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Urinary Excretion of Testosterone in Idiopathic Hirsutism. (30334)

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It is generally accepted that hair growth may be related both to hormonal factors, especially testosterone(1,2) and to genetic factors(1). In iodopathic hirsutism in women, it seems clear that genetic factors play a role: hirsutism is more common in Mediterranean peoples than in other Europeans, and is much less common in oriental women than in Caucasians. A role for hormonal factors is not so evident, however. The urinary excretion of 17 ketosteroids, frequently taken as an index of "androgenic status," is on the average, slightly higher than normal, but individual values cover a wide range(3,4). There does not appear to be a consistent, abnormal pattern of metabolites within the 17 KS group (5). However, since only a small portion of 17 KS is derived from testosterone, the possibility remains that there is an increase in testosterone production in this condition. The further possibility then exists that genetic differences in hair growth are mediated by differences in androgen production(6). Since the metabolism of this hormone in hirsute women is normal(7), urinary excretion of endogenous testosterone should be a reliable index of hormone secretion. The present report deals with urinary excretion of testosterone in normal Caucasian and oriental women and in women with idiopathic hirsutism.

Materials and methods. The subjects studied were 6 patients with idiopathic hirsutism, aged 18-55, whose history and physical examination, including pelvic examination, and other investigations, revealed no abnormalities to account for hirsutism, and 15 normal healthy women aged 20-40 of oriental (Chinese, Thai, Philippino) and occidental origin (Canadian, Swiss), who had a normal working life. Urine was collected for 24 hours without preservative, during the luteal phase of the menstrual cycle. The specimen, if not processed immediately, was kept frozen, under N_2.

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TABLE I. Systems Used for Thin-Layer Chromatography.

No.	Supporting medium	Solvent system	Running time
1.	Silica gel G.	Benzene:EtOAc, 3:2	55-65 min
2.	" " "	Hexane:EtOAc, 1:1	50-60 " (27)
3.	Aluminum oxide G.	Benzene:Ether, 1:1	34-45 " (")

Steroids were extracted following enzymatic hydrolysis of glucuronides and solvolysis, by the method of Goldzieher and Axelrod for fractionation of 17-ketosteroids(8). Testosterone-4-C^{14}, 0.4 μc, was added after glucuronide hydrolysis, to serve as an indicator for recovery of endogenous testosterone. The final extract was dried under reduced pressure at a temperature $<40°C$. The residue was dissolved in 10 ml ethanol, of which 0.1-0.2 ml was taken for total 17 KS determination and the remainder was taken to dryness for testosterone measurement. The dried residue was dissolved in 2 ml of 70% methanol and partitioned against 0.4 ml of hexane. The methanol layer was evaporated to a small volume for application on thin-layer plates. The separation of testosterone was achieved by 3 TLC systems in sequence (Table I) using chromatoplates 20 $\times$ 20 $\times$ 0.1 cm.

Testosterone on the chromatoplates was located by scanning for radioactivity with an end-window Geiger counter (window width 3 mm) and by fluorescence under UV irradiation (254 mμ). The area containing testosterone was eluted and the eluate reduced to a small volume and applied to the next plate. The testosterone obtained from the third plate was oxidized to Δ^4 androstenedione by overnight incubation at room temperature, with 2% chromic trioxide (0.2 ml) and glacial acetic acid (0.3 ml)(9). Distilled water (0.2 ml) was added and the steroid extracted with a total of 3 volumes of ethyl acetate. The ethyl acetate was evaporated and the residual Δ^4 androstenedione was purified by TLC using system 1. After elution, the Δ^4 androstenedione was measured by the micro-Zimmermann reaction(10). The spectrum was read in a recording spectrophotometer and corrected to "true" optical density(11). Total 17 KS were measured by the modified Zimmermann reaction(10), using DHA as the standard, and corrected as above. Final measurement of radioactivity, to calculate recovery, was made in a liquid scintillation counter using toluene, POPOP, and PPO. The recovery through the entire procedure was 45-75%. All the values were corrected for loss.

Ethyl acetate, ethanol, and ether were purified(12-14). Benzene, methanol and ethyl acetate were redistilled. N-hexane was spectrograde quality or else reagent grade, purified (15). Water for preparation of thin-layer plates was glass-distilled and silica gel G. was washed(16). Silica gel G. and aluminum oxide G. were from Research Specialties Corp. Sources of standards were as follows: testosterone-4-C^{14}, specific activity 22 mc/millimole (New England Nuclear Corp.); DHA, androsterone, etiocholanolone, and Δ^4 androstenedione (Sigma Chemical Co.); testosterone (CIBA); 11 ketoandrosterone and 11 ketoetiocholanolone (Southeastern Biochemicals, Inc.); 11-OH androsterone and 11-OH etiocholanolone (gift from Prof. W. Klyne, Westhill College, London). Test tubes and pipettes used in the Zimmermann reaction were cleaned with alcoholic KOH, then rinsed with tap water and redistilled water.

Results. The values of urinary testosterone and total 17 KS are shown in Table II. By analysis of variance, there were no significant differences in testosterone or total 17 KS excretion among the three groups studied (analysis of variance, $F>0.1$).

For the whole group, there was a correlation coefficient of .446 between urinary testosterone and total 17 KS ($P = .05$). It is apparent (Fig. 1) that in general, for a given level of testosterone, the hirsute patients tend to have higher 17 KS than the others.

TABLE II. Urinary Excretion of Testosterone and 17-Ketosteroids.

	Avg age	Testosterone (μg)	17-Ketosteroids (mg)
Normal-Occidental (7)	26	11.1 $\pm$ 6.2	4.3 $\pm$ 2.4
Normal-Oriental (8)	29	7.7 $\pm$ 3.3	3.3 $\pm$ .1
Hirsute-Occidental (6)	27	9.6 $\pm$ 6.4	6.6 $\pm$ 2.2

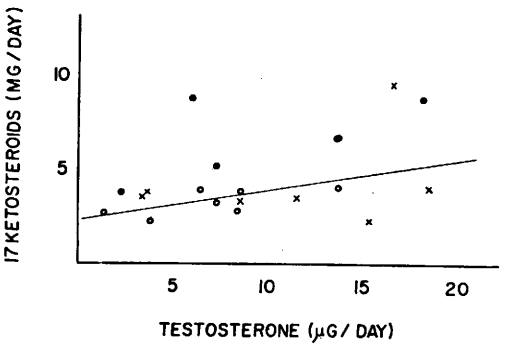

FIG. 1. Relation between testosterone and 17-ketosteroid excretion. Normal occidental ×; normal oriental ○; hirsute occidental ●. Regression line is for combined group of normal subjects.

Discussion. The use of TLC permitted a good separation of testosterone from DHA, androsterone, etiocholanolone, 11 oxy 17 KS, Δ^4 androstenedione, and isotestosterone in a short time. However, it did not remove all of the non-specific urinary chromogens which interfere with the quantitative spectrophotometric methods commonly employed, *viz.*, UV absorption in methanol, ethanol, or conc. H_2SO_4(17) and color development with concentrated sulfuric acid reagents(18-20). Oxidation of testosterone to Δ^4 androstenedione and measurement of the latter by the Zimmermann reaction proved suitable.

Urinary testosterone excretion by normal women in this study was within the previously reported range(9,17,21,22), although the variation was somewhat greater. Subjects with idiopathic hirsutism had essentially normal urinary testosterone values. During this study, several reports appeared on the measurement of testosterone in plasma and urine. Urinary excretion measured in one patient with idiopathic hirsutism was found to be elevated(9). Plasma values were elevated in some patients, but in many they fell well within the normal range(23-26). In the present work, all values were within the normal range.

Since there were no differences in urinary testosterone or total 17 KS excretion rates between normal occidental and oriental women, it is unlikely that genetic factors related to the incidence of hirsutism have a role in controlling testosterone production. The prevalence of hirsutism in occidental women is therefore probably due to genetically-determined differences in the hair follicles.

In agreement with the results obtained by others, the total 17 KS in idiopathic hirsutism overlapped the normal range. Though the correlation between urinary testosterone and total 17 KS for the whole group of subjects was significant at 5%, there was considerable scatter. This relatively low correlation reemphasizes the view that total 17 KS excretion is not a reliable index of testosterone or androgen production in the normal range. The finding that hirsute patients had higher 17 KS values relative to testosterone than the other subjects may indicate an increase in the production of 17 KS from other sources.

During the course of this study, a 57-year-old woman being treated for facial hirsutism was found to have a 17 KS value of 7.4 mg/24 hours with a testosterone excretion of 72 μg. Repeated examination ultimately revealed the presence of a small ovarian tumor which was removed surgically and found to be a hilum-cell tumor. Since surgery, there has been a distinct decrease in the rate of growth of the facial hair.

Summary. Testosterone and total 17 ketosteroids were measured in 24-hour urine samples of normal Caucasian women, normal oriental women, and patients with idiopathic hirsutism. Testosterone excretion ranged from 3-18 μg/day and did not vary significantly from group to group. The total 17 ketosteroid excretion was higher, on the average, in the hirsute patients, but the difference was not statistically significant. There was a low correlation between testosterone excretion and 17 ketosteroid excretion for the total group.

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Coccidioidin Induction of Skin Sensitivity and Positive Serology.* (30335)

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In a study designed to compare skin test activity of coccidioidins, guinea pigs were rendered skin sensitive by a single intraperitoneal (ip) injection of coccidioidin. Sera, however, studied by micro-complement fixation and agar gel double diffusion techniques, both plate and tube, were negative (1). Further studies using an Ouchterlony technique devised to demonstrate blocking reactions in the guinea pig sera gave equivocal results.

Rabbits are also skin sensitized by a single subcutaneous (sc) injection. Sera from rabbits skin test positive after one and multiple sc injections of coccidioidin with and without adjuvants have been studied by agar gel techniques.

It is the purpose of this report to elucidate the relationship between skin sensitization and acquisition of demonstrable humoral antibodies in rabbits given parenteral injections of coccidioidin.

Materials and methods. Male New Zealand white rabbits, purchased locally, were maintained on the usual pelleted food. They were reference bled from the marginal ear vein, pre-injection, and weekly or bi-weekly thereafter. Merthiolate was added to the sera in a final dilution of 1:10,000. Sera were stored at —20°C until tested.

All rabbits were skin test negative to undiluted coccidioidin at the initiation of the study. They were skin tested at varied intervals with 2 coccidioidins, Smith's Lot 64D4 and Huppert's Lot XVB52F, received through the courtesy of Dr. Milton Huppert of the San Fernando VAH. No two skin tests were given in the same area. Test coccidioidins were injected intradermally (id) into a cleanly shaven site. Disposable tuberculin sy-

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