THE CHARACTERIZATION OF POLAR CORTICOSTEROIDS IN THE URINE OF THE MACAQUE MONKEY (MACACA FASCICULARIS) AND THE BABOON (PAPIO HAMADRYAS)

By

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ABSTRACT

Computerised gas chromatography-mass spectrometry was employed in the identification of polar corticosteroid metabolites excreted in the urine from the macaque monkey (Macaca fascicularis) and the baboon (Papio hamadryas). The following steroids were identified in significant amounts in the urine from both species: 3α,17α,20α,21-tetrahydroxy-5β-pregnan-11-one; 3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one; 5β-pregnane-3α,11β,17α,20α,21-pentol; 5β-pregnane-3α,11β,17α,20β,21-pentol; 5α-pregnane-3β,11β,17α,20β,21-pentol. 11β,17α,21-Trihydroxy-4-pregnene-3,20-dione (cortisol), 11β,17α,20β,21-tetrahydroxy-4-pregnen-3-one and 11β,17α,20β,21-tetrahydroxy-5β-pregnan-3-one were identified in macaque monkey urine. Two steroids, 17α,20β,21-tri hydroxy-4-pregnan-3,11-dione and 17α,20α,21-tri hydroxy-4-pregnene-3,11-dione were excreted as major C₂₁ metabolites in the baboon but were not identified in the urine from the macaque monkey. 3β-Hydroxy-5α-pregnan metabolites were identified in the urine from both species. All these steroids were excreted conjugated to glucuronic acid, evidenced by their recovery after hydrolysis with β-glucuronidase enzyme.

An efficient 20β-reduction of corticosteroids in both species is apparent, and the excretion pattern of polar steroid metabolites in the two species was shown to be similar.
In spite of the widespread application of sub-human primates as models for man in endocrinological research, the data available concerning the detailed metabolism and excretion of many principal hormones is extremely limited. Efforts to characterise completely all of the metabolites excreted in urine have probably been limited by the lack of specific and sensitive techniques.

In a previous study employing gas chromatography-mass spectrometry, Setchell & Shackleton (1975) have described the major metabolites of cortisol and corticosterone in the macaque monkey (Macaca fascicularis). Although the excretion of significant amounts of polar corticosteroids was reported, it was evident that additional metabolites were excreted by this species which were not identified.

Gontscharow et al. (1972a,b,c) have identified many corticosteroids in baboon urine (Papio hamadryas) using infrared spectroscopy following extensive purification and separation by paper and thin layer chromatography. In these studies, two zones in the paper chromatograms gave rise to compounds which could not be identified by infrared spectroscopy alone.

The present paper describes the results of further studies and is devoted to the characterization of additional polar metabolites not previously identified in the urine from these two species of sub-human primates.

**MATERIALS AND METHODS**

**Chemicals**

All solvents were of reagent grade and were re-distilled before use. Hexamethyldisilazane and trimethylchlorosilane (Applied Science Laboratories, State College, Pa., USA) were re-distilled. Methoxamine hydrochloride was from Eastman Organic Chemicals (Rochester, N.Y., USA), and trimethylsilylimidazole from the Pierce Chemical Co. (Rockford, Ill., USA). Amberlite XAD-2 was obtained from Rohm and Haas (Pa., USA) and washed in a large column with water, ethanol, acetone and water. This was stored in water until required. Sephadex LH-20 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) and Lipidex 5000 from Packard-Becker Chemicals (Groningen). Ketodase (β-glucuronidase enzyme) was obtained from Warner-Chilcott Laboratories (Morris Plains, New Jersey, USA).

**Reference steroids**

Reference steroids were obtained from the Medical Research Council Steroid Reference Collection (curator Dr. D. N. Kirk), Westfield College, Hampstead, London.

**Abbreviations**

MO-TMS: O-Methyloxime-trimethylsilyl ether

TMS: Trimethylsilyl ether

a. m. u.: Atomic Mass Units

536
tR: Retention Time relative to 5α-Cholestan
TheH: 3α,17α,21-Trihydroxy-5β-pregnan-a,20-dione
THF: 3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one
α-Cortolone: 3α,17α,20α,21-Tetrahydroxy-5β-pregnan-11-one
β-Cortolone: 3α,17α,20β,21-Tetrahydroxy-5β-pregnan-11-one
3β-Cortolone: 3β,17α,20α,21-Tetrahydroxy-5α-pregnan-11-one
α-Cortol: 5β-Pregnan-3α,11β,17α,20α,21-pentol
β-Cortol: 5β-Pregnen-3α,11β,17α,20β,21-pentol
3β-Cortol: 5α-Pregnan-3β,11β,17α,20α,21-pentol
20α-Dihydrocortisol: 11β,17α,20α,21-Tetrahydroxy-4-pregnen-3-one
20β-Dihydrocortisol: 11β,17α,20β,21-Tetrahydroxy-4-pregnen-3-one
20α-Dihydrocortisone: 17α,20α,21-Trihydroxy-4-pregnen-3,11-dione
20β-Dihydrocortisone: 17α,20β,21-Trihydroxy-4-pregnen-3,11-dione
Cortisol: 11β,17α,21-Trihydroxy-4-pregnen-3,20-dione

Methodology

Urine (24 h) collections were obtained from (i) two adult female macaque monkeys (Macaca fascicularis), body weight 3.5 kg and (ii) eight adult female baboons (Papio hamadryas), body weight between 5–7 kg. All animals were housed in individual metabolism cages in a temperature controlled environment. After collection of urine, the polar steroids excreted in the two species were isolated by different procedures.

(i) Isolation of polar steroids from macaque monkey urine. – Details of the method have been described previously (Setchell & Shackleton 1975). Briefly, steroids were extracted on columns of Amberlite XAD-2 resin. The unconjugated steroids were removed by partition between methylene chloride and water and the steroid conjugates hydrolysed with a β-glucuronidase enzyme. A repeated extraction on Amberlite XAD-2 was carried out prior to fractionation of the hydrolysed glucuronides into six fractions of increasing polarity on Sephadex LH-20 (Setchell & Shackleton 1973). Fractions 5 and 6 contained the highly polar corticosteroid metabolites, which were then analysed by gas chromatography-mass spectrometry after the preparation of suitable derivatives.

(ii) Isolation of polar steroids from baboon urine. – Details of the procedure have been reported previously (Gontscharow et al. 1972a). Steroids were extracted from urine (24 h) by shaking with chloroform (10 volumes) for 30 min. The chloroform was removed, the aqueous phase acidified and then extracted with ethyl acetate (2 volumes) by shaking for 10 min. The two organic phases were pooled and taken to dryness. The extract was then hydrolysed with β-glucuronidase enzyme. The hydrolysed steroids were then extracted with ethyl acetate, spotted on paper and chromatographed in a Bush B1 system for 15 h. The zone corresponding to an Rf value of cortisol in this system was eluted from the paper with methanol and re-chromatographed on paper in a Bush B5 system for 16 h. The paper was then divided into five zones of increasing polarity. The area corresponding to 2.0–8.0 cm from the origin and having a polarity similar to that of 20-dihydrocortisol was called Zone 1, and this was taken for analysis. The area corresponding to 21.5–27.0 cm from the origin was called Zone 3, and had a polarity between that of THF and THE and this was also taken for analysis. Analysis of Zones 2, 4 and 5 has been reported previously (Gontscharow et al. 1972a,b,c). The compounds were eluted from the paper with methanol and derivatised prior to analysis by gas chromatography-mass spectrometry.
Preparation of derivatives for gas chromatography

Aliquots of the polar steroid fractions were taken and the following derivatives prepared:

O-Methyloxime-trimethylsilyl ethers, MO-TMS (persilylated). – The method was essentially as described by Thenot & Horning (1972) but the additional modification described by Axelson & Sjövall (1975, in press) included the purification and removal of reagents on small columns of Lipidex 5000 (hydroxyalkoxypropyl Sephadex). Methoxamine hydrochloride (5 mg) was added to the dried steroid extract, followed by 50 µl of dry pyridine. This was then incubated at 60°C for 15 min. The sample was then dried over a stream of nitrogen, trimethylsilylimidazole (50 µl) added and the sample left to react for 2 h at 110°C. Small columns of Lipidex 5000 (250 mg) were prepared in the solvent system, hexane/pyridine/hexamethyldisilazane (98:1:1). After reaction, the sample was transferred to the Lipidex 5000 column with 0.5 ml of the solvent using a Pasteur pipette. The sample tube was then washed with three successive 0.5 ml volumes of solvent, which were then transferred and passed through the Lipidex 5000 column. The eluent (2.0 ml) was collected in glass tube. Prior to gas chromatography the solvent was evaporated to dryness and the derivatised sample re-dissolved in hexane (50 µl).

Trimethylsilyl ethers. – These were prepared basically according to Makita & Wells (1968). The dried steroid extract was dissolved in re-distilled dry pyridine (250 µl). Hexamethyldisilazane (200 µl) and trimethylchlorosilane (5 µl) were added, the tube stoppered and left to react for 4 h at room temperature. The sample was then taken to dryness over nitrogen and the steroid derivatives extracted into hexane (0.5 ml). By this technique all but tertiary hydroxyl groups become silylated.

O-Methyloxime-trimethylsilyl ethers (partially silylated). – The methyloxime was first prepared according to Fales & Luukkainen (1965). To the dried steroid extract was added 200 µl of a 1% solution of methoxamine hydrochloride in pyridine and this was left to react at room temperature for 16 h. The silylation procedure was then carried out as described above.

Acetonide derivative. – This was prepared essentially as described by McCloskey & McClelland (1965). The dried steroid extract was dissolved in re-distilled acetone (2 ml) and anhydrous copper sulphate (50 mg) added. The tube was stoppered and left for 2 h at 50°C after which the copper sulphate was removed by filtration through a glass wool plug. The acetone was evaporated and the derivative dissolved in hexane.

Gas chromatography

Gas chromatography was carried out using a Pye 104 gas chromatograph equipped with flame ionisation detection and housing a glass column (3 m length, 4 mm i.d.) packed with 1.5% silicone SE-30 on Chromosorb W. H. P. (80–100 mesh). Isothermal operation at 230°C was employed. Retention times were calculated relative to 5α-cholestanate, which gave a retention time of approximately 6 min using these conditions. A semi-quantitative estimation of the steroids excreted was made by addition of cholesteryl butyrate (10 µg) to the sample prior to derivatisation and comparison of the area of the steroid peaks against this standard in the gas chromatogram.
Computerised gas chromatography-mass spectrometry

Low resolution gas chromatography-mass spectrometry was carried out using an LKB 9000 combined gas chromatograph-mass spectrometer, housing a 1.5% SE-30 column. Modifications of the instrument and the methods for computerised evaluation of the mass spectral data have been described previously (Reimendal & Sjövall 1972). Operating conditions were as follows: temperature of column 230°C isothermal; separator temperature 250°C; temperature of ion source 290°C; energy of bombarding electrons 22.5 eV; ionising current 60 μA; accelerating voltage 3.5 kV. Repetitive scanning was carried out over the mass range 0–760 a.m.u. and a delay of between 3–5 min allowed after injection of the sample and prior to the start of the scanning.

The identification of a steroid was based upon the retention time relative to 5α-cholestan (tR), the mass spectrum and the fragment ion current chromatograms (FIC) constructed of the characteristic ions given by the derivative of the steroid.

RESULTS

Analysis of macaque monkey urine

Fig. 1 shows the gas chromatography-mass spectrometric analysis of the compounds isolated in Sephadex LH-20 fraction 5 from one of the animals. A search was made for polar corticosteroid metabolites and a computer constructed fragment ion current chromatogram plotted of m/e values characteristic of derivatised structures present in MO-TMS (persilylated) of cortolone, cortol, cortisol, 20-dihydrocortisol, and tetrahydrocortisol.

By this technique eight distinctive steroids were identified, the identification being based upon the combined information of retention time, mass spectrum and FIC chromatograms when compared against reference compounds.

Four cortolone isomers were indicated in the FIC chromatograms; of these three were positively identified as α-cortolone (tR = 1.80), β-cortolone (tR = 1.99) the major compound and 3β-cortolone (tR = 3.38). The fourth is probably a cortolone with a 3α-hydroxy-5α-pregnane configuration (allo-cortolone) but standards were not available to enable confirmation.

β-Cortol was identified from the mass spectrum taken at retention time relative to 5α-cholestan of 1.99. The persilylated β-cortol has the same retention time as the persilylated TMS ether of β-cortolone, however, the FIC chromatograms readily enabled differentiation of these two compounds.

Two isomers of tetrahydrocortisol were identified at retention times relative to 5α-cholestan for the MO-TMS ethers of 1.71 and 2.70. The compounds produced similar mass spectra (Fig. 2). The compound eluted earlier had a molecular ion (M) at m/e 683, and a prominent ion at m/e 652 representing a loss of 31 a.m.u. due to the fragmentation of a methylxime structure. Four hydroxyl groups were indicated by the presence of intense ions at m/e 562 (M-(31 + 90)), m/e 472 (M-(31 + 2 × 90)), m/e 382 (M-(31 + 4 × 90)) representing
consecutive losses of 90 a.m.u. due to fragmentation of the derivatised TMS hydroxyl structures. After comparison against the authentic steroid, the compound eluted at tR = 1.77 was identified as 3α,11β,17α,21-tetrahydroxy-5β-pregn-20-one (THF). The mass spectrum of the other isomer (tR = 2.70) also gave a molecular ion at m/e 683 and an ion at m/e 652 due to the presence of a derivatised carbonyl group. The presence of four derivatised hydroxyl groups was indicated by the ions at m/e 580 (M-103) formed by the loss of a primary silylated hydroxyl structure, m/e 490 (M-(90 + 103)), m/e 400 (M-(2 × 90 + 103)) and m/e 310 (M-(3 × 90 + 103)). The loss of 205 a.m.u. from the molecular ion (cleavage between carbon atoms 17 and 20) giving rise to an intense ion at m/e 478, indicated a silylated 17,20,21-triol structure. The base peak was at m/e 243 and this ion is characteristic of this type of structure, being formed from carbon atoms 15–17, the side chain and two TMS groups. From the mass spectrum this compound was identified as 11β,17α,20α,21-tetrahydroxy-5-pregn-3-one.

Cortisol (11β,17α,21-trihydroxy-4-pregnene-3,20-dione) was identified from both the mass spectrum (Fig. 3) and the FIC chromatograms. The retention time relative to 5α-cholestan for the MO-TMS derivative was 3.47, and this was not completely separated from 3β-cortolone under the gas chromatographic conditions used.

A 20-dihydrocortisol was also indicated from the FIC chromatograms. The retention time relative to 5α-cholestane was 3.73. The mass spectrum (Fig. 3) showed the molecular ion to be at m/e 681. The base peak was at m/e 243 characteristic for silylated 17,20,21-triol structures. This was supported by the presence of an intense ion at m/e 476 (M-205). The presence of four silylated hydroxy groups was indicated by the ions at m/e 578 (M-103), m/e 488 (M-(90 + 103)), m/e 398 (M-(2 × 90 + 103)), and m/e 308 (M-(3 × 90 + 103)), in addition to the intense ion at m/e 296 (M-(2 × 90 + 205)). From the mass spectrum and the retention time this compound was identified as 11β,17α,20β,21-tetrahydroxy-4-pregn-3-one. The FIC chromatograms also indicated the 20α isomer to be present in a very small amount.

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**Fig. 1.**

Partial ion current chromatogram (m/e 200–800, top recording) and fragment ion current chromatograms constructed by the computer for MO-TMS derivatives of steroids from macaque monkey urine, isolated in Sephadex LH-20 fraction 5. M/e values characteristic of the derivatives of isomers of the following steroids were selected: 3,11,17,21-tetrahydroxy-5-pregn-20-one (tetrahydrocortisol), 3,17,20,21-tetrahydroxy-5-pregn-11-one (cortolone), 5-pregnane-3,11,17,20,21-pentol (cortol), 11,17,20,21-tetrahydroxy-5-pregn-3-one, 11,17,21-trihydroxy-4-pregnene-3,20-dione (cortisol), 11,17,20,21-tetrahydroxy-4-pregn-3-one (20-dihydrocortisol). For the purposes of illustration the intensities of the ions were multiplied by factors shown to the right of the m/e values.

541
Mass spectra of the MO-TMS derivatives of 3α,11β,17α,21-tetrahydroxy-5β-pregn-20-one (top panel) and 11β,17α,20ξ,21-tetrahydroxy-5ξ-pregn-3-one (lower panel) isolated in Sephadex L-20 fraction 5 from macaque monkey urine.
Mass spectra of the MO-TMS derivatives of 11β,17α,21-trihydroxy-4-pregnene-3,20-dione (cortisol, top panel) and 11β,17α,20β,21-tetrahydroxy-4-pregnene-3-one (20β-dihydrocortisol, lower panel) isolated in Sephadex LH-20 fraction 5 from macaque monkey urine.
Partial ion current chromatogram (m/e 200–800, top recording) and fragment ion current chromatograms constructed by the computer for MO-TMS derivatives of steroids from macaque monkey urine, isolated in Sephadex LH-20 fraction 6. M/e values characteristic of the derivatives of isomers of 3,11,17,21-tetrahydroxy-5-pregnan-20-one (tetrahydrocortisol) and 5-pregnane-3,11,17,20,21-pentol (cortol) are shown, and for the purpose of illustration the intensities of the ions were multiplied by the factors indicated.

Analysis of the more polar Sephadex LH-20 fraction 6 is illustrated in Fig. 4, in which the FIC chromatograms indicated three cortols and one tetrahydrocortisol structure. Mass spectrum and retention data compared with authentic steroids showed these compounds to be 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one (THF, tR = 1.71), 5β-pregnan-3α,11β,17α,20β,21-pentol (β-cortol (tR = 1.99), 5β-pregnan-3α,11β,17α,20α,21-pentol (α-cortol, tR = 2.24), and 5α-pregnan-3β,11β,17α,20β,21-pentol (3β-cortol, tR = 3.04).

Analysis of baboon urine

Fig. 5 shows the FIC chromatograms of the compounds isolated in Zone 1 from the final paper chromatography in the Bush B5 system. Analysis of this zone indicated an almost identical pattern of excretion of polar steroids to that found in the macaque monkey. Predominant amounts of cortols and corto-
Fig. 5.
Partial ion current chromatogram (m/e 200-800, top recording) and fragment ion current chromatograms of MO-TMS derivatives of the steroids isolated by paper chromatography (Zone 1) from baboon urine. M/e values characteristic of the derivatives of 3,17,20,21-tetrahydroxy-5-pregnan-11-one (cortolone) and 5-pregnane-3,11,17,20,21-pentol (cortol) are shown and the intensities of the ions were multiplied by the factors indicated.

Iones were identified in this fraction. From the mass spectrum, the retention times and the FIC chromatograms the following steroids were identified:

- 3α,17α,20α,21-tetrahydroxy-5β-pregnan-11-one (α-cortolone, t_R = 1.85);
- 3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one (β-cortolone, t_R = 1.99);
- 5β-pregnane-3α,11β,17α,20β,21-pentol (β-cortol, t_R = 1.99);
- 5β-pregnane-3α,11β,17α,20α,21-pentol (α-cortol, t_R = 2.21) and
- 5α-pregnane-3β,11β,17α,20β,21-pentol (3β-cortol, t_R = 3.04).

Quantitatively the major compounds excreted in this zone were the 20β isomers of cortol and cortolone.

Gas chromatographic analysis of the material eluted from Zone 3 in the final Bush B5 paper chromatography system indicated two major compounds with retention times relative to 5α-cholestane of the MO-TMS ethers (persilylated) of 3.27 and 3.45 respectively. The mass spectrum of the two compounds was identical and therefore indicated two isomers of the same compound. The mass...
Fig. 6.

546
spectrum of the persilylated MO-TMS derivative (Fig. 6) indicated a molecular ion at \( m/e \) 607 and a base peak at \( m/e \) 402 (M-205), suggesting the presence of a silylated 17,20,21-triol structure. In addition, the presence of a methyloxime structure was indicated by the ion at \( m/e \) 576 (M-31). Positive identification was difficult on the basis of this spectrum alone, although it did indicate both compounds to be 20-dihydrocortisone. This was later confirmed after the preparation of three further derivatives, the MO-TMS ether (partially silylated), the TMS ether (partially silylated) and the acetonide derivative. The mass spectra of these derivatives are shown in Fig. 6.

Gas chromatography of the MO-TMS (partially silylated) derivative gave two peaks with retention times relative to 5α-cholestane of 4.13 and 4.25 respectively and the mass spectra of both compounds were identical. The mass spectrum of this derivative gave a molecular ion at \( m/e \) 535 and a characteristic fragmentation of the methyloxime resulted in an ion at \( m/e \) 504 (M-31).

The loss of a primary silylated hydroxy group was indicated by the ion at \( m/e \) 432 (M-103). The base peak at \( m/e \) 329 (M-206) is formed by the loss of carbon atoms 20 and 21, the silylated hydroxyls and a hydrogen. This fragmentation also gives rise to the base peak in the corresponding 11-deoxy steroid. The prominent ion at \( m/e \) 116 is frequently seen in 17α,20β,21-triol structures with two TMS groups and an underivatised hydroxyl group (Brooks et al. 1966). This configuration of the side chain is also supported by the presence of the peak at \( m/e \) 402 (M-133) the formation of which is due to migration of the TMS group of C-20 to the C-17 position and loss of carbon atoms 20 and 21 together with one silylated and one underivatised hydroxyl group (Bailie et al. 1972).

Gas chromatography of the TMS derivative showed two distinct peaks of retention times relative to 5α-cholestane of 3.84 and 4.17. The mass spectra taken at both these retention times were similar. The molecular ion was at \( m/e \) 506 and the base peak at \( m/e \) 116. A prominent ion at \( m/e \) 373 is formed by the loss of 133 a.m.u.

Finally gas chromatography of the acetonide derivative of these compounds showed only one peak with a retention time relative to 5α-cholestane of 3.01. It is probable that the two isomers of this derivative were not resolved from each other using the described conditions. The molecular ion of this derivative was at \( m/e \) 402. The base peak, \( m/e \) 301 (M-101) is probably formed by cleavage between C-17 and C-20, and loss of the acetonide structure and this also gives rise to the prominent ion at \( m/e \) 101. In addition, an ion at \( m/e \) 487 (M-15) is formed by the loss of one of the methyl groups in the acetonide structure. Finally the presence of the 3,11-dione structure is indicated by the ion at \( m/e \) 122, which is formed by a simple fission of the C₆-C₇ and C₉-C₁₀ bonds, a fragmentation which is greatly influenced by the type of group in the C-11 position (Shapiro & Djerassi 1964).
On the basis of the evidence it was concluded that the two compounds isolated in this zone by paper chromatography were 17α,20α,21-tri-hydroxy-4-pregnene-3,11-dione and 17α,20β,21-trihydroxy-4-pregnene-3,11-dione respectively, and the ratio of the 20β:20α isomers was 3:1. Preliminary results indicated the excretion of these two steroids to be 10 and 3.4 µg/kg body weight/24 h.

**DISCUSSION**

In previous reports the identification of the major corticosteroid metabolites excreted by the macaque monkey (Setchell & Shackleton 1975) and the baboon (Gontscharow et al. 1972a,b,c) has been described.

In the present study gas chromatography-mass spectrometry was employed and the characterization of additional polar steroids was aided by the preparation of the more suitable persilylated MO-TMS derivatives (Thenot & Horning 1972), together with specific computerised evaluation of the mass spectral data recorded. A summary of the polar corticosteroids identified in the urine from both species and a preliminary semi-quantitative estimation of the amounts excreted is given in Table 1.

In the human the metabolism of cortisol involves (a) reduction of ring ‘A’ to form the tetrahydro derivatives, (b) oxidation of the 11β-hydroxyl group to form the corresponding 11-oxo compounds, (c) reduction of the 20-oxo group to yield the 20α and 20β hydroxy compounds, (d) side chain cleavage to give rise to 11-oxygenated-17-oxosteroids and (e) conjugation to glucuronic acid.

Evidence for all of these reactions occurring in both the macaque monkey and the baboon is apparent from the complex mixture of polar corticosteroids identified in the urine. However, the relative importance of the different pathways differs somewhat from that in the human. In the human the principal metabolites of cortisol are THE and THF formed by ring ‘A’ reduction (Fukushima et al. 1960). In the macaque monkey the levels of THE and THF have been reported to be relatively low: 9–13 µg/kg body weight/24 h (Setchell & Shackleton 1975). Similar figures have also been reported for the rhesus monkey (Macaca mulatta) and the baboon (Gontscharow et al. 1972a).

In both the macaque monkey and the baboon the 20β-reduction is very efficient resulting in the formation of considerable amounts of β-cortol, β-cortolone and 20β-dihydro metabolites. From this study the ratio of 20β:20α metabolites was of the order of 10:1 for the macaque monkey and 4:1 for the baboon while for the human this ratio is approximately 0.81:1.00 (Setchell et al., in prep). There appears to be no previous reports of the identification of cortol, cortolone and 20-dihydrocortisone in baboon, but preliminary results from this study indicate these metabolites to be excreted in significant amounts.
Table 1.
A summary of polar corticosteroids excreted in the urine of baboon and macaque monkeys.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>t_R</th>
<th>Baboon (Papio hamadryas)</th>
<th>Macaque (Macaca fascicularis)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Isolated</td>
<td>Isolated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg/kg body wt/24 h</td>
<td>µg/kg body wt/24 h</td>
</tr>
<tr>
<td>3α,17α,21-tri-hydroxy-5β-pregnane-11,20-dione (THE)</td>
<td>-</td>
<td>(a)</td>
<td>5.4</td>
</tr>
<tr>
<td>3α,11β,17α,21-tetrahydroxy-5β-pregnane-20-one (THF)</td>
<td>1.71</td>
<td>(a)</td>
<td>4.7</td>
</tr>
<tr>
<td>3α,11β,17α,21-tetrahydroxy-5α-pregnane-20-one (allo-THF)</td>
<td></td>
<td>(a)</td>
<td>(c)</td>
</tr>
<tr>
<td>11β,17α,20α,21-tetrahydroxy-5α-pregnane-3-one</td>
<td>2.65</td>
<td>-</td>
<td>Not identified</td>
</tr>
<tr>
<td>11β,17α,20β,21-tetrahydroxy-4-pregnene-3,11-dione (20β-DHE)</td>
<td>3.55</td>
<td>(b)</td>
<td>(2.2)</td>
</tr>
<tr>
<td>17α,20β,21-tri-hydroxy-4-pregnene-3,11-dione (20α-DHE)</td>
<td>3.27</td>
<td>P. C. Zone 3</td>
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<tr>
<td>17α,20α,21-tri-hydroxy-4-pregnene-3,11-dione (20α-DHE)</td>
<td>3.45</td>
<td>P. C. Zone 3</td>
<td>3.4</td>
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<tr>
<td>3α,17α,20α,21-tetrahydroxy-5β-pregnane-11-one (α-cortolone)</td>
<td>1.81</td>
<td>P. C. Zone 1</td>
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<td>3α,17α,20β,21-tetrahydroxy-5β-pregnane-11-one (β-cortolone)</td>
<td>1.99</td>
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<td>3α,17α,20α,21-tetrahydroxy-5α-pregnane-11-one (cortolone)</td>
<td>NA</td>
<td>-</td>
<td>Not identified</td>
</tr>
<tr>
<td>3β,17α,20α,21-tetrahydroxy-5α-pregnane-11-one (3β-cortolone)</td>
<td>NA</td>
<td>-</td>
<td>Not identified</td>
</tr>
<tr>
<td>5β-pregnane-3α,11β,17α,20α,21-pentol (α-cortol)</td>
<td>2.24</td>
<td>P. C. Zone 1</td>
<td>1.0</td>
</tr>
<tr>
<td>5β-pregnane-3α,11β,17α,20β,21-pentol (β-cortol)</td>
<td>1.99</td>
<td>P. C. Zone 1</td>
<td>4.0</td>
</tr>
<tr>
<td>5α-pregnane-3β,11β,17α,20β,21-pentol (3β-cortol)</td>
<td>3.08</td>
<td>P. C. Zone 1</td>
<td>0.5</td>
</tr>
<tr>
<td>11β,17α,21-tri-hydroxy-4-pregnene-3,20-dione (cortisol)</td>
<td>3.47</td>
<td>(a)</td>
<td>(1.0)</td>
</tr>
</tbody>
</table>

(a) Values taken from Gontscharow et al. (1972a)
(b) Values taken from Gontscharow et al. (1972b)
(c) Value taken from Setchell & Shackleton (1975)
NA – Reference steroid not available
* Retention time of authentic steroid MO-TMS derivative relative to 5α-cholestane at 230°C isothermal on SE-30 (1.5%).
In both the macaque monkey and the baboon the formation of $3\beta$-hydroxy-5a-pregnane metabolites is in distinct contrast to the lack of such metabolites in the human.

In conclusion, the pattern of excretion of polar corticosteroids in the baboon and macaque monkey is very similar, but differs significantly from the pattern of excretion for the human.

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