Urinary Excretion of Progesterone Metabolites in Pregnant Rhesus Monkeys[1] (36312)

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The excretion of progesterone metabolites in the urine of pregnant monkeys has not been extensively studied. Moreover, the limited data, which are available, are conflicting. Early studies failed to detect 5β-pregnane-3α,20α-diol, one of the major urinary metabolites of progesterone in pregnant women (1, 2), while more recently this metabolite was identified in the urine of both pregnant and progesterone-treated rhesus monkeys (3, 4). Further studies indicated that, in contrast to humans, androsterone rather than pregnanediol was the major urinary metabolite of progesterone-injected *Macaca mulatta* and *Macaca nemestrina* (5, 6).

Although the primary purpose of this study was to determine if pregnanediol was present in the urine of pregnant rhesus monkeys and if a specific excretion pattern existed during gestation, pregnant monkey urine samples were also qualitatively analyzed and compared to the urine of progesterone-treated animals.

Materials and Methods. Eight mature female *Macaca mulatta* with uncomplicated pregnancies were used in this study. The animals were housed, fed, and the urine was collected during the last 2 months of pregnancy as previously described (7). To support the identification of progesterone metabolites, two ovariectomized animals were injected with progesterone (5 mg/kg im) for 7 consecutive days; urine was collected daily, before, during, and after treatment and analyzed along with the pregnancy samples.

Aliquots (50 ml) of urine samples were enzymatically hydrolyzed employing Ketodase (Warner Chilcott, Laboratories) as recommended by Cawley *et al.* (8), and then extracted with ethyl ether (3 $\times$ 75 ml). The pooled urine extracts were evaporated under reduced pressure after being washed with a concentrated solution of $NaHCO_3$ (3 $\times$ 50 ml) and distilled water (3 $\times$ 50 ml). The dry residues were then dissolved in redistilled toluene (50 ml) and extracted with 1 *N* NaOH (3 $\times$ 20 ml) to remove the phenolic fraction. The toluene phase, containing the nonphenolic fraction, was washed with distilled water (3 $\times$ 20 ml) and evaporated under reduced pressure. Ethanol (10 ml) was added and then reevaporated to remove any traces of water. The dry extracts were taken up in 15 ml of ethanol, divided into aliquots and evaporated under nitrogen at 56°. These aliquots were used for: qualitative thin-layer chromatography (TLC), gas liquid chromatography (glc), and TLC followed by elution and glc of the eluates.

Urine samples, water blanks, and aliquots of these, containing added quantities of 5β-pregnane-3α,20α-diol (Mann Research Laboratories), were treated in the same manner throughout the procedure.

Extracts and standards, dissolved in ethanol, were spotted on thin-layer plates coated with Adsorbosil P_1 or P_2 (Applied

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[2] The following trivial names are used: androsterone (3α-hydroxy-5α-androstan-17-one), DHEA or dehydroepiandrosterone (3β-hydroxy-5-androsten-17β-ol), etiocholanolone (3α-hydroxy-5β-androstan-17-one), pregnanediol (5β-pregnan-3α,20α-diol), and pregnanolone (3α-hydroxy-5β-pregnan-20-one).

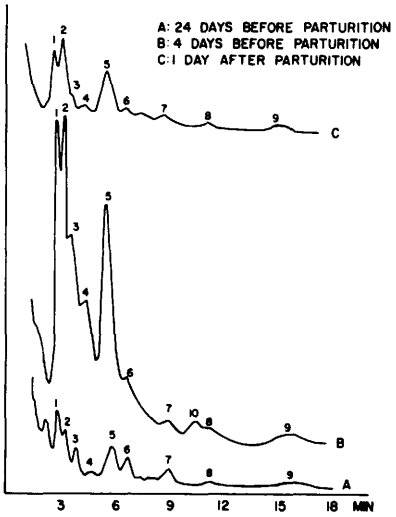

FIG. 1. Gas chromatograms of the TMSi derivatives of the urinary nonphenolic fraction during pregnancy and postpartum: (A, B, and C) 1/100 of 24 hr urine samples. (1) pregnanediol; (2) androsterone; (3) etiocholanolone; (4) DHEA; (5) pregnanolone; (6) 5α-pregnane-3β-ol,20-one; (7, 8, 9, and 10) not identified. Column: Anakrom ABS + 3% XE 60; temp, 215°; argon pressure, 32 lb/cm².

Science Laboratories) or on silica gel F-254 precoated plates (E. Merck, AG) which were activated at 100°. The solvent systems used were: chloroform–acetone (9:1, v/v) and chloroform–ethyl acetate (8:2, v/v) (9).

The following detecting systems were employed sequentially using the same plate: UV light, phosphoric acid reagent spray, followed by phosphomolybdic acid. The spotting, as well as the detecting procedures, have been previously described (7).

The TLC and elution system described by Brooks *et al.* (10), employing the sylil-derivatives of steroids and chloroform as solvent, was used for elution and further glc quantification.

A Barber Colman Model 10 gas chromatograph equipped with a Sr-90 argon ionization detector and glass U-shaped columns (6 ft long × 5 mm i.d.) was used for glc. The metabolites of progesterone were qualitatively analyzed as free steroids on columns packed with Gas Chrom Q coated with 3% QF_1 (Applied Science Laboratories) (Gas Chrom Newsletter, 1964), and as trimethylsilyl (TMSi) derivatives on Anakrom ABS coated with 3% XE 60 (Applied Science Laboratories) (11, 12), or on Gas Chrom Z coated with 2% XE 60 + 1% SE 30 (13).

Quantitative studies were performed using Anakrom ABS + 3% XE 60 columns. The pregnanediol concentration was calculated by triangulation and comparison with pregnanediol standard curves (area vs μg), and then corrected for the proper dilution factors. Recoveries throughout the different steps of the procedure were: hydrolysis and extraction 94 ± 0.7%, TLC elution 82 ± 4% and 77.5 ± 2.8% for the entire procedure.

Results. Ten different steroids were detected in the urinary nonphenolic fraction of pregnant monkeys by both TLC and glc. The concentration of these steroids increased throughout the last month of pregnancy, started to decrease close to parturition, and on the day following delivery the levels were essentially the same as those found in early pregnancy (Fig. 1). The peak area of some of these steroids increased significantly during gestation, while in others only moderate increases were observed.

The parallel study, in which the urine of progesterone-treated ovariectomized animals was analyzed along with the pregnancy samples, showed that the TLC and glc data were analogous in both cases (Fig. 2). The only exception was the absence of one compound (peak 5) in the urine of the progesterone-treated animals.

A partial attempt to identify the progesterone metabolites was made. The combined chromatographic data distinctly revealed that one of the 10 steroids had the same migration (r_F) and color characteristics of authentic 5β-pregnane-3α,20α-diol in each of the different TLC systems, as well as identical retention time (*Rt*) in glc. Other compounds chromatographically behaved as androsterone, etiocholanolone, DHEA, 5β-pregnane-3α-ol,20-one and 5α-pregnane-3β-ol,20-one (Fig. 1) when compared with the authentic steroids.

Quantitative analysis revealed that the daily excretion of pregnanediol increased during the last 6 weeks of pregnancy, declined moderately 72 to 24 hr before parturition, fol-

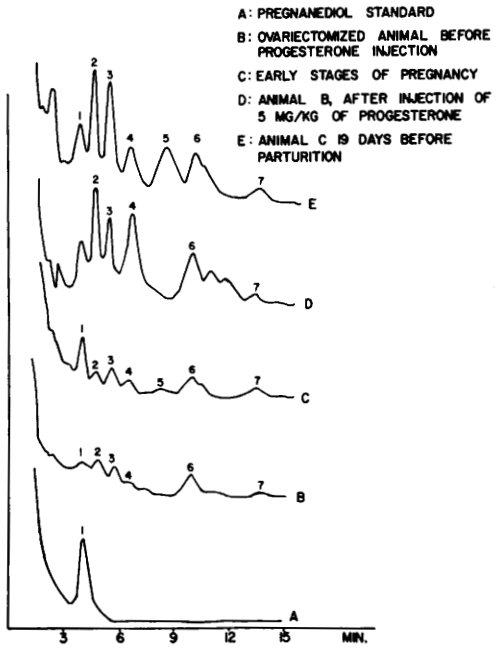

FIG. 2. Gas chromatograms of the urinary nonphenolic fractions of pregnant and progesterone-treated monkeys: Each chromatogram represents 1/100 of 24 hr samples. Peak 1 has the *Rt* of authentic pregnanediol; peaks 8, 9, and 10 are not shown. Column: Anakrom ABS + 3% XE 60; temp, 207°; argon pressure, 32 lb/cm².

lowed by a sudden decrease on the day of delivery and remained low thereafter (Fig. 3).

5β-Pregnane-3α,20α-diol, however, did not represent the most important progesterone metabolite (Figs. 1 and 2) but it had an excretion curve which paralleled most of the other metabolites. Although pregnanediol levels were different among the eight monkeys and the peak values close to parturition varied from 100 to 900 μg/24 hr, the same excretion profiles were seen in all animals.

Discussion. In contrast to some previous reports (1, 2, 14), but in agreement with more recent studies (3, 4), the results of this study revealed that 5β-pregnane-3α,20α-diol is present in the urine of both pregnant and progesterone-treated *Macaca mulatta*.

The urinary levels of pregnanediol and other progesterone metabolites increased during the last weeks of pregnancy and in those animals injected daily with progesterone. In addition to pregnanediol, four of these metabolites were identified as androsterone, etiocholanolone, DHEA, and 5β-pregnane-3α-ol, 20-one, which were also detected in pregnant monkey urine by Chamberlain *et al.* (3). A sixth metabolite, 5α-pregnane-3β-ol,20-one, was also identified by Ainsworth *et al.* (15) in *in vitro* studies.

The low urinary pregnanediol levels probably reflect the low concentration of progesterone in peripheral blood (16) and in the placenta (14). Furthermore, the pregnanediol excretion pattern during the last weeks of pregnancy was similar to the circulating pro-

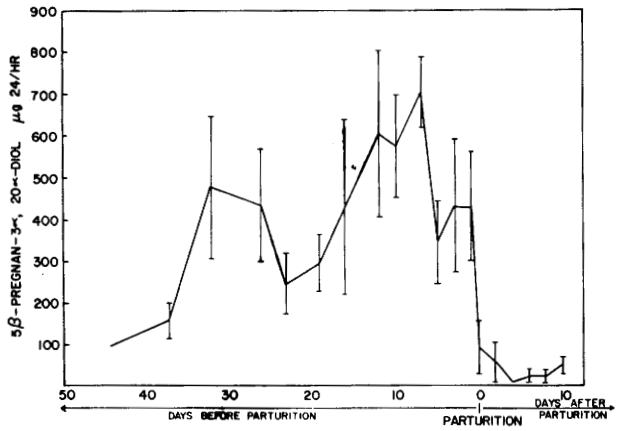

FIG. 3. Daily urinary pregnanediol levels during pregnancy and postpartum (mean values of eight animals ±SE).

gesterone pattern; both were moderately elevated toward the end of pregnancy and decreased suddenly to nonpregnant values on the day of, or following, delivery (16). The pregnanediol excretion, however, started to decine gradually 72 to 24 hr prior to parturition.

In contrast to humans, pregnanediol did not quantitatively represent one of the major urinary progesterone metabolites. Irrespective of the stage of pregnancy, other metabolites were excreted in equal or higher amounts.

It has been reported that some subhuman primates (pig-tailed monkey and baboon) excreted androsterone as the major urinary metabolite of progesterone (5, 6, 17). Our study demonstrated that androsterone was also an important progesterone metabolite in the urine of rhesus monkeys (Figs. 1 and 2).

Comparison of the available data regarding urinary pregnanediol levels in pregnant subhuman primates indicates that the excretion in the rhesus monkey is similar to that of the baboon (18) and the pig-tailed monkey (Liskowski, unpublished information); but is significantly lower than that of the pregnant chimpanzee (19). In contrast to these species, it is of interest that neither 5β-pregnane-3α,20α-diol nor androsterone can be detected in pregnant squirrel monkey (*Saimiri sciureus*) urine (Liskowski, unpublished information).

Summary. Ten different steroids were detected in the nonphenolic fraction of pregnant monkey urine. Of these 5β-pregnane-3α-20α-diol, androsterone, DHEA, etiocholanolone, 5β-pregnane-3α-ol,20-one, and 5α-pregnane-3β-ol,20-one were identified.

Corroborating evidence that these steroids represent urinary metabolites of progesterone was obtained by analyzing the urine of ovariectomized progesterone-treated rhesus. Both pregnant and injected animals excreted the same metabolites with the exception of one, which was present only in pregnant monkey urine.

The excretion of 8 of the 10 steroids increased during the last weeks of pregnancy; however, at no time did 5β-pregnane-3α, 20α-diol represent the major progesterone metabolite. Although the individual pregnanediol levels in eight monkeys were different, the peak value in each monkey was reached 72 to 24 hr before parturition (100 to 900 μg/24 hr), then decreased to trace or nondetectable amounts on the day of parturition, and remained low thereafter.

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