STEROID EXCRETION AND BIOSYNTHESIS, WITH SPECIAL REFERENCE TO 16-UNSATURATED C\textsubscript{19} STEROIDS, IN CASES OF TESTICULAR FEMINIZATION AND IN A MALE PSEUDOHERMAPHRODITE

By

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ABSTRACT

The concentrations of the following steroids were measured in the urine of four patients with testicular feminization (TF) syndrome and of one male pseudohermaphrodite: 5\textalpha-androst-16-en-3\textalpha-ol (3\textalpha-androstenol), 5\beta-androst-16-en-3\textalpha-ol (aetiocholenol), 5,16-androstadien-3\beta-ol (androstadienol), androsterone, aetiocholanolone, dehydroepiandrosterone (DHA) and testosterone. Elevated levels of 3\textalpha-androstenol, androsterone, aetiocholanolone and DHA were found in the urine of two TF patients studied. The third TF patient and the male pseudohermaphrodite were excreting normal amounts for adult females of the three 16-unsaturated C\textsubscript{19} steroids, although androgen output was raised. Decreases in the excretion of these steroids occurred after removal of the testes. Administration of human chorionic gonadotrophin (HCG) and corticotrophin (ACTH) to one TF patient resulted in two- to four-fold increases in urinary 16-unsaturated C\textsubscript{19} steroids. The excretion of the three 17-oxosteroids measured, particularly androsterone and aetiocholanolone, increased after HCG and to a lesser extent after ACTH administration.

In preparations of testes removed from three TF patients androstadienol (1–8 %) was formed from pregnenolone. 4,16-Androstadien-3-one (androstadienone) was obtained in greater yield (0.2–1.33 %) from progesterone than from pregnenolone (0.1–0.48 %) although in the histologically normal testis from the pseudohermaphrodite, pregnenolone proved to be the more efficient precursor of androstadienone. Testosterone gave rise to no 16-unsaturated C\textsubscript{19} steroids when incubated under identical conditions.
The biosynthesis of testosterone in TF testis preparations was achieved normally from both pregnenolone and progesterone, in keeping with a defect in target organ insensitivity rather than in androgen formation. The possible significance of these findings and of the formation of large amounts (19%) of DHA from pregnenolone in TF testis is discussed.

The clinical and biochemical aspects of the testicular feminization (TF) syndrome have been the subject of some recent reviews (Southren 1965; Prunty 1967; Edwards 1970; Polani 1970; Raiti 1970). In the complete form of the disease (Morris 1953) the levels of plasma and urinary testosterone and 17-oxosteroids (17-OS) are in the male range and show a marked decrease after gonadectomy. Administration of human chorionic gonadotrophin (HCG) causes an increase in the testosterone production rate (Jeffcoate et al. 1968) and in urinary 17-oxosteroids (Desphande et al. 1965) and oestrogens (Gwinup et al. 1966).

Although indirect evidence has suggested that in TF skin, the conversion of testosterone to 5α-dihydrotestosterone (5α-DHT) is defective (Mauvais-Jarvis et al. 1969), the latter was biosynthesized in vitro normally in skin taken from TF patients (Wilson & Walker 1969; Jenkins & Ash 1971). The defect in this syndrome therefore appears to lie at the cellular level since administration of testosterone (French et al. 1966) or 5α-DHT (Strickland & French 1969) does not result in virilization nor in the anticipated anabolic effect. Moreover, it appears that it is the binding of 5α-DHT to specific receptors that is defective (Gehring et al. 1971).

Although the urinary excretion of 16-unsaturated C19 steroids is now well-documented in normal individuals and in patients with endocrine abnormalities (Brooksbank & Haslewood 1961; Brooksbank & Gower 1970; Gower 1972), these compounds have not hitherto been studied in the TF syndrome nor in pseudohermaphrodites. This paper is concerned with the excretion and testicular biosynthesis of this group of steroids and, in one patient, the effect of corticotrophin (ACTH) and HCG has also been studied on urinary 16-unsaturated C19 steroids and on individual 11-deoxy-17-OS. A preliminary report of these investigations has already been published (Bicknell & Gower 1971a).

**CASE REPORTS**

*Miss L. R.* - (aged 18) was investigated at the Clinical Endocrinology Research Unit, Edinburgh and found to be a typical case of testicular feminization syndrome with 46 XY chromosome complement. Breast development occurred normally at puberty but there was no sexual hair. No other hirsutes were present. Laparotomy revealed that a vagina was absent and the inguinal testes were removed. Urine (48 h samples) was collected during control periods and during the administration of HCG (5000 IU im per day for 5 days) and of ACTH (40 units im per 12 h for 4 days). Some
of these urine samples were obtained by us through the courtesy of Dr. A. A. A. Ismail.

*Miss R. F.* – (aged 33) had normal breast development but no axillary or pubic hair. She presented with primary amenorrhea and was found to be chromatin negative with 46 XY chromosome complement. Laparotomy was performed and the intra-abdominal testes removed. No uterus or Fallopian tubes were found.

*Miss J. M.* – (aged 18) also presented with primary amenorrhea and was found to have minimal breast development. Her chromosome complement was 46 XY. As in the case of R. F., laparotomy was performed and the testes removed.

*Miss S. D.* – (aged 37) was another case of testicular feminization, closely resembling Miss R. F. in clinical details.

In the patients, L. R., R. F., J. M. and S. D. the external genitalia were normal. Histological examination of gonads from these patients revealed that they were primitive testes containing immature Sertoli cells and plentiful interstitial tissue compatible with testicular feminization.

*Miss F. N.* – (aged 28) a graduate of normal intelligence, had been brought up as a female whose libido was directed exclusively towards males. However, she was of masculine appearance with facial hirsutes and no breast development. The vagina was small and blind and internal female genitalia were absent. Testes were present in the groins and were removed at operation. The left testis was grossly enlarged and proved to be a loculated hydrocoele. The right testis was histologically normal and was used for biosynthetic studies. The diagnosis made was that of male pseudohermaphrodisim.

**MATERIALS AND METHODS**

Solvents and alumina used for chromatography were as described by *Gower & Ahmad* (1967). [7α-3H] Androstadienone* (specific activity 120 mCi/mm) was synthesized and purified by *Wilkinson et al.* (1970). [4-14C] DHA sulphate, specific activity 58.8 mCi/mm was obtained from New England Nuclear Corp., Frankfurt/M., Germany; [4-14C] pregnenolone, progesterone and testosterone (specific activities 55.7, 60.0 and 29.2 mCi/mm respectively) were purchased from The Radiochemical Centre, Amersham, Bucks. U. K. Urinary 16-unsaturated steroids, androsterone, aetiocholanolone and DHA were measured by the method of *Brooksbank & Gower* (1970) slightly modified as follows: To each urine sample (30 ml) was added [7α-3H] androstadienone (105 dpm) and [4-14C] DHA sulphate (5.5 × 10⁴ dpm). Hydrolysis of steroid conjugates was achieved by incubation at 37°C for 48 h after adding *Helix pomatia* (0.5 ml) (Boehringer Corporation, London, W.5.) and 10 ml of sodium acetate buffer (0.2 M, pH 5.0). Liberated steroids were extracted by gentle swirling for 20 min periods with ether (3 × 20 ml). The pooled extract was dried (Na₂SO₄), evaporated to dryness and the residue subjected to column chromatography on alumina, partially deactivated with water 4-5% (v/w) (*Gower & Ahmad* 1967). Three fractions were eluted containing (1) 3α-androstenol; (2) aetiocholenol plus androstenol and (3) androsterone, aetiocholanolone plus DHA, as described earlier (*Brooksbank & Gower* 1970). Tritiated androstadienone was eluted in fraction (1) and the radioactivity in this fraction thus

*Abbreviations used: Androstadienone, 4,16-androstadien-3-one; 3α-androstenol, 5α-androst-16-en-3α-ol; 3β-androstenol, 5α-androst-16-en-3β-ol; aetiocholenol, 5β-androst-16-en-3α-ol; androstenol, 5,16-androstadien-3β-ol; DHA, dehydroepi-androsterone.*
Table 1.
Urinary 16-unsaturated C₁₉ steroids (μg/g creatinine), 17-oxosteroids (mg/24 h) and testosterone (μg/24 h) in testicular feminization patients before and after bilateral gonadectomy.

<table>
<thead>
<tr>
<th>Patient</th>
<th>3α-Androstenol</th>
<th>Aetiocholenol</th>
<th>Androstadienol</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. R. (pre-operative)</td>
<td>207 (130–330)</td>
<td>6.9 (3.0–10.4)</td>
<td>29.0 (12.5–46.0)</td>
</tr>
<tr>
<td>L. R. (post-operative)</td>
<td>N. M.</td>
<td>N. M.</td>
<td>N. M.</td>
</tr>
<tr>
<td>R. F. (pre-operative)</td>
<td>1280</td>
<td>11.7</td>
<td>59.0</td>
</tr>
<tr>
<td>R. F. (post-operative)</td>
<td>463</td>
<td>6.3</td>
<td>21.0</td>
</tr>
<tr>
<td>S. D. (pre-operative)</td>
<td>612</td>
<td>1.8</td>
<td>9.67</td>
</tr>
<tr>
<td>S. D. (pre-operative)</td>
<td>650</td>
<td>1.0</td>
<td>3.24</td>
</tr>
<tr>
<td>S. D. (post-operative)</td>
<td>525</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>S. D. (post-operative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. D. (post-operative)</td>
<td>146</td>
<td>nil</td>
<td>4.1</td>
</tr>
<tr>
<td>S. D. (post-operative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. N.¹</td>
<td>339</td>
<td>5.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Normal values for</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult women</td>
<td>320 (230–420²)</td>
<td>4.8²</td>
<td>20.7²</td>
</tr>
</tbody>
</table>

¹) Male pseudohermaphrodite.
Ranges are given in parenthesis. N. M. = not measured.
²) (Brooksbank & Gower 1970).

gave an estimate of losses incurred in extraction up to this stage in the analysis. Losses of 11-deoxy-17-OS were estimated by measurement of the ¹⁴C content of fraction (3). The mean recoveries of ³H-androstadienone and ¹⁴C-DHA were 62% and 59% respectively.

16- Unsaturated C₁₉ steroids were estimated in fractions (1) and (2) by GLC, after conversion to the chloromethyl dimethyl (CMDS) ethers (Brooksbank & Gower 1970; Gower & Thomas 1968) and 11-deoxy-17-OS by GLC of trimethylsilyl (TMS) ethers (Kirschner & Lipsett 1963). Urinary testosterone was estimated by the method of Brooks (1964) modified and urinary total 17-OS measured by the method of Few (1961) modified by James & de Jong (1961).

Incubation of testis tissue. - Testis tissue was obtained from some patients R. F., J. M., S. D. and F. N. after gonadectomy, and minces (approximately 500 mg wet wt.) and/or homogenates (5% w/v in Tris-HCl buffer (0.05 M, pH 7.5)) were prepared. The tissue preparations (minces suspended in Tris-HCl buffer, 2 ml) or homogenates (2 ml) were incubated for 40–60 min at 38°C with [⁴-¹⁴C] pregnenolone, [⁴-¹⁴C] progesterone or [⁴-¹⁴C] testosterone as indicated in Table 2. No cofactors were added to the incubations of minced tissue but NADPH (0.8 mg) was added to homogenates
<table>
<thead>
<tr>
<th>Androsterone</th>
<th>Aetiocholanolone</th>
<th>DHA</th>
<th>Total 17-OS</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 (3.1–7.4)</td>
<td>4.4 (2.5–6.6)</td>
<td>1.8 (0.3–3.6)</td>
<td>15.2 (11.8–17.7)</td>
<td>251 (49.5–500)</td>
</tr>
<tr>
<td>N. M.</td>
<td>N. M.</td>
<td>N. M.</td>
<td>5.3 (3.9–6.2)</td>
<td>29.0 (16.3–50)</td>
</tr>
<tr>
<td>2.93</td>
<td>3.77</td>
<td>2.57</td>
<td>–</td>
<td>50.0</td>
</tr>
<tr>
<td>1.35</td>
<td>1.08</td>
<td>0.72</td>
<td>5.3</td>
<td>6.0</td>
</tr>
<tr>
<td>2.5</td>
<td>N. M.</td>
<td>3.72</td>
<td>15.8</td>
<td>N. M.</td>
</tr>
<tr>
<td>2.9</td>
<td>N. M.</td>
<td>7.25</td>
<td>13.2</td>
<td>N. M.</td>
</tr>
<tr>
<td>1.03</td>
<td>N. M.</td>
<td>2.4</td>
<td>8.5</td>
<td>3.0</td>
</tr>
<tr>
<td>0.6</td>
<td>N. M.</td>
<td>2.84</td>
<td>10.3</td>
<td>8.0</td>
</tr>
<tr>
<td>2.6</td>
<td>9.6</td>
<td>3.0</td>
<td>11.5 (10.6–12.5)</td>
<td>61.5 (46–96)</td>
</tr>
<tr>
<td>1.4 (0.9–2.6³)</td>
<td>1.8³ (0.6–2.2)</td>
<td>0.56³ (0.2–1.13)</td>
<td>4-15⁴</td>
<td>&lt;20⁵</td>
</tr>
</tbody>
</table>

³) (Kirschner & Lipsett 1964).
⁵) (Brooks 1964) modified.

as the conversion of pregnenolone to androstanediol is NADPH-dependent (Katkov & Gower 1970; Katkov 1971). After incubation, carrier steroids were added and extraction performed with ethyl acetate (4 X 2 ml) as described by Katkov & Gower (1970) and the dried, pooled extracts evaporated to dryness. The residues (dissolved in small volumes of chloroform) were then subjected either to thin-layer chromatography (TLC) in benzene-ether (9:1, v/v) run twice (Gower 1964) or to two-dimensional TLC (Bicknell & Gower 1971b). Using the latter technique a separation of the very non-polar 16-unsaturated C₁₉ steroids from more polar steroids, such as pregnenolone or testosterone, was achieved on one plate (further details of this separation are given in the Legend to Fig. 4.) After development the plates were dried and radiography performed using Kodirex X-ray film (Kodak, Ltd., London) for 36–48 h. Δ⁴-3-Oxosteroid carriers were located in UV light (wavelength, 254 nm) and most other steroids of interest (including the 16-unsaturated C₁₉ steroid³) were detected by spraying with a saturated solution of iodine in light petroleum (Gower 1964).

³) Authentic 16-unsaturated C₁₉ steroids were generously supplied by Dr. C. L. Hewitt, Organon Ltd., Lanarkshire, Scotland.
Effect of HCG and ACTH on urinary 3α-androstenol in testicular feminization patient (L. R.). HCG (5000 IU/day for 5 days) and ACTH (40 units/12 h for 4 days) were administered separately as indicated. The mean level of 3α-androstenol during control periods is represented by ---.

**Identification of metabolites.** After elution from TLC plates, tentatively identified 16-unsaturated C_{19} steroids were purified by TLC on AgNO₃-impregnated Kieselgel G (Lisboa & Palmer 1967) using benzene-ethyl acetate (1:2, v/v) (Katkov & Gower 1970) as solvent. Previous work (Katkov 1971) has shown that these procedures result in a satisfactory purification of 16-unsaturated steroids and particularly of androstadienol and 3β-androstenol. Other metabolites were purified by TLC in benzene-acetone (4:1, v/v) or benzene-ethanol (95:5, v/v) as described previously (Gower & Stern 1969).

**Specific activity measurements.** Whenever possible radiochemical purity of metabolites was established by measurement of specific activity after repeated TLC in various systems (as above) or by repeated gas-fraction collection. The latter was performed essentially as described by Brookesbank & Wilson (1970) using a gas-chromatographic column fitted at one end with a 25:1 stainless steel stream splitter (Pye-Unicam, Cambridge, U.K.). Steroid fractions were collected in cooled (~60°C) glass U-tubes, filled with fat-free cotton wool. After elution with chloroform, the weights of carrier steroids were determined by gas-liquid chromatography (GLC): 16-unsaturated steroids were measured as described by Gower & Thomas (1968); other steroids, such as testosterone, pregnenolone, progesterone (eluted from TLC plates), using a 92 cm column of silanized Suprasorb (B. D. H. Ltd., Poole, Dorset, U.K.), coated with XE-60 (3% w/w) and maintained at temperatures in the range 220–230°C.
Effect of HCG and ACTH on urinary actiocholenol (□) and androstadienol (□) in testicular feminization patient (L. R.). The mean levels of actiocholenol and androstadienol during control periods are represented by --- and -------- respectively. For further details see legend to Fig. 1.

RESULTS

Analysis of urinary steroids. — The results of these analyses are summarized in Table 1.

16-Unsaturated C₁₉ steroids. — In two patients (R. F. and S. D.) 3α-androstenol excretion was raised above the level for normal adult women (in patient R. F. the value of 1280 µg/g creatinine was well into the adult male range). In patient L. R. and in the male pseudohermaphrodite (F. N.), however, urinary 3α-androstenol was within the normal adult female range. R. F. was also excreting greater than normal amounts of actiocholenol and androstadienol before gonadectomy but the other patients studied excreted normal or lower-than-normal amounts of these two steroids. After gonadectomy there was a marked decrease in the urinary 3α-androstenol (patients R. F. and S. D.). Allowing for day-to-day fluctuations there was also a tendency for the urinary actiocholenol and androstadienol to decrease. The effect of HCG and ACTH administration to patient L. R. is depicted in Figs. 1 and 2. Urinary 3α-androstenol increased above the mean basal level by four-fold after HCG and by three-fold after ACTH (Fig. 1). Increases of two- to three-fold were noted in the actiocholenol and androstadienol excretion (Fig. 2).
Effect of HCG and ACTH on urinary androsterone, aetiocholanolone and DHA in testicular feminization patient (L. R.). ■ and □ represent the mean levels (with S. E.) of steroids during control periods before and after treatments with HCG or ACTH respectively; ■ and □ represent steroid levels during HCG and ACTH administration respectively. For further details see legend to Fig. 1.

17-Oxosteroids and testosterone
The urinary excretion of androsterone, aetiocholanolone and DHA was markedly raised in all the patients studied but the relative proportions of the three compounds were normal (cf. David et al. 1965). Urinary testosterone was also elevated in two patients (L. R. and R. F.). A fall in the three urinary 17-OS to normal adult female levels occurred after gonadectomy (cf. Morris & Mahesh 1963; French et al. 1965), except in patient S. D. whose urinary DHA was still elevated seven days after operation. It is conceivable that this DHA arose from the adrenals which may have been stressed due to the surgical procedures.

Radioautographs of two-dimensional thin-layer chromatograms of 14C-steroids obtained from the incubation of feminizing testis (from patient S. D.) with (a) [4-14C] pregnenolone and (b) [4-14C] progesterone. Carrier steroids were added to the incubations before extraction; after chromatography, Δ4-3-oxosteroids were located with UV light, 254 nm (○) and most other steroids of interest by spraying with iodine in light petroleum. I = testosterone plus 17α-hydroxyprogesterone (subsequently resolved by further TLC); II = 4-androstenedione; III = progesterone; IV = androstadienone; 1 = 17α-hydroxyprogrenenolone; 2 = DHA; 3 = pregnenolone; 4 = androstadienol. Plates were developed in benzene-ether (9:1, v/v) run twice, as the first solvent followed by benzene-methanol (9:1, v/v) as the second.

Fig. 3.

Fig. 4.
Fig. 4.
Table 2.

Biosynthesis of 16-unsaturated C19 steroids in human testicular feminization testis preparations. All preparations were of minced tissue except in the case of S. D. when homogenates were prepared.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Precursor</th>
<th>Percentage yields (corrected for analytical losses).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Androstadienone</td>
</tr>
<tr>
<td>R. F.</td>
<td>Pregneneolone</td>
<td>0.22 (70,77)</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>0.86 (286,230)</td>
</tr>
<tr>
<td>J. M.</td>
<td>Pregneneolone</td>
<td>0.1 (345,322)</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>0.2</td>
</tr>
<tr>
<td>S. D.&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>Pregneneolone</td>
<td>0.48 (130,127)</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>1.33 (716,720)</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>nil</td>
</tr>
<tr>
<td>F. N.&lt;sup&gt;2)&lt;/sup&gt;</td>
<td>Pregneneolone</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>trace</td>
</tr>
</tbody>
</table>

<sup>1</sup> See also Table 3.

<sup>2</sup> F. N. was a male pseudohermaphrodite with histologically normal testes.

<sup>3</sup> Tentative identification only.

<sup>4</sup> Approximate values.

Figures in parenthesis are specific activities (cpm/μg) measured by repeated thin-layer chromatography or gas-fraction collection after initial purification by conventional and/or argentation TLC.
Table 3.
Metabolism of C21 and C19 steroids in homogenates of feminizing testis (patient S. D.).

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Progesterone</th>
<th>17α-OH-pregnenolone</th>
<th>17α-OH-progesterone</th>
<th>DHA</th>
<th>Androstenedione</th>
<th>Testosterone</th>
<th>5α-Androstan-3β,17β-diol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnenolone</td>
<td>0.4</td>
<td>16</td>
<td>N. M.</td>
<td>19.6 (5375,5450)</td>
<td>0.1</td>
<td>3.3 (460,398)</td>
<td>N. M.</td>
</tr>
<tr>
<td>Progesterone</td>
<td>32.0</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>trace</td>
<td>2.3 (457,492)</td>
<td>N. M.</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.45 (1420,1220)</td>
<td>71.5</td>
<td>0.5 (84.5,94.0)</td>
</tr>
</tbody>
</table>

Figures in parenthesis are specific activities (cpm/μg) measured by repeated thin-layer chromatography or gas-fraction collection after initial purification by two-dimensional and one-dimensional TLC (see Methods Section).

N. M. = not measured.
HCG administration to L. R. (Fig. 3) resulted in increased excretion of the three major urinary 17-OS especially androsterone and aetiocholanolone but only small increases were recorded after ACTH.

In vitro studies. — Metabolites of labelled precursors were separated initially by two-dimensional TLC (Bicknell & Gower 1971b) as described in the Methods section. Fig. 4 compares the pattern of metabolites formed from pregnenolone and from progesterone. After elution and further purification, a number of metabolites were characterized or tentatively identified and the percentage yields calculated after allowance for analytical losses. Testosterone was not a precursor of 16-unsaturated C19 steroids but androstadienol was formed from pregnenolone in appreciable yields (1–8 %) in all incubations (Table 2). Androstadienone was also formed, progesterone being a more efficient precursor than pregnenolone in the TF tissue preparations. In the pseudohermaphroditic tissue (F.N.) incubation, however, the yield of androstadienone was greater from pregnenolone than from progesterone, a finding noted earlier with boar testis preparations (Ahmad & Gower 1968). Only very small quantities of 3α-androstenol and aetiocholenol were formed from the C21-precurors used (Table 2). The analysis of other metabolites in experiment S.D. (Table 3) revealed that testosterone was formed from both pregnenolone and progesterone. The presence of reductive enzymes was shown by the conversion of testosterone to a small yield of 5α-androstan-3β,17β-diol.

DISCUSSION

There appears to be no clear-cut correlation between urinary 16-unsaturated steroids and androgens in the limited number of testicular feminization patients and the male pseudohermaphrodite in this study (Table 1). The relationship between these two groups of steroids remains obscure; in breast cancer patients, a positive correlation was found between urinary 3α-androstenol and androsterone, aetiocholanolone and DHA (Bulbrook et al. 1963) but no correlation was observed in women with simple hirsutism (Brooksbank & Gower 1970). Moreover, there is no evidence either in vivo or in vitro to show that 16-unsaturated C19 steroids are derived from C19 steroids in any more than trace amounts (for references see Gower 1972). In the present work, testosterone did not serve as a precursor of 16-unsaturated C19 steroids (Table 2, patients F. N. and S. D.).

Sites of biosynthesis of 16-unsaturated C19 steroids.

The data in Table 2 clearly show that androstadienol is formed in appreciable quantities in human testis preparations. This compound is also formed in high yields from pregnenolone in boar testis preparations (Katkov & Gower
1970; Loke & Gower 1971) and occurs to a limited extent in boar spermatid
vein blood (Gower et al. 1970b). In accord with these observations, a study of
the metabolism of pregnenolone in healthy men and women (Brooksbank &
Wilson 1970) revealed that urinary androstadienol (but not 3α-androstenol)
was derived from this circulating C21 steroid. In boar testis, androstadienol
is converted to androstadienone in the presence of NAD+ (Katkov & Gower
1970) but whether this transformation can also occur in human testis has not
yet been ascertained. Androstadienone is certainly formed from pregnenolone
in such preparations (Table 2) and it is of considerable interest that more
is formed from progesterone than from pregnenolone in TF tissue whereas
in the pseudohermaphroditic (F.N.) testis (which was histologically normal)
and also in boar testis (Ahmad & Gower 1968) pregnenolone is by far the
better precursor. Preliminary evidence suggests that the difference may not
be explicable on the basis of different cofactor additions or whether the pre-
paration was made from fresh tissue or tissue that had been previously stored
at −20°C. The significance, if any, of this finding remains to be determined.

The decrease in urinary androstadienol after gonadectomy (Table 1) is in
keeping with the testicular biosynthesis of this compound (Table 2), so also
is the increase in urinary androstadienol after HCG administration (Fig. 2).
However, the increase noted after ACTH administration in the same patient
(Fig. 2), could suggest an adrenal origin for androstadienol. The biosynthesis
of this steroid in normal human adrenal tissue has not been studied but is
known to occur in adrenal carcinoma tissue (Gower & Stern 1969; Gower
et al. 1970a).

The increases in urinary 3α-androstenol after ACTH and HCG admini-
stration (Fig. 1) would also suggest both an adrenal and testicular origin for
this steroid or, possibly, its immediate precursor(s). Similar increases have
been reported for healthy men after both ACTH and HCG but the effect
in women normally occurs only after ACTH administration, there being no
response to HCG (Brooksbank 1962; Cleveland & Savard 1964). The in vitro
results (Table 2) however, show that pregnenolone and progesterone give rise
to little or no 3α-androstenol and it seems unlikely that androstadienone,
formed in appreciable quantities, could be subsequently converted by reduction
to 3α-androstenol because [7α-3H] androstadienone, administered intravenously
to two men and one woman (Brooksbank et al. 1972), gave rise to only minute
quantities of tritiated 3α-androstenol in the urine.

The results of urinary steroid assays in the male pseudohermaphrodite (F. N.)
are essentially in accord with those obtained by Rice et al. (1967) with a
similar patient, whose urine, spermatid vein plasma and peripheral plasma
contained testosterone and 17-OS in amounts normally found in adult males.

In testis taken from the TF patient (S. D.) the biosynthesis of testosterone
from both pregnenolone and progesterone appeared to be occurring normally
(Table 3). Other workers have reported similar findings (see David et al. 1965 and reviews by Polani 1970 and Raiti 1970) in keeping with a defect, not in the biosynthesis of androgens but in their effect on target organs. The formation of large quantities of 17α-hydroxypregnenolone (16%) from pregnenolone and of 17α-hydroxyprogesterone (14%) from progesterone has also been recorded by David et al. (1965) (18.3% and 15.5% respectively) but the conversion of pregnenolone to progesterone in our incubations was very small. The high yield (19%) of DHA from pregnenolone is probably in accord with the elevated urinary DHA in this patient (3.72 to 7.25 mg/24 h., Table 1). The testicular biosynthesis in TF patients of large yields of DHA (normally a product of the adrenal) has been described by David et al. (1965) and moreover Morris & Mahesh (1963) have reported raised plasma DHA sulphate levels and significant quantities of free DHA in gonadal vein blood in three TF patients. Also in keeping with a testicular origin for DHA is the increased excretion of this steroid in patient L. R. in response to HCG (Fig. 3) (cf. David et al. 1965). The fact that ACTH resulted in a small increase indicates that the adrenals in this patient were also secreting DHA. It is conceivable that the actual increases were greater than those observed due to the fact that, in this patient, urine was collected for 48 h periods instead of the normal 24 h periods.

The increase in urinary androsterone and aetiocholanolone after HCG administration to the same patient (L. R. Fig. 3) are very similar to those reported by David et al. (1965) and by Deshpande et al. (1965) but, allowing for day-to-day fluctuations, there were very much smaller increases after ACTH. This suggests that the bulk of these 11-deoxy-17-OS had a testicular origin.

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