STUDIES ON SEX HORMONE EXCRETION IN NORMAL AND HIRSUTE WOMEN

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ABSTRACT

Serial assays of testosterone, epitestosterone, oestrogens and pregnanediol in urine have been conducted in 11 hirsute patients and the results compared with those in 14 normally menstruating women. Overall mean levels for testosterone and epitestosterone were significantly higher in the hirsute group suggesting that excessive androgen production is of importance in the pathogenesis of this condition; significant differences in mean oestrogen and pregnanediol output between the two groups were not noted. In the hirsute subjects significant differences in urinary steroid excretion were not found between patients suffering from simple hirsutism and those in whom the excessive hair growth was associated with other pathological conditions. In both normal and hirsute groups steroid levels differed significantly between subjects and in individual women at different times. The incidence of anovulatory cycles was considerably higher in the hirsute than in the normal group.

Both endocrinological and genetic factors are believed to be involved in the development of hirsutism in human subjects (see Hamilton & Terada 1965 and references therein). Since the growth of sexual hair is generally regarded as a secondary sex characteristic, it is reasonable to anticipate that the gonadal hormones are mainly responsible, and in this connection it has been suggested that hirsutism might result either from an overproduction of testosterone and related C19 steroids or from a relative lack of antiandrogenic hormones, such as the oestrogens.

At the time of writing the majority of endocrine studies in hirsute women have been confined to the estimation of total or fractionated 17-oxosteroids or
of the parent hormone, testosterone, in blood and urine (Mauvais-Jarvis & Baulieu 1962; Lamb et al. 1964; Mansuwan & Kalant 1965; Lloyd et al. 1966; France & Knox 1967; Jayle et al. 1961; Bardin & Lipsett 1968; Casey & Nabarro 1967 and references therein). Little attempt has been made either to investigate hormonal interrelationships in this condition or to conduct serial rather than isolated determinations of hormones and their metabolites. The aim of the present communication, a preliminary account of which has been given elsewhere (Ismail et al. 1969), is to extend knowledge in this field by comparing the absolute levels of a number of steroids in hirsute patients with those in normally menstruating women of comparable age. In both groups of subjects assays of testosterone, epitestosterone, oestrone, oestradiol, oestriol and pregnanediol were performed, and in all the women serial estimations were conducted for periods of time of not less than four weeks.

MATERIALS AND METHODS

In the present study the control subjects are numbered from 1 to 14 and the hirsute patients from 15 to 25.

1. Controls

Fourteen normally menstruating women ranging in age from 21 to 35 years were investigated. All were healthy and normally active during the period of study. None had a previous history either of hirsutism or of menstrual abnormalities. The mean (± S.E.M.) duration of their cycles was 27 ± 3.2 and of menstrual bleeding 4 ± 1.2 days.

2. Hirsute subjects

There were 11 patients in this group, the age range being from 18 to 37 years. All showed hirsutism affecting the face, limbs, abdomen and chest, the hair growth being of masculine distribution. In one (15) the hirsutism was relatively mild, and the excessive hair growth was mainly confined to the perineal region and the thighs.

Five subjects (15, 20, 21, 24 and 25) had reasonably regular menstrual cycles, varying in length from 27 to 40 days; the duration of bleeding ranged from 3 to 7 days in individual cases. In one woman (23) the hirsutism was associated with grossly irregular cycles with variable menstrual loss. Two subjects (17 and 19) suffered from oligomenorrhoea as well as hirsutism; in one (19) menstruation occurred at intervals of 3–7 months and was associated with dysmenorrhoea on the first day of the period, while in the other (17) oligomenorrhoea and infertility were the presenting features. Two patients (18 and 22) gave a history of secondary amenorrhoea, the duration being 24 and 18 months respectively.

In all these 10 hirsute subjects, estimations of total urinary 17-oxosteroids and total 17-hydroxycorticosteroids were within the normal range. Clinical investigations failed to reveal a specific cause for the hirsutism except in patient 24 in whom bilaterally enlarged ovaries could be palpated. The last subject in this series (16) differed from

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the remainder in that the hirsutism and irregular menstrual cycles were associated with stigmata of virilism and with lesbianism.

3. Methods of assay

In the early part of the study, estimations of urinary testosterone were performed by the method of Ismail & Harkness (1966). Subsequently, the modified method of Ismail et al. (1968) for the determination of testosterone and epitestosterone was employed. It should be pointed out that when both methods are applied to the same urine sample the results with respect to testosterone are virtually the same. In all cases [4-14C] testosterone was added as an internal standard following hydrolysis to correct for losses occurring during the performance of the method.

Estimations of urinary pregnanediol and of oestrone, oestradiol and oestriol, were performed using the techniques of Klopper et al. (1955) and Brown (1955) respectively.

Estimations of urinary testosterone, epitestosterone and oestrogens were made on 48 h pools of urine, and the results were expressed as µg/24 h urine sample. In the case of urinary pregnanediol, assays were performed on 72 h pools of urine, and the results were expressed as mg/24 h urine sample.

RESULTS

With none of the urinary steroids estimated were significant differences found between patients suffering from simple hirsutism with regular cycles, and those in whom the hirsutism was associated with menstrual irregularities and other pathological conditions. Accordingly, the comparison made in the present study is between the normally menstruating women on the one hand and all the hirsute subjects on the other. In both groups, levels of all steroids were found to differ significantly between subjects (P < 0.01 – < 0.001).

Testosterone

Data in relation to this hormone are shown in Fig. 1.

The mean value in the normal group was 7.3 ± 0.36 and in the hirsute women 22.6 ± 0.8 µg/24 h. These values differed significantly (P < 0.001). The range in the normal group was from 1.0 to 21.0 and in the hirsute women from 4.7 to 87.3 µg/24 h.

In patient 15 who suffered from mild hirsutism in association with localised hair growth in the perineal area and thighs, the mean testosterone output (8.6 ± 0.4 µg/24 h) was the lowest in the group. Nevertheless, the figure was still significantly higher than the mean value (7.8 ± 0.86) in the control series (P < 0.02).

Epitestosterone

Values for this steroid are shown in Fig. 2.

The mean in the normal series was 8.9 ± 0.67 and the corresponding value in the hirsute group 15.7 ± 0.83 µg/24 h. These figures differed significantly
Mean testosterone excretion in normal and hirsute women.

The open columns are values in the control subjects while the crosshatched columns are those in the hirsute patients. The height of the column is the mean excretion value in each woman, and the vertical line is the appropriate standard error (S.E.M.). The solid columns are the overall means of the two groups, the enclosed figure indicating the number of observations on which the mean with its standard error is based.

\( P < 0.001 \). The range in the control series was from 3.3 to 23.4 and in the hirsute patients from 2.7 to 84.8 \( \mu g/24 \) h.

**Oestrogens**

A satisfactory correlation was noted between excretion values for the three oestrogens measured in both normal and hirsute groups. Accordingly, only the data in relation to the primary hormone, oestradiol, are described; these are shown in Fig. 3.

The mean oestradiol excretion in the normally menstruating women was 3.53 ± 0.27 and in the hirsute group 3.99 ± 0.27 \( \mu g/24 \) h; these values did not differ significantly. The range in the controls was from 0 to 11.5 and in the hirsute patients from 0 to 25.5 \( \mu g/24 \) h. The percentage of undetectable readings (\(< 1.5 \mu g/24 \) h) in the hirsute women was significantly higher \( P < 0.01 \) than in the controls (21 as compared with 8.9).
In patient 16 in whom the hirsutism was associated with virilism and lesbianism the mean oestradiol excretion \((0.14 \pm 0.07 \mu g/24 \text{ h})\) was significantly lower than the lowest mean value in the normal series \((P < 0.001)\). In subject 15 in whom the excessive hair growth was especially marked on the perineum and thighs the mean value \((9.1 \pm 1.36 \mu g/24 \text{ h})\) was significantly higher than in any of the normal subjects \((P < 0.001)\).

**Pregnanediol**

Data in relation to this steroid are illustrated in Fig. 4.

The overall mean in the normal subjects was \(1.24 \pm 0.10\) and in the hirsute group \(1.42 \pm 0.03 \text{ mg/24 h}\); these figures did not differ significantly. The range in the former group was from 0.15 to 3.9 and in the latter from 0.4 to 6.6 mg/24 h. In patient 20 the mean pregnanediol output \((3.6 \pm 0.21 \text{ mg/24 h})\) was significantly higher than that in the normal series \((P < 0.001)\); all the other mean pregnanediol levels in the hirsute group did not differ significantly from normal.

In the 14 cycles in the normally menstruating women the pattern of pregnanediol output was definitely ovulatory in character in 13 and equivocal in one. On the other hand, in the 14 cycles in the hirsute women with reasonably regular menstruation, a luteal phase rise in pregnanediol output occurred in only 7 (50%).
DISCUSSION

As already indicated there is now much evidence to support the view that androgenic hormones, especially testosterone, stimulate hair growth, while the oestrogens tend to inhibit this process (see Greenblatt 1965). Hair growth is now regarded as being essentially phasic in nature, each follicle passing through repeated cycles of resting and activity. The growth of an individual follicle is not synchronised with that of its immediate neighbour, and the total population of follicles is believed to represent a random mosaic of activity and resting structures (Ebling et al. 1964 and references therein). A change in the
balance of the sex hormones within the body is capable of altering the ratio of active to resting follicles and of producing the condition of hirsutism. In the present investigation a comparison of excretion values for testosterone, epi-testosterone, oestrogens and pregnanediol has been made between normal and hirsute women, and the results obtained for the individual steroids will be discussed.

Testosterone (T)

At the time of writing estimations of this hormone in body fluids represent the most satisfactory method for assessing androgenic function in health and disease. Reliable assay methods are now available for the quantitative determination of T in plasma and urine, and on the basis of these determinations production and clearance rates for the hormone can be calculated. The advantages and limitations of the various approaches to the estimation of T in clinical conditions have been reviewed elsewhere (Ismail & Loraine 1968).

In the present investigation the mean T output in the hirsute patients was shown to be significantly higher than in the normally menstruating women. This finding is in general agreement with that of Bardin & Lipsett (1968) who
demonstrated that despite normal plasma T levels in a proportion of their subjects, T production rates were invariably higher in hirsute women than in controls. The serial assays reported in the present paper have indicated that, although T readings are generally elevated in hirsute women, values within the normal range can be obtained for limited periods of time during the period of study.

The reason for the elevated urinary T levels in hirsute patients is not entirely clear. It could be due to the direct secretion of the hormone itself, to increased secretion of precursors such as androstenedione, and to increased peripheral conversion of androstenedione to T. A combination of these possibilities could also exist. On balance, it appears most likely that the elevated T levels in the hirsute subjects reflect direct secretion of the hormone by steroid-producing tissues, and this view is in keeping with that put forward by Bardin & Lipsett (1968). Ismail & Loraine (1968) and others have emphasised that when the major portion of the T produced arises as a result of direct secretion by the endocrine glands, urinary measurements correlate in a satisfactory manner with assays in blood and provide a valid estimate of androgenic function in human subjects.

Epitestosterone (epiT)

Mean excretion values for this steroid are significantly higher in hirsute women than in controls. However, it is worthy of comment that in some of the former group elevated T readings coincided with normal epiT values. This is well illustrated by patient 25 in whom all measurements of epiT were within the normal range while T readings were the highest in the series (Fig. 2). Another point which emerges from the present study is that high epiT levels always appear to be associated with elevated T values. A typical example of this is subject 23 in whom the mean epiT level (30.9 ± 4.5 μg/24 h) was the highest in the series and in whom T levels were also grossly elevated (mean 30.5 ± 1.2 μg/24 h). Although the evidence in this paper indicates that epiT excretion in hirsute subjects is higher than in controls, the degree of elevation of this steroid in the former group is less marked than that previously reported by de Nicola et al. (1966), the results of our study being more in keeping with those presented by France & Knox (1967).

The source and precursors of epiT in the body are not yet known with certainty, and the precise role of this steroid in physiological and pathological processes in man remains to be elucidated (see Ismail & Loraine 1968). The view that the formation of epiT represents a mechanism by means of which the biological activity of T is neutralised is no longer tenable because of the low interconversion rate of the two steroids. Another possibility is that epiT might directly affect pituitary gonadotrophic activity and alter functional relationships between the pituitary gland and the gonads (Allois & Hoffman 1959);
at the time of writing there is no definite evidence either to support or refute this hypothesis.

Oestrogens

In both the hirsute and the normal women oestrone, oestradiol and oestriol were measured and showed a significant correlation with one another and with their sum. Mean values for the three oestrogens differed significantly between subjects, but there were no significant differences between the means of the normal and hirsute groups. This is in agreement with observations previously reported in the literature (Cudmore et al. 1967 and references therein). The mean oestrogen output in patient 16 in whom the hirsutism was associated with virilism and lesbianism, was significantly lower than the normal mean value, while her mean T excretion was only slightly elevated as compared with the controls. It is of interest to note that, in spite of the relatively low T readings, virilisation occurred, and this must presumably have been due to the even lower secretion of oestrogens by the patient. As mentioned previously the excessive hair growth in subject 15 was mainly confined to the perineum and the thighs. This patient's oestradiol excretion was significantly higher than that in the normally menstruating women, while her T output was the lowest in the hirsute group; these findings might suggest that in her case the excessive hair growth resulted from oestrogenic rather than from androgenic stimulation.

Pregnanediol (P)

Although pregnanediol is not the sole urinary metabolite of progesterone and is not derived entirely from the parent hormone, it is generally accepted that P assays in urine do reflect in a reasonably satisfactory manner the secretion of progesterone by the ovary and by the adrenal cortex (see Loraine & Bell 1966). In the present investigation no significant difference was detected between the overall mean P excretion values of the normal and hirsute women. However, in one hirsute subject (20) the mean excretion of this steroid was significantly higher than the highest average value in the normal group. In this subject levels of urinary T and epiT were also above the normal range, and it is possible that the hirsutism resulted from increased conversion of progesterone to C₁₉ steroids such as androstenedione and T probably via 17α-hydroxyprogesterone.

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REFERENCES


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