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IDENTIFICATION OF 5α-ANDROSTANE-3β,17β-DIOL IN HUMAN URINE

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SUMMARY

An assay for the identification and measurement of 5α-androstane-3β,17β diol in human urine is described. Identity of this testosterone metabolite in urine has been proved by gas–liquid chromatography (GLC), GLC-mass spectrometry (GLC-MS) and crystallization to constant specific activity. Epimers ratio (3α/3β) was found to be 5·1 in male urines and 3·1 in female urines. The physiological importance of this testosterone metabolite is discussed.

INTRODUCTION

A 3β-HYDROXYSTEROID dehydrogenase activity has been characterized in androgen human and animal target tissues. *In vitro* testosterone transformation to 5α-androstane-3β,17β-diol was shown in human benign prostatic hypertophy tissues homogenates by Chamberlain *et al.*[1] and in rat prostate tissue culture by Baulieu *et al.*[2]. Morfin *et al.*[3, 4] have produced evidence for *in vivo* 5α-androstane-3β,17β-diol formation by human and canine prostates after infusion of labelled testosterone in the arterial blood supply of the glands.

Baulieu *et al.*[5, 6] studied the effects of this testosterone metabolite on rat prostate: tissue culture experiments showed this steroid to maintain growth and secretion in epithelial cells. Physiological importance of this dihydroxysteroid was then underlined by Baulieu[7] and Ofner[8].

5α-andostane-3β,17β-diol has been characterized as a disulfate conjugate in plasma[9] and both as a sulfate conjugate[10] and unconjugated[11] in human faeces. This dihydroxysteroid has not been yet identified in human urines.

Actual development of gas–liquid chromatography (GLC) techniques allowed us to investigate the presence of 5α-androstane-3β,17β-diol in human urines. This report presents some estimations of yields, proofs of identity by crystallisation to constant specific activity and GLC-mass spectrometry, and discusses the physiological importance of this testosterone metabolite.

EXPERIMENTAL

Materials

Solvents and reagents were purified before use as previously described[13] and the solvents purity checked by GLC.

Tritiated acetic anhydride (100 mCi/mmol) was purchased from CEA (France); propanediol from Merck; 3-chloroperbenzoic acid from Fluka; N,O-bis silyl trifluoroacetamide (BSTFA) from Regis Chemical Co, and 5α-androstane-3β,17β-diacetate from the Sigma Chemical Co.

Pyridine (Merck) was distilled over baryum oxide before use.

Fluid for liquid scintillation consisted in PPO (2,5 diphenyl-oxazol) (Merck) 4·5 g, POPOP (1,4-bis[2-(5 phenyloxazolyl)]benzene) (Merck) 0·055 g/l toluene.

5α-androstane-3β,17β-diol-[4-^{14}C] was synthetized by potassium borohydride reduction of 5α-dihydrotestosterone obtained by reduction of testosterone [4-^{14}C](9,3 mCi/mmol from NEN) according to Birch[12].

Methods

Extraction and purification procedures of urinary steroids[13] were previously described. The modified technique presented in Fig. 1 includes epoxidation reaction and paper chromatography which were added to increase specificity of the previous method.

Epoxidation. After column chromatography on alumina the urinary extract was dryed and weighed and dissolved in anhydrous benzene. 3-chloroperbenzoic acid was added to the solution in the ratio of one mg per mg of the dried extract and the reaction mixture left overnight at room temperature. Anhydrous ether (10 ml) was added after completion of the reaction and the mixture washed in turns with N sodium hydroxide (1 ml) and water (1 ml × 2). Excess water was removed by filtration through sodium sulfate and the dried filtrate spotted on Whatmann paper.

Paper chromatography. Whatmann paper number 1, washed in purified methanol then in anhydrous benzene for each 24 h, was dried at 60°C. Paper damping with propanediol was done as described by Baulieu and Jayle[14]. Spotting on the damped paper of the urinary extracts in methanol (100 μl × 3) was done under a stream of cold air. Chromatography was started for 34 h at 19 ± 1°C in the system cyclohexane–benzene (1 : 1, v/v) after equilibration for 6 h. Radioactive tracers were located on the chromatograms with a Packard Scanner model 7201 to direct elutions with chloroform–methanol (1 : 1, v/v) mixture.

Thin layer chromatography. Precoated silica-gel F_{254} thin layer plates (Merck) were utilized with a methylene chloride-ethyl acetate (8:2, v/v) solvents system. Details on the technique were given previously[13].

Gas–liquid chromatography. GLC was done on a Pye 104-84 gas-chromatograph equipped with a flame ionization detector. Two types of column packing were utilized with the following settings:

Hi-Eff 8 BP (or CHDMS) 1% stationary phase on Gas Chrom Q 100–120 mesh was packed in a 1·4 m × 4 mm i.d. silanized glass column. Silanization was

Urine 24 h
  Enzymatic hydrolysis (H. pomatia)
Ethyl ether extraction
  NaOH N washing → phenolic steroids
Neutral steroids
  Girard's T reagent → ketone steroids
Alcoholic steroids
  Adsorption chromatography on alumina
C_{19} and C_{21} dihydroxysteroids
  Epoxidation
  Paper chromatography → unsaturated steroids
Thin layer chromatography on Silicagel F_{254}

Measurement and identification of
5α-androstane-3β,17β-diol by GLC, GLC-MS
and crystallization to constant specific activity.

Fig. 1. General purification procedure.

by 5% dimethyldichlorosilane in toluene. Filtration technique[15] was followed for coating. Temperature settings were 196°C for the column and 220°C for the detector. Carrier gas flow was 30 ml/min at 1·8 bar.

OV-1 0·2% stationary phase on Corning glass beads (Corning GLC 110) was packed in a 1·5 m × 4 mm i.d. glass column. The filtration technique[16] was utilized for coating. Carrier gas flow was 20 ml/min at 1·8 bar and column temperature set at 214°C.

Steroids contained in the purified urinary extracts were injected on the OV-1 stationary phase either as free compounds or as acetate or trimethylsilyl ether (TMSi) derivatives. TMSi derivatives were only injected during the Hi Eff 8 BP stationary phase.

Solid injection was automated by the Pye Auto solid injector.

Retention times and peak areas were measured by an electronic integrator (Infotronic CRS 104) coupled with the chromatograph electrometer.

Trimethylsilyl ethers derivatives. TMSi derivatives were prepared according to Luukainen *et al.*[17] and injected as carbon disulfide solutions. Solid injections with capillary glass tubes required a different procedure: an aliquot part of the purified urinary extract was injected at the bottom of a capillary glass tube and placed for 18 h at 50°C in a 250 ml vial containing P_2O_5 and saturated by N,O-bis silyl trifluoroacetamide vapours. This technique proved to be satisfactory.

Acetate derivatives. The sample to be acetylated was dried in calcium chloride for 24 h and dissolved in 40 μl distilled pyridine. 20 μl acetic anhydride (20% in benzene) were added and the mixture was left in the dark for 24 h. The reaction was stopped by addition of 0·5 ml water containing 25% methanol. The reaction products were extracted in chloroform (3 ml) and ethanol was added to the extract before drying at 40°C with a stream of nitrogen.

Double isotopic dilution and crystallization to constant specific activity. Urinary extract containing the 5α-androstane-3β,17β-diol and pure 5α-androstane-3β,17β-diol[4-^{14}C] was dried and labelled by acetylation with tritiated acetic anhydride.

The extracted reaction mixture was chromatographed on a silica-gel F_{254} thin layer plate and developed twice in n-hexane/ethyl acetate (5 : 1 v/v). The radioactive zone corresponding to 5α-androstane-3β,17β-diol diacetate was located by scanning of the plate and eluted from the gel in 4 × 2 ml chloroform/methanol (2 : 1, v/v) mixture.

An aliquot was saved for radioactivity determinations and non-radioactive 5α-androstane-3β,17β-diol diacetate carrier was added to the remaining portion. The carrier was then crystallized and specific activity determined in crystals and mother liquors fractions. Specific activity determinations were made according to Morfin[18]: weight measurements were obtained by determination of peaks heights after injection of an aliquot in the gas chromatograph.

Radioactivity was determined by counting an aliquot in a Packard Tricarb 3375 liquid scintillation spectrometer; sufficient counts were accumulated to give counting errors of less than 0·5% at the 99% confidence level.

Constant specific activity was considered to be reached when two consecutive crystal fractions and the last mother liquor fraction presented the same specific activity within 5% and the same $^3H/^{14}C$ ratio.

Gas–liquid chromatography-mass spectrometry. Tests were done an AEI-MS12-mass spectrometer coupled with a F & M Scientific 802 gas chromato-

graph. The mass spectrometer source was set at 250°C and the ionizing current was 100 μA. The electron energy was 70 eV. The gas chromatograph was equipped with a glass column (1·80 m × 2 mm i.d.) packed with 1% OV-7 on Gas Chrom Q 100–120 mesh. Coupling with the mass spectrometer was by a Biemann-Watson type molecular separator. Oven and molecular separator temperatures were set at 210°C, injector's was 220°C and introduction line's at 250°C.

TMSi derivatives of urinary extracts were injected and resolution followed on the monitogram. Mass spectra were taken when maximal peaks height was reached.

RESULTS

GLC analysis. Human male and female urinary extracts were injected on OV-1 and Hi-Eff-8 BP stationary phases as free compounds, acetates or TMSi derivatives. In all cases (Fig. 2) two main peaks were observed.

Preliminary identification of peak A was based upon its relative retention time with respect to 5α-cholestane and comparison with those of authentic reference steroids (Table 1). In all cases, peak A presented relative retention times identical to those of authentic 5α-androstane-3β,17β-diol within the limits of standard error.

Double isotopic dilution and crystallization to constant specific activity. Two urinary extracts containing known amounts of 5α-androstane-3β,17β-diol-4-[^{14}C] were separately acetylated with tritiated acetic anhydride and the doubly labelled diacetate isolated by thin layer chromatography (see methods). Pure 5α-androstane-3β,17β-diol diacetate carrier was added to each eluate and the mixture

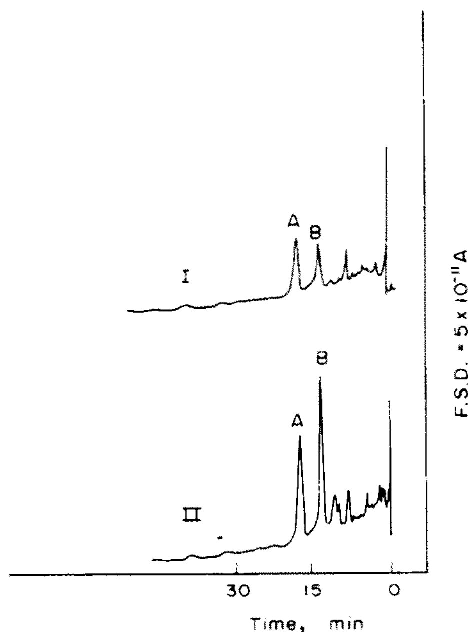

Fig. 2. Gas chromatogram of purified urinary extracts. Chromatograms I and II were obtained respectively from female and male urinary extracts. Peak A retention time coincides with this of standard 5α-adrostane-3β, 17β-di-TMSi. Peak B has not yet identified. Chromatograms were obtained on an Hi-Eff 8 BP column utilizing the automatic solids injector.

Table 1. Relative retention time of reference steroids on two stationary phases

Steroids	1% Hi-Eff 8 BP* TMSi at 196°C	0·2% OV-1 at 214°C† free	diacetate	TMSi
5α-androstane-				
-3α,17α-diol	0·208±0·002	0·390	0·705	0·362
-3α,17β-diol	0·245±0·003	0·396	0·780	0·477
-3β,17β-diol	0·404±0·004	0·402	0·793	0·483
-3β,17α-diol	0·316±0·003	0·400	0·780	0·407
5β-androstane-				
-3α,17α-diol	0·216±0·002	0·370	0·632	0·326
-3α,17β-diol	0·340±0·003	0·374	0·770	0·490
-3β,17β-diol	0·250±0·003	0·340	0·732	0·458
-3β,17α-diol	0·183±0·002	0·337	0·650	0·350
5-androstene-				
-3β,17β-diol	0·415±0·004	0·378	0·925	0·525
-3β,17α-diol	0·335±0·004	0·376	0·783	0·515
peak A	0·404±0·005	0·406	0·795‡	0·482
peak B	0·308±0·003			0·435

Relative retention times were calculated on the basis of 5α-cholestane retention time (1·00).

*In these conditions 5α-cholestane retention time was 60 min. Solid injections were done with automatic reseting of the integrator coupled with the gas chromatograph.

†In these conditions 5α-cholestane retention time was 24 min.

‡Peaks A and B are not separated (see Fig. 4).

crystallized to constant specific activity. Results of both diacetates derivatives crystallizations are reported in Fig. 3.

In each case, constancy was reached at the third crystallization. The drop in the specific activity of original mixtures may be due to incomplete separation of radioactive contaminants on the thin layer plate. This double isotopic dilution technique led to the calculation of the amount of acetylated 5α-androstane-3β,17β-diol. Yields obtained in these two cases are reported in Table 2 and compared with those obtained by GLC measurements. Good agreement of these results is a further proof in urinary 5α-androstane-3β,17β-diol identification by both methods. The 5α-androstane-3α,17β-diol/5α-androstane-3β,17β-diol ratios were respectively 5·1 and 3·1 for males and females (Table 2).

Mass spectrometry. TMSi derivatives of urinary extracts were analyzed on an OV-7 column. Mass spectrum was taken at the tops of peaks. This mass spectrum presented identical fragmentation pattern with these of 5α-androstane-3β,17β and 3β,17α-diol TMSi derivatives (Table 3).

In each case the molecular peak was at m/e = 436 and the base peak at m/e = 129 corresponding with the fragment $(CH_3)_3$-Si-O=CH-CH=CH_2[19]. Fragments providing structural information are m/e = 421 (M-CH_3), 346 (M-90), 331 (M-90-15), 319 (M-90-27), 256 (M-90-90), 241 (M-90-90-15), 217 (M-90-129), 215 (M-90-90-41), 305 (M-90-41). Other important peaks are at m/e = 73 and 75 corresponding respectively with $(CH_3)_3$-Si^+ and $(CH_3)_2$-Si^+=OH fragments. Loss of two trimethylsilanol fragments of mass 90 confirm the presence of two hydroxyls in peak A compound. The principal cleavage in ring D involves cleavage of the C-13-C-17 bond and either C-15-C_{16} (M-90-27) corresponding with the fragment m/e = 319 or C-14-C_{15} (M-90-41) corres-

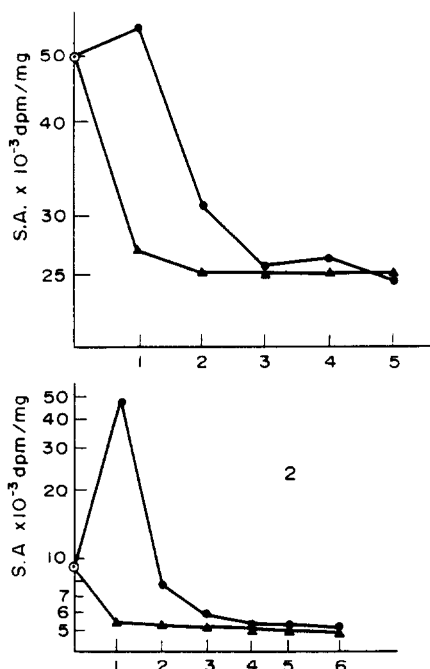

Fig. 3. Two cases of crystallization to constant specific activity of doubly labelled ($^3H/^{14}C$) 5α-androstane-3β,17β-diacetate. 5α-androstane-3β,17β-diol was isolated and purified from subjects 1 and 2 prior to acetylation. Solvents systems used for the crystallisations were 1: acetone/water; 2: methanol/water; 3: acetone/pentane; 4: methanol/water; 5: ether/pentane; 6: acetone/water. The symbols denote: ▲ crystals; ● mother liquors; ⊙ calculated specific activity.

Table 2. Excretion rates of 5α-androstane-3β,17β-diol and 5α-androstane-3α,17β-diol. Quantities are expressed as μg/24 h.

		5α-androstane-3β,17β-diol				
Subject	Sex	GLC 1% Hi Eff 8 BP	GLC 0·2% OV-1	Double isotopic dilution	5α-androstane-3α,17β-diol*	3α/3β ratio
1	female	14·2	14	14·2 ± 3	49	3·47
2	male	40·7	43·6	50·8 ± 8	248	5·89
3	female	20·7	19·5	–	56	2·78
4	male	39·5	40·2	–	216	5·42
5	male	34	36·5	–	140	3·97

*These values were obtained by a previously described method[13].

ponding with the fragment m/e = 305[20–21–22]. The peak m/e = 149 corresponds with the androstane skelet fragmentation[19].

The silyl derivatives of 5α-androstane-3,17-diol isomers present very small differences (see Table 3) in relative intensities of important fragments in mass spectrometry[23, 24]. Consequently these epimers have to be sufficiently separated by GLC to facilitate their respective identification. Retention index (25) on OV-7 stationary phase are presented in Table 4 for TMSi derivatives of

Table 3. Relative intensities of 5α-androstane-di-TMSi isomers fragments in mass spectrometry

m/e	5α-A 3β,16β	5α-A 3β,16α	5α-A 3β,17β	5α-A 3β,17α	Peak A	Fragments
436	13	15	21	30	13	M
421	51	52	33	47	21	M-15 (CH_3)
346	22	22	30	39	20	M-90[(CH_3)-Si-OH]
331	13	16	16	21	13	M-90-15
319	–	–	5	7	4	M-90-27 (ring D)
305	9	12	6	6	4	M-90-41 (ring D)
256	28	39	20	26	19	M-(2 × 90)
241	16	15	31	35	22	M-(2 × 90)-15
217	16	17	19	21	17	M-90-129
215	14	13	25	26	18	M-(2 × 90)-41
149	8	8	28	32	29	
148	7	7	21	25	20	
144	55	59	–	–	–	
129	11	12	100	100	100	$(CH_3)_3$-Si-$\overset{+}{O}$=CH-CH=CH_2
107	25	23	32	26	31	
95	20	20	23	20	31	
93	24	23	26	21	25	
75	100	100	92	78	98	$(CH_3)_2$-Si=$\overset{+}{O}$-H
73	54	64	94	65	98	$(CH_3)_3$-Si^+

androstanediols epimers. Among those, 5α-androstane-3β,16β- and 3β,16α-diols and 5-androstene-3β,17β-diol and 4-androstene-3β,17β-diol presented retention index close to that of 5α-androstane-3β,17β-diol. Epoxidation eliminated 4-ene and 5-ene steroids; furthermore, if any remained, mass spectrometry would show a molecular peak at m/e = 434. It is shown in Table 3 that on mass spectrometry 5α-androstane-3β,16β and 3β,16α-diols present fragmentation patterns different from those obtained both with 5α-androstane-3β,17β-diol and peak A compound.

DISCUSSION

The identification of urinary 5α-androstane-3β,17β-diol is based upon GLC analysis, crystallization to constant specific activity and combined GLC-MS. TMSi derivatives of the urinary extract were chosen because the free compounds and the diacetate derivatives gave on GLC analysis one peak which was not homogeneous (Fig. 4) TMSi derivatives injected either on a polar or a non polar stationary phase gave similar results, thus suggesting peak A to be homogeneous. In these cases, columns with at least 2500 theorical plates were necessary to obtain a resolution of peaks A and B about 98%.

Further identification proofs were achieved by crystallization to constant specific activity of the tritiated diacetate derivative and mass spectrometry of urinary extract after TMSi derivative formation. Purity of the material subjected to derivative formation and identification tests was based upon paper and thin-layer chromatographies. Chromatography of the epoxidation reaction product on propanediol damped paper in the system cyclohexane–benzene (1 : 1 v/v) allowed separation of polar epoxides and of many dihydroxysteroids. Data obtained are presented in Table 4 and are in good agreement with these published elsewhere [26].

Thin-layer chromatography of the eluted zone corresponding to 5α-androst-

Table 4. Separation of androstane and pregnane-diols isomers on thin-layer, paper and gas–liquid chromatographies

Steroids	Thin-layer chromatography on silica-gel F_{254}, R_f^*	Paper chromatography	Retention index on OV-7 2·8% at 220°C
5α-androstane-3α,17α-diol	0·44	2·10	2510
5α-androstane-3α,17β-diol	0·90	1·65	2580
5α-androstane-3β,17β-diol	1·00	1·00	2670
5α-androstane-3β,17α-diol	0·84	1·26	2621
5α-androstane-3β,16β-diol	1·00	–	2650
5α-androstane-3β,16α-diol	0·69	–	2650
5β-androstane-3α,17α-diol	0·23	1·40	2480
5β-androstane-3α,17β-diol	0·48	1·08	2580
5β-androstane-3β,17β-diol	1·12	1·40	2562
5β-androstane-3β,17α-diol	0·76	1·87	2565
5-androstene-3β,17β-diol	1·02	0·90	2665
5-androstene-3β,17α-diol	0·92	0·75	2620
4-androstene-3β,17β-diol	1·10	1·20	2660
4-androstene-3β,17α-diol	0·93	1·44	2610
4-androstene-3α,17β-diol	0·62	1·00	2587
4-androstene-3α,17α-diol	0·29	1·10	2515
5α-pregnane-3α,20α-diol	0·89	1·40	–
5α-pregnane-3α,20β-diol	1·03	–	–
5α-pregnane-3β,20β-diol	1·02	–	–
5α-pregnane-3β,20α-diol	1·02	–	–
5β-pregnane-3α,20α-diol	0·50	1·65	–
5β-pregnane-3α,20β-diol	0·64	–	–
5β-pregnane-3β,20β-diol	1·14	–	–
5β-pregnane-3β,20α-diol	1·10	1·80	–
5-pregnene-3β,20β-diol	1·06	–	–
5-pregnene-3β,20α-diol	1·00	–	–
4-pregnene-3β,20β-diol	1·17	–	–
epoxides	0·10 ± 0·05	–	–
Peak B	–	–	2595
Peak A	–	–	2670

*Androstane and pregnane-diols R_f were calculated on the basis of 5α-androstane-3β,17β-diol migration (R_f = 1·00).

Paper chromatography was in the system propanediol-cyclohexane/benzene 1 : 1 v/v. Thin-layer chromatography was on Silica-gel F_{254} in the system methylene-dichloride/ethyl acetate 8 : 2 v/v, two migrations.

Retention data in GLC are retention index according to Kováts. A 7 feet × 4 mm i.d. glass column packed with 2·8% OV-7 on Gas Chrom Q 100-120 mesh was used; oven temperature was 220°C and carrier gas flow was 30 ml/min at 1·8 bar.

ane-3β,17β-diol allowed separation of many other isomers like the 5β-androstane-3α,17β-diol which is found in urines[13, 27]. Epoxidation reaction of the crude extract was necessary for 5-androstene-3β,17β-diol elimination. This unsaturated dihydroxysteroid interfers with 5α-androstane-3β,17β-diol in many chromatographic systems (see Tables 1 and 4) and is present in relatively large quantities in urines[28, 29]. Epoxidation of 5-androstene-3β,17β and 3β,17α-diols yields a reaction product containing 60% of the 5α-6α-epoxide and 40% of the 5β-6β-epoxide[30]. Infrared spectra of reaction products agrees with these structures. Yields of epoxidations were controlled in each case by calculating the recovery of added [7α-^{3}H]-5-androstene-3β,17β-diol. This labelled marker was obtained by

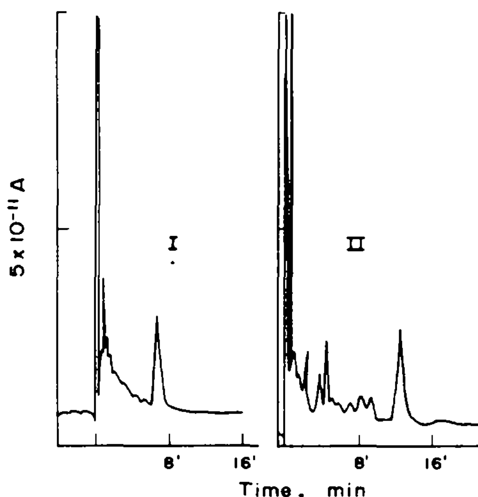

Fig. 4. Gas–liquid chromatogram of purified urinary extracts from normal human males. Acetylated (I) and free (II) extracts were injected on a 5 ft × 4 mm i.d. glass column containing glass beads Corning GLC-110 coated with 0·2% OV-1. Oven temperature was set at 214°C. Peaks contain a mixture which is resolved when TMSi derivatives are formed.

potassium borohydride reduction of [7α-^{3}H]-3β-hydroxy-5-androsten-17-one (40 Ci/mmol from NEN). Yields were of 99±2% and epoxide removal was completed by thin-layer chromatography.

Through all purification procedures, losses in 5α-androstane-3β,17β-diol occured. Recovery at the final stage could be quantitated by counting the remaining ^{14}C labelled 5α-androstane-3β,17β-diol which was added in known quantity to the crude extract. Presence of this labelled marker was also of help in the double isotopic dilution technique. A blank was carried through the entire procedure and consisted of one liter of distilled water instead of urine. The blank showed non contaminant which could interfere with the 5α-androstane-3β,17β-diol (Fig. 5.).

This report is the first presenting evidences and proofs of the presence of the 5α-androstane-3β,17β-diol in human urine. Mauvais-Jarvis *et al.*[31] showed this diol to appear in small quantities in human urine after injection of labelled 5α-androstane-3β,17β-diol or 5α-androstane-3α,17β-diol. Their work suggested that the 5α-androstane-3β,17β-diol was converted *in vivo* essentially into the 3α epimer and 17 ketosteroids, thus explaining the low quantities of the recovered 5α-androstane-3β,17β-diol.

Our results (Table 2) show urinary 5α-androstane-3β,17β-diol to be excreted both in males and females and the patterns to be sex dependent but to a lesser extent than those of the 3α epimer. Excretion rate was 4 to 5 times higher in males than in females for the 5α-androstane-3α,17β-diol but only double for the 3β epimer.

This determined the epimers ratio 3α/3β to average respectively 5·1 and 3·1 for males and females. These results are in good agreement with data published by Mahoudeau *et al.*[32]. These authors have shown conversion of plasma 5α-dihydrotestosterone to 5α-androstane-3α,17β-diol/conversion to 5α-androstane-3β,17β-diol ratio to be 4·90 in men and 3·70 in women. Differences in these

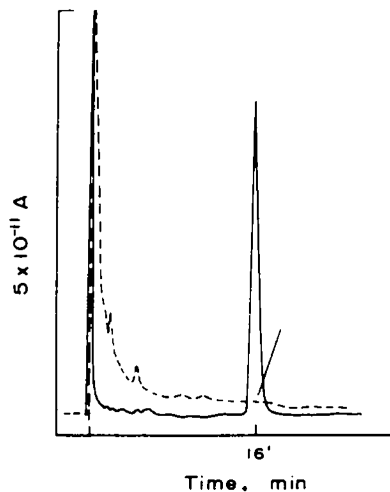

Fig. 5. Gas chromatogram of the blank. Blank was obtained by replacing urine by one liter distilled water through the entire procedure (dashed chromatogram). The other chromatogram (solid line) shows this blank when adding 40 μg standard 5α-androstane-3β,17β-diol to distilled water. Chromatography was performed on an Hi-Eff 8 BP column.

ratios are related to sex and there are published data to support the fact that 5α-androstane-3β,17β-diol would be retained in males after intravenous administration.

Mauvais-Jarvis *et al.*[31] recovered in the urine of two males over a 3 day period 13% of the labelled 5α-androstane-3β,17β-diol injected intravenously. Only 0·5% of the injected radioactivity was still associated with 5α-androstane-3β,17β-diol. Since 5α-androstane-3α,17β-diol injection led to nearly a three times higher recovery of radiosteroids and since in that case the obtained 5α-androstane-3β,17β-diol amounted 0·19% of the injected radioactivity, one can argue that most of the injected 5α-androstane-3β,17β-diol (at least the difference observed between recoveries after injections of 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol) was either retained in the body after 3 days or further transformed to unknown metabolites.

This could also explain why, after injection of labelled testosterone or 17β-hydroxy-5α-androstan-3-one, no labelled 5α-androstane-3β,17β-diol could be found in urines; limited transformations into this metabolite and further transformations into this metabolite and further transformation or binding to target sites would explain its absence in urines.

The 3α/3β ration of 3·1 that we found in female urines stresses this point as one would think females to have less target tissues to androgens than males. In other words and in relation to a much lower production of androgens, females excrete relatively more 5α-androstane-3β,17β-diol than males. This suggests 5α-androstane-3β,17β-diol to have androgenic potencies either as a substrate for metabolism to more androgenic compounds or by itself in the role it might play once in the target cell. Robel *et al.*[7] have shown this diol to have effectively androgenic potencies.

All these facts suggest that 5α-androstane-3β,17β-diol is not an end product

in testosterone metabolism and that it may be retained and/or further metabolized in human target tissues. This is correlated by the works of Ofner *et al.*[8] and Morfin *et al.*[4] who showed *in vivo* that after infusion of testosterone in arterial blood supply of cancerous and hyperplasic human prostate the recovered 5α-androstane-3α,17β-diol predominated above the 3β epimer: epimers ratios 3α/3β were respectively found to be 1·6 and 1·4.

Since our results obtained from male urines give ratios of 5·1 one can deduct that prostate contains relatively more 5α-androstane-3β,17β-diol than what is found in urine and therefore that this dihydroxysteroid is retained by the target tissue.

Physiological significance and mecanism of action of the 5α-androstane-3β,17β-diol has not yet been established. Indentification and dosage of the 5α-androstane-3β,17β-diol in urine and establishment of 3α/3β ratios should help future investigations of its physiological importance.

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