BIOSYNTHESIS OF TESTOSTERONE BY A STEIN-LEVENTHAL OVARY

By
Nelson Leon,* Manuel Neves e Castro**
and Ralph I. Dorfman

ABSTRACT

A Stein-Leventhal ovary from a 23-year-old virilized woman who was excreting a normal amount of 17-ketosteroids incubated with acetate-1-14C formed testosterone-14C. The possibility that certain types of hirsutism, end even virilism in women, may be due to a small increase in testosterone production insufficient to produce a significant increase in urinary 17-ketosteroids is discussed.

It has been suggested that the hirsutism or virilism associated with various types of ovarian hyperactivity of tumours in which minimum or no changes in 17-ketosteroids are observed may be due to excessive testosterone production (Sauvad et al. 1961; Kase et al., 1961). The data reported in this communication concerning a patient with the Stein-Leventhal Syndrome is consistent with this hypothesis.

Case Report. D. A. C. (H. C. 441767), a 23 year-old white woman, was first admitted (Medical School of Sao Paulo) on May, 1956. She had been moderately obese as a child and continued to gain excessive weight after puberty. At the age of 18 she noted the first signs of her disease. On May 21, 1956,

Supported in part by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.
* Present address: Department of Endocrinology, 1st Medical Clinic, Hospital das Clinicas Sao Paulo, Brazil.
** Gulbenkian Foundation Fellow (Portugal). Present address: Department of Endocrinology, Portuguese Inst. of Oncology, Palhava-Lisbon, Portugal.
at the age of 21 she was seen by one of us (N. L.). Menarche was established at 12 followed by normal menstruations of 3-days duration, although she had the impression that the quantity of the menstrual flow had been scarce. Her general condition was satisfactory. Her past history was not significant. She had had mumps as a child. There was no hirsutism in the family. She had acne.

At physical examination, the presence of excessive hair was noted on the chin, in the mandibular area, this being less along her superior lip. Long and firm hair was present on her breasts and the abdominal hair followed a masculine pattern. She had normal axillary hair and her pubic hair spread to the anal region. Some slight white striae were present at both sides of the abdomen. Her musculature and bone configuration were normal. The thyroid gland was palpable but of normal consistency. Examination of the head, heart, lungs, lymphatic system, extremities and neurologic system yielded nothing unusual.

The external genitalia were normal, no atrophy was seen on the labia majora or the labia minora. Her clitoris was small. Palpation was negative. At rectal examination, a small uterus was found.

Height: 158 cm; weight: 63 kg; blood pressure: 130 × 80 mm Hg.

Laboratory Findings. Slight oestrogenic activity was noted by vaginal smears (30 % of acidophilic cells at the 13th day of menstruation). She had trichomiasis.

Blood electrolytes: Na 140 mval/l; K 4.1 mval/l; P 4.16 mg/100 ml; Ca 9.75 mg/100 ml; Cl 96 mval/l.

Glucose tolerance test: 87, 141, 114, 101, 109 mg/100 ml.

Insulin tolerance test: 94, 49, 98, 101, 91 mg/100 ml.

There was a diminished iodine uptake (20.6 %) and a urine excretion of iodine of 61.9 %.

The basal metabolic rate was 2.2.

Blood cholesterol: 149 mg/100 ml (Sheftel 1944). Urinary gonadotrophins: between 10 and 20 m. u. in 24 hours (Klinefelter et al. 1943).

17-Ketosteroids: 11.4 mg/24 hours (Drektar et al. 1952; Callow et al. 1938).

11-Oxysteroids: 1.2–2.2 mg/24 hours (Heard & Sabel 1946).

Oestrogenic substances: more than 16 r. u. (Allen & Doisy 1923).

On November 13, 1957, the patient complained that her menstruations became more scarce and her hirsutism was more accentuated. A laparoscopy was done the next day and bilateral ovarian enlargement was noted, the ovaries being pale in colour, suggesting Stein-Leventhal Syndrome. The basal temperature was monophasic. The vaginal smears did not show more than 30 % of acidophilic cells.

On July 16, 1958, a wedge-resection was done on both ovaries. The right ovary was enlarged (4 × 3 cm) and had many microcysts on its surface. The left ovary was 3 × 2 cm and also had microcysts. Microscopic examination showed a thickened tunica albuginea, some fibrosis of the stroma, but follicles
in all stages and some corpora albicantia were present. The 17-ketosteroids of the patient continued to be normal after the operation:

<table>
<thead>
<tr>
<th>Date</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 18, 1957</td>
<td>8.3 mg/24 h</td>
</tr>
<tr>
<td>December 18, 1957</td>
<td>8.1 mg/24 h</td>
</tr>
<tr>
<td>July 8, 1958</td>
<td>11.0 mg/24 h</td>
</tr>
<tr>
<td>September 5, 1958</td>
<td>11.4 mg/24 h</td>
</tr>
</tbody>
</table>

(Drekter et al. 1952)

When the patient was seen two months after the operation, the menstruation was more regular. The menstrual flow was more abundant and accompanied by abdominal pain. The temperature curves had become diphasic. She lost some weight after the operation. Vaginal smears done after the operation was as follows:

- August 4, 1958: 70% acidophilic cells (menstruation on July 17)
- August 12, 1958: 20% acidophilic cells
- September 2, 1958: 40% acidophilic cells

(15th day since the menstruation)

**Tissue Incubation.** Tissue slices from the resected portion of the Stein-Leventhal ovary were incubated with 0.5 mc of sodium acetate-1-14C, 10 ml of the patient's serum, 5 mg of glucose, 0.01 mmol of sodium fumarate with phosphate buffer at 37°C for 3.5 hours under oxygen. The mixture was homogenized, extracted successively with ethyl acetate, ether-chloroform (4:1), and acetone. The combined extracts were concentrated to dryness under a stream of nitrogen, 10 mg each of testosterone and androst-4-ene-3,17-dione were added to the dried residues. The residue, containing the added steroids, was partitioned between 90% methanol and ligroin and methanolic fraction contained 95,000 counts per minute. The neutral fraction, prepared by partition between toluene and 1 N sodium hydroxide, contained 37,500 counts per minute.

The testosterone and androst-4-ene-3,17-dione fractions were obtained by paper chromatography in the ligroin-propylene-glycol system. Radiochemical purity was not achieved with the eluted crystalline material having the mobility of androst-4-ene-3,17-dione. The testosterone fraction was chromatographed on a silica gel column and in a Bush system (petroleum ether, benzene-methanol-water) and 0.5 mg of testosterone was obtained with a specific activity of 80 c/min/mg which was considered to be radiochemically pure. This material was acetylated, chromatographed on paper and the testosterone acetate was recrystallized to a constant specific activity of 79 c/min/mg (calculated as testosterone).

**DISCUSSION**

It is clear that testosterone can be formed by normal human ovaries as has been demonstrated by Kase et al. (1961). Preliminary reports by O'Donnell &
\textit{McCaig} (1960) and \textit{Goldzieher \& Axelrod} (1960) have indicated that testosterone may be produced by abnormal human ovaries. The qualitative detection of newly formed testosterone in this study, therefore, does not set off this Stein-Leventhal ovary from a normal ovary. The finding herein reported can only be said to be consistent with the idea that hirsutism and even virilism related to abnormal ovaries may be due to a relatively small increased production of testosterone insufficient to produce a significant rise in urinary 17-ketosteroids. \textit{Lanthier \& Sandor} (1960) have studied the formation of androst-4-ene-3,17-dione from pregnenolone in normal and Stein-Leventhal type ovaries and have concluded that the abnormal ovaries produce an increased quantity of this androgen. It seems rather unlikely that this androgen would produce the clinical symptoms because of its low androgenicity in humans (\textit{Savard et al.} 1961).

\textbf{REFERENCES}

\textit{Allen E. \& Doisy E. A.:} J. Amer. med. Ass. 81 (1923) 819.

Received on April 14th, 1961.