	301	0
9	195	3.6	312	0
10	195	3.6	342	0
11	195	3.6	376	0
12	195	3.6	415	0
TREATMENT EVERY 6 DAYS: 8 TO 18 INJECTIONS, EACH 0.2 MGM.				
Box II				
1	190	1.6	245	+
2	190	1.8	259	0
3	190	2.0	296	0
4	190	2.0	296	0
5	190	3.2	310	0
6	190	3.2	310	0
7	190	3.6	357	0
8	190	3.6	364	+
9	190	3.6	370	0
10	190	3.6	371	0
11	190	3.6	421	0
12	190	3.6	421	0

* Testosterone propionate for these experiments was generously furnished by the Schering Corporation; Roche-Organon, Incorporated; and Ciba Pharmaceutical Products, Incorporated; progesterone by Roche-Organon, Incorporated, and by E. R. Squibb and Sons.

mgm. A mammary tumor appeared in a fourth mouse 2 months after the injections had been completed.

Twenty-four young female mice (Table II, Boxes III and IV) 8 weeks old received 6 mgm. of progesterone in divided doses during 8 weeks (June 11, 1943, to August 9, 1943). The progesterone was dissolved in peanut oil in the proportion of 1 mgm. to 0.1 cc. of oil. The first death of a tumor-free mouse occurred at 7 months of age. The last death occurred at 20 months, and a tumor was found in the left axilla. Another mouse developed a perivulvar tumor at

TABLE II: PROGESTERONE IN FEMALE RIII MICE

TREATMENT EVERY 10 DAYS: 6 INJECTIONS, EACH 1.0 MGM.

Box III	Age at beginning of treatment, days	Dose, mgm.	Survival, days	Tumor
1	62	6.0	327	0
2	62	6.0	344	0
3	62	6.0	487	0
4	62	6.0	511	0
5	62	6.0	511	0
6	62	6.0	518	0
7	62	6.0	518	0
8	62	6.0	568	+
9	62	6.0	594	+
10	62	6.0	628	+
11	62	6.0	628	0
12	62	6.0	628	0

TREATMENT EVERY 10 DAYS: 6 INJECTIONS, EACH 1.0 MGM.

Box IV				
1	55	6.0	231	0
2	55	6.0	231	0
3	55	6.0	375	0
4	55	6.0	494	0
5	55	6.0	494	0
6	55	6.0	501	0
7	55	6.0	501	0
8	55	6.0	551	0
9	55	6.0	551	0
10	55	6.0	551	0
11	55	6.0	577	0
12	55	6.0	611	+

18 months of age; a third showed a small growth in the left axilla; and a fourth in the left groin. These last 2 animals were over 20 months of age. Though the injections were given between the second and fourth months of life, the tumors appeared in these 5 mice more than 1 year after the progesterone was administered; yet in this strain 54 to 90 per cent of the females develop spontaneous mammary cancer at from 6 to 12 months of age, as has been said.

Thus, of 48 mice treated with progesterone alone only 8, or 16.6 per cent, developed mammary cancer, and in 4 instances the animals were over 1 year of age. The average survival period of the injected animals was more than 1 year, and 19 survived 14 months without the appearance of tumors.

Another series of 12 animals, 6 months old, received progesterone in doses of 0.2 mgm. in 0.1 cc. oil and 0.2 mgm. of testosterone propionate in 0.1 cc. oil, both every 3 days (Table III, Box III). One developed a tumor 9 weeks after the first injection was given and it had received 2 mgm. of progesterone and testosterone propionate. The second showed a tumor 5 months after injections were begun, having received 3.4 mgm. of progesterone and testosterone propionate.

A second series of 12 mice, 6 months old, received the same amount of progesterone and testosterone propionate in the same time (Table III, Box IV). The first tumor appeared 3 weeks after injections were started. Eleven animals dead between the tenth and 13th months of age were negative for tumors.

Twenty-four female mice, 8 to 11 weeks old, received weekly during 8 weeks 6 mgm. progesterone alternating with 6 mgm. of testosterone propionate, both dissolved in sterile peanut oil so that 1 mgm. was contained in 0.1 cc. (Table IV, Boxes VII and VIII). The first died at 5 months and the last at 20 months of age. No tumors appeared in these animals.

Thus of 48 mice treated with testosterone propionate and progesterone only 3, or 6.2 per cent, developed mammary cancer, as compared with 16.6 per cent in those treated with progesterone only. Microscopic examination of the 11 spontaneous carcinomas in the 96 mice of these 2 groups showed no variation from those appearing spontaneously in untreated control animals.

Thirty-six RIII females were spayed when 8 weeks old. Twelve received 7 mgm. of progesterone divided into 7 subcutaneous weekly injections. Twelve others were given 6 mgm. each of testosterone propionate and progesterone divided into 6 subcutaneous doses. Twelve were untreated. One of the spayed controls developed a sarcoma of the lower jaw, a spontaneous tumor rarely seen in this strain, perhaps as a result of trauma. None of the other treated or control mice in this group had tumors of any sort.

The effect of testosterone propionate and progesterone was then studied in 18 mice with transplanted malignant tumors. Six dba female mice were injected weekly with 6 mgm. of testosterone propionate and 6 mgm. of progesterone. Two days after the last injection subcutaneous implants of mammary carcinoma RC (Taylor) were made. Growths appeared in the 6 animals on the seventh day as usual.

Six C57 black females were similarly injected with the same hormones. Transplants of mammary carcinoma 755 (Bagg) introduced subcutaneously grew in all after the seventh day.

Sarcoma 180 grew in 6 female RIII mice into which

TABLE III: PROGESTERONE AND TESTOSTERONE PROPIONATE ALTERNATELY IN FEMALE RIII MICE

TREATMENT EVERY 3 DAYS: 4 TO 34 INJECTIONS, EACH 0.2 MGM.

Box III	Age at beginning of treatment, days	Progesterone, mgm.	Testosterone propionate, mgm.	Survival, days	Tumor
1	184	2.0	2.0	246	+
2	184	2.2	2.2	262	0
3	184	3.4	3.4	325	+
4	184	3.4	3.4	343	0
5	184	3.4	3.4	360	0
6	184	3.4	3.4	360	0
7	184	3.4	3.4	360	0
8	184	3.4	3.4	360	0
9	184	3.4	3.4	382	0
10	184	3.4	3.4	382	0
11	184	3.4	3.4	382	0
12	184	3.4	3.4	382	0
Box IV					
1	195	0.4	0.4	204	+
2	195	3.2	3.2	300	0
3	195	3.4	3.4	338	0
4	195	3.4	3.4	345	0
5	195	3.4	3.4	345	0
6	195	3.4	3.4	356	0
7	195	3.4	3.4	356	0
8	195	3.4	3.4	381	0
9	195	3.4	3.4	381	0
10	195	3.4	3.4	381	0
11	195	3.4	3.4	387	0
12	195	3.4	3.4	387	0

TABLE IV: PROGESTERONE AND TESTOSTERONE PROPIONATE IN FEMALE RIII MICE

TREATMENT EVERY 7 DAYS: 12 ALTERNATING INJECTIONS, EACH 1.0 MGM.

Box VII	Age at beginning of treatment, days	Progesterone, mgm.	Testosterone propionate, mgm.	Survival, days	Tumor
1	78	6.0	6.0	395	0
2	78	6.0	6.0	398	0
3	78	6.0	6.0	400	0
4	78	6.0	6.0	414	0
5	78	6.0	6.0	460	0
6	78	6.0	6.0	460	0
7	78	6.0	6.0	460	0
8	78	6.0	6.0	460	0
9	78	6.0	6.0	474	0
10	78	6.0	6.0	524	0
11	78	6.0	6.0	587	0
12	78	6.0	6.0	600	0
Box VIII					
1	58	6.0	6.0	142	0
2	58	6.0	6.0	234	0
3	58	6.0	6.0	234	0
4	58	6.0	6.0	348	0
5	58	6.0	6.0	367	0
6	58	6.0	6.0	433	0
7	58	6.0	6.0	480	0
8	58	6.0	6.0	502	0
9	58	6.0	6.0	513	0
10	58	6.0	6.0	555	0
11	58	6.0	6.0	555	0
12	58	6.0	6.0	555	0

it had been transplanted after similar hormonal injections.

The growth of these 3 transplantable tumors in animals that had received testosterone and progesterone proves that the hormones had no inhibiting effect on already established mammary cancer. This is further verified by the appearance of spontaneous mammary tumors in treated female mice shortly after a few injections had been given. Microscopic tumors were probably established before treatment was begun.

DISCUSSION

Histologic studies of mammary gland from animals treated with testosterone and progesterone revealed a few compressed alveoli and ducts surrounded by fibrotic connective tissue. The cells lining the acini and ducts were small, little secretion was seen in the lumina, and desquamation was evident. The nipples were flat and involuted.

The hormones employed probably reduced the pituitary gonadotropic fraction and this deficiency, in turn, was followed by a suppression of ovarian secretion. It is known that in cancer-susceptible strains of mice the ovarian estrogens may stimulate the mammary gland and act as one factor in the production of cancer.

The normal fluctuations in the functional activity of the gland during estrus and pregnancy were probably moderated by the male hormone administered in these experiments, so that circulatory changes and mammary engorgement were diminished, a neutralizing effect that has been described by Korenchevsky and Hall (10). It may be noted that sterility occurred in the animals receiving only testosterone (8); when it was given with progesterone, the mice littered, but had few young. It will be recalled that in 4 of the mice treated only with progesterone the tumors appeared about 1 year after the injections had been completed, when the indirect effect on the normal mammary tissue had probably disappeared. It is possible that this hormone in combination with testosterone prevents the initiation of mammary cancer in the Paris RIII strain by causing involution of the mammary tissue, as the histologic findings suggest, but the synergistic effect of the two hormones is obscure. The reduction in cancer incidence brought about by treatment with testosterone, progesterone, or both, is corroborated by the fact that spayed and treated females of this strain did not develop any mammary tumors, though both control and injected mice survived 16 to 24 months.

Those who criticize the use of testosterone in women for the prevention of mammary cancer have pointed out the danger of masculinization and sterility, but the small doses now employed in conjunction with progesterone have not been sufficient to produce these effects. Furthermore, the literature contains numerous reports on the beneficial action of testosterone propionate alone in large doses without any untoward results (3, 4, 14, 16).

It has been suggested in a previous article (8), therefore, that testosterone be given in prophylactic doses to women with a family history of cancer who complain of mammary disturbances. No untoward result has been noted in 16 women who have received these injections at the hands of the author for over 6 years, and who previously had complained of pain, swelling, and tenseness in the mammary glands. The synergistic effect of progesterone makes possible the reduction of testosterone.

CONCLUSION

1. Subcutaneous injections of progesterone in 48 brother-to-sister bred RIII female mice between the ages of 2 and 6 months reduced the incidence of spontaneous mammary adenocarcinoma from 54 per cent to 16.6 per cent.

2. Subcutaneous injections of testosterone propionate with progesterone further reduced the incidence to 6.25 per cent in 48 treated female mice between 2 and 6 months of age, the progesterone acting as an adjuvant.

3. Preliminary subcutaneous injections of these two hormones did not prevent the growth of transplanted mouse carcinoma and sarcoma.

4. Spayed females injected with testosterone and progesterone did not develop any mammary tumors. One control spayed female developed a sarcoma of the lower jaw, apparently unrelated to the experiment.

5. The injection of testosterone propionate and progesterone is suggested as a prophylactic measure in women with a family history of cancer.

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