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# DETERMINATION OF URINARY 17-HYDROXYPREGNANOLONE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

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SUMMARY

The excretion of pregnantriol, pregnantriolone and 17-hydroxypregnanolone of eight male and twelve female children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency has been studied by gas chromatography on open tubular glass capillary columns. Individual steroids were identified by mass spectrometry. The results indicate that beside pregnantriol and pregnantriolone, being well established characteristics of this disease, urinary 17-hydroxypregnanolone is of comparable diagnostic value.

The adrenogenital syndrome due to defective 21-hydroxylase-activity is characterised by greatly elevated secretion rates of 17-hydroxyprogesterone (17-hydroxy-4-pregnene-3,20-dione). This precursor of cortisol is metabolised via 17-hydroxypregnanolone (3α,17-dihydroxy-5β-pregnen-20-one) into pregnantriol (5β-pregnane-3α,17α,20α triol). If 17-hydroxyprogesterone is hydroxylated in 11β-position 21-Deoxycortisol (11β,17-dihydroxy-4-pregnene-3,20-dione) is formed which gives rise to elevated levels of urinary pregnantriolone (3α,17,20α-trihydroxy-5β-pregnen-11-one). The presence of 17-hydroxypregnanolone in the urine of patients with congenital adrenal hyperplasia was first reported by Lieberman *et al.* [1, 2] and later confirmed by Fukushima and Gallagher [3]. In a gas chromatography study Kecskes *et al.* observed 3α,17α-dihydroxy-17β-methyl-D-homoetiocholanolone, which originated from 17-hydroxypregnanolone after acetylation and was only detectable in the urine of patients suffering from the disease under discussion [4]. We investigated the urinary excretion of 17-hydroxypregnanolone and took advantage of the gas chromatography and mass spectrometry techniques recently described by Völlmin *et al.* [5] and Hornig *et al.* [6]. These procedures allow qualitative and quantitative determination of individual urinary steroids in one single chromatogram without structural alterations (see Fig. 1).

After hydrolysis with β-glucuronidase/arylsulphatase (*Helix pomatia*, Boehringer, Mannheim, Germany) for 24 h (Ph 5, 40°C) analytical samples of human urinary steroids (24 h collections) were converted into methoxy trimethylsilyl (MO-TMS) derivatives by a modified version of the method described by Horning *et al.* [6]. Gas chromatograms were run on a Carlo Erba (Milan, Italy) gas chromatograph model Fractovap 2101 equipped with 30 m glass capillary columns coated with OV 101 (H. Jaeggi, Trogen, Switzerland). Mass spectral data were obtained using a Varian MAT-311 system (Varian MAT, Bremen, Germany). Quantification of steroids occurred by relating their peak heights to the height of 5-androstane-3β-17β diol which served as internal standard. The recovery was proved to be constant by subsequent injection of reference steroid mixtures. Mass spectra (70 eV) of the steroids separated from pathological urine by g.l.c. on open tubular glass capillaries directly coupled to the mass spectrometer were compared with those obtained from authentic steroids. Major fragments of the third metabolite beside pregnantriol and pregnantriolone, which was found to be characteristically elevated in urines of patients with CAH are identical with those recorded from 17α-hydroxypregnanolone-MO-TMS ($m/e$ = 435, 404, 396, 314, 296).

The data listed in Table 1 show that pregnantriol and its precursor 17-hydroxypregnanolone are excreted in comparable amounts and predominate the pregnantriolone production.

Pregnantriol arises not only from 17-hydroxyprogesterone but also from 17-hydroxypregnenolone without the intervention of 17-hydroxyprogesterone [7]. Thus 17-hydroxypregnanolone and not pregnantriol should be regarded as the exclusive metabolite of 17-hydroxyprogesterone, whose elevated serum levels are evidence for a failure of 21-hydroxylation.

* Clinical application of gas chromatography on open tubular glass capillaries. Part I. Supported by Deutsche Forschungsgemeinschaft, SFB 51, C 10.

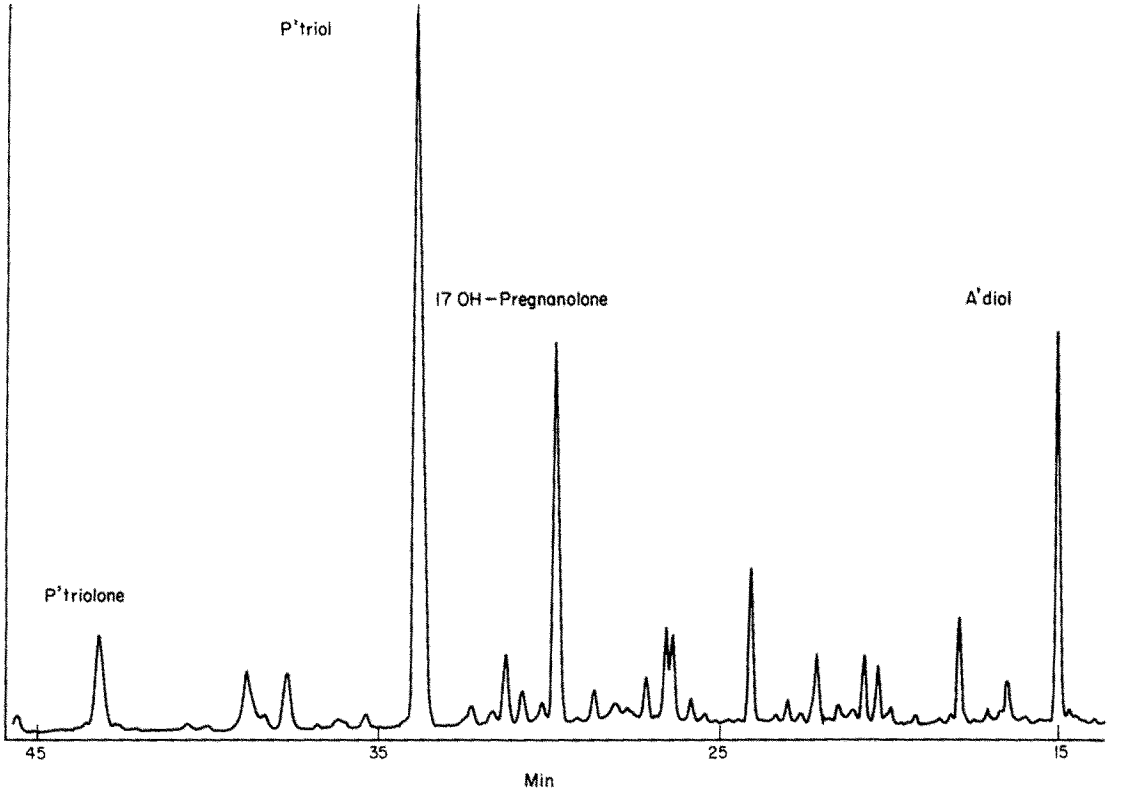


Fig. 1. Typical urinary steroid profile of an untreated girl (18 yrs) with congenital adrenal hyperplasia due to an 21-hydroxylase deficiency. Experimental data: carrier gas: hydrogen; splitless sample injection. Temperature program: 80°C for 2 min; 200°C for 12 min, 1°C/min up to 240°C. The coefficients of variation were found to be 13% (17-hydroxypregnanolone), 10% (pregnantriol) and 14.5% (pregnantriolone). (PregnanTRIOL, PregnanTRIOLONE, 17-OH PREGNANOLONE, and AndrostanDIOL, which served as internal standard).

Table 1. Daily excretion rates of three urinary steroids which are of diagnostic significance for 21-hydroxylase deficiency. Urines are taken from patients before treatment

| Patient | yrs./sex | 17-OH-pregnanolone mg/d | Pregnantriol mg/d | Pregnantriolone mg/d |
|---|---|---|---|---|
| A.S. | 7/f | 28.0 | 42.0 | 6.8 |
| H.O. | 8/f | 3.5 | 16.2 | 7.1 |
| H.C. | 9/f | 10.8 | 11.2 | 3.6 |
| Z.B. | 10/f | 31.2 | 24.0 | 6.4 |
| K.R. | 12/f | 18.0 | 25.0 | 3.7 |
| V.A. | 16/f | 28.0 | 56.0 | 6.4 |
| S.F. | 16/f | 6.4 | 10.2 | 0.6 |
| A.S. | 18/f | 16.0 | 22.0 | 5.8 |
| E.Z. | 18/f | 10.5 | 9.2 | 1.3 |
| K.S. | 18/f | 44.0 | 56.0 | 8.0 |
| K.M. | 19/f | 17.5 | 29.4 | 7.7 |
| I.R. | 20/f | 14.6 | 17.1 | 3.9 |
| R.H.-J. | 6/m | 15.2 | 19.1 | 6.5 |
| F.R. | 10/m | 5.4 | 5.7 | 2.6 |
| M.L. | 10/m | 5.3 | 10.5 | 3.0 |
| W.E. | 11/m | 4.8 | 7.5 | 1.5 |
| S.S | 12/m | 6.8 | 14.5 | 4.7 |
| W.E. | 12/m | 15.0 | 14.6 | 4.6 |
| M.A. | 13/m | 26.8 | 36.0 | 16.4 |
| W.F. | 14/m | 4.0 | 3.0 | 2.6 |

In contrast to 17-hydroxypregnanolone levels in blood the urinary amounts of 17-hydroxypregnanolone, pregnantriol and pregnantriolone do not underlie circadian rhythms and are rapidly obtained in one single chromatogram. Therefore the GC-method described here seems to be most convenient in management of CAH.

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