Preliminary Notes

PN 1203

Studies in steroid metabolism
XV. The rapid determination of urinary pregnanediol by gas chromatography*

Recent reports from this laboratory have shown the applicability of gas-chromatographic separation and detection systems to the determination of the classic estrogens in crude urine extracts[1]. The separation of certain progesterone metabolites on a gas-chromatographic column has been reported[2,3], but no utilization of this technique for the analysis of such compounds in biological material is as yet described.

It has been possible to devise a rapid method for the determination of pregnanediol in crude urine extracts using essentially the principles of analysis described before[1].

Urine aliquots (50 ml) were refluxed with 10 vol % of HCl and toluene, as described by KLOPPER[4], for 10 min. After cooling and extraction with more toluene, the organic layers were washed with alkali and water. The toluene layer was rapidly evaporated *in vacuo* in a rotating still. The residue was acetylated as outlined by KLOPPER[4] using acetyl chloride and benzene for 1 h. After washing with $NaHCO_3$ and water the residue was evaporated and dissolved in 100 μl of acetone.

An 0.5-μl aliquot was injected into a gas-chromatograph equipped with a flame ionization detector. The 6 ft, 1/4 in. column was packed with 3 % SE-30 on 80–100

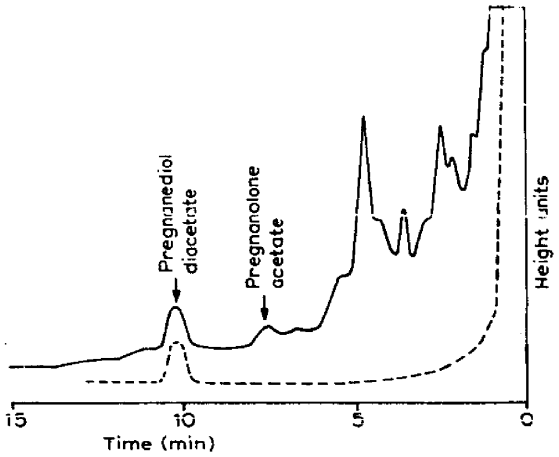

Fig. 1. GLP – Chromatogram of a 1/2800 aliquot of urine from a female in the proliferative phase of the cycle. Total pregnanediol excretion 430 μg/24 h. Column, 6 ft, 1/4 in.; absorbancy, 3 % SE-30 on 80–100 mesh Anakrom ABS; temp., 245°; pressure, 30 lb/in.^{2}.

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mesh diatomaceous earth (Anakrom AB85). The detector was operated under 12 lb of hydrogen pressure and 8 lb of compressed air.

Fig. 1 shows a typical chromatogram on a urine extract from a female in the proliferative phase. The area under the peak corresponding exactly to that of pure pregnane-3α,20α-diol diacetate was trapped in liquid nitrogen and infrared comparison of this material with authentic pregnanediol diacetate showed no significant differences. The large peak appearing at about 5 min was tentatively identified as a mixture of androsterone and etiocholanolone acetates.

TABLE I

EXCRETION OF PREGNANEDIOL DURING PREGNANCY AS MEASURED BY GLPC

Patient	*Month*	*Pregnanediol (mg/24 h)*
R.C.	7½	32
H.J.	7½	24
R.C.	8	41
R.C.	8¼	37
S.F.	8¼	29
S.F.	8½	38
A.C.	8½	19
A.C.	8¾	26
M.Z.	9	36
A.R.	9	43

Six aliquots each of three urine specimens were analyzed showing a reproducibility of ± 6 %. The lower limit of sensitivity at the 25 % levels was found to be 50 μg/24 h of urine, accepting a peak of 3 times the height of the background as the minimum readable concentration. Recovery of steroids added to male urine was found to be ± 2.4 % at high levels (5000 μg/24 h) and ± 6 % at low levels (50 μg/24 h).

In Table I are shown some data obtained on eighth and ninth months pregnancy urines.

The values obtained are well within the limits described by other accepted methods. Further studies, particularly on pregnanediol excretion during normal menstrual cycles will shortly be published in detail.

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