STUDIES ON THE URINARY STEROIDS IN BILATERAL GYNECOMASTIA*

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It has been known that gynecomastia is often associated with numerous clinical conditions, i.e., normal puberty, hypergonadotropic hypogonadism, tumors of the testis, disorders of sex, bronchogenic carcinoma, adrenocortical carcinoma, diseases of the thyroid gland and pituitary, diseases of the liver, malnutrition, after the period of sexual decline (Wallach et al., 1957; Treves, 1958; Hall, 1960; Kessel et al., 1963). But the etiology of gynecomastia seems to remain obscure. The histological approaches made so far in clarifying the mechanism of gynecomastia appear to be unsuccessful, because microscopically gynecomastia shows similar findings of ductal hyperplasia and an increase in collagenous fibres in all cases of various clinical conditions.

An attempt was made with a newly devised chromatographic system to estimate individual estrogens, 17-ketosteroids and 17-hydroxycorticosteroids present in the urine of normal subjects and patients with bilateral gynecomastia in order to make an etiological pursuance of the disease. As a result it was generally found that there were certain differences in the patterns of urinary estrogens and/or 17-ketosteroids between a normal and a diseased group.

MATERIALS AND METHODS

Material

Twenty one cases of gynecomastia and 20 cases of mastopathia admitted to the university hospital were studied. Cases with a unilateral breast enlargement were excluded from the present study so that the elimination of breast tumor cases might be assured. As controls, 24 normal males (25~50 years old), 6 male cases of arrested tuberculosis (20~35 years old) and 10 normal females of luteal phase were studied. These controls were selected at random.

Solvents and reagents

The solvents and reagents used were those of analytical grade except for sodium chloride

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which was of chemically pure grade.

Collection of urine

Twenty-four hrs. urine samples were collected without preservative and stored at -10°C if necessary.

Quantitative determination of estrogens

The technique used in this study involved acid hydrolysis of 200 ml of urine, extraction with ether containing toluene, separation of the phenolic fraction from the neutral fraction, purification of the phenolic fraction by the method of Bauld (1956), separation of individual estrogens by elution chromatography on partially esterified Amberlite IRC-50 using a mixture of ethanol and water (3:2 by vol.) as the eluant, and fluorometric estimation (Matsumoto and Seki, 1963a and b.)

Quantitative determination of urinary 17-ketosteroids and 17-hydroxycorticosteroids

A 300 ml portion of urine was adjusted to pH 4.7 with 2N acetic acid solution and then 60,000 units of limpet β-glucuronidase (Levvy et al., 1957) and 30 ml of acetate buffer (M, pH 4.7) were added. After incubation for 48 hrs. at 37°C, the urine was extracted with ethyl acetate (2×300 ml). The extract was washed with N-NaOH (2×30~4×30 ml), with 0.5% acetic acid solution (1×30 ml), with water (2×15 ml) and evaporated to dryness in vacuo below 40°C. The residue contained steroids which were present in urine as free and as β-glucosiduronate.

The aqueous phase after extraction with ethyl acetate was brought to pH 2 with 17N sulfuric acid and extracted with ethyl ether (1×300 ml). The aqueous phase was again brought to pH 1 to 0.5 with 17N sulfuric acid, 60 g of NaCl was dissolved and extracted with ethyl acetate (1×300 ml). After standing for 48 hrs. at 25°C, the ethyl acetate extract was washed with N-NaOH (4×15 ml), 0.5% acetic acid solution (2×15 ml), water (2×10 ml) and evaporated to dryness in vacuo below 40°C. The residue contained steroids which were present in urine as sulfate.

Chromatographic separation of the individual 17-ketosteroids and 17-hydroxycorticosteroids present in the residues described above was performed by elution chromatography on partially esterified Amberlite IRC-50 using a mixture of ethanol, methanol and water (9:3:8 by vol.) as the eluant (Seki and Matsumoto, 1963). The 17-ketosteroids and 17-hydroxycorticosteroids present in the effluent fractions were determined photometrically by the Zimmermann reaction and the Porter-Silber reaction. The separation of individual 17-hydroxycorticosteroids was not satisfactory (Fig. 1), e.g., tetrahydrocortisol, tetrahydrocortisone and cortisol were eluted together into the same fractions and allotetrahydrocortisol overlapped with cortisone. A search for optimal conditions for successful separation of these steroids is under way.

RESULTS AND DISCUSSION

Urinary 17-ketosteroids and estrogens in cases of gynecomastia and normal males were quantitatively determined (Table 1). None of the cases examined showed abnormalities of liver function on routine tests. A mean value of total urinary 17-hydroxycorticosteroids in cases of gynecomastia was somewhat lower than that of normal males, although it was within a normal range. No remarkable findings were obtained from the present analytical data of 17-hydroxycorticosteroids, e.g., the ratio of tetrahydrocortisol plus tetrahydrocortisone plus hydrocortisone to allotetrahydrocortisol plus cortisone was within a normal range.

It is to be noted that there were differences observed between normal males
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<th>KetoET</th>
<th>OHAn</th>
<th>DHA</th>
<th>Et</th>
<th>epiAn</th>
<th>An</th>
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Table 1. Analytical data of urinary estrogens and 17-ketosteroids in bilateral gynecomastia and mean value of 10 normal males (µg/24 hrs.)


* According to Hall (1960) Grade I: Breast development is obvious at a glance but is not readily recorded in a photograph.
Grade II: Breast development is obvious in a photograph.
Grade III: Breast enlargement is so marked as to appear adolescent female organ.
and cases of gynecomastia in the urinary patterns of estrogens and 17-ketosteroids, the details of which will be discussed.

**Estrogens**

The total amounts of estrogens in 24 hrs. urine and the ratios of estriol plus 16-epiestriol to estradiol-17β plus estrone in 10 normal males, 10 normal females of luteal phase, 21 cases of gynecomastia and 20 cases of mastopathia are shown in Figures 2 and 3. In about half the cases of gynecomastia, the total amounts of urinary estrogens were within a normal range and in the other half, the amounts of estrogens exceeded a normal range. The urinary patterns of estrogens in gynecomastia investigated could be divided into 3 types. Those cases which

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**Fig. 1. Elution patterns of 17-OHCS and 17-KS obtained from the urine (300 ml) of a gynecomastia case and a normal male by hydrolysis with β-glucuronidase**

- **a**: Gynecomastia (No. 13 of Table 1)
- **b**: Normal male

**Adsorbent**: partially esterified Amberlite IRC-50

**Eluant**: methanol-ethanol-water 3:9:8 by vol.

**Column size**: 0.8 × 110 cm

**Room temperature**: 23 ± 1°C

**One fraction**: 40 drops

**Flow rate**: 50 fractions/24 hrs.

**17-OHCS**: 17-hydroxycorticosteroid

- solid line: optical density at 410 mμ

**17-KS**: 17-ketosteroid

- dashed line: optical density at 520 mμ
excreted less than a normal upper limit of estrogens are classified as type A gynecomastia (9 cases). The upper limit of the estrogen value of normal males was tentatively designated as 11.4 μg per 24 hrs. (mean value plus standard deviation for 10 normal males i.e., 8.8 ± 2.6 μg). Those cases in which the excretion of estrogens exceeded 11.4 μg per 24 hrs. are classified as type B gynecomastia (9 cases). Type C gynecomastia (3 cases) is designated for the cases in which the excretion of estrogens exceeded 11.4 μg per 24 hrs. and the ratio of estriol plus 16-epiestriol which are almost biologically inactive, to estradiol-17β plus estrone which are biologically active, was less than 0.8. A normal range of this ratio varied from 0.8 to 8. In 1 case of type C gynecomastia, the ratio was only slightly lower
than a normal value, but in the other 2 cases it was much lower. In the 3 cases of type C, bilateral breast enlargement was so marked as to appear feminine. The low inactivation of estrogens observed in type C gynecomastia may play an important role in the pathogenesis in some cases of gynecomastia. A rather low inactivation of estrogens was also observed in 1 case of mastopathia among the 20 cases studied. In type A gynecomastia the amount of each estrogen excreted was too small to secure accurate determination, so that the ratio of estriol plus 16-epiestriol to estrone plus estradiol-17β thus calculated will remain rather questionable. The analytical method adopted in this study does not permit the determination of ring D α-ketolic estrogens (Givner et al., 1960), 18-hydroxyestrone and estrogens hydroxylated or methoxylated at carbon 2 (Givner et al., 1960;
Fishman, 1963). Therefore it seems impossible to evaluate a possible contribution of metabolic pathways which have been suggested by Fishman (1963), to the inactivation of estrogens in type A or C gynecomastia.

17-Ketosteroids

The excretion of 17-ketosteroids of 24 normal males and 6 male cases with arrested minimal tuberculosis (Figs. 6 and 7) showed the following characteristics: (1) the ratio of etiocholanolone plus androsterone to 11-oxygenated 17-ketosteroids varied from 3 to 10, and (2) the ratio of etiocholanolone to androsterone varied from 0.5 to 2. Since etiocholanolone and androsterone are formed by the steroids secreted from both adrenals and gonads, and 11-oxygenated 17-ketosteroids by the steroids secreted from adrenals, the ratio of the amounts of the former two steroids to the latter may indicate a relative dominance of activity between the gonads and the adrenals. Such indication may be justified from the observation that the ratio decreased following castration, although Vande Wiele et al. (1962) reported that etiocholanolone and androsterone were mostly derived from de-
Fig. 5. Elution patterns of estrogens obtained from the urine of gynecomastia

a: type A (case No. 17 of Table 1)
b: type B (case No. 16 of Table 1)
c: type C (case No. 15 of Table 1)

Adsorbent: partially esterified Amberlite IRC-50
Eluant: ethanol-water 3:2 by vol.
Column size: 0.42×100 cm
Room temperature: 23±1°C
One fraction: 10 drops
Flow rate: 50 fractions/24 hrs.
hydroepiandrosterone, a steroid secreted from the adrenals. The meaning of the ratio of etiocholanolone to androsterone seems to have been left unclarified, although it is suggested that the ratio may be adopted as a kind of measure for the inactivation of androgens.

It is a significant finding that the ratio of etiocholanolone plus androsterone to 11-oxygenated 17-ketosteroids was less than 3 in 10 cases out of the 17 cases of gynecomastia studied (Fig. 8). A typical elution pattern of neutral fraction of β-glucuronidase hydrolysate of urine is shown in Figure 1. A total amount of 17-ketosteroids was below a normal range in half the cases of gynecomastia studied.

In 2 out of the 17 cases, the amount of etiocholanolone excreted was 3 to 5 times larger than that of androsterone (Fig. 9).

Thus, an increased inactivation of androgens and/or a decreased rate of secretion of testicular androgens and dehydroepiandrosterone seem to constitute a causal factor in some cases of gynecomastia.
Recently, it was shown by Wallach and Garcia (1962) that the amount of urinary estrogens, 17-ketosteroids and 17-hydroxycorticoids excreted was within a normal range in familial gynecomastia and an inherited heightened tissue sensitivity to a normal endocrine environment was postulated to explain the reported familial gynecomastia. It must be admitted that the etiology of gynecomastia in many cases cannot be stated with certainty because the sensitivity of mammary tissues to a given stimulus appears to vary from one individual to another and with age. However, it is suggested that estrogens represent a sort of final common factor through which breast stimulation is brought about in most cases of gynecomastia and a decreased androgen activity may enhance the action of estrogens on mammary tissues in some cases.
It is generally accepted that the priming with estrogen is necessary for trophic hormone such as ACTH, growth hormone, prolactin and gonadotrophin to promote the development of gynecomastia. Therefore, it is suggested that an abnormal secretion and/or disturbed metabolism of estrogens and androgens might be a primary causative factor for many cases of bilateral gynecomastia.

**SUMMARY**

Studies were made on the urinary estrogens and 17-ketosteroids in bilateral gynecomastia. Twenty one cases of gynecomastia were classified into 3 types by estrogen excretion patterns. The daily excretion of estrogen was less than 11.4 μg (normal upper limit) in type A (9 cases) and over 11.4 μg in type B (9 cases); a partial blocking was observed in the conversion of estradiol-17β and estrone to estriol and 16-epiestriol in type C (3 cases).
In 10 out of 17 cases of gynecomastia, the ratio of etiocholanolone plus androsterone to 11-oxygenated 17-ketosteroids was less than 3. A normal value of the ratio varied from 3 to 10. The ratio of etiocholanolone to androsterone was more than 3 in 2 cases. A normal value ranged from 0.5 to 2. A total amount of 17-ketosteroids was less than a normal range in half the cases of gynecomastia studied.

Thus, in some cases of gynecomastia, it is suggested that breast stimulation is brought about by an increased secretion and/or a decreased rate of inactivation of estrogens, and a decreased secretion of gonadal androgens and/or dehydroepiandrosterone may enhance the action of estrogens on mammary tissues.
REFERENCES