Estrogen and 17-Ketosteroid Excretion in Patients with Breast Carcinoma*

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Investigations of the hormone content of the urine of cancer patients have been made repeatedly in the search for abnormalities that might reflect a disturbance in the endocrine physiology of the tumor-bearing person. Certain early reports suggested that excessive quantities of estrogens were excreted by cancer patients (8), especially women with genital tumors (32, 36, 50, 51, 56). Although these earlier observations on total estrogens have not proved correct, it has more recently been reported that special abnormalities occur in the relations of the different estrogen fractions to each other (44), particularly following the administration of estrone to women with uterine cancer (43).

The investigation of the urinary estrogens was begun in this laboratory eight years ago on patients with chronic cystic mastitis, a disease that seemed especially promising for this type of study on account of the associated menstrual abnormalities and other signs of endocrine disorder (62, 63). In the type of chronic mastitis termed adenofibrosis, the estrogen excretion patterns were found to be consistently normal; while in the type associated with nonpuerperal lactation, abnormalities in excretion were sometimes found, but these were compatible with the menstrual abnormalities also present and were not specific for the breast disease itself (63). Gynecomastia associated with cirrhosis of the liver has been studied by Glass, Edmondson, and Soll (25) with reported findings of low androgens and increased "free" estrogen in the urine. In breast cancer normal estrogen and androgen excretion has been found in both males (67) and females (46).

The plan of investigation adopted by us for a study of estrogen and androgen excretion in breast cancer included two parts: First it was necessary to compare the spontaneous excretion of estrogens and androgens of breast cancer patients with normal persons in comparable physiologic epochs. Secondly in a search for some peculiarity in the capacity of breast cancer patients to utilize, destroy, or eliminate estrogens it was determined to study the effects on urinary excretion rates of overloading the system with artificially administered hormone. These two methods of approach, designed to study the possibilities of somewhat different concepts of the relationship of the estrogens and androgens to breast cancer, form separate portions of the present communication.

METHODS

Hydrolysis.—Hydrolysis was carried out according to the method described by Callow and his group (2, 4), in which 40 cc. of concentrated hydrochloric acid is added to each liter of urine and the mixture boiled under a reflux condenser for one-half hour. A series of 20 urines run by us in duplicate to compare the Callow method with that recommended by Smith and Smith (54), the latter consisting of 10 minute boiling with 15 per cent hydrochloric acid by volume, failed to demonstrate any consistent difference in the yield of either the 17-ketosteroids or the estrogens.

Extraction.—The cooled hydrolyzed urine was extracted for 24 hours in a continuous ether extractor. The Kutscher-Steudel extraction apparatus for use with solvents lighter than water was used. Great efficiency was obtained by the employment of a sintered glass plate, through which the solvent was forced in minute bubbles, to afford maximum contact with the urine.

Separation of androgens from estrogens.—The ethereal solution was washed 2 or 3 times with saturated sodium bicarbonate and then extracted 5 times with 2 N sodium hydroxide. The neutral ethereal fraction was washed with water, filtered, and reduced to dryness on the steam bath and in the desiccator before determination of the androgens. The alkaline aqueous fraction was made acid to Congo paper with concentrated hydrochloric acid and extracted with ether. This ether extract was washed with water, filtered, and reduced to dryness on the steam bath and in the desiccator before determination of the androgens. The alkaline aqueous fraction was made acid to Congo paper with concentrated hydrochloric acid and extracted with ether. This ether extract was washed with water, filtered, and reduced to dryness, the residue being finally taken up in an amount of sesame oil suitable for the biologic assay of the estrogens.

In a previous investigation we had used benzene as the solvent for the extraction of the estrogens. In the belief that work with benzene constituted a definite hazard, ether was substituted. Recovery experiments, carried on during the course of the clinical studies, showed that estrogens and androgens were completely

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* This investigation was aided by a grant from the Commonwealth Fund.
removed from urine by the ether. Although extraction of the phenolic estrogens from ether by sodium hydroxide has been a widely used method (64, 20, 46), we found toward the end of our clinical work that ether is not a satisfactory solvent at this stage, for some estrogenic activity remained behind even after 5 or 6 extractions with the alkali. The lower estrogen excretion rates here reported, as compared to figures noted in a previous study on chronic mastitis (63), may be attributed to this loss of estrogen into the neutral fraction. From a short trial with other solvents it now appears that, if benzene is not to be used, carbon tetrachloride is the best medium from which to separate the phenolic fraction by means of extraction with alkali.

Bioassay.—Bioassay of the extracts was carried out upon spayed adult white mice. These animals were standardized before each test by determining that they reacted with full estrus to a single priming dose of 2.5 gamma estrone given a week before their use. In the actual assay each mouse received one subcutaneous injection of 0.1 cc. of the unknown extract dissolved in sesame oil on each of 2 successive days, vaginal smears being made at 48, 72, and 96 hours after the first injection. Readings were made from a previously constructed dose response curve, based upon the injection of 300 mice with various amounts of crystalline estrone. Ten animals were injected for each final assay, and if from 30 to 70 per cent were positive the estrogen content was read directly from the curve. An average of 50 per cent of the animals in this laboratory have rather consistently shown full estrus when injected under these conditions with 0.1 gamma of estrone.

Fractionation of the estrogens.—The work of Pincus and Graubard (45) has suggested that peculiarities in estriol-estrone excretion ratios might characterize patients with cancer, particularly of the uterus. For this reason the original plans of this experiment called for a separation of the total estrogens of the urine into the estrone, estradiol, and estriol fractions. The procedure planned was the separation of the estradiol as a strong phenol from the estrone and estriol by means of weak alkali (5, 57) and the determination of the estradiol of the second fraction after inactivation of the estrone by semicarbazide. A complete separation of the "strong" from the "weak" phenols did not, however, prove successful in this laboratory. It was found, for example, that with the use of solutions of pure estradiol a minimum of 6 per cent was extracted by the weak alkali into the "estriol" fraction. This would not represent a great error if chemical determinations were depended upon, but such an error is enormously magnified by the bioassay. In our laboratory the amount of each estrogen required to produce estrus in the mouse was found to be as follows: 0.035 gamma of estradiol, 0.1 gamma of estrone, and 7.0 gamma of estriol. Dingemanse and Laqueur (9) noted a somewhat similar relationship. It is evident, then, that a trace of estradiol or estrone in the "estriol" fraction when assay is made with white mice will give greatly distorted estriol figures. For this reason the attempt to separate the three estrogens in single urine specimens was abandoned.

Assay of the androgens.—Androgens were determined according to the colorimetric method of Callow (1, 4), which had been adapted to reading on the Pulfrich photometer. This method is based on the Zimmermann reaction (68, 69), which is specific for all 17-ketosteroids, including substances not androgenically active. Bioassays with a slight modification of the Frank (15, 16, 35) baby chick comb method were also undertaken on a limited number of specimens. For a period of 7 days, 0.05 cc. of an oily solution was applied daily to the base of the combs of 1 day old, White Leghorn, female chicks in groups of 20. The ratio of body weight to comb weight was calculated for each chick and the biological content of a urinary extract was determined in terms of androstenedione by reference to a standard curve.

Table I: Estrogen and Androgen Excretion in Young Women with Regular Menses

<table>
<thead>
<tr>
<th></th>
<th>Total estrogens</th>
<th>17-Ketosteroids by colorimetric determination</th>
<th>Androgens by bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
<td>Number of tests</td>
<td>Daily average in milligram equivalents of estrone</td>
</tr>
<tr>
<td>Normal controls</td>
<td>3</td>
<td>22</td>
<td>3.4</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>2</td>
<td>20</td>
<td>5.0</td>
</tr>
</tbody>
</table>

PART 1

Spontaneous Excretion of Estrogens and 17-Ketosteroids

The first part of the investigation consisted in the estimation of the total estrogen and total androgen excretion of patients with breast cancer, developing under four distinct physiologic conditions, each controlled by similar cases without breast cancer. The persons representing these four distinct physiologic conditions were the following: (a) young menstruating women; (b) young amenorrheic women; (c) women in the menopause; (d) men.

(a) Young women with regular menses (Table I).—Forty-two specimens, based on 24- or 72-hour urine
collections, were studied for total estrogens and a somewhat larger series for 17-ketosteroids. Since the daily estrogen excretion ranged from 0 to 34.0 gamma of estrone equivalents, the difference noted in average daily excretion between normal and cancer patients appears to be not significant. The excretion of 17-ketosteroids as well as of the biologically determined androgens was higher in the cancer groups, largely because of the very high figures of several specimens of ketosteroid excretion that did not differ from that of younger women. No difference existed between the normal women and those with cancer.

(c) Males with breast disease (Table IV).—These men showed no characteristic differences in daily excretion of estrogens. A relatively low excretion rate for 17-ketosteroids was noted both in cases of gynecomastia and in cancer of the male breast. Similar differences were present in the excretion rates of comb-stimulating substances. These observations suggest the possibility that gynecomastia and cancer of the male breast arise in the same type of person.

Excretion of Estrogens and 17-Ketosteroids after Hormone Administration

Although differences in the spontaneous excretion rates of total estrogens and 17-ketosteroids characteristic of breast cancer seem not to be present, it remains possible that these substances may be less efficiently handled by the cancer patient or the person susceptible to cancer development. Such a deficiency might be made apparent by burdening the organism with excess-

### Table II: Estrogen and Androgen Excretion in Young Women with Amenorrhea

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of patients</th>
<th>Number of tests</th>
<th>Daily average in gamma equivalents of estrone</th>
<th>Number of patients</th>
<th>Number of tests</th>
<th>Daily average in milligram equivalents of androsterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amenorrhea and tuberculosis</td>
<td>1</td>
<td>13</td>
<td>0.5</td>
<td>1</td>
<td>13</td>
<td>9.3</td>
</tr>
<tr>
<td>Amenorrhea and breast cancer</td>
<td>4</td>
<td>27</td>
<td>0.3</td>
<td>4</td>
<td>27</td>
<td>4.6</td>
</tr>
</tbody>
</table>

### Table III: Estrogen and Androgen Excretion in the Menopause

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of patients</th>
<th>Number of tests</th>
<th>Daily average in gamma equivalents of estrone</th>
<th>Number of patients</th>
<th>Number of tests</th>
<th>Daily average in milligram equivalents of androsterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2</td>
<td>13</td>
<td>0.4</td>
<td>2</td>
<td>16</td>
<td>8.1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>2</td>
<td>10</td>
<td>0.4</td>
<td>2</td>
<td>10</td>
<td>7.8</td>
</tr>
</tbody>
</table>

### Table IV: Excretion of Estrogens and Androgens in Male Breast Disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of patients</th>
<th>Number of tests</th>
<th>Daily average in gamma equivalents of estrone</th>
<th>Number of patients</th>
<th>Number of tests</th>
<th>Daily average in milligram equivalents of androsterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal males</td>
<td>2</td>
<td>7</td>
<td>0.7</td>
<td>5</td>
<td>19</td>
<td>21.2</td>
</tr>
<tr>
<td>Gynecomastia</td>
<td>10</td>
<td>10</td>
<td>0.9</td>
<td>10</td>
<td>10</td>
<td>11.9</td>
</tr>
<tr>
<td>Males with breast cancer</td>
<td>2</td>
<td>6</td>
<td>0.7</td>
<td>2</td>
<td>6</td>
<td>10.6</td>
</tr>
</tbody>
</table>

(b) Young women with amenorrhea (Table II).—In terminal cases of breast cancer, as well as in other serious protracted illness, menstruation ceases and the excretion of estrogenic substances diminishes. The very low rate of 17-ketosteroid excretion is probably also a reflection of the general ill health and is not specific for cancer. Nevertheless, it is noteworthy that in these advanced cases with a maximum quantity of cancer tissue in the body there is certainly no elevation of 17-ketosteroid excretion. A pronounced fall in androgenically active material likewise appears to take place.

(c) Women in the menopause (Table III).—These showed little estrogen excretion but an average 17-
sive quantities of these substances artificially administered. Were a deficiency of this type present it might then manifest itself clinically by a susceptibility to disorders in the ovarian cycles, by unusual percentages of excretion of the injected hormone, or by disturbance in the excretion of other hormones.

Two women, one recently operated upon for breast cancer and a normal one of approximately the same age, submitted all urine excreted for seven successive menstrual cycles. The first cycle was devoted to the determination of the spontaneous excretion of estrogens and 17-ketosteroids. Thereafter two cycles each were assigned to a study of the effects of the injection of estrone, testosterone propionate, and progesterone respectively. Two cycles were devoted to each of the three hormones since it was known that the effects might be different depending upon whether administration was made before or after the time of ovulation. Care was taken to give the hormone injections on identical days of the cycle in the normal woman and cancer patient.

Clinical material.—Brief clinical histories of the two women follow:

M.P.—The normal one was a divorced woman of 37 with one child, 16 years of age, and a history of one spontaneous abortion 15 years prior to the study. There was no history of serious illness in the patient or record of cancer in the family. Menstruation, which had begun at the age of 11, occurred as a rule at 30 day intervals, lasted for 4 days, and was accompanied by slight dysmenorrhea. Breasts and pelvic organs were normal.

C.G.—The cancer patient was an unmarried girl of 27 with no history of previous serious illness or of cancer in her family. Menstruation occurred at 26 to 30 day intervals, lasted 3 days, was accompanied by moderate cramps, and preceded by some breast pain and swelling. A radical mastectomy for carcinoma of the breast with axillary metastases had been performed 4 months before these studies were undertaken. At the time of the tests there was no clinical evidence of cancer and the patient has remained well to the present time. The case was selected as one which might disclose physiologic peculiarities perhaps predisposing to cancer and not one on which to investigate disorders of steroid metabolism due to existent tumor tissue.

A comparison of the estrogen and 17-ketosteroid excretions and of cycle length in response to various hormone injections is shown for the two women in a series of seven charts. These are constructed to show average daily excretion of these substances as based on 72-hour urine collections. There are in addition two tables (Tables V and VI), which show whether the excretion rate of estrogens or 17-ketosteroids in the 9 or 10 day period during which injections were given was increased or decreased as compared with the same days of the cycle in the same patient before any injections had been started.

Control Periods before Hormone Administration

The total excretion of estrogen for a menstrual cycle has been reported to amount to from as low as 1,500 mouse units (14) to as high as 3,000 rat units (55), with many figures in between (6, 10, 18, 27, 51, 64, 66). Observations from this laboratory have previously shown normal monthly excretion rates of 3,000 to 6,000 international units. The monthly curve is known to show a peak of excretion at the time of ovulation, when as much as 800 international units or more may be excreted in a single day (26, 40). A second and less pronounced rise may develop shortly before menstruation.

The daily androgen excretion for normal women shows slight if any variations that can be correlated with the menstrual cycle. Werner (64) has recently reported normal 17-ketosteroid values for women from 5.4 to 19.6 mgm. daily, and Fraser and his associates (17) have reported an average of 9 mgm. daily.

In the control period the women of this study both exhibited a longer menstrual interval than they had reported as usual for themselves, the normal one having a 31 and the cancer patient a 34 day cycle (Fig. 1). The total estrogen excretion in both was lower than that previously found by us for normal women, the low figures being apparently a result of technical differences previously noted. Each showed typical peaks of estrogen excretion, the cancer patient exhibiting this rather late in her cycle. The 17-ketosteroid excretion averaged a little over 10 mgm. a day in her, a little below that in the normal control. All these figures lie within the normal range.

Administration of Estrone

The administration of estrogens to women has an effect on certain physiologic characteristics of menstruation as well as on the rates of steroid hormone excretion. Menstruation may be delayed for periods varying from 7 to 70 days, and ovulation prevented with subsequent elimination of dysmenorrhea at the next period (60).

When an estrogen is injected there is a rise in the estrogenic activity of the urine amounting, it is said, to only 6 per cent or less of the injected material (33, 34, 38, 45, 61). Since any special estrogen after its injection is converted at least in part to other forms of differenf biologic activity (65), bioassay of the urine without fractionation of the estrogens cannot give a figure that can be directly related to the amount of
injected material. In this report, then, percentages of recovery are only relative and used in comparing one case with the other.

### Table V: Effect of Hormone Injection on Estrogen Excretion

<table>
<thead>
<tr>
<th>Time and type of hormone injections</th>
<th>Time of urine collection, days</th>
<th>Estrogen excretion in gamma equivalents of estrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone: Pre-ovulation</td>
<td>7th to 18th</td>
<td>Case: Normal 28.6, Control: 145.0, Increase: 116.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 26.3, Control: 111.8, Increase: 85.5</td>
</tr>
<tr>
<td>Estrone: Post-ovulation</td>
<td>17th to 26th</td>
<td>Case: Normal 22.0, Control: 125.0, Increase: 103.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 85.5, Control: 105.0, Increase: 19.5</td>
</tr>
<tr>
<td>Testosterone Propionate: Pre-ovulation</td>
<td>8th to 16th</td>
<td>Case: Normal 19.3, Control: 25.2, Increase: 5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 20.4, Control: 18.8, Increase: 1.6</td>
</tr>
<tr>
<td>Testosterone Propionate: Post-ovulation</td>
<td>14th to 22nd</td>
<td>Case: Normal 24.9, Control: 87.0, Increase: 62.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 43.8, Control: 36.8, Increase: 7.0</td>
</tr>
<tr>
<td>Progesterone: Pre-ovulation</td>
<td>8th to 16th</td>
<td>Case: Normal 19.3, Control: 36.8, Increase: 17.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 20.4, Control: 48.5, Increase: 28.1</td>
</tr>
<tr>
<td>Progesterone: Post-ovulation</td>
<td>17th to 25th</td>
<td>Case: Normal 22.0, Control: 24.4, Increase: 2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 85.5, Control: 49.5, Increase: 35.6</td>
</tr>
</tbody>
</table>

### Table VI: Effect of Hormone Injection on 17-Ketosteroid Excretion

<table>
<thead>
<tr>
<th>Time and type of hormone injections</th>
<th>Time of urine collection, days</th>
<th>17-Ketosteroid excretion in milligrams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone: Pre-ovulation</td>
<td>7th to 18th</td>
<td>Case: Normal 108.6, Control: 87.6, Increase: 21.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 120.0, Control: 162.3, Increase: 42.3</td>
</tr>
<tr>
<td>Estrone: Post-ovulation</td>
<td>17th to 25th</td>
<td>Case: Normal 84.1, Control: 65.1, Increase: 19.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 95.3, Control: 90.9, Increase: 4.4</td>
</tr>
<tr>
<td>Testosterone Propionate: Pre-ovulation</td>
<td>8th to 16th</td>
<td>Case: Normal 77.9, Control: 116.6, Increase: 48.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 91.0, Control: 156.6, Increase: 65.6</td>
</tr>
<tr>
<td>Testosterone Propionate: Post-ovulation</td>
<td>14th to 22nd</td>
<td>Case: Normal 83.6, Control: 103.5, Increase: 19.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 94.2, Control: 117.0, Increase: 22.8</td>
</tr>
<tr>
<td>Progesterone: Pre-ovulation</td>
<td>8th to 16th</td>
<td>Case: Normal 77.9, Control: 79.2, Increase: 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 91.0, Control: 77.4, Increase: 13.6</td>
</tr>
<tr>
<td>Progesterone: Post-ovulation</td>
<td>17th to 25th</td>
<td>Case: Normal 84.1, Control: 55.8, Increase: 28.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case: Cancer 95.3, Control: 94.3, Increase: 1.0</td>
</tr>
</tbody>
</table>

A decrease in the androgen content of the urine in women has also been reported to follow estrogen administration (28, 29).

**Estrogens before ovulation** (Fig. 2).—Estrone was first given in 4 doses of 20,000 international units from the 8th to the 15th day. Both women responded by a great prolongation of the menstrual cycle, to 38 days in the cancer patient, to 45 days in the control. In each case a definite increase was found in the estrogenic activity of the urine, amounting in terms of biologic
given during the 3rd month, in 4 doses of 20,000 international units each, from the 17th to the 24th day. Both women returned toward a shorter cycle type, and although the normal one menstruated on her 34th day and the cancer patient on the 26th day, these dates might be interpreted as within those on which spontaneous menstruation might have occurred.

The estrogen activity found in the urine during the periods of injection were identical in the two subjects. This represented a greater increase for the normal woman on account of the low values found in her control period. The 17-ketosteroid excretion was not significantly affected in either case by the estrone administration after the time of ovulation.

**Effects of Administration of Testosterone Propionate**

Testosterone in large doses has a profound effect on the function of the ovary and on the rhythm of menstruation. The action is said to be primarily an inhibition of the gonadotropic function of the anterior pituitary gland (22, 47). There follows as a result an inhibition of ovulation and corpus luteum formation with a delay in menstruation for longer or shorter periods of time (19, 21, 37, 41, 48). The critical dose, above which such effects occur, has been placed at 500 mgm. given during the pre-ovulatory phase (23). If it is given later in the month menstruation may occur, since ovulation has not been inhibited.

A rise in the urinary excretion rate of androgens occurs after the oral or intramuscular administration of testosterone propionate (13). The elevation of the subnormal values of male castrates and eunuchoids has been repeatedly observed (3, 11, 31). With small doses of testosterone propionate slight rises in androgen excretion have also been noted in female castrates (39).

A somewhat surprising observation has been that the administration of the male hormone causes a rise in estrogen excretion (3, 12, 31, 39, 58, 59). Various explanations have been offered, including the suggestion that the excreted estrogens were degradation products of testosterone or that the effect was due to certain androgens having some estrogenic qualities (7). It must be remembered that such increases in estrogen excretion rates have been observed chiefly in males and with moderate doses. With the administration to females of quantities sufficient to inhibit the activity of the anterior pituitary, and so prevent ovulation, a pronounced decrease in estrogen production and excretion may be expected.

**Androgen administration before ovulation (Fig. 4).**

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Fig. 1.—Excretion of estrogens and androgens. No treatment.
the 8th to 14th days. This relatively small amount was selected since it was hoped to study excretion rates without producing major physiologic distortions of the ovarian cycle.

With 200 mgm. given before ovulation the response of the two women was completely dissimilar. The normal subject, apparently missing an entire period, had no bleeding for a total of 59 days. The cancer patient began to menstruate 4 days after her last injection, or on the 18th day of her cycle. Both women experienced a rise in their androgen excretion rates, corresponding to a recovery of about 15 per cent of the injected testosterone propionate in each instance. The estrogen excretion was essentially unaffected in either case.

**Androgen administration after ovulation (Fig. 5).—** Testosterone propionate was given in the same dosage from the 15th through the 21st day. Differences in response were again noted, the control having her period on the 30th, the cancer patient on her 21st day. A considerable increase in estrogen excretion occurred in the normal woman through the days of injection, while the cancer subject showed a slight but probably insignificant decrease.

The 17-ketosteroids of the urine also rose sharply in both cases during the period of administration but in

![Diagram](image-url)

**Fig. 2.**—Excretion of estrogens and androgens after estrone injections administered before ovulation.

x = 2 mgm. of administered estrone.

The Effects of the Administration of Progesterone

The effects of the injection of corpus luteum are apparently quite different from those which follow the administration of estrogens or androgens. When given before the time of ovulation in doses of 20 to 50 mgm., progesterone has been reported to produce almost im-
mediate uterine bleeding, which may be a “pseudo-menstruation” \((70, 71, 72)\) or a premature menstruation \((24)\). Progesterone given at or after the time of ovulation is said to produce no effect on the menstrual cycle \((71)\).

Progesterone has also been reported to increase the excretion of estrogens \((42, 52, 53)\), although more recent investigation has indicated that progesterone has no effect on either estrogen or androgen excretion rates \((49)\).

**Progesterone before ovulation (Fig. 6).—**Forty milligrams of progesterone was given in 4 divided doses, case was the probable date of the period affected, menstruation occurring on the 34th day in the control, on the 31st day in the cancer patient.

No increase in estrogen excretion was notable when progesterone was given at this time in the month, perhaps because a maximum corpus luteum effect was already present at this time. There was a decrease in rate of androgen excretion in the normal case.

**DISCUSSION**

The setting up of a controlled investigation of the urinary excretion of the sex steroids is enormously

![Graphs showing excretion of estrogens and androgens after estrone injections administered after ovulation.](image)

injected from the 8th to the 14th day. The two subjects again responded quite differently. The control showed no sign of bleeding until her 32nd day, when she went through what appeared to be a normal menstrual cycle. The cancer patient developed typical “intracyclic bleeding” or premature menstruation on her 18th day.

A considerable increase in estrogen excretion was noted during the days of progesterone administration when compared with the corresponding days of the control cycle in both cases. The effect of 17-ketosteroids was inconsistent and probably not significant.

**Progesterone after ovulation (Fig. 7).—**Forty milligrams of progesterone was given to each subject in 4 equal doses from the 8th to the 14th day. The two subjects again responded quite differently. The control showed no sign of bleeding until her 32nd day, when she went through what appeared to be a normal menstrual cycle. The cancer patient developed typical “intracyclic bleeding” or premature menstruation on her 18th day.

A considerable increase in estrogen excretion was noted during the days of progesterone administration when compared with the corresponding days of the control cycle in both cases. The effect of 17-ketosteroids was inconsistent and probably not significant.

**Fig. 3.—**Excretion of estrogens and androgens after estrone injections administered after ovulation. \(x = 2 \text{ mgm. of injected estrone.}\)
Fig. 4.—Excretion of estrogens and androgens after testosterone propionate injections administered before ovulation. x = 50 mgm. of testosterone propionate.

Fig. 5.—Excretion of estrogens and androgens after testosterone propionate injections administered after ovulation. x = 50 mgm. of testosterone propionate.
Fig. 6.—Excretion of estrogens and androgens after progesterone injections administered before ovulation. 
\( x = 10 \text{ mgm. of progesterone.} \)

Fig. 7.—Excretion of estrogens and androgens after progesterone injections administered after ovulation. 
\( x = 10 \text{ mgm. of progesterone.} \)
considered characteristic for some pathologic condition such as breast cancer, the deviation must be demonstrable against controls in as nearly identical physiologic states as possible; it must be consistent; and it must be of considerable magnitude. From the studies here presented and from a review of the literature it appears to us most unlikely that any measurable disturbance exists in the spontaneous excretion of total estrogens or total 17-ketosteroids in the presence of breast cancer.

In the second part of the investigation the attempt was made to expose some disturbance in steroid metabolism by observing the effects produced from the administration of excessive quantities of hormones to two women, one normal and one who had been treated for breast cancer. By the methods employed it was hoped that physiologic peculiarities in the patient susceptible to breast cancer might manifest itself in one of several ways; namely, in the excretion rates of administered hormones, in secondary effects on the excretion of other hormones, or in specific disturbance in menstrual function.

Although about 150 specimens were collected, and their content of estrogens and 17-ketosteroids determined, the investigation was confined to one cancer patient and one normal control. For this reason, the work cannot be considered in any sense final but rather exploratory to determine the most promising directions for further work.

The investigation led to certain observations of interest in the field of general sex hormone physiology as well as in that of cancer research. In particular the belief that the time of the cycle in which hormone injection is made will greatly influence not only cycle length but hormone excretion rate was amply confirmed.

With regard to cycle length, injection of neither estrone, testosterone propionate, nor progesterone after the time of ovulation seems to affect the date of the next menstrual period to any great extent. Given before ovulation, estrone postponed the onset of the next period, while with the other hormones inconsistent effects were produced. It seems possible that the determination of the threshold dosage of a given hormone required to produce certain characteristic disturbances of menstrual rhythm could be developed into a test of ovarian or anterior pituitary function.

The influence of hormone administration on urinary excretion rates was somewhat confused by the obscuring effects of the physiologic change in ovarian function resulting from the injection. A few points may be stressed.

Estrone administration caused a rise in the estrogenic activity of the urine regardless of the phase of the cycle in which it was given. The 17-ketosteroid excretion seemed little affected by estrone injections, although a slight rise may have occurred in the cancer patient when estrogen was given before ovulation.

When testosterone propionate was injected a considerable rise in 17-ketosteroid excretion was noted, but the percentage recovery in these menstruating women was not as high as that reported for similar experiments in males. The estrogen excretion when androgens were given before ovulation was reduced or unaffected. This may be explained on the basis of an indirect inhibition of the patient's own ovary by the androgens, which would more than compensate for any possible conversion of androgens to estrogenic material. After ovulation androgen injections were followed in one instance by a rise in estrogenic activity of the urine similar to that reported for males and female castrates.

Progesterone injections before ovulation were followed by a slight rise in estrogen excretion similar to that reported by Smith and Smith (52, 53). When they were given after ovulation no such rise occurred, perhaps because the administered progesterone had an insignificant physiologic effect in the presence of the patient's own actively functioning corpus luteum.

As for evidence of a functional predisposition to cancer nothing final can be said from this study. No striking contrasts in the rates of elimination of injected hormones were noted. Certain differences in hormone excretion were found, but these were not significant when only two cases were being compared. The response of the cycle length to the injections gave more definite contrasts, for the patient with cancer appeared consistently more susceptible to a shortening of the cycle than did the control.

CONCLUSIONS

1. The spontaneous excretion of total estrogens and 17-ketosteroids is not abnormal in breast cancer.

2. When estrone, testosterone propionate, and progesterone are injected into women, definite alterations occur in the rates of estrogen and 17-ketosteroid excretion in the urine and in the duration of the menstrual cycle. The exact alterations that occur depend to a considerable extent upon the time of the cycle in which the hormone is administered.

3. Certain contrasts in the response to hormone injection were noted in the seven month study of one woman with breast cancer and one normal control.

4. These differences in response undoubtedly reflect some physiologic differences in the two women and suggest clinical methods by which a physiologic predisposition to breast cancer may be sought for in further work.
REFERENCES


Estrogen and 17-Ketosteroid Excretion in Patients with Breast Carcinoma

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