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EXCRETION OF STEROIDS IN TWO WOMEN WITH VIRILIZING ADRENO-CORTICAL TUMORS

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SUMMARY

The results of fractionation and individual determination of urinary steroids during the control period, after ACTH stimulation, and after surgery, are presented in two virilized women with adreno-cortical tumors. The data indicate that the increased excretion of 5-pregnene-3β,17α,20α-triol and pregnanetriol along with elevated levels of dehydroepiandrosterone, testosterone-glucuronide and tetrahydro-11-deoxycortisol, may be a most helpful test in evaluating the virilizing adrenal tumors.

INTRODUCTION

In view of the recent demonstrations concerning the peripheral metabolism of [^{3}H]-17α-hydroxypregnenolone in normal subjects, it has been established that the urinary metabolite 5-pregnene-3β,17α,20α-triol is liberated in significant amounts by β-glucuronidase hydrolysis or by solvolysis [1]. Therefore, it is assumed that sequential application of the hydrolytic and solvolytic techniques gives an accurate assessment of the urinary 5-pregnenetriol* [2].

In adrenal carcinoma [2, 3], the excretion of 5-pregnenetriol was markedly increased and the major portion was found in the solvolyzed fraction, although some was also released by β-glucuronidase.

Using a suitable technique for measurement of 5-pregnenetriol, the urinary excretion of 5-pregnenetriol, in addition to that of other major steroid metabolites, was studied in two women with virilizing adrenal carcinoma before and after surgical removal of the tumors.

EXPERIMENTAL

Case report

Two young female patients (R. E., 22 yr old, and B. A., 21 yr old) with a marked and progressive hirsutism of the face and extremities, with episodes of amenorrhea, but without marked changes in body configuration or breast size, were operated for removal of tumors, which upon microscopic examination revealed adrenal carcinoma. After surgery the two patients showed a marked diminution of the hirsutism and the menses became regular.

**Trivial names and abbreviations used in this paper*: Pregnanediol (PG): 5β-pregnane-3α,20α-diol; Pregnanetriol (PGT): 5β-pregnane-3α,17α,20α-triol; 5-Pregnenetriol (Δ_5-triol): 5-pregnene-3β,17α,-20α-triol; Pregnenolone (Δ_5-P): 3β-hydroxy-5-pregnen-20-one; 17α-Hydroxypregnenolone (17α-OH-Δ_5-P): 3β,17α-dihydroxy-5-pregnen-20-one; Dehydroepiandrosterone (DHA): 3β-hydroxy-5-androsten-17-one; Testosterone-glucuronide (TG): 3-oxo-4-androsten-17β-y1-β-D-glucopyranosiduronate; Tetrahydro-11-deoxycortisol (THS): 3α,17α,21-trihydroxy-5β-pregnan-20-one; 17-Ketosteroids (17 KS); Porter-Silber chromogens (17 OHCS).

Materials and methods

The excretion of steroids in the urine for 24 h was analyzed (1) during the control period, (2) after ACTH stimulation (a single injection of 40 U gel-ACTH) and (3) after removal of the adrenal virilizing tumors.

Analysis of total urinary 17-ketosteroids (17 KS) and Porter–Silber chromogens (17 OHCS) was performed by the methods of Drekter [4] and Porter–Silber (5); the corticosteroids were measured by the method described by Pasqualini [6] and modified in our laboratory. Chromatographic fractionation of the individual 17 KS metabolites was carried out by the method of Baulieu [7].

The following methods were used for the quantitative determination of the major urinary steroids:
The method of Szereday and Sachs [8] for testosterone glucuronide.
The method of Jayle *et al.* [9] for pregnanediol and pregnanetriol.
The method of Jayle *et al.* [10] for total estrogens and that of Brown [11] for their chromatographic fractionation.

The procedure for the separation and the measurement of the 3β-hydroxy-Δ_5-steroids in adult urine is outlined schematically in Fig. 1.

Urinary Δ_5-steroids were separated by the method described by Shackleton [12]; his procedure involves sequential hydrolysis and solvolysis and identification of 5-pregnenetriol on thin-layer silica-gel chromatograms.

For the quantitative determination of the Δ_5-steroids we used elution from silica-gel with ethanol and subsequent colorimetric assay: the Allen reaction for 5-pregnenetriol, the Pettenkofer reaction for other 3β-hydroxy-Δ_5-steroids, and the Talbot reaction for pregnanetriol.

The quantity of each Δ_5-steroid was expressed in terms of the respective authentic standard using standard curves obtained under the same conditions.

The average recovery in 8 experiments was 80%. Δ_5-Steroids were localized by means of authentic compounds run (single or multiple runs) alongside on thin-layer chromatograms and stained with antimony trichloride; 5-pregnenetriol was oxidized *in situ* on silica-gel films with periodate ($NaIO_4$) and the oxidized product rechromatographed on thin layer with authentic DHA; the spots were localized with Zimmermann reagent, and DHA and the oxidized product of 5-pregnenetriol exhibited the same R_F value [13].

RESULTS

Table 1 summarizes the levels of urinary steroids during the control period, after ACTH stimulation and after removal of the adrenal virilizing tumors. In

Precipitation of sulfate ions by 10% $BaCl_2$
Enzyme hydrolysis (*succus Entericus* of Helix pomatia)
Solvolysis by ethyl acetate at 38°
TLC silica-gel Hf 254 Merck
  System E × 1:cyclohexane–ethyl acetate (5 : 5, v/v)
  System B × 2: chloroform–absolute ethanol (19 : 1, v/v)
  System D × 2: benzene–absolute ethanol (24 : 1, v/v)
Elution with ethanol
Quantitation: Δ_5-triol: Allen reaction
  Other 3β-OH-Δ_5-steroids: Pettenkofer reaction
  PGT: Talbot reaction

Fig. 1. Separation and measurement of urinary 3β-hydroxy-5-steroids in adult urine.

Table 1. Summary of urinary steroid results in two virilized women with adrenocortical tumors under adrenal stimulation and after total left adrenalectomy

Subject Sex–age	Conditions	17 KS Drekter	17 KS Baulieu	DHA	17 OHCS Porter–Silber	Cortico-steroids Pasqualini	THS	PG Jayle	PGT	Δ_5-triol Schac–kleton	TG Szereday–Sachs	Estrogens Jayle–Brown
		mg/24 h									μg/24 h	
R.E. f.22	Control	191	126	99	7·7	6·6	0·7	x	x	10	465	$E_1 = 8$
	ACTH	165	105	73	23	11·3	1·3	x	x	12	482	$E_2 = 2$
												$E_3 = 4$
	Post. adrx.	6·0	3·0	0·2	5·2	1·5	0·03	2·6	1·6	0·2	63	12·5
B.A. f.21	Control	343	194	170	11·6	6·8	1·4	3·7	7	18	174	345
	ACTH	213	232	210	17·6	7·5	1·7	4·8	5	20	273	212
	Post. adrx.	1·3	1·2	0·1	3·4	1·0	0·07	1·6	0·3	0·4	60	14

*Because of interfering material, determinations could not be carried out.

nine normal subjects (one male and eight females) the excretion of 5-pregnenetriol varied from 0,100 to 0,900 mg/24h [14].

Urinary C_{19}-steroids. The excretion of 17 KS was markedly elevated over normal ranges in both cases, with DHA accounting for 78% and 90% respectively of the sum of individual 17 KS. Excretion of testosterone-glucuronide was markedly increased. ACTH stimulation had relatively little influence upon C_{19}-steroid excretion.

Serial determinations of the 17 KS at various time intervals after adrenalectomy showed, in both cases, values within the normal range. The excretion of testosterone-glucuronide, though considerably decreased after surgery, remained at distinctly higher levels than normal female range.

These findings were associated with a clinical remission.

Urinary corticosteroids. In the reported cases, 17 OHCS (Porter–Silber) and total corticosteroids in the control collections were elevated; the corticosteroid pattern is characterized by increased excretion of tetrahydro-11-deoxycortisol.

In case R.E., ACTH stimulation resulted in the significant increase of 17 OHCS, total corticosteroids, and tetrahydro-11-deoxycortisol. In case B.A., ACTH stimulation had relatively little influence upon urinary corticosteroids. After surgical removal of the tumors, 17 OHCS, total corticosteroids and tetrahydro-11-deoxycortisol decreased to normal levels in both patients.

Urinary 5-pregnenetriol was considerably elevated in both patients (10 and 18 mg/24h respectively) and showed no response to ACTH. After surgery, urinary 5-pregnenetriol decreased to normal levels.

Urinary pregnanediol and pregnanetriol excretion was increased in patient B.A. with a prompt and progressive fall after surgery. In the other patient, the determinations of pregnanediol and pregnanetriol could not be carried out because of interfering material.

The very high values of the *urinary total estrogens* in patient B.A. fell after surgery. In case R.E., the basal line of estrogens was in the normal range.

DISCUSSION

Urinary 5-pregnenetriol was measured along with the major urinary steroid metabolites-including 17 KS, DHA-sulfate, corticosteroids, tetrahydro-11-deoxycortisol, pregnanediol and pregnanetriol, testosterone-glucoronide and estrogens-in two young female patients with adrenal carcinoma before surgical treatment, after ACTH stimulation and after surgery.

Preoperatively, in both patients studied, an abnormally large fraction of the increased excretion of 17 KS consisted of DHA-sulfate. Both young females had an increased excretion of urinary testosterone-glucuronide.

Our findings confirmed elevation of total 17 KS and DHA-sulfate reported by others in adrenal carcinoma [15–17] and in adrenocortical tumors [18] and also confirmed the increased excretion of testosterone-glucuronide reported in adrenocortical tumors [18] and the high levels of tetrahydro-11-deoxycortisol reported by Cost [19] in adrenal carcinoma.

It is interesting to note the marked increase in urinary 5-pregnenetriol in both patients studied. A significant elevation in the urinary excretion of 5-prenenetriol has been reported in adrenal cancer [2, 3, 20–22].

An increase in urinary 5-pregnenetriol has also been found in the Stein–Leventhal syndrome [23, 24] and in congenital adrenal hyperplasia [2, 21, 25].

In normal urine, sixty per cent of the 5-pregnenetriol was found in the glucoronide fraction [1, 2]. Similar distribution was observed after ACTH administration and in congenital adrenal hyperplasia [2]. A different distribution was observed in adrenal carcinoma. In the present study, in both cases, the conjugate of 5-pregnenetriol is predominantly the sulfate and suggests that 5-pregnenetriol sulfate must be derived directly from 17-hydroxypregnenolone sulfate—a significant secretion product in adrenal tumors. These results are in agreement with those published for similar cases by Gallagher [3] and by Riley [2].

High values of 5-pregnenetriol in the urine of patients with adrenal carcinoma are considered to stem from an overproduction of pregnenolone as well as from insufficient utilization of intermediates at several stages of steroid biosynthesis. In this respect, the high secretion rate of 17-hydroxypregnenolone and its peripheral conversion to 5-pregnenetriol and pregnanetriol has been reported in virilizing adrenal tumor [26].

Furthermore, the lack of Δ_5-3β-hydroxysteroid-dehydrogenase was demonstrated in adrenal carbinoma by histochemical study [27].

Another investigation from our laboratory [28] deals with diminished activity of the 3β-hydroxy-steroid–dehydrogenase system for C_{21}-steroids in neoplastic adrenal tissue.

Our present results clearly reflect the diagnostic value of increased urinary 5-pregnenetriol sulfate which must be considered along with elevated levels of pregnanetriol, DHA-sulfate, testosterone-glucoronide and tetrahydro-11-deoxycortisol in cases of virilizing adrenal carcinoma.

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