STEROIDS IN PRIMATES: EXCRETION OF 3β-HYDROXY-Δ^5 STEROIDS BY A NEWBORN CHIMPANZEE (*PAN TROGLODYTES*)

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1. Introduction

The human newborn, compared with adults, excretes relatively large amounts of 3β-hydroxy-Δ^5 steroids [1] and it is probable that these are formed as a result of a relative inactivity of 3β-hydroxysteroid oxidoreductase enzymes in the adrenal glands of the foetus and newborn. The major steroids contain 16α- and 16β-hydroxyl groups due to the presence of an active 16-hydroxylase system and the most important ones are 3β,16α (and 16β)-dihydroxy-5-androsten-17-one, 3β,17β-dihydroxy-5-androsten-16-one, 5-androstene-3β,16α,17β-triol and 3β,16α-dihydroxy-5-pregnen-20-one [1–5].

The present investigation of steroids in the newborn chimpanzee was undertaken in an endeavour to find an animal species which had a urinary steroid pattern similar to that of the human in the perinatal period. Lanman in 1957 [6] reported that there were many similartities in the morphology of the foetal and infant adrenal glands of the human and chimpanzee, particularly as they both have a large foetal zone which degenerates in the latter part of pregnancy and after birth. In the human, this zone is probably largely responsible for the production of the 3β-hydroxy-Δ^5 steroids, which decreases as the zone degenerates during the weeks after birth [2, 7]. It was considered that the structural similarities in the adrenal glands of human and other primates, particularly chimpanzees, may result in a similar pattern of steroid excretion by newborn infants of the species.

2. Material and methods

The sources of reference steroids used during this study have been reported previously [4]. 7α-^{3}H-3β-Hydroxy-5-androsten-17-one sulphate was obtained from the Radiochemical Centre, Amersham, England. Amberlite XAD-2 and Amberlyst A-26 were obtained from British Drug Houses Ltd., Poole, Dorset, England. Sephadex LH-20 was obtained from AB Pharmacia, Uppsala, Sweden.

Urine samples were collected from an infant chimpanzee during periods of four hr on the 11th, 12th and 13th days after birth. The pooled volume of urine was 18 ml. Following addition of labelled 3β-hydroxy-5-androsten-17-one sulphate, steroids were extracted by a method described previously [8] using a column of Amberlite XAD-2 resin (10 g). The steroids were adsorbed by the resin and were removed by elution with methanol. The methanol extract was dried and chromatographed on a Sephadex LH-20 column (10 g) using methanol–chloroform (1:1, made 0.01 M with respect to sodium chloride) as the eluant. Fractions of volume 2.5 ml were collected and a small portion of each fraction was removed for measurement of radioactivity. 7α-^{3}β-Hydroxy-5-androsten-17-one sulphate appeared in 80–110 ml of effluent and the fractions between 60 and 220 ml were pooled to form a "monosulphate" fraction. After evaporation of the solvent the residue was dissolved in 2 ml of 0.5 M acetate buffer (pH 4.5) and steroid conjugates were

hydrolyzed by sulphatases and glucuronidases from the snail *Helix pomatia*. The liberated steroids were extracted with Amberlite XAD-2 resin and acidic impurities were removed by passing the methanol eluate through the anion exchanger Amberlyst A-26 in the bicarbonate form.

The methanol extract was dried, trimethylsilyl ethers were prepared [9] and the samples were analyzed by gas chromatography and combined gas chromatography–mass spectrometry. QF-1 and SE-30 stationary phases were used for the analyses, and retention times were determined relative to 5α-cholestane (t_R).

A semi-quantitative estimation of some of the major steroids in the infant chimpanzee urine was carried out by the addition of an internal standard, 5β-cholane-3α, 24-diol, to the samples prior to formation of trimethylsilyl ethers.

3. Results and discussion

The gas-chromatographic separation of steroids (as trimethylsilyl ethers) present in the "monosulphate" fraction of infant chimpanzee urine is illustrated in fig. 1. A steroid was considered identified when it had the same retention times and mass spectrum (as trimethylsilyl ether) as the reference compound. The steroids so identified are listed in table 1 together with their relative retention times and approximate rate of excretion (μg/24 hr, calculation based on a 12 hr collection period). Mass spectra of the steroids identified have been published previously following investigations into steroid excretion by human infants.

The four major steroids identified were 3β,16α-dihydroxy-5-androsten-17-one, 3β,17β-dihydroxy-5-androsten-16-one, 5-androstene-3β,16α,17β-triol and 3β,16α-dihydroxy-5-pregnen-20-one and it is significant that these are also four of the principal steroids excreted by newborn humans. Other similarities were noted between the excretion of steroids by human and chimpanzee infants. Newborn humans excrete little 3β-hydroxy-5-androsten-17-one and this was also shown to be true for the chimpanzee. Before excretion, this compound is probably extensively metabolized

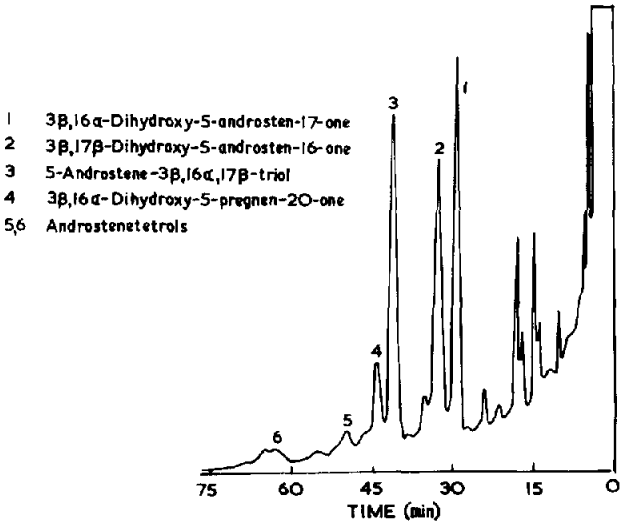

Fig. 1. Gas chromatographic separation of trimethylsilyl ethers of steroids in the "monosulphate" fraction of infant chimpanzee urine. Column: 3% SE-30 on Gas-Chrom Q at 225°.

Table 1

Comparison between the relative retention times of trimethylsilyl ethers of steroids isolated from the "monosulphate" fraction of infant chimpanzee urine and the corresponding reference compounds. The approximate excretion of these steroids (μg/24 hr) is also given.

Steroid	SE-30, 225°		QF-1, 210°		μg/24 hr (approx.)
	Reference	Urinary	Reference	Urinary	
3β,16α-Dihydroxy-5-androsten-17-one	0.84	0.83	1.46	1.49	240
3β,17β-Dihydroxy-5-androsten-16-one	0.94	0.93	1.88	1.88	190
5-Androstene-3β,16α,17β-triol	1.24	1.21	1.04	1.01	300
3β,16α-Dihydroxy-5-pregnen-20-one	1.30	1.29	2.16	2.20	60

by 16-hydroxylation. Two androstenetetrols were detected in the urine from the newborn chimpanzee and although their structures are still uncertain, the mass spectra and t_R:s indicate that they are identical to two of the tetrols detected in urine from newborn infants [5, 10].

It is evident that 16-hydroxylation is as prominent in the steroid metabolism of the newborn chimpanzee as in that of the newborn human. Humans, gorillas [11] and chimpanzees [12] all excrete oestriol as the predominant oestrogen during pregnancy. In contrast, oestriol is not as important a urinary steroid in Rhesus monkeys [13] and no 16-hydroxylated steroids have been isolated from foetal tissues following injection of radioactive progesterone into the foeto-placental unit of such monkeys [14]. It has recently been shown by Heinrichs and Colás [15] that 3β-hydroxy-5-androsten-17-one is 16-hydroxylated to a greater extent by liver microsomes from adult from newborn or foetal Rhesus monkeys (*Macaca mulatta*). Recent studies indicate that 5-androstene-3β,16α,17β-triol and androstene-3,16,17-triols are important steroids excreted during pregnancy by *Macaca irus* (unpublished observation) but in view of the findings by Heinrichs and Colás these steroids may be of maternal rather than foetal origin.

There is thus evidence that the steroid metabolism in the greater and lesser primates are quite different in the perinatal period. The results of the present investigation suggest that the formation of steroids by the newborn chimpanzee is similar to that of the human and that this species may be use as a model for the study of steroid biosynthesis and metabolism by the human newborn and foeto-placenta unit.

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