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PROGESTERONE AND OESTROGEN METABOLISM IN THE PREGNANT MARMOSET (*CALLITHRIX JACCHUS*)

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SUMMARY

The steroid metabolites excreted by two marmosets in the third trimester of pregnancy have been studied by gas chromatography-mass spectrometry. It was found that pregnancy in the marmoset is associated with a relatively high excretion of progesterone and oestrogen metabolites, a situation not found in many other primate species.

The major progesterone metabolites were identified as 3α,6β-dihydroxy-5α-pregnan-20-one (about 500 μg/24 h) and 3α,16α-dihydroxy-5α-pregnan-20-one (about 100 μg/24 h) although small amounts of pregnanediols and pregnanetriols were also excreted.

Oestradiol (about 300 μg/24 h) was the major oestrogen identified accounting for about 75% of the total oestrogen fraction. Oestrone, 16α-hydroxyoestrone and oestriol were also identified.

INTRODUCTION

The search for an animal model suitable for the study of foetal-placental steroid production continues, and non-human primates are becoming increasingly important in these studies. Some of the recent investigations are well reviewed in a World Health Organization publication [1].

Previous investigations have described the steroid metabolism in pregnant apes [2–4], baboons [5–8] and various species of macaque monkeys [8–14], but to the author's knowledge little is known of the pregnancy steroids of New World monkeys.

A species of New World monkey, the marmoset, is being increasingly used in medical research because of their small size and their quick adaptation to living and breeding in captivity. This study describes an investigation into the excretion of progesterone and oestrogen metabolites during pregnancy by two animals of this species.

EXPERIMENTAL

Twenty-four hour urine samples were collected from two pregnant female marmosets (14 and 16 weeks gestation respectively) housed in metabolism cages. The urine samples were diluted to 25 ml with water and brought to pH 4·5 with 2·5 ml 5 M acetate buffer. The steroid conjugates were hydrolysed for 48 h by enzymes present in the digestive juice of the snail (*Helix pomatia*). The freed steroids were extracted by Amberlite XAD-2 resin [15] and separated into groups by Sephadex LH-20 chromatography [16]. Silyl ethers* and oxime–silyl ethers were prepared of the steroid fractions [17], which were then analysed by gas-chromatography and combined gas chromatography-mass spectrometry.

Semi-quantitation of the major steroids was achieved by addition of cholesteryl butyrate as internal standard to the steroid fractions prior to the formation of steroid derivatives.

RESULTS

The gas chromatograms illustrated in Fig. 1 clearly show the difference between the steroid profiles of non-pregnant and pregnant female animals, the excretion of steroids by the non-pregnant animals being very low. The major steroids identified are indicated, but a full list of all those characterized in the individual fractions from Sephadex chromatography, with the approximate daily excretion rate, is given in Table 1.

The identification of the urinary steroids was based on the similarity of their gas chromatographic retention volumes (methylene units, MU) [18] and mass spectra compared with those of authentic reference steroids. It would be impractical to publish the mass spectra of all the steroids identified. All those listed in Table 1 give fragmentation patterns virtually indis-

* *Trivial names and abbreviations used*: **DHA**, 3β-hydroxy-5-androsten-17-one; **16α-hydroxy DHA**, 3β,16α-dihydroxy-5-androsten-17-one; **pregnanediol**, 5β-pregnan-3α,20α-diol; 6β-hydroxypregnenolone, 3α,6β-dihydroxy-5α-pregnen-20-one; **silyl ether**, trimethylsilyl ether; **oxime-silyl ether** (MO-TMS), 0-methyloxime-trimethylsilyl ether.

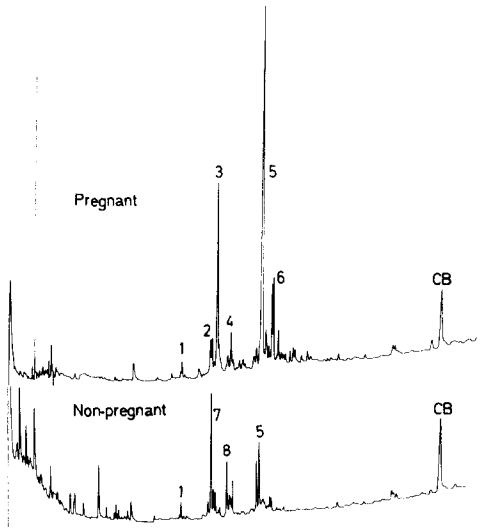

Fig. 1. Gas chromatographic separation of the steroids (as silyl and oximesilyl ethers) excreted by pregnant and non-pregnant marmosets. The following steroids are indicated: **1**, androsterone; **2**, oestrone and 11-oxo-androsterone; **3**, oestradiol; **4**, 16α-hydroxyoestrone and 11β-hydroxyandrosterone; **5**, 6β-hydroxypregnanolone; **6**, 16α-hydroxypregnanolone; **7**, 11-oxo-androsterone; **8**, 11β-hydroxyandrosterone. The internal standard used was cholesteryl butyrate (CB).

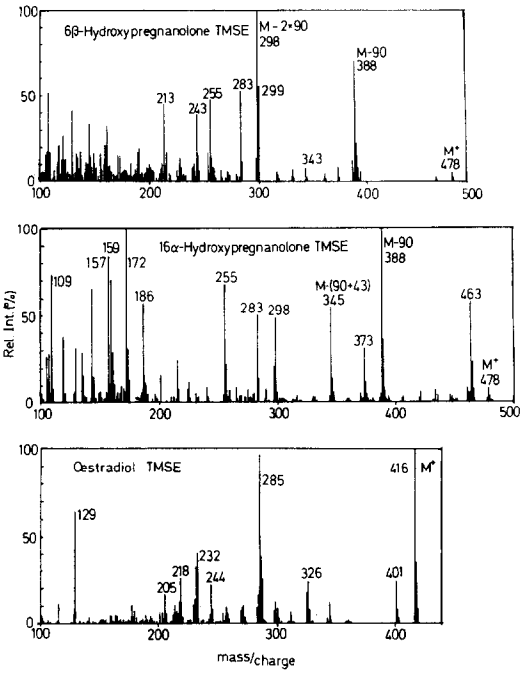

Fig. 2. Mass spectra of the silyl ethers of the three principal steroids excreted by pregnant marmosets, 6β-hydroxypregnanolone, 16α-hydroxy-pregnanolones and oestradiol.

tinguishable from reference compounds. However, three steroids account for about 60% of the total daily steroid excretion and because of their importance a detailed discussion of their identity is presented.

6β-Hydroxypregnanolone

The mass spectrum of the silyl ether of urinary 6β-hydroxypregnanolone is illustrated in Fig. 2. The parent ion is at *m/e* 478 and the base peak at *m/e* 298 [M-(2 × 90)]. Prominent peaks are also seen at *m/e* 299 [M-(90 + 89)] and 255 [M-(2 × 90)] + 43]. The loss of 43 mass units is due to the fragmentation of the side chain.

Because of similarity of the mass spectra of the epimeric 6-hydroxypregnanolones, identification had to be made by retention volumes on gas chromatography.

Table 1. The steroids identified in urine from two pregnant marmosets (14 and 16 weeks gestation respectively) and their approximate quantitative importance* (μg/24 h)

Steroid	Marmoset 1	Marmoset 2	Steroid	Marmoset 1	Marmoset 2
Androsterone	20	25	6β-Hydroxypregnanolone	560	520
11-Oxo-androsterone	45	55	16α-Hydroxypregnanolone	150	110
11β-Hydroxyandrosterone	30	25	Pregnane-3,16,20-triol†	35	45
			Pregnane-3,17,20-triol†	45	40
			Pregnane-2,3,20-triol†	30	35
Oestrone	50	40	Cortisone	45	25
Oestradiol	410	160	Tetrahydrocortisone	40	30
16α-Hydroxyoestrone	18	16	17α,21-Dihydroxypregnane-3,11,20		
Oestriol	9	6	trione	‡	‡
			17α,20,21-Trihydroxy-4-pregnene-3,11,dione†	85	55
			17α,20,21-Trihydroxypregnane-3,11-dione†	70	45
			α-Cortolone	55	45
			β-Cortolone	60	40

* The figures have not been corrected for losses occurring during analysis.

† The stereochemical configuration of one of the functional group in these steroids was not ascertained.

‡ Identified only, not measured.

The retention volumes for the major urinary steroid (TMSE 27·26 MU; MO-TMSE 28·00 MU) were less than any of the 6α-hydroxypregnanolone reference steroids indicating 6β-hydroxylation for the urinary steroid. The retention volumes of the authentic 3α,6β-dihydroxy-5β-pregnan-20-one derivative (TMSE 27·63 MU; MO-TMSE 28·32 MU) were greater than those of the major urinary steroid although a small amount of this epimer is present in urine. From data of the retention volumes of pregnanolones it may be deduced that the 3α-hydroxy-5α epimer would have a shorter retention time than the 3α-hydroxy-5β epimer and the two 3β-hydroxy epimers would have longer retention times. It was therefore considered that the 6β-hydroxypregnanolone had the structure 3α,6β-dihydroxy-5α-pregnan-20-one.

16α-Hydroxypregnanolone

The mass spectrum of the silyl ether of urinary 16α-hydroxy-pregnanolone is also illustrated in Fig. 2. The parent ion is at *m/e* 478 and important peaks are seen at *m/e* 388 (M-90) and *m/e* 298 [M-(2 × 90)]. The fragments at *m/e* 345 [M-(90 × 43)] and *m/e* 255 [M-(2 × 90 × 43)] are due to the loss of silyl groups and the side chain. The peaks at *m/e* 109, 157, 159, 172 and 186 are specific for 16-hydroxylated steroids with unsubstituted side chain [19]. The retention volumes of the derivatives of the urinary steroid (TMSE 27·80 MU; MO-TMSE 20·30 MU) were identical to those found for authentic 3α,16α-dihydroxy-5α-pregnan-20-one (TMSE 27·75 MU; MO-TMSE 28·32 MU).

Oestradiol

The lower diagram in Fig. 2 illustrates the mass spectrum of urinary oestradiol trimethylsilyl ether. The parent ion and base peak are at *m/e* 416 and strong peaks are seen at *m/e* 285 (M-127) and *m/e* 129. These fragments are probably formed by loss of the D-ring containing the trimethylsilyl group.

DISCUSSION

About 75% of the oestrogen compounds excreted by marmosets in the third trimester of pregnancy is accounted for as oestradiol. Oestrone, oestriol and 16α-hydroxyoestrone were also identified but were present in urine in low concentration. In most other species of monkeys where pregnancy oestrogens have been investigated, oestrone or oestriol are the dominant oestrogens [2, 4, 5, 11]. In the higher apes, oestriol is most important [4], in the old world monkeys (macaques and baboons), oestrone is dominant [2, 5, 11] and it now seems probable that in the New World monkeys oestradiol is largely excreted unmetabolized. Oestrogen synthesis in the higher primates is carried out by the foetal–placental unit, the foetus supplying essential neutral steroid precursors (DHA and 16α-hydroxy-DHA) for the placental synthesis of oestriol [20, 21, 22] but it is not known whether a foetal-placental unit is functional in New World monkeys. It is possible that the foetal marmoset does provide DHA and 16α-hydroxy-DHA for conversion to the oestradiol, 16α-hydroxy oestrone and oestriol which appear in the maternal urine, but no proof is available. However, as in man, large amounts of oestrogen are produced during pregnancy which is not the case for many old world monkeys [2, 4, 5, 10]. Therefore, both in human and marmoset pregnancy there appears to be a requirement for a high production of oestrogen.

The major metabolite of progesterone found in the urine of the pregnant marmoset was identified as 6β-hydroxypregnanolone although 16α-hydroxypregnanolone was also quantitatively important. It is of interest that 6- and 16-hydroxylation are important metabolic transformations undergone by progesterone during human pregnancy [23, 24] even though pregnanediol is the major metabolite.

Although the excretion rates of C_{21} steroid metabolites of progesterone by pregnant women, apes [5, 6] and the marmoset are high, species of macaque monkey (e.g. Rhesus monkey) have a very different metabolism of progesterone, C_{19} steroids being the major metabolites [25, 26], although urinary pregnanediol has been found [9, 12]. The metabolism of progesterone by baboons appears to be intermediate between the macaque monkeys and the higher primates, since pregnanediol and androsterone are both important [8]. In all these species only low levels of progesterone metabolites are found in the urine.

It was of particular interest that the levels of urinary progesterone and oestrogen metabolites in the pregnant marmoset were of similar order of magnitude to man (when related to body weight) since this has also been found to be true for the levels during the reproductive cycle [27]. Marmosets are the only species of monkeys so far investigated which have plasma sex steroid levels similar to man in the non-pregnant state.

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