Urinary Excretion of Pregnanetriol and Δ^5-Pregnenetriol in Two Forms of Congenital Adrenal Hyperplasia

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ABSTRACT Although congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency generally reveals a predominance of Δ^5-3β-hydroxysteroids, on occasion substantial quantities of pregnanetriol have been found as well. It appears that the latter steroid more often occurs in the subjects who have survived beyond infancy. The use of the measurement of pregnanetriol alone may therefore not be relied upon as a sole determinant of the specific form of defective steroidal biogenesis. It is more characteristic of the 21-hydroxylase deficiency. However when both Δ^5-pregnenetriol and pregnanetriol are measured the ratio of the former to the latter is always considerably below 1.0 in 21-hydroxylase deficiency and always above 1.0 in 3β-hydroxysteroid dehydrogenase. Furthermore, 11-ketopregnanetriol has been found only in the urine of subjects with the 21-hydroxylase deficiency. Thus, these two forms of defective steroidal biogenesis may be distinguished by the measurement of these three urinary steroidal metabolites.

INTRODUCTION

In our original description of congenital adrenal hyperplasia due to a deficiency of 3β-hydroxysteroid dehydrogenase (HSD),[1] one case excreted significant quantities of pregnanetriol in the urine in addition to the assortment of Δ^5-3β-hydroxysteroids characteristic of this condition (1). At the time, it was believed that this single instance might represent a double enzyme defect involving the 21-hydroxylase (21-H) as well. However, it is to be noted that a second case in this original series also excreted pregnanetriol in small quantities (1). More recent reports of further instances of HSD deficiency with the expected basic pattern of Δ^5-3β-hydroxysteroids in the urine also include patients who excreted substantial quantities of pregnanetriol (2–5). On examining the assortment of reported cases, it appears that older subjects more often excrete pregnanetriol. In our original study most patients were infants. A double enzyme defect in this increasing number of cases now seems unlikely. The matter has been reinvestigated by examining the urines of 16 subjects with 21-H and 9 with HSD deficiency with particular attention to the urinary metabolites, Δ^5-pregnenetriol, pregnanetriol, and 11-ketopregnanetriol. One of the purposes of this study was to find a criterion based on these metabolites for the clear delineation of the two types.

METHODS

Urines were obtained before the initiation of any treatment and stored at $-10°C$. The subjects and their ages are listed in Table I. Portions of 10–25 ml were sequentially hydrolyzed by mammalian glucuronidase and solvolysis as described earlier (1). After extraction, the products of each method of hydrolysis were examined separately. The extracts were partially purified before gas-liquid chromatography (GLC) as follows. They were applied in benzene to columns of neutral alumina (80–200 mesh, 4% water)

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[1] *Abbreviations used for this paper:* GLC, gas-liquid chromatography; 21-H, 21-hydroxylase; HSD, 3β-hydroxysteroid dehydrogenase; pregnanetriol, (5β-pregnane-3α,17α,20α-triol); Δ^5-pregnenetriol, (preg-5-ene-3β,17α,20α-triol); 11-ketopregnanetriol, (3α,17α,20α-trihydroxy-5β-pregnan-11-one); dehydroepiandrosterone, (3β-hydroxyandrost-5-en-17-one); 5β-androsterone, (3α-hydroxy-5β-androstan-17-one); 11-keto-5β-androsterone, (3α-hydroxy-5β-androstane-11,17-dione); 17α-hydroxypregnenolone, (3β,17α-dihydroxypregn-5-ene-20-one).

TABLE I

Urinary Steroids (mg/24 hr) in Adrenal Hyperplasia

	21-Hydroxylase deficiency						3β-HSD deficiency				
	Glucuronides			Sulfates			Glucuronides			Sulfates	
Age	Tr'one	P'triol	Δ⁵Triol	P'triol	Δ⁵Triol	Age	Tr'one	P'triol	Δ⁵Triol	P'triol	Δ⁵Triol
1 wk	1.6	6.9	0	0	0	1 wk	0	0	0	0	1.8
2 wk	0.1	0.3	0	0	0	2 wk	0	0	0	0	4.0
2 wk	2.0	0.3	0	1.5	0	3 wk	0	2.0	0	0	5.0
2 wk	0	1.8	0	0	0	7 wk	0	0	0	0	6.8
3 wk	3.0	1.0	0	0	0.4	8 wk	0	0	0	0	6.5
1 month	0.2	5.1	0	5.0	0	3 months	0	0	0	0	7.0
2 months	5.2	2.9	0	0	0.4	6 yr	0	1.7	0	0	11.0
6 months	0.5	1.8	0	0.1	0.1	9¾ yr	0	7.9	0.8	3.1	11.6
6 months	0.8	4.1	0	1.5	0	13 yr	0	19.7	3.2	1.8	23.5
14 months	1.5	1.0	0	0	0						
18 months	2.9	8.3	0	0	2.3						
2¼ yr	—	10.0	0	0	2.7						
4 yr	—	12.1	0	0	1.8						
7 yr	7.0	26.5	0	2.7	0						
9 yr	8.6	60.0	2.9	1.3	0.9						
20 yr	2.6	97.8	5.1	1.7	0.3						

Tr'one, pregnanetriolone; P'triol, pregnanetriol; Δ⁵Triol, Δ⁵-pregnenetriol; (—), indicates not measured.

1 × 10 cm and elution was carried out by gradient employing increasing concentrations of ethanol. The fraction eluted between 1–4% ethanol in benzene was employed for further studies. Prior trials revealed that authentic samples of the steroidal compounds sought were found in this region and that this eluate was satisfactory for gas chromatography.

The trimethylsilyl acetamides were prepared by treating the residues with *N,O*-bis-trimethylsilyl acetamide in pyridine. The pyridine was evaporated at 60°C under a stream of nitrogen and this residue was redissolved in benzene for application to gas-liquid chromatography.

GLC was carried out in a Barber-Coleman gas chromatograph (division of Nuclear-Chicago Corp., Des Plaines, Ill.), model 5000 with a hydrogen flame ionization detector and a chart recorder. Glass columns of 6 feet length and 3 mm I.D. packed with 100–120 mesh Gas-Chrom Q (Applied Science Labs, Inc., State College, Pa.) coated with QF-1 were employed. The temperature used was 202°C. The pressure of the carrier gas, nitrogen, was 14 psi.

Identification of the pregnanetriol and Δ⁵-pregnenetriol by GLC was according to the following criteria. The retention times of the trimethylsilyl acetamides was required to be identical with that of derivatives of authentic compounds on QF-1. Chromatography was then repeated adding small quantities of reference steroids to each urinary residue and direct superimposition was found in each instance wherein the results have been reported. A portion of each residue was removed before silylation and oxidized with periodic acid whereupon it was required that equivalent quantities (within 5%) of the expected products were identified by GLC, both as the free compound and as the acetates. The derivatives were chromatographed on OV-1 at 232°C and 22 psi nitrogen. The retention times of the derivatives differ significantly on OV-1 from those of the free steroids. The products were dehydroepiandrosterone for Δ⁵-pregnenetriol, 5β-androsterone for pregnanetriol, and 11-keto-5β-androsterone for 11-ketopregnanetriol. Correspondence was found throughout for those results shown in Table I. Quantitation of the compounds was by planimetry from the tracings of the trimethylsilyl acetamides. Recoveries of authentic samples of pregnanetriol and pregnenetriol extracted from water and run through the entire procedure 10 times was 87 ±5%.

Two adult normal male volunteers were given 50–60 mg of 17α-hydroxypregnenolone (1 mg/kg body weight) orally. Two 24-hr urine collections were obtained before and three after the administration of the steroid.

RESULTS

The findings in subjects with congenital adrenal hyperplasia are shown in Table I and Fig. 1.[2] The excretory products found in the two normal human volunteers studied are indicated in Fig. 2.

Although most of the pregnanetriol and 11-ketopregnanetriol were present in the fraction released by glucuronidase and the Δ⁵-pregnenetriol in that released after solvolysis, there was overlap in some instances. Pregnanetriol and 11-ketopregnanetriol predominate in 21-H deficiency in all instances although occasionally respectable but considerably smaller amounts of Δ⁵-pregnanetriol were found. In HSD deficiency pregnanetriol was found, at times in large quantity, usually in the older subjects. Nonetheless when the results are expressed as ratios of pregnenetriol/pregnanetriol as shown in Fig. 1 there is no overlap between these two forms of the disorder and it is possible to distinguish them on this basis. The ratio is well below 1.0 in 21-H deficiency and greater than 1.0 in HSD deficiency. Furthermore although 11-ketopregnanetriol was usually

[2] Normal values for pregnanetriol in this laboratory are: birth-5 yr, generally absent; 5 yr-puberty, 0.1–1.0 mg per day; adults, 0.8–3.1 mg per day. Normal values for Δ⁵-pregnenetriol: birth-3 months, 0.2–0.8 mg per day; 4 months-puberty, 0.0–0.2 mg per day; adults, 0.1–0.4 mg per day.

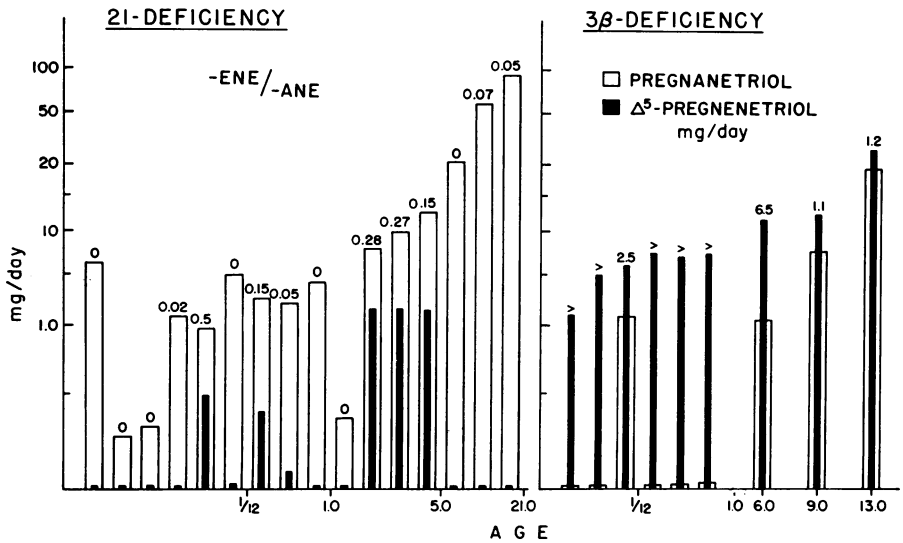

FIGURE 1 Illustration of the relative quantities of pregnanetriol and Δ^5-pregnenetriol in the urine of subjects with 21-hydroxylase deficiency (21-deficiency) and 3β-hydroxysteroid deficiency (3β-deficiency). The ratio of the quantity of Δ^5-pregnenetriol/pregnanetriol is shown at the top of each column. The symbol O indicates that virtually no Δ^5-pregnenetriol was detected. The symbol > indicates that virtually no pregnanetriol was present. The age is shown in years.

present in 21-H deficiency it was at no time detectable in HSD deficiency. The two normal volunteers eliminated most of the 17α-hydroxypregnenolone as pregnanetriol with considerably smaller amounts of Δ^5-pregnenetriol.

DISCUSSION

It is apparent that pregnanetriol may be found in the urine of subjects with congenital adrenal hyperplasia due to HSD deficiency and its detection or moderate elevation therefore does not mitigate against this diagnosis.

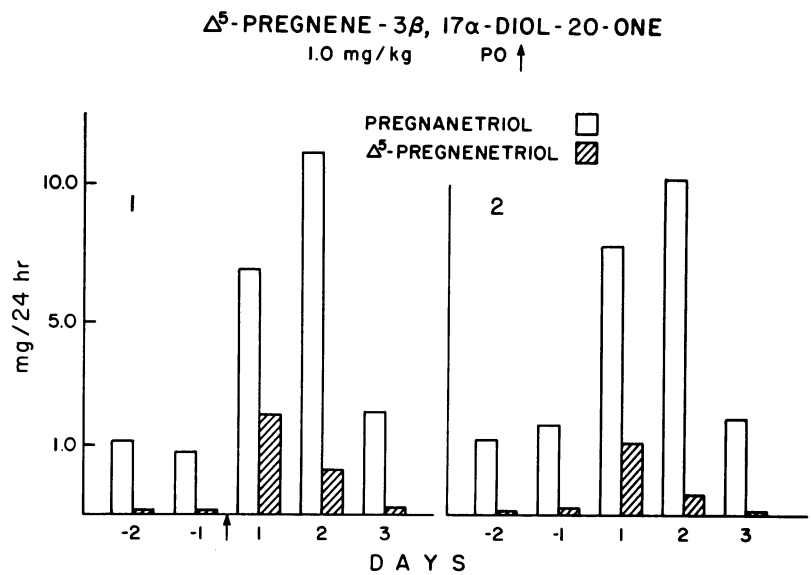

FIGURE 2 The excretion of pregnanetriol and Δ^5-pregnenetriol before and after the oral administration of Δ^5-pregnene-3β,17α-diol-20-one (3β,17α-dihydroxy-pregn-5-20-one). The designations -1 and -2 represent two control specimens before the administration and the subsequent values are for each of 3 consecutive days beginning at the time of the administration of the steroid.

The measurement of pregnanetriol and the 17-ketosteroids in the urine are not reliable in themselves for distinguishing between HSD and 21-H deficiency. It appears that the presence of pregnanetriol is more common in the older subjects with HSD deficiency and less usual in infancy. However a measurement of both pregnanetriol and Δ^5-pregnenetriol will distinguish between these two types of defective steroidogenesis. In addition, the finding of increased quantities of various other Δ^5-3β-hydroxy-steroids characteristic of HSD deficiency as detailed earlier (1) provides further substantiation. But the measurement of these two compounds and their relative quantities should provide a useful screening procedure. In addition our results indicate that 11-ketopregnanetriol is to be found only in 21-H deficiency.

It is probable that pregnanetriol arises from peripheral extra-adrenal conversion of Δ^5-3β-hydroxysteroids in HSD deficiency. A likely precursor for such a transaction is 17α-hydroxypregnenolone. In two normal subjects the administration of this compound led to the excretion of more pregnanetriol than Δ^5-pregnenetriol and it is likely that the conversion occurred in extra-adrenal sites. Fukushima, Bradlow, Hellman, and Gallagher (6, 7) provided evidence that urinary pregnanetriol arose from 17α-hydroxypregnenolone in a subject with metastatic adrenal carcinoma, and further showed that a normal subject excreted substantial quantities of pregnanetriol which was derived from the administration of isotopically labeled 17a-hydroxypregnenolone. Roberts, Vande Wiele, and Lieberman (8) have provided similar evidence in a patient with an adrenal adenoma. Fotherby and Love (9) have also noted the conversion of administered nonisotopic 17α-hydroxypregnenolone to urinary pregnanetriol in five human subjects. Our results reveal an even greater conversion to pregnanetriol than was reported in these other studies. We administered the precursor orally whereas the others did so intravenously. This suggests that the liver may be the extra-adrenal site of conversion perhaps through hepatic 3β-hydroxysteroid dehydrogenase which may be under genetic control different from that of the adrenals. These extra-adrenal enzymes may mature slowly after birth so that the observation of elevated urinary pregnanetriol in HSD deficiency is to be more commonly found after infancy.

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