THE URINARY EXCRETION OF TESTOSTERONE AND EPITESTOSTERONE IN FEMALES FOLLOWING INTRAVENOUS INFUSIONS OF TESTOSTERONE BEFORE AND DURING TREATMENT WITH AN ANTI-ANDROGEN (CYPROTERONE)

By

J. Tamm and W. Beischer*)

ABSTRACT

Intravenous infusions of 10 mg testosterone (T) in two females (one of them adrenalectomized for Cushing's syndrome) resulted in an increased urinary excretion of T-glucuronide, whereas the excretion of epitestosterone (ET)-glucuronide was not altered significantly. The excretion of T-sulphate increased only slightly. During treatment with 100 mg Cyproterone p. d. a marked elevation of urinary T and ET excretion occurred. A slight increase in unconjugated T and ET was also observed. A second T infusion during Cyproterone treatment did not lead to a further increase of urinary T-glucuronide, but there was a slight increase in urinary excretion of ET-glucuronide and of T-sulphate.

It has been demonstrated previously that treatment with the antiandrogen Cyproterone (1,2α-methylene-6-chloro-Δ⁴,6-pregnadiene-17α-ol-3,20-dione) brings about a reasonable increase in the urinary excretion of testosterone (T) and epitestosterone (ET) in normal men (Voigt et al. 1968 a; Apostolakis et al. 1968). Recent results (Voigt et al. 1968 b) obtained after the administration of labelled Cyproterone showed that the increase in urinary T and ET was not due to the excretion of Cyproterone itself or some of its metabolites, but rather to a stimulation of T production. This assumption was confirmed by the fact

* Part of the doctoral thesis.
that the combined administration of the antiandrogen and human chorionic
gonadotrophin (Apostolakis et al. 1968) resulted in an additional increase in
urinary T excretion. Whereas the pattern of urinary 17-ketosteroids was not
uniform (Bettendorf et al. 1968; Laschet & Laschet 1967) the urinary excre-
tion of oestrogens remained essentially unchanged during treatment with
Cyproterone (Voigt et al. 1968a; Bettendorf et al. 1968). In this paper the
effect of Cyproterone on the excretion of T and ET in urine following in-
fusions of T is described.

METHODS AND MATERIALS

The experiments were carried out in two female patients. Pat. No. 1 (28 years of age)
suffered from an anovulatory menstrual cycle. Pat. No. 2 (37 years of age) was
bilaterally adrenalectomized two years previously for Cushing's syndrome. Her men-
strual cycle was regular. The experimental regimen is shown in Tables 1 and 2.
10 mg of T in 5 ml 50% ethanol were diluted in 300 ml 0.9% saline. The solution
was administered intravenously during a period of 3 hours.

The methods used have been outlined in detail previously (Voigt et al. 1964;
Tamm et al. 1966). In order to separate unmetabolized Cyproterone from T and ET
an additional paper chromatogram in Zaffaroni's system hexane : propylene glycol was
introduced (running time after impregnation of the paper strips with 23% propylene
glycol in methanol, 24 hours). R\textsubscript{S} values of Cyproterone 0.35, T 0.62, ET 0.96 (de-
oxycorticosterone = 1.0). In the fraction of unconjugated steroids, treatment with
Cyproterone resulted in the appearance of an \(\alpha,\beta\)-unsaturated substance slightly more
polar than androstenedione (A). By means of a rechromatography in system Bush A
on the same strip, this unidentified substance X could be clearly separated from A
\(R_F\) values of X 0.35, A 0.5.

RESULTS

The results obtained are summarized in Tables 1 and 2. The sulphates of T
and ET were estimated only in pat. No. 2. In this patient the control values of
T- and ET-glucuronide in urine were found to be in the normal range of
menstruating women (Apostolakis et al. 1966). In pat. No. 1 the excretion of T
and ET during the control day was found to be in the upper range of the
luteal phase of normal menstruating women (Apostolakis et al. 1966). The
intravenous infusion of 10 mg T resulted in an increase of urinary T-glucuro-
nide of 93 \(\mu\)g/24 h in pat. No. 1 and of 65 \(\mu\)g/24 h in pat. No. 2. These data
correspond to 0.93 and 0.65%, respectively, of the administered amount of T.
On the day of infusion the excretion of ET-glucuronide showed no significant
deviation from the control values. A very small increase if any was observed
in the free fraction. In the sulphate fraction (pat. No. 2) the T infusion also
caused only a very small increase in T and ET. On the days following the
infusion the steroid excretion returned to control levels.
Table 1.
Urinary Excretion of Steroids Before and During Treatment with Cyproterone.
Patient No. 1, 28 years.

<table>
<thead>
<tr>
<th>Day of Experiment</th>
<th>Day of C. Treatment (100 mg p. d.)</th>
<th>Remarks</th>
<th>Unconjugated Fraction (µg/24 h)</th>
<th>Glucuronide Fraction (µg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T     ET  A</td>
<td>T     ET</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>sample lost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3.</td>
<td>10 mg T Infusion</td>
<td>ND</td>
<td>ND</td>
<td>1.6</td>
</tr>
<tr>
<td>4.</td>
<td>Control</td>
<td>ND</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>5.</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10.</td>
<td>5. Control</td>
<td>2.9</td>
<td>6.5</td>
<td>–</td>
</tr>
<tr>
<td>11.</td>
<td>6. Control</td>
<td>4.6</td>
<td>8.2</td>
<td>–</td>
</tr>
<tr>
<td>12.</td>
<td>7. Control</td>
<td>2.5</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td>13.</td>
<td>8. 10 mg T Infusion</td>
<td>7.6</td>
<td>4.6</td>
<td>3.0</td>
</tr>
<tr>
<td>14.</td>
<td>9. Control</td>
<td>2.4</td>
<td>ND</td>
<td>3.3</td>
</tr>
</tbody>
</table>

C = Cyproterone, T = Testosterone, ET = Epitestosterone, A = Androstenedione, ND = Not detectable.

Table 2.
Patient No. 2, 37 years.

<table>
<thead>
<tr>
<th>Day of Experiment</th>
<th>Day of C. Treatment (100 mg p. d.)</th>
<th>Remarks</th>
<th>Unconjugated Fraction (µg/24 h)</th>
<th>Glucuronide Fraction (µg/24 h)</th>
<th>Sulphate Fraction (µg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T     ET  A</td>
<td>T     ET</td>
<td>T    ET</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>1.9</td>
<td>ND</td>
<td>1.8</td>
<td>5.9</td>
</tr>
<tr>
<td>2.</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
<td>10.2</td>
</tr>
<tr>
<td>3.</td>
<td>10 mg T Infusion</td>
<td>1.2</td>
<td>ND</td>
<td>3.0</td>
<td>73.3</td>
</tr>
<tr>
<td>4.</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>1.7</td>
<td>9.1</td>
</tr>
<tr>
<td>5.</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5.0</td>
</tr>
<tr>
<td>10.</td>
<td>5. Control</td>
<td>5.2</td>
<td>2.7</td>
<td>2.2</td>
<td>293.7</td>
</tr>
<tr>
<td>11.</td>
<td>6. Control</td>
<td>6.9</td>
<td>2.0</td>
<td>2.2</td>
<td>307.2</td>
</tr>
<tr>
<td>12.</td>
<td>7. 10 mg T Infusion</td>
<td>6.8</td>
<td>2.5</td>
<td>3.0</td>
<td>305.9</td>
</tr>
<tr>
<td>13.</td>
<td>8. Control</td>
<td>10.3</td>
<td>3.6</td>
<td>1.4</td>
<td>294.4</td>
</tr>
<tr>
<td>14.</td>
<td>9. Control</td>
<td>7.4</td>
<td>3.1</td>
<td>2.3</td>
<td>313.9</td>
</tr>
</tbody>
</table>

Legend see Table 1.
Treatment with Cyproterone was followed in both cases by a marked increase in the urinary excretion of mainly T- and ET-glucuronide. A slight but measurable elevation was also observed in the fraction of unconjugated T and ET. The excretion of A remained essentially unchanged. The same was true for the sulphate fraction in pat. No. 2. The results of the second T infusion were as follows: The elevated excretion of T-glucuronide remained unaltered, whereas the urinary levels of ET-glucuronide increased by 11 and 16 µg/24 h, respectively. A very small increase of free T in the urine was seen in pat. No. 1. On the days following the second T infusion, the excretion of ET-glucuronide was found to be in the preinfusion range. In pat. No. 2 the excretion of T-sulphate was increased on the two days after the infusion. A slight increase of unconjugated T and ET was also seen on the day following the administration of T.

**DISCUSSION**

It is beyond doubt that treatment of males and females with Cyproterone is followed by an increase in the urinary excretion of T- and ET-glucuronide (Voigt et al. 1968 a; Apostolakis et al. 1968; Ismail, personal communication). This has also been confirmed by the present experiments.

Before the administration of Cyproterone the percentage conversion of intravenously administered T into urinary T-glucuronide was in agreement with the results reported in the literature following the injection of tracer doses of this steroid (Mauvais-Jarvis & Baulieu 1965). The percentage amount of urinary T-glucuronide derived from exogenous T remained rather constant even after stimulation by HCG of the endogenous T-production (Horton et al. 1965). However, the elevated urinary excretion of T-glucuronide following treatment with Cyproterone did not increase further after the infusion of T. The mechanism by which Cyproterone inhibited any further increase in the T-glucuronide excretion in the urine is not known. It has been reported recently (Baulieu 1968) that Cyproterone displaces T from the binding sites of its specific transport globuline in plasma. By this means the metabolic transformation of T may probably be accelerated.

There was a slight but definite increase in the urinary excretion of ET-glucuronide following the second infusion of T, which was missed after the control infusion. This indicates that during treatment with Cyproterone the epimerization of T into ET played a more important role in peripheral T metabolism. Compared with the control infusion, more T-sulphate was excreted in urine during treatment with the antiandrogen. The delayed increase of this conjugate after the second infusion may in part be due to the very low metabolic clearance rate of T-sulphate (Wang et al. 1967).
ACKNOWLEDGEMENTS

These experiments have been supported by a grant of the Deutsche Forschungsgemeinschaft. Cyproterone has been generously supplied by Schering A. G., Berlin. The skilful technical assistance of Miss Ursula Volkwein is gratefully acknowledged.

REFERENCES


Received on March 2nd, 1968.