Action of Testosterone Propionate on the Output of Pituitary Gonadotrophins in Mice*

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Ovarian tumors can be induced by a disturbance of the hormonal balance in animals of several species.

Ovaries transplanted in castrated animals to a region drained by the portal system (e.g., spleen, pancreas) become tumorous. The liver partly inactivates the estrogens formed by the transplanted ovary. The low level of circulating estrogens results in a higher output of pituitary gonadotrophins, which is presumably responsible for the initiation of the tumors in the ovaries (4, 5, 11, 16—2.0, 2.2., 2.4, 2.6, and 2.7).

Exogenous gonadotrophins may accelerate the development of these tumors (3). Hypophyseal transplants tend to have the same effect (28), as does union in parabiosis with two castrates (25).

Tumors do not form if the ovarian graft adheres to the parietes. The estrogens formed by the graft find their way into the general circulation, and their level remains high enough to control the secretion of pituitary gonadotrophins. Tumor formation also does not occur if one normal gonad is present, or if the animal is given estrogens or androgens.

Mice exposed to adequate doses of x-rays acquire similar tumors (1, 7, 10, 12, 14, 15, 21, and 30). In this case two factors could be involved: a primary local action and a secondary effect due to the hormonal imbalance caused by the irradiation. The primary local action can be excluded, since unilaterally irradiated ovaries do not become tumorous. The disturbed hormonal balance seems to be important here also, since the existence of one normal ovary or the injection of estrogens prevents tumor formation (13, 14).

As mentioned above, androgens (testosterone propionate) given to mice with intrasplenic ovarian transplants prevent the growth of tumors in these grafts. However, comparable doses of androgen failed to prevent ovarian tumor formation in x-irradiated mice (18). This is an interesting difference, and the purpose of this study was to determine whether or not the doses of testosterone suppress the gonadotrophic hormone output equally effectively under both experimental conditions. Both irradiated and ovariectomized mice were used in this study. Since the production of gonadotrophins can be followed in parabiotic animals, we decided to use that technic.

MATERIALS AND METHODS

In the first series of experiments young adult female mice of the C57BR strain were divided into two groups. One group was castrated and the other given a total-body irradiation of 175 r.¹ Six to 8 weeks after the castration or x-radiation the mice were brought into parabiosis (6) with untreated female partners of the same strain. Each group was now subdivided into three. The first subgroup served as control; the second and third each received four weekly subcutaneous injections of testosterone propionate (TP)² in oil of 1.25 and 2.5 mg., respectively. The injections were begun 2 weeks after the union, and the animals were sacrificed 1 week after the last injection. Forty-seven pairs of parabiotic animals survived the experiment (see Table 1 for distribution over the different groups).

Two pairs of animals were kept together as a group in a small cage; they received Purina Fox Chow and water ad libitum. Vaginal smears were taken daily. At the autopsy the weights of the ovaries and the uterine horns cut off directly proximal of the cervix were recorded. These organs were fixed in Bouin’s fluid and stained with hematoxylin and triosin for microscopic examination.

¹ 200 kv.; 15 ma.; 45 cm. distance; filter, 0.5 mm. Cu and 1 mm. Al; HVL-1.1 mm. Cu; output, 78 r per minute.
² Supplied by Ciba Pharmaceutical Products, Summit, N.J.

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* Part of this paper was presented before the annual meeting of the American Association for Cancer Research, New York City, April 11—18, 1959 (abstract published in Cancer Research, 12:234, 1959).

Supported by a grant from The Anna Fuller Fund, The Jane Coffin Childs Memorial Fund, and the National Cancer Institute, United States Public Health Service.

Received for publication December 3, 1954.
In a later series of experiments, 1.25 mg. TP was injected weekly starting immediately following castration or x-radiation of the mice and continued for a period of 4–10 months. Thereafter, the treated animals were brought into parabiosis with untreated females of the same age. The pairs were sacrificed 12 days after the union to determine the level of pituitary gonadotrophic activity at that time. In these experiments mice of the C57BL strain were used. Fifty-two pairs of parabiotic animals were available for analysis (Table 2).

**RESULTS**

**SHORT-TERM EXPERIMENTS**

**Estrus.**—About 1 week after the union in parabiosis the normal female partners in both the castrated and the irradiated groups showed cornified vaginal smears. The cornified smears persisted. The castrated and x-rayed partners showed anestrus or diestrum. When testosterone propionate was injected into the castrated or x-rayed mouse, the persistent vaginal cornification of the partner stopped after an average of 10 days, and the vaginal smears showed diestrum during the rest of the experiment (3–4 weeks).

**Ovarian and uterine weights.**—The average weight of the ovaries of ten normal, young, adult mice of this strain was 7.8 ± 0.8 mg. Parabiosis itself caused a slight loss of ovarian weight (7.1 ± 0.6 mg.). The ovaries and uteri of the intact mice in parabiosis with castrated or x-rayed mice weighed 16.8 ± 0.8 mg. and 16.0 ± 2.8 mg., respectively.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose of testosterone or roentgen rays</th>
<th>No. of pairs</th>
<th>Av. ovarian wt., intact partner (mg.)</th>
<th>Av. ovarian wt., treated partner (mg.)</th>
<th>Av. uterine wt., intact partner (mg.)</th>
<th>Av. uterine wt., treated partner (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovariectomy</td>
<td>1.25 mg/wk</td>
<td>10</td>
<td>16.8 ± 0.8*</td>
<td></td>
<td>147 ± 20</td>
<td></td>
</tr>
<tr>
<td>2. Ovariectomy</td>
<td>2.6 mg/wk</td>
<td>10</td>
<td>16.6 ± 0.6</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>3. Ovariectomy</td>
<td>1.25 mg/wk</td>
<td>6</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>4. X-ray</td>
<td>175 r</td>
<td>7</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>5. X-ray</td>
<td>175 r</td>
<td>7</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>6. X-ray</td>
<td>175 r</td>
<td>6</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>7. Ovariectomy</td>
<td>1.25 mg/wk</td>
<td>5</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>8. Ovariectomy</td>
<td>2.6 mg/wk</td>
<td>5</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
<tr>
<td>9. Ovariectomy</td>
<td>1.25 mg/wk</td>
<td>5</td>
<td>16.0 ± 0.8</td>
<td></td>
<td>160 ± 20</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean.

Significance: ovarian weights intact partners:
- Groups 1 and 2: P < .01
- Groups 1 and 3: P < .001
- Groups 2 and 3: P < .001
- Groups 2 and 4: P < .001
- Groups 2 and 5: P < .001
- Groups 2 and 6: P < .001
- Groups 3 and 4: P < .001
- Groups 3 and 5: P < .001
- Groups 3 and 6: P < .001
- Groups 4 and 5: P < .001
- Groups 4 and 6: P < .001
- Groups 5 and 6: P < .001

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose of testosterone propionate</th>
<th>Duration (months)</th>
<th>No. of pairs</th>
<th>Av. ovarian wt., intact partner (mg.)</th>
<th>Av. ovarian wt., treated partner (mg.)</th>
<th>Av. uterine wt., intact partner (mg.)</th>
<th>Av. uterine wt., treated partner (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. X-ray</td>
<td>175 r</td>
<td>4.5</td>
<td>5</td>
<td>12.5 ± 0.6*</td>
<td>9.0 ± 0.3</td>
<td>28 ± 4.6</td>
<td>28 ± 4.6</td>
</tr>
<tr>
<td>2. X-ray</td>
<td>175 r</td>
<td>7</td>
<td>5</td>
<td>10.5 ± 0.9</td>
<td>2.6 ± 0.3</td>
<td>32 ± 4.0</td>
<td>32 ± 4.0</td>
</tr>
<tr>
<td>3. X-ray</td>
<td>175 r</td>
<td>10</td>
<td>5</td>
<td>9.9 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>32 ± 4.0</td>
<td>32 ± 4.0</td>
</tr>
<tr>
<td>4. X-ray</td>
<td>175 r</td>
<td>4–5</td>
<td>7</td>
<td>6.0 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>28 ± 3.5</td>
<td>28 ± 3.5</td>
</tr>
<tr>
<td>5. X-ray</td>
<td>175 r</td>
<td>7.5</td>
<td>11</td>
<td>6.8 ± 0.4</td>
<td>1.9 ± 0.1</td>
<td>32 ± 4.0</td>
<td>32 ± 4.0</td>
</tr>
<tr>
<td>6. X-ray</td>
<td>175 r</td>
<td>10</td>
<td>5</td>
<td>6.9 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>32 ± 4.0</td>
<td>32 ± 4.0</td>
</tr>
<tr>
<td>7. Ovariectomy</td>
<td>175 r</td>
<td>5–6</td>
<td>6</td>
<td>21.1 ± 3.2</td>
<td>132 ± 11.0</td>
<td>30 ± 1.4</td>
<td>30 ± 1.4</td>
</tr>
<tr>
<td>8. Ovariectomy</td>
<td>175 r</td>
<td>4–5</td>
<td>7</td>
<td>8.0 ± 0.7</td>
<td>49 ± 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Ovariectomy</td>
<td>175 r</td>
<td>10</td>
<td>5</td>
<td>7.1 ± 0.3</td>
<td>30 ± 1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean.

Significance: ovarian weight intact partners:
- Groups 1 and 2: P < .01
- Groups 3 and 4: P < .01
- Groups 3 and 5: P < .01
- Groups 3 and 6: P < .01
- Groups 4 and 5: P < .01
- Groups 4 and 6: P < .01
- Groups 5 and 6: P < .01
respectively (Table 1 and Chart 1). The stimulated ovaries contained many large or cystic follicles. Corpora lutea were absent; the interstitial cells were hypertrophic. The uteri showed estrogenic stimulation, and the vaginal epithelium was cornified. The ovaries of the irradiated partners were similar to those of irradiated mice which were not in parabiosis: follicles and corpora lutea were lacking, the ovaries consisted mainly of interstitial cells with small peripheral anovular follicles. The uteri of the x-rayed partners were atrophic, the vaginae in diestrum.

When the castrated or irradiated partner received testosterone propionate, the ovarian weight of the intact partner did not increase (Table 1 and Chart 1). The ovaries contained growing follicles, but many of the secondary follicles were atretic. Corpora lutea were usually present; the interstitium was poorly developed. No further indication of unusual stimulation was present. The continuous cornification of the vagina of the intact parabiont disappeared after about 10 days; at autopsy, the uteri were atrophic and the vaginae in diestrum or anestrus.

**Long-Term Experiments**

**Estrus.**—Vaginal smears were made during the period of parabiosis. During the 2d week after the union all intact partners of the castrated and x-rayed mice were in estrus and remained so until the autopsy. The x-rayed partners showed anestrus and diestru in the vaginal smears. The intact partners in parabiosis with testosterone propionate-treated castrated or irradiated mice were for the greater part in diestrum during the 2d week; some showed an uninhibited cycle.

**Ovarian and uterine weights.**—The ovaries and uteri of the normal partners in parabiosis with castrated or x-rayed mice were increased in weight (Table 2). From the groups of x-rayed mice these weights were far below those of the castrated mice. Also, there was a gradual decrease in ovarian stimulation in the x-rayed groups according to the time the animals were allowed to live after the irradiation. The ovaries and uteri of the normal partners in parabiosis with castrated or x-rayed mice treated with testosterone propionate were of normal weight (Table 2).

**DISCUSSION**

The mice usually lost a few grams in weight in the first few days after the parabiosis, and the average body weight loss of ten pairs after 12 days was 6.1 gm., or about 3 gm/mouse. However, the average ovarian weights did not decrease significantly; in a group of ten animals the average ovarian weight was 7.8 ± 0.8 mg. before parabiosis and a similar group 7.1 ± 0.6 after parabiosis.

Subsequent to castration or x-radiation of the young adult mouse, the output of gonadotrophic hormone of the pituitary increased (29). This fact was confirmed in our control series with castrated and x-rayed mice joined with intact females. The ovaries of the intact females were stimulated excessively by the augmented gonadotrophin secretion from their castrated or x-rayed partners. The ovaries weighed 16.8 ± 0.8 and 16.0 ± 2.8 mg., respectively, in the short-term experiment (Table 1).

The resultant high level of estrogen in the intact parabiont was evidenced by the permanent estrus and the increased uterine weights of these animals: average weight, 147 ± 20.0 and 110 ± 12.6 mg., respectively. The increase of uterine weight can be detected as early as the 11th day after parabiosis (8, 9). In our experiment it was recorded at 7 weeks after parabiosis.

The increase of ovarian and uterine weight in the long-term experiment was also very striking in the castrated group but less striking in the x-rayed group. In the castrated group the average ovarian weight was 21.1 ± 3.2 mg. and the average uterine weight 182.0 ± 11.0 mg. after 5-6 months. In the x-rayed groups there was a gradual decrease of ovarian stimulation: the average ovarian weight was 12.5 ± 1.6 mg. after 4-5 months; 10.5 ± 0.9 mg. after 7 months; and 9.9 ± 0.4 after 10 months. The uterine weights for these groups were 93 ± 7.1, 88 ± 12.0, and 84 ± 9.0 mg., respectively. These findings indicate that a definite but different degree of ovarian stimulation occurred in the castrated and x-radiated groups. This may be partly explained by the fact that x-radiation produces a less complete castration than surgery.

Estrogens injected into the castrated or x-rayed
mice reduced the gonadotrophic output of the pituitaries of these animals (2, 8, 9, and 23); the ovarian weights of their parabiotic partners were not increased. Testosterone propionate (1.25 mg. weekly in our experiments) produced the same effect. In the short-term experiments the ovarian weights returned to normal after 5 weeks; the estrogen level became low and the cornified vaginal smears disappeared after 2 days. The weights of the uteri were normal at the end of the experiment (27.2 ± 4.8 and 85.0 ± 8.0). The larger dose of testosterone propionate (2.5 mg/week) produced no different effect. Thus, testosterone propionate in doses of 1.25 mg. weekly, in our opinion, is sufficient to suppress a detectable increase in the output of pituitary gonadotrophins and to reduce high levels of gonadotrophins to normal.

In the long-term experiments the castrated and x-rayed mice received weekly injections of testosterone propionate for 4–10 months, to assure the continuous action of testosterone throughout the period of observation. If the animals had become refractory to the testosterone, or if the pituitary of the x-radiated animal responded differently to testosterone than the castrated, this would become evident as soon as the mice were brought into parabiosis with intact females. In our experiments, however, the weights of the ovaries and uteri of the intact mice did not increase. Thus, no elevated secretion of the gonadotrophins occurred during the 10 consecutive months of testosterone administration. It seems reasonable to assume that the pituitary gonadotrophic function would not change significantly if these animals were maintained for a longer period than 10 months in the same condition, after which period ovarian tumors usually begin.

In view of the fact that ovarian tumors developed in the testosterone-treated x-radiated female mouse but not in the testosterone-treated castrated female mouse with an intrasplenic ovarian graft (19), and in view of our present finding that testosterone was equally effective in suppressing the hypersecretion of pituitary gonadotrophin resulting from castration or x-radiation, it is postulated that other factors than overproduction of gonadotrophins were responsible for ovarian tumor formation in the x-radiated mice.

SUMMARY AND CONCLUSION
Testosterone propionate suppressed the augmented gonadotrophic hormone output of the pituitary of both x-radiated and gonadectomized mice, as determined by the response of intact parabionts.

Two series of experiments involving 47 and 52 pairs of mice in parabiosis were used. In a short-term experiment testosterone propionate (1.25 mg. weekly) restored the high output of pituitary gonadotrophins in castrated and x-rayed animals to normal levels. In a long-term experiment the same dose of testosterone propionate given weekly kept the gonadotrophic output within normal limits for periods up to 10 months.

Testosterone inhibited the abnormal release of pituitary gonadotrophins both after castration or x-radiation of mice. Why ovarian tumors still occur in x-rayed mice which are regularly injected with testosterone propionate cannot be explained by assuming an elevated exposure of the ovaries to gonadotrophins. In these animals other factors than overproduction of gonadotrophins must be responsible for the tumor formation.
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