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URINARY STEROID EXCRETION AND CONJUGATION BY THE BABOON (*PAPIO HAMADRYAS*)—A COMPREHENSIVE STUDY

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SUMMARY

Ion exchange chromatography on a lipophilic ion exchange gel, diethylaminohydroxypropyl Sephadex LH-20 was used to separate steroids extracted from the urine of adult male baboons (*Papio hamadryas*) into four conjugate groups; neutral steroids, glucuronides, monosulphates and disulphates. Analysis of these groups using computerised gas chromatography-mass spectrometry showed there to be a total of 103 steroids which comprised 34 neutral steroids, 44 glucuronides, 22 monosulphates and 3 disulphates. Relatively large amounts of highly polar corticosteroid metabolites were found, in particular 6-hydroxylated steroids. 3α,6β,11β,17α,21-Pentahydroxy-5β-pregnan-20-one and 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one were quantitatively the major steroids excreted in the urine of this species.

INTRODUCTION

Although there have been a number of investigations of steroid metabolism and excretion in several species of sub-human primates [1–21], to date there has been no detailed study of the pattern of steroid conjugation in urine or on how the steroid structure influences the type of conjugate formed.

Recent speculation on the potential of the baboon as an animal model for man in the investigation of steroid metabolism, particularly relating to pregnancy [11, 12, 19–21], led us to make a comprehensive study of steroid excretion by this animal.

Lipophilic ion exchange gels synthesised from Sephadex LH-20 [22, 23] were used to separate steroids extracted from baboon urine according to their mode of conjugation [24]. The steroids were then quantified by gas chromatography (GC) on open-tubular glass capillary columns, and characterised using computerised gas chromatography–mass spectrometry (GC–MS).

EXPERIMENTAL

Urine collections

Daily collections were obtained from 4 adult male baboons (*Papio hamadryas*) weighing 20–30 kg. The animals were kept individually in metabolism cages in a temperature controlled environment. The samples were frozen immediately following collection and were stored until required for analysis.

Methodology

The general scheme of analysis is illustrated in Fig. 1. Complete details and the evaluation of the techniques have been described elsewhere [24], consequently these will be briefly outlined as follows:

Steroids were extracted from urine on columns of the neutral resin Amberlite XAD-2. This extract was then passed through a column of the cation exchange gel, SE-LH-20, prior to separating the steroids according to their mode of conjugation using the anion exchange gel DEAP-LH-20. Neutral steroids passed directly through the column in the solvent 72% ethanol, and stepwise elution of the glucuronide, monosulphate and disulphate conjugates was achieved with the solvent systems, 0.25 M formic acid

The following trivial names and abbreviations are used in this paper: TMS, Trimethylsilyl ether; MO-TMS, Methyloxime-trimethylsilyl ether; t_R, retention time relative to 5α-cholestane; *m/e*, mass/charge ratio; M^+, molecular or parent ion; a.m.u., atomic mass units; Androsterone, 3α-hydroxy-5α-androstan-17-one; Aetiocholanolone, 3α-hydroxy-5β-androstan-17-one; Epiandrosterone, 3β-hydroxy-5α-androstan-17-one; Dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one; 11β-Hydroxyandrosterone, 3α,11β-dihydroxy-5α-androstan-17-one; 11β-Hydroxyaetiocholanolone, 3α,11β-dihydroxy-5β-androstan-17-one; THE (tetrahydrocortisone), 3α,17,21-trihydroxy-5β-pregnane-11,20-dione; THB (tetrahydrocorticosterone), 3α,11β,21-trihydroxy-5β-pregnan-20-one; *allo*-THB (*allo*-tetrahydrocorticosterone), 3α,11β,21-trihydroxy-5α-pregnan-20-one; THF (tetrahydrocortisol), 3α,11β,17,21-tetrahydroxy-5β-pregnan-20-one; *allo*-THF (*allo*-tetrahydrocortisol), 3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one; α(β)-Cortolone, 3α,17,20α(β),21-tetrahydroxy-5β-pregnan-11-one; α(β)-Cortol, 5β-pregnane-3α,11β,17,20α(β),21-pentol; 20-Dihydrocortisol, 11β,17,20,21-tetrahydroxy-4-pregnen-3-one; the terms pregnane-tetrol-one, pregnane-pentol, etc., indicate general steroid structures and do not imply any specific configuration.

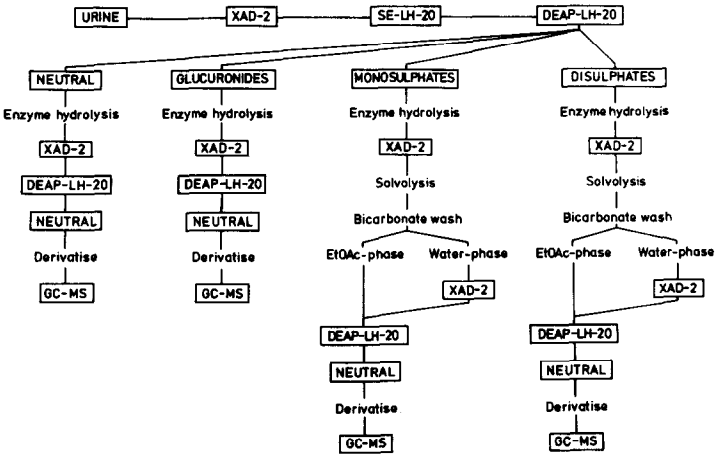

Fig. 1. The general scheme of analysis of baboon urine.

in 72% ethanol, 0.3 M acetic acid–potassium acetate in 72% ethanol (pH 6.3) and 0.5 M potassium acetate solution in 72% ethanol (pH 10) respectively. The steroid conjugates were hydrolysed by enzymes and solvolysis [25] and the liberated steroids were purified and finally isolated after passing through a column of DEAP-LH-20.

Preparation of derivatives for GC

O-Methyloxime-trimethylsilyl ether derivatives were prepared essentially as described previously [26] after the addition of the internal standards 5α-androstane-3α,17α-diol and stigmasterol. The derivatised samples were purified by passage through a Lipidex 5000 column [27] prior to analysis by GC.

Gas liquid chromatography (GC)

GC was carried out on either a Pye 104 gas chromatograph or a Becker 409 gas chromatograph, both equipped with flame ionisation detectors and housing 25 metre open-tubular glass capillary columns coated with OV-1. Samples were introduced through solid injection systems and nitrogen was the carrier gas at an approximate flow rate of 1 ml/min through the column. Both temperature programmed and isothermal operation were employed.

Computerised gas chromatography–mass spectrometry (GC–MS)

GC–MS was carried out using a modified LKB 9000 instrument, housing a 25 metre open-tubular glass capillary column coated with OV-1 and connected *via* a single stage adjustable jet separator. The operating conditions have been described previously [24]. Repetitive magnetic scanning was carried out over the mass range 0–800 a.m.u. Methods for the computerised evaluation of the mass spectral data recorded on magnetic tape have been described previously [28, 29].

Identification and quantification of steroids

The identification of a steroid was based upon the retention time relative to 5α-cholestane at isothermal conditions (t_R), the complete mass spectrum and interpretation of fragment ion current (FIC) chromatograms constructed of characteristic ions given by the steroid derivatives. The *m/e* values which were chosen in this study in order to give a relatively unbiased analysis have been listed in a previous publication [24].

The steroids were quantified as the methyloxime-trimethylsilyl ether derivatives, using 5α-androstane-3α,17α-diol and stigmasterol as internal standards. A line was drawn between the peak heights produced from equal amounts of these standards and the peak height of the steroids was measured relative to this line. Mixtures of known amounts of reference steroids were analysed in the same way and factors (between 0.76–1.70) were calculated to correct for the differences in mass/peak height relationships. When pure reference compounds were not available the factor 1.0 was used.

RESULTS

A list of the steroids identified in the conjugate groups isolated from baboon urine is given in Table 1. In those cases where the stereochemistry was not ascertained, a trivial name is given together with the retention time relative to 5α-cholestane. A total of 103 steroids were identified by this technique and these comprised 34 neutral steroids, 44 glucuronide conjugates, 22 monosulphates and 3 disulphates.

Neutral fraction

Quantitatively the major steroids identified in this fraction and the amounts excreted are summarised in Table 2. The mass spectra of the majority of the common steroids identified in this fraction have been described elsewhere and only those of particular interest will be discussed in detail.

Following GC–MS analysis computer constructed molecular ion and fragment ion current (FIC) chromatograms were made of *m/e* values characteristic of MO-TMS derivatives of polar corticosteroids having

Table 1. List of steroids identified from the urine of the adult male baboon (*Papio hamadryas*)

No.	t_R[a]	Steroid structure[b]	Conjugate fraction[c]			
1	0.51	3α-Hydroxy-5-androsten-17-one	N	G	—	—
2	0.53	3α-Hydroxy-5α-androstan-17-one	N	G	M	—
3	0.55	3α-Hydroxy-5β-androstan-17-one	N	G	M	—
4	0.55	5β-Androstane-3α,17β-diol	N	G	M	—
5	0.63	3β-Hydroxy-5-androsten-17-one	—	—	M	—
6	0.65	3β-Hydroxy-5α-androstan-17-one	—	—	M	—
7	0.66	5-Androstene-3β,17β-diol	—	—	M	D
8	0.66	5α-Androstane-3β,17β-diol	—	—	—	D
9	0.67	3β,7α-Dihydroxy-5-androsten-17-one	—	—	M	—
10	0.67	3α-Hydroxy-5α-androstane-11,17-dione	N	G	—	—
11	0.67	Androstenediolone	—	G	—	—
12	0.69	3α-Hydroxy-5β-androstane-11,17-dione	N	G	—	—
13	0.74	Androstanediolone	—	G	—	—
14	0.75	Androstanediol-11-one	—	G	—	—
15	0.79	Androstanediolone	—	G	—	—
16	0.82	3α,11β-Dihydroxy-5α-androstan-17-one	N	G	—	—
17	0.84	3α,11β-Dihydroxy-5β-androstan-17-one	N	G	—	—
18	0.91	3β,16α-Dihydroxy-5-androsten-17-one[d]	—	G	—	—
19	0.91	Pregnenediol	—	G	—	—
20	0.91	Androstanediolone	—	G	—	—
21	0.92	Androstane-3,16,17-triol	N	G	—	—
22	0.94	3β,16α-Dihydroxy-5-androsten-17-one[d]	—	G	—	—
23	0.94	3β,7β-Dihydroxy-5-androsten-17-one	—	—	M	—
24	0.95	Androstane-3,16,17-triol	—	G	—	—
25	0.95	Androstane-3,16,17-triol	N	—	—	—
26	0.97	Pregnanetriol	N	—	—	—
27	1.04	5β-Pregnane-3α,17,20α-triol	N	G	—	—
28	1.06	Pregnenetriol	—	G	—	—
29	1.08	Pregnanetriol	N	G	—	—
30	1.12	Pregnanetriolone	—	G	—	—
31	1.26	5-Androstene-3,16,17-triol	—	—	M	—
32	1.39	Pregnene-3,16,20-triol	—	G	—	—
33	1.49	Pregnene-3,17,20-triol	N	G	M	—
34	1.60	5-Pregnene-3β,17,20α-triol	—	—	M	D
35	1.63	3α,17,21-Trihydroxy-5β-pregnane-11,20-dione	N	G	—	—
36	1.63	3α,21-Dihydroxy-5β-pregnane-11,20-dione	—	G	—	—
37	1.67	(Pregnadiene-tetrolone)[e]	N	G	M	—
38	1.78	3α,11β,21-Trihydroxy-5β-pregnan-20-one	—	G	—	—
39	1.86	3α,11β,21-Trihydroxy-5α-pregnan-20-one	N	—	—	—
40	1.92	3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one	N	G	M	—
41	2.00	3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	N	G	M	—
42	2.03	3α,6β,17,21-Tetrahydroxy-5β-pregnane-11,20-dione	N	G	M	—
43	2.07	3α,17,20α,21-Tetrahydroxy-5β-pregnan-11-one	N	G	M	—
44	2.24	5β-Pregnane-3α,11β,17,20β,21-pentol	N	G	M	—
45	2.24	3α,17,20β,21-Tetrahydroxy-5β-pregnan-11-one	N	G	M	—
46	2.29	3α,6β,11β,17,21-Pentahydroxy-5α-pregnan-20-one	N	G	M	—
47	2.32	3,17,20,21-Tetrahydroxy-pregnan-11-one	N	G	—	—
48	2.32	3α,6β,11β,17,21-Pentahydroxy-5β-pregnan-20-one	N	G	M	—
49	2.53	5β-Pregnane-3α,11β,17,20α,21-pentol	N	G	M	—
50	2.54	3,X,17,20,21-Pentahydroxy-pregnan-11-one[f]	N	G	—	—
51	2.65	3,X,17,20,21-Pentahydroxy-pregnan-11-one[f]	N	G	—	—
52	2.68	Pregnenetetrolone	N	G	—	—
53	3.47	11β,17,21-Trihydroxy-4-pregnene-3,20-dione[d]	N	G	M	—
54	3.55	11β,17,20β,21-Tetrahydroxy-4-pregnen-3-one[d]	N	G	—	—
55	3.59	6β,11β,17,21-Tetrahydroxy-4-pregnene-3,20-dione[d]	N	—	—	—
56	3.63	11β,17,20α,21-Tetrahydroxy-4-pregnen-3-one[d]	N	G	—	—

[a] Retention time of the TMS or MO-TMS derivative relative to that of 5α-cholestane at 230°C on a 25 metre open-tubular glass capillary column coated with silicone OV-1.

[b] Where the exact stereochemistry has not been ascertained a trivial name is given.

[c] N = neutral; G = glucuronide; M = monosulphate; D = disulphate.

[d] The MO-TMS derivative of these steroids give two peaks.

[e] Possible structure based on mass spectrometry.

[f] X denotes unknown position.

Table 2. Levels of the principal steroids excreted in the urine of 4 normal male baboons (*Papio hamadryas*)

Steroid	Excretion (μg/kg body wt/24 h)			
Neutral steroids:				
3α-Hydroxy-5α-androstan-17-one	21.7	27.8	19.1	26.8
3α-Hydroxy-5β-androstan-17-one	11.2	7.5	6.8	6.8
3α-Hydroxy-5α-androstane-11,17-dione	10.8	3.6	—	—
3α-Hydroxy-5β-androstane-11,17-dione	8.5	5.3	3.7	3.9
3α,11β-Dihydroxy-5α-androstan-17-one	21.9	16.6	3.4	10.8
3α,11β-Dihydroxy-5β-androstan-17-one	18.0	7.7	5.0	7.4
5β-Pregnane-3α,17,20α-triol	9.9	2.7	3.7	2.6
3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one	9.5	2.6	3.0	3.9
3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	16.8	7.2	5.8	18.8
3α,6β,17,21-Tetrahydroxy-5ξ-pregnane-11,20-dione	16.0	4.2	7.1	12.3
3α,6β,11β,17,21-Pentahydroxy-5β-pregnan-20-one	13.7	17.9	16.0	37.2
11β,17,21-Trihydroxy-4-pregnene-3,20-dione	4.4	3.3	3.6	2.1
11β,17,20β,21-Tetrahydroxy-4-pregnen-3-one	15.8	10.3	5.3	3.3
11β,17,20α,21-Tetrahydroxy-4-pregnen-3-one	17.9	14.1	7.1	3.9
17,20β,21-Trihydroxy-4-pregnene-3,11-dione	7.6	4.6	4.6	2.7
Glucuronide conjugates:*				
3α-Hydroxy-5α-androstan-17-one	—	4.6	29.7	15.3
3α-Hydroxy-5β-androstan-17-one	—	0.2	3.0	3.0
3α-Hydroxy-5α-androstane-11,17-dione	—	—	3.0	2.0
3α-Hydroxy-5β-androstane-11,17-dione	—	0.8	3.7	—
3α,11β-Dihydroxy-5α-androstan-17-one	—	2.7	12.9	4.1
3α,11β-dihydroxy-5β-androstan-17-one	—	0.3	11.8	3.7
5β-Pregnane-3α,17,20α-triol	—	2.1	2.6	2.3
3α,17,21-Trihydroxy-5β-pregnane-11,20-dione	—	4.4	7.4	6.6
3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one	—	1.4	10.5	2.4
3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	—	11.5	32.4	11.0
3α,17,20α,21-Tetrahydroxy-5β-pregnan-11-one	—	0.6	7.2	1.0
3α,17,20β,21-Tetrahydroxy-5β-pregnan-11-one 5β-Pregnane-3α,11β,17,20β,21-pentol	—	1.5	5.7	1.9
5β-Pregnane-3α,11β,17,20α,21-pentol	—	1.1	3.6	3.0
Monosulphate conjugates:				
3β-Hydroxy-5-androsten-17-one	7.4	3.8	2.4	5.5
3β-Hydroxy-5α-androstan-17-one	1.9	0.5	0.2	1.0
5-Androstene-3β,17β-diol	1.3	0.6	0.5	0.5
5-Androstene-3β,16α,17β-triol	1.1	3.1	1.1	0.5

* One sample lost.

the general structure, pregnane-pentol-one, pregnane-tetrol-dione (11-one), pregnane-pentol, pregnane-triol-dione (11-one), pregnane-pentol-11-one and pregnane-tetrol-one (Fig. 2). By this procedure several previously unidentified steroids were detected.

The complete mass spectrum of the MO-TMS derivative of the major steroid (compound 11, Fig. 2) in this fraction ($t_R = 2.32$) showed the molecular ion (M^+) to be at *m/e* 771. Following purification, isolation on Sephadex LH-20 and extensive chemical and mass spectrometric analysis, this compound was identified as 3α,6β,11β,17,21-pentahydroxy-5β-pregnan-20-one (6β-hydroxy-THF) [30]. Compound 10 (Fig. 2) eluted at a retention time relative to 5α-cholestane of 2.30 had an almost identical mass spectrum to that of compound 11 and it was shown to have the structure 3α,6β,11β,17,21-pentahydroxy-5α-pregnan-20-one [30].

Compound 8 eluted at a retention time relative to 5α-cholestane of 2.03 was shown from the FIC chromatograms (Fig. 2) to have the general structure of an MO-TMS derivative of pregnane-tetrol-dione with an underivatised carbonyl at the C-11 position. The mass spectrum was similar to that of the MO-TMS derivative of THE except that the molecular ion and fragment ions were 88 a.m.u. greater in magnitude due to the presence of an additional derivatised hydroxyl group. This compound was identified as 3α,6β,17,21-tetrahydroxy-5ξ-pregnane-11,20-dione (6β-hydroxy-THE) on the basis of its retention time and mass spectrum and substantiated by the demonstration of other 6-hydroxylated corticosteroid metabolites, in particular the large amounts of 6β-hydroxy-THF already shown to be present in the urine of this species.

Two other polar corticosteroid metabolites were also apparent in this fraction (Fig. 2, compounds 3 and 4). The mass spectrum of both compounds was similar. The molecular ion was at *m/e* 742 indicating a pregnane-pentol-11-one structure. The ion at *m/e* 537 is formed by the loss of 205 a.m.u. due to cleavage between carbon atoms 17 and 20 and the loss of two silylated hydroxyl groups and this fragmentation is characteristic of all steroids with a glycerol side-chain.

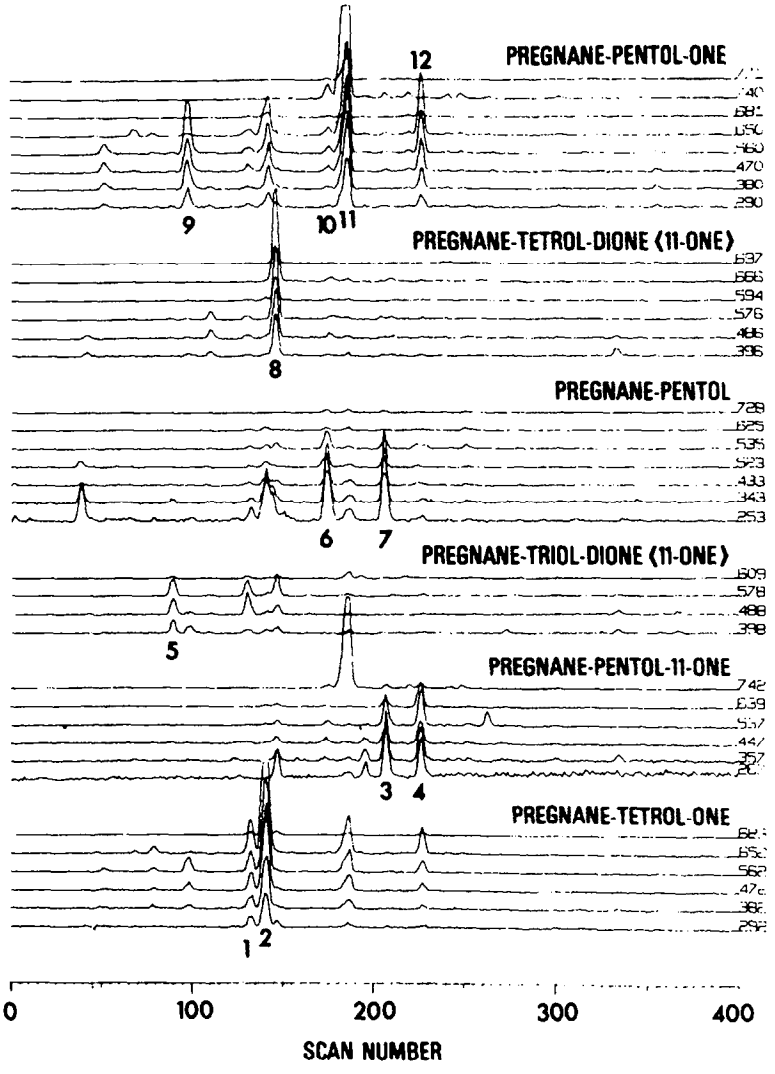

Fig. 2. Molecular ion and fragment ion current chromatograms constructed by the computer for MO-TMS and TMS derivatives characteristic of steroids with the general structures as follows: pregnane-pentol-one, pregnane-tetrol-dione (underivatised 11-one), pregnane-pentol, pregnane-triol-dione (underivatised 11-one), pregnane-pentol-11-one and pregnane-tetrol-one. The steroids indicated are: *1.* 3α,11β,17,21-tetrahydroxy-5β-pregnan-20-one (THF), *2.* 3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one (*allo*-THF), *3.* and *4.* 3α,X,17,20,21-pentahydroxy-11-one where X is probably position 6, *5.* 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (THE), *6.* 5β-pregnane-3α,11β,17,20β,21-pentol (β-cortol), *7.* 5β-pregnane-3α,11β,17,20α,21-pentol (α-cortol), *8.* 3α,6β,17,21-tetrahydroxy-5β-pregnane-11,20-dione (6β-hydroxy-THE), *9.* pregnadiene-triol-one (tentative structure), *10.* 3α,6β,11β,17,21-pentahydroxy-5α-pregnan-20-one (6β-hydroxy-*allo*-THF), *11.* 3α,6β,11β,17,21-pentahydroxy-5β-pregnan-20-one (6β-hydroxy-THF), *12.* pregnene-tetrol-one.

The presence of three other derivatised hydroxyl groups is indicated by the ions at *m/e* 537, 447, 357 and 267. Although positive identification has not yet been possible, these steroids are tentatively suggested to be 6-hydroxylated derivatives of α-cortolone and β-cortolone.

Compounds 1, 2, 5, 6 and 7 (Fig. 2) were identified as 3α,11β,17,21-tetrahydroxy-5β-pregnan-20-one (THF), 3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one (*allo*-THF), 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (THE), 5β-pregnane-3α,11β,17,20β,21-pentol (β-cortol) and 5β-pregnane-3α,11β,17,20α,21-pentol (α-cortol) respectively. Although these steroids were quantitatively important metabolites in this animal, details of their mass spectra have been omitted since they are well documented elsewhere.

Two compounds, the structures of which have yet to be determined, were apparent following construction of FIC chromatograms. Compound 9 (Fig. 2) gave rise to a molecular ion at *m/e* 591 and a base peak at *m/e* 560, formed by the loss of 31 a.m.u. due to a derivatised carbonyl group. Three derivatised hydroxyl groups were indicated by the ions at *m/e* 470, 380 and 290, formed by the loss of 90 a.m.u. from

the base peak. Based on this information it is possible that this compound could have the general structure of a pregnadiene-triol-one, however, this is very speculative. Compound 12 (Fig. 2) was identified from FIC chromatograms to have the structure of an MO-TMS derivative of a pregnene-tetrol-one. From the complete mass spectrum, the molecular ion was shown to be at *m/e* 681, and the presence of a derivatised carbonyl group was indicated by the ion at *m/e* 650 (M-31) which was also the base peak in this spectrum. Four derivatised hydroxyl groups were apparent from the ions formed at *m/e* 560, 470, 380 and 290, due to consecutive losses of 90 a.m.u. from the base peak. Although all of these ions are present in the mass spectrum of the MO-TMS of 20-dihydrocortisol, the retention time is somewhat different. Positive identification of this compound has not yet been possible and is further complicated by the difficulty in resolving it from other polar steroids in spite of using open-tubular glass capillary columns.

Glucuronide fraction

A typical gas chromatographic profile of the glucuronide fraction is illustrated in Fig. 3. A list of those steroids present as glucuronide conjugates is given in Table 1. Quantitatively the major steroid excreted as a glucuronide conjugate was *allo*-THF (Table 2) and a striking feature was the predominance of steroids with a 3α,5α configuration compared with the respective 3α,5β isomers.

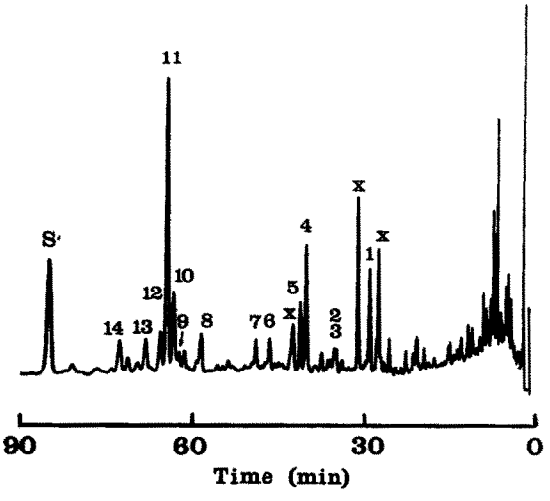

Fig. 3. The gas chromatogram of the MO-TMS derivatives of steroids isolated as glucuronide conjugates from the urine of the baboon (*Papio hamadryas*). Gas chromatography was carried out on a 25 m open-tubular glass capillary column coated with silicone OV-1. Temperature programmed operation from 180°C in increments of 1°C/min was used. The following steroids are indicated: *1.* 3α-hydroxy-5α-androstan-17-one, *2.* 3α-hydroxy-5α-androstane-11,17-dione, *3.* 3α-hydroxy-5β-androstane-11,17-dione, *4.* 3α,11β-dihydroxy-5α-androstan-17-one, *5.* 3α,11β-dihydroxy-5β-androstan-17-one, *6.* 5β-pregnane-3α,17,20α-triol, *7.* pregnene-3,16,20-triol, *8.* 3α,17,21-trihydroxy-5β-pregnane-11,20-dione, *9.* 3α,11β,21-trihydroxy-5α-pregnan-20-one, *10.* 3α,11β,17,21-tetrahydroxy-5β-pregnan-20-one, *11.* 3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one, *12.* 3α,17,20α,21-tetrahydroxy-5β-pregnan-11-one, *13.* 3α,17,20β,21-tetrahydroxy-5β-pregnan-11-one and 5β-pregnane-3α,11β,17,20β,21-pentol, *14.* 5β-pregnane-3α,11β,17,20α,21-pentol. S = stigmasterol (internal standard), X = impurities from the solvents and silylated reagents.

Monosulphate fraction

Dehydroepiandrosterone was quantitatively the major steroid excreted as a monosulphate conjugate (Table 2). Most of the steroids identified possessed a 3β-hydroxy-5-ene configuration and are commonly occurring steroids. A number of corticosteroids were also identified but at levels too low to enable satisfactory quantitation.

Disulphate fraction

Only three steroids were identified in significant amounts as disulphates; 5-androstene-3β,17β-diol, 5α-androstane-3β,17β-diol and 5-pregnene-3β,17α, 20α-triol.

DISCUSSION

Using recently developed techniques [24] the pattern of steroid excretion and conjugation was determined in 4 adult male baboons. Steroids were separated according to their mode of conjugation [24] by column chromatography using a lipophilic ion exchange gel, diethylaminohydroxypropyl-Sephadex LH-20 (DEAP-LH-20) synthesised from Sephadex LH-20 [22, 23]. The conjugate fractions were then subjected to either enzyme hydrolysis or combined enzyme hydrolysis/solvolysis procedures to liberate the respective free steroids.

Qualitative and quantitative analysis of the steroids from each conjugate group was achieved by gas chromatography using open-tubular glass capillary columns and computerised gas chromatography–mass spectrometry. Recent progress with open-tubular glass capillary columns has illustrated their potential in the multicomponent analysis of steroids. In fact the mixture of steroids in urine is so complex that a satisfactory analysis cannot be achieved by gas chromatography using conventional packed columns. At present GC–MS offers the highest degree of specificity and for this reason was chosen for the characterisation of steroids. Using repetitive magnetic scanning operation over the mass range 0–800 a.m.u. a large number of scans were recorded, thereby necessitating computerised evaluation of the data. From this data, fragment ion current (FIC) chromatograms were constructed by the computer of *m/e* values characteristic of steroid TMS and MO-TMS derivatives. A series of *m/e* values was chosen to allow the detection of many types of steroid structures, in addition to a series typical of specific conformations either in the steroid derivative or produced in the ion source as a result of fragmentation [24]. Using this approach a relatively unbiased analysis was achieved.

While the major pathway for the elimination of steroid hormones in man is by excretion in the urine [31], it would appear from the large amount of compounds identified that this is probably also the case for the baboon (*Papio hamadryas*).

The distribution of steroids within the conjugate groups was found to be somewhat related to their structure. The neutral and glucuronide fractions contained mainly steroids with a 3α,5α or 3α,5β configuration in the 'A' ring, while the sulphate fractions generally contained compounds with a 3-hydroxy-5-ene structure. These observations are consistent with those for the human [24, 32, 33].

In the neutral and glucuronide fractions steroids with a 3α,5α configuration were generally present in greater amounts than their respective 3α,5β isomers. The glucuronide conjugate fraction showed striking qualitative and quantitative similarities to human urine. Relatively large amounts of C_{19} steroids and corticosteroid metabolites were found, in particular, androsterone, 11β-hydroxyandrosterone, THE, THF, *allo*-THF, cortols and cortolones; the daily excretion being comparable to that for man when calculated on a body weight basis. These findings differ somewhat from those obtained for the macaque monkey, also a species of Old World Monkey, in which very small amounts of C_{21} steroids are excreted because the major pathway for the elimination of corticosteroids in this species is by 20β-reduction and side-chain cleavage giving rise to 11-oxygenated-17-oxo-steroids [18].

The principal steroids excreted in the monosulphate fraction were dehydroepiandrosterone, 5-androstene-3β,17β-diol and epiandrosterone, while the disulphate fraction contained only three identifiable steroids. Corticosteroid metabolites were identified in the monosulphate fraction in trace amounts, and this is consistent with the previously reported existence of sulphated corticosteroid metabolites in both man [34] and baboon (*Papio hamadryas*) [11].

The neutral fraction was found to be qualitatively and quantitatively the most important one. This is in contrast to the human in which little steroid is excreted in uncharged form when urine is analysed by the same procedure [24]. Degradation of steroid conjugates by hydrolysis due to bacteria has been proposed as an explanation for the large amount of free steroids excreted by the baboon and it was suggested that until this was proven otherwise, any study of conjugation was not feasible [35]. In view of these comments, strict precautions were taken to minimise the possibility of bacterial degradation. In this experiment the urine was collected directly into containers surrounded by dry-ice. In this way the urine was frozen immediately and kept at below −70°C after excretion. It is difficult to envisage degradation occurring under these conditions, and for this reason it is felt that our extensive study of conjugation of steroids by this animal is fully justified.

It is most probable that these neutral compounds are excreted as 'free' steroids, since in subsequent experiments they have been shown to be readily extractable by solvent partition with ethyl acetate. Although the existence of steroids conjugated to neutral sugar derivatives has been demonstrated [36], it seems most unlikely that this is the case for the baboon, since a striking feature of the neutral fraction from this animal was the presence of highly polar cortisol and corticosterone metabolites, and which by virtue of their polarity are most probably excreted as unconjugated ('free') steroids in urine.

The occurrence of highly polar corticosteroid metabolites in this species has been recognised previously in studies of cortisol metabolism in normal female baboons [11], pregnant baboons [12] and from our earlier observations in male baboons [17]. The composition of several of these metabolites was not established, although a suggestion that they were hydroxylated metabolites of THF and THE was proposed [11]. The major steroid excreted by the baboon (*Papio hamadryas*) in this study was shown to have the structure 3α,6β,11β,17,21-pentahydroxy-5β-pregnan-20-one (6β-hydroxy-THF) [30], while trace amounts of the 3α,5α epimer were also detectable using computerised GC–MS. Several other polar steroids were detected; of these a relatively high concentration was found for a steroid identified as 3α,6β,17,21-tetrahydroxy-5β-pregnane-11,20-dione (6β-hydroxy-THE). In addition, hydroxylated derivatives of 20-reduced corticosteroids were also detected and on the basis of the extensive 6-hydroxylation these are most probably 6-hydroxy derivatives. In spite of the large number of steroids identified there were two important compounds which have yet to be characterised. From the initial findings it is probable that one of these could be an unsaturated tetrahydrocortisol, while the other appears to have the general structure of a pregnadiene-triol-one.

To conclude, although this species excretes significant amounts of polar corticosteroids, in particular 6-hydroxylated metabolites, the results of this study corroborate earlier reports of the suitability of the baboon as an animal model for studies of human corticosteroid metabolism.

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