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ANDROGEN METABOLISM IN THE BABOON: A COMPARISON WITH THE HUMAN*

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SUMMARY

To study the metabolism of androgens in the baboon, *Papio cyanocephalus*, and for a comparison with that in a human, a mixture of [^{3}H]-testosterone (T) and [^{14}C]-androst-4-ene-3,17-dione (A) was injected intravenously into (1) a male and a female baboon with biliary (cannulated) fistulas and catheterized for collection of urine, and (2) a normal 59 year old man. The rates and patterns of excretion of both labels were almost identical in baboon and human urine. The latter fluid contained 60% of the administered dose with an additional 20% of the label appearing in baboon bile. The major conjugate types in the urine after fractionation by DEAE-Sephadex A-25 column chromatography were glucosiduronates and sulfates, with a small percent (<2%) being unconjugated metabolites. Minor amounts of sulfo-glucosiduronates and disulfates were also present in the bile. In baboon urine and bile, glucosiduronates, sulfates and diconjugates, respectively, represented 82–89%, 4–10% and 2–3% of the labeled compounds. The urinary conjugate pattern of the human resembled that of the baboon. The aglycones of the glucuronide fractions were separated by Lipidex 5000 column-, thin layer- and paper chromatography and identified by co-crystallization with standard compounds. The following compounds were identified: androsterone, etiocholanolone, T, epi-testosterone (epi-T), 5α-androstane-3α,17β-diol, 5α-androstane-3α,17α-diol and 5β-androstane-3α,17α-diol. Other polar compounds were not identified. In urine of both baboons, androsterone predominated (both labels) with 5β-androstane-3α,17β-diol being second highest. In bile, the [^{3}H]-labeled latter compound predominated; androsterone was the predominant [^{14}C]-compound. In human urine, etiocholanolone predominated (both labels) with androsterone being second highest (both labels). Thus, with minor exceptions, the excretion, conjugation and quantitative appearance of aglycone metabolites after administration of androgens were similar in baboon and man.

INTRODUCTION

Recent determinations of the endogenous levels and nature of androgens in the blood of human, baboon and Rhesus monkey point to the close resemblance of these parameters in the human and these two primates[1, 2]. These reports and the fact that androgen metabolism has not been extensively studied in non-human primates, prompted us to study the urinary and biliary excretory patterns and androgen metabolism in the baboon, *Papio cyanocephalus*.

In the present work, we injected i.v. a mixture of [^{3}H]-T and [^{14}C]-A into a male and female baboon, from which bile and urine were collected; and into a normal human male from whom urine was obtained. The excretion, conjugation and aglycone metabolites of the two androgens injected were analyzed in these samples.

MATERIALS AND METHODS

The experimental and analytical methods were carried out according to the methods previously reported[3]; they are briefly described here. Radioactive T-1β,2β-[^{3}H] (S.A. = 40 Ci/mM, 138 mCi/mg) and A-[4-^{14}C] (S.A. = 57 mCi/mM, 200 μCi/mg) were purchased from New England Nuclear Co., Boston, MA, and their purity was checked by paper chromatography before use. These labeled steroids were injected into 2 baboons; an adult female weighing 18 kg received 62.2 μCi of [^{3}H] and 17.6 μCi of [^{14}C], and an adult male, weighing 24 kg received 67.5 μCi of [^{3}H] and 10.6 μCi of [^{14}C]. Bile and urine were collected at intervals for 7–8 h in the two animals, through cannulated biliary fistulas and urinary catheters. [^{3}H]-T (57 μCi) and [^{14}C]-A (17 μCi) were injected i.v. into a normal 59 year old male and urine was collected at intervals for 8 h.§

After the excretion in bile and urine was determined in each collection, the conjugate types were separated by filtration through DEAE-Sephadex A-25 columns. Three columns (Pharmacia K9/60, K9/30 and K9/15) were used in series[3]. The conjugate types were defined by single (β-glucuronidase) or sequential enzyme hydrolysis[3] and/or solvolysis; the latter was carried out according to the method of Burstein and Lieberman[4]. The aglycones obtained from the glucosiduronate fraction were separated into metabolite groups by Lipidex 5000 column chromatography[3]. For the elution of this column, a linear gradient of 500 ml of hexane and 20% benzene:hexane (400 ml)–hexane (400 ml) followed by a wash with a polar solvent mixture (hex-

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§ Even though the metabolism of T and A has been studied extensively in man, the present study was performed as a check on the new methodologies used and for comparison with data obtained in the past in many other (including our) laboratories.

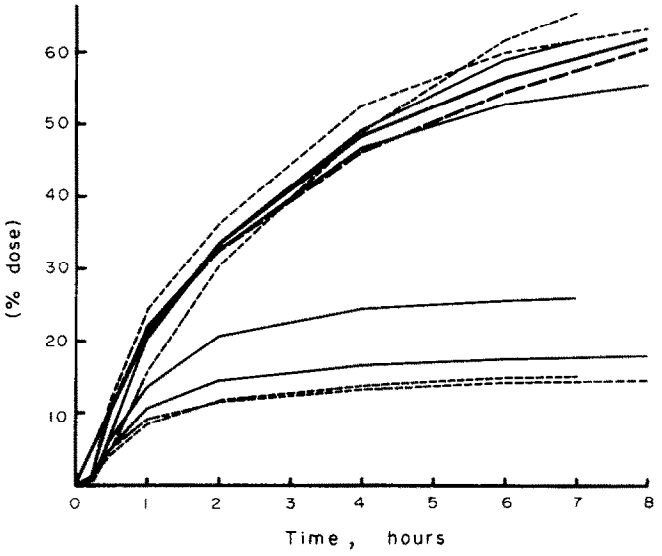

Fig. 1. Cumulative excretion pattern of radioactivity in urine and bile following intravenous injection of [^{3}H]-testosterone (solid line) and [^{14}C]-4-ene-androstenedione (dotted line). Narrow line (female baboon, 0–480 min.; male baboon, 0–360 min.). Thick line (human, 0–480 min.).

ane–chloroform–methanol: 70:20:10; 150 ml) was used. The metabolites were further separated and/or purified by thin layer or paper chromatography and finally identified by co-crystallization methods.

RESULTS

1. *Excretion and baboon bile and urine*

Figure 1 presents the cumulative excretion of radioactivity following i.v. injection of mixtures of [^{3}H]-T and [^{14}C]-A into a female and a male baboon. The rates of excretion of the [^{3}H] and [^{14}C] labels were very similar. Nearly 80% of the injected dose was recovered in 8 h, with extensive excretion being observed in the initial 4 h (Fig. 1). The recovery of radioactivity in the urine was 2–3 times greater than that in bile. Some quantitative differences in excretion were observed between [^{3}H] and [^{14}C]; the excretion of [^{3}H] was lower in urine and higher in bile than that of ^{14}C. The urinary excretion pattern is similar to that of the human (Fig. 1), who excreted nearly 60% during 8 h of collection.

2. *Conjugates in baboon bile and urine*

Baboon bile and urine were applied to DEAE-Sephadex columns and the conjugates fractionated. The chromatographic elution patterns of bile and urine are shown in Figs 2 and 3, respectively. A summary of the various conjugates in baboon bile is given in Table 1, as determined by hydrolyses described under "Methods".

F-I (tubes 4–8), from bile (Fig. 2), eluted with distilled H_2O, consisted of uncharged compounds, 60–70% of which were ether extractable. F-II, eluted in tubes 36–51, was the first fraction appearing after starting the NaCl linear gradient (see "Methods"). This fraction was the largest among the peaks separated and consisted of two peaks. The total separation of these two peaks was subsequently accomplished using a large column system[3] with a lower concen-

Table 1. Characterization of conjugate forms in baboon bile

	Fractions							
	I	II	III	IV	V	VI	VII	VIII
β-Glucuronidase hydrolysis	*	†	–	–	–	–	–	*
Solvolysis	*	*	‡	†	–	–	–	+
Sequential hydrolysis	*	*	*	*	‡	§	–	*
Conjugate form determined	Uncharged	GLU	SUL	SUL	S-G	S-G		DS

Fraction I = uncharged compounds, 60–70% are ether extractable.
* = Not carried out. † = More than 80% hydrolyzed. ‡ = 50–80% Hydrolyzed. § = 30–50% Hydrolyzed. – = Negative. GLU = glucuronide. SUL = sulfate. S-G = sulfo-glucuronide. DS = disulfates.

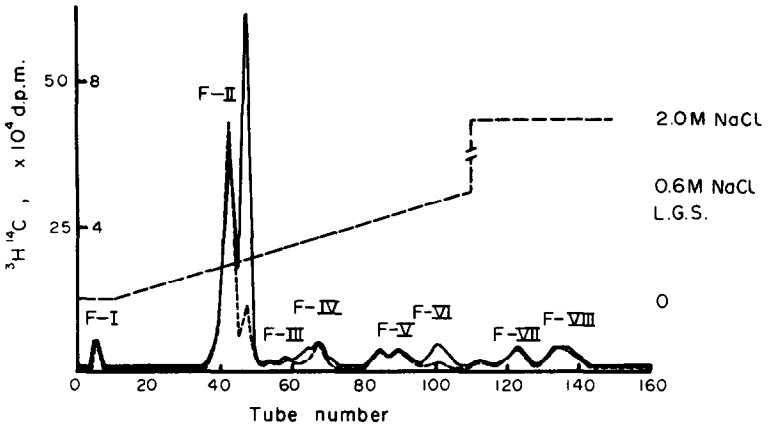

Fig. 2. DEAE-Sephadex A-25 column chromatographic fractionation of androgen conjugates in baboon bile collected following intravenous injection of [^{3}H]-testosterone and [^{14}C]-4-ene-androstenedione. The types of conjugates in fractions are: F-I, uncharged; F-II, glucosiduronates; F-III and F-IV, sulfates; F-V and F-VI, sulfo-glucosiduronates; F-VII, unknown; F-VIII, disulfates.

tration linear gradient elution (0–0.4 M NaCl). F-II was hydrolyzed with β-glucuronidase. The percentage of extractable label with ether from control, enzyme and enzyme inhibited incubations was:

	^{3}H	^{14}C
Control	8%	9%
Enzyme	95%	96%
Enzyme + Inhibitor	13%	16%

Fractions F-II to F-VI were eluted with the linear NaCl gradient up to 0.6 M, and fractions F-VII and F-VIII were eluted with a 2.0 M NaCl wash, suggesting that they were highly charged conjugates. The fractions from F-III to F-VIII, which were rather small peaks compared with F-II, were not hydrolyzable with β-glucuronidase, and upon solvolysis, three of these fractions were hydrolyzed as follows:

	^{3}H	^{14}C
F-III (tubes 55–61)	47%	57%
F-IV (tubes 62–73)	80%	80%
F-VIII (tubes 129–143)	67%	68%

Sequential β-glucuronidase hydrolyses and solvolyses were carried out on fractions F-V, F-VI and F-VII with the following percent hydrolysis:

	^{3}H	^{14}C
F-V (tubes 80–95)	60%	76%
F-VI (tubes 96–106)	20%	26%
F-VII (tubes 118–127)	—	—

Fraction F-VII was not hydrolyzed by either of the above methods.

In contrast to bile, uncharged (F-I), glucosiduronates (F-II) and sulfates (F-III) were the main fractions obtained from baboon urine (Fig. 3). ^{3}H and ^{14}C conjugate elution patterns were not significantly different in urine.

The change of conjugate composition as a function of time was determined in baboon bile and urine and is summarized in Fig. 4, showing the ^{3}H label only for clarity, since there was no significant difference between the ^{3}H and ^{14}C data. Total recovery of radioactivity in the various fractions as a percent of the injected dose is given in Table 2. Glucosiduron-

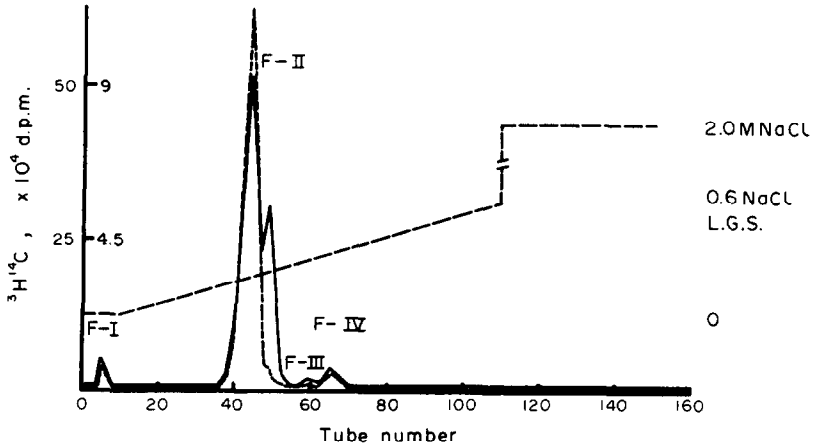

Fig. 3. DEAE-Sephadex A-25 column chromatographic fractionation of androgen conjugates in baboon urine collected following intravenous injection of [^{3}H]-testosterone and [^{14}C]-4-ene-androstenedione. The types of conjugates in fractions are: F-I, uncharged; F-II, glucosiduronates; F-III and F-IV, sulfates.

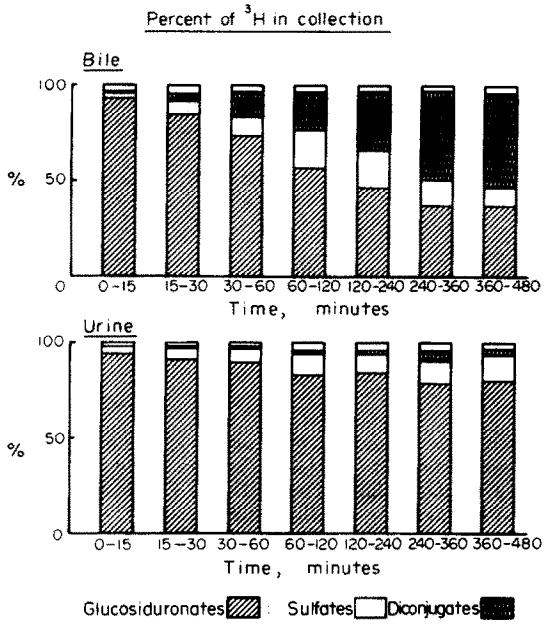

Fig. 4. Change of conjugate composition as a function of time in female baboon bile and urine. Diconjugates include sulfo-glucosiduronates, disulfates and undetermined high charged conjugates (F-VII). [^{3}H]-labeled data of the female baboon were used.

ates were the major conjugates and their excretion in bile decreased remarkably with time, whereas this decrease was insignificant in urine. As a consequence, the total percentage (Table 2) of glucosiduronates was somewhat higher in urine (48.07% and 56.01% in female and male, respectively) than in bile (11.96% and 20.84% in female and male, respectively) for ^{3}H. The preponderant excretion of this conjugate took place in the initial two hours of bile collection and in the initial four hours of urine collection.

Quantitatively, the sulfates represented the second highest fraction excreted among the four conjugates determined, but their excretion was quantitatively less when compared to that of the glucosiduronates. The highest excretion of sulfates was observed in the initial two hours of collection (Fig. 4).

Diconjugate excretion was less than that of sulfates, i.e. less than 3% of the total recovery, with more being found in bile than urine (Table 2). There was a remarkable increase with time of the amount of diconjugates (Fig. 4).

3. *Metabolites*

The uncharged fraction contained 1–2% of the administered dose, 60–70% of which was unconjugated in both urine and bile. In this fraction, some metabolites including mono- and di-hydroxy androgens, polar metabolites and diones were separated by thin layer chromatography, but their rigorous identification was not carried out.

The aglycones obtained upon hydrolysis of the peaks were fractionated by chromatography on Lipidex 5000 columns; a typical elution pattern is shown in Fig. 5. No significant quantitative or qualitative differences were found between bile and urine samples. Seven peaks, P1 to P7, were obtained. P1 (tubes 5–15) was eluted as a few small peaks in the area where compounds less polar than mono-hydroxy androgens usually occur. P2 (tubes 21–29), P3 (tubes 30–38) and P4 (tubes 39–50) were eluted in hexane and contained the mono-hydroxy metabolites. Upon further separation by paper chromatography, the aglycones were identified by co-crystallization, as follows: P2 = androsterone, P3 = etiocholanolone, P4 = epi-T and T. P5 (tubes 75–95) and P6 (tubes 96–116) were eluted using a hexane–20% benzene–hexane linear gradient. When chromatographed on thin layer, P5 was further separated into two peaks which were identified as 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol. P6 consisted mostly of ^{3}H and was shown to be 5α-androstane-3α,17β-diol. P7, obtained in the wash using a polar solvent mixture, was separated into 5 peaks on thin layer chromatography in the system of chloroform–ethanol (9:1); they were not further identified.

Quantitation of metabolic conversion (expressed as percent of administered dose) in the glucosiduronate fraction is given in Tables 3 and 4 for the female and male baboons, respectively. Androsterone and etiocholanolone were identified (doubly labeled), both being derived from [^{3}H]-T and [^{14}C]-A. Tables 3 and 4 show that androsterone was one of the major metabolites and this conversion was higher in the male (total urine + bile: ^{3}H = 26% and ^{14}C = 40%) than in the female baboon (total urine + bile: ^{3}H = 11% and ^{14}C = 16%). The formation of etiocholanolone, on the other hand, was smaller (3–4%) than that of androsterone; the androsterone/etiocholanolone ratio was 3.7 for the ^{3}H label and 4.3 for

Table 2. Percent recovery of radioactivity in conjugate fractions of baboon bile and urine and human urine

Time min.	Subject	% Of injected dose in bile							
		F-I		Glucosiduronate		Sulfate		Diconjugate	
		[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]
0–480	Female baboon	0.1	0.1	11.9	9.3	2.2	1.4	2.7	2.9
0–420	Male baboon	0.6	0.3	20.8	11.9	1.7	0.9	2.1	1.6
		% Of injected dose in urine							
0–480	Female baboon	0.7	0.9	48.0	55.3	4.9	7.7	1.3	1.6
0–420	Male baboon	1.5	1.1	56.1	60.0	3.0	3.0	0.2	0.3
0–480	Human	1.4	2.6	89.0	88.0	5.2	6.8	—	—

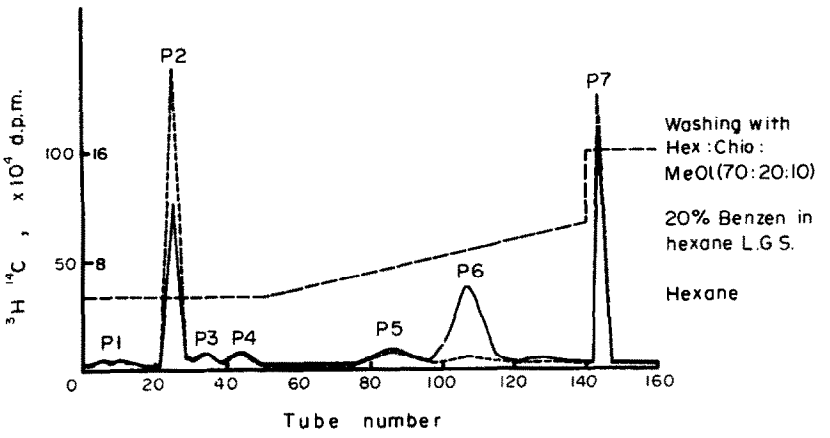

Fig. 5. Typical Lipidex 5000 column chromatographic elution pattern of androgen metabolites following β-glucuronidase hydrolysis of the glucosiduronate fraction (F-II) of baboon urine and bile, collected following intravenous injection of [^{3}H]-testosterone (solid line) and [^{14}C]-4-ene-androstenedione (broken line). The metabolites identified in peaks are: P1, artifacts; P2, androsterone; P3, etiocholanolone; P4, testosterone and epi-testosterone; P5, 5α-androstane-3α,17β-diol and 5α-androstane-3α,17α-diol; P6, 5β-androstane-3α,17β-diol; P7, polar metabolites (unidentified).

the ^{14}C label in the female baboon, and 10 for the ^{3}H and 15 for the ^{14}C label in the male baboon. The isotope ratio, ^{3}H/^{14}C, of either etiocholanolone or androsterone was smaller than that of the injected mixture. T was identified in the male baboon, but the conversion from ^{14}C was small; 0 in urine and 0.02% in bile. On the other hand, more ^{14}C-epi-T was formed in the male baboon as the ^{3}H/^{14}C ratio indicates in Table 3. It is of interest that 5α-androstane-3α,17α-diol exceeded 5α-androstane-3α,17β-diol in quantity, but the total 5α-diol formation was not high (4–5% of the injected dose, Tables 3 and 4). One of the major conversions from [^{3}H]-T was to 5β-androstane-3α,17β-diol; 8% and 20% in the female

Table 3. Percent recovery of identified metabolites in glucuronide fraction of urine and bile from the female baboon

	Urine			Bile			Total urine + bile	
Metabolites	[^{3}H]	[^{14}C]	Ratio	[^{3}H]	[^{14}C]	Ratio	[^{3}H]	[^{14}C]
Androsterone	11.3	16.2	2.4	1.4	2.1	2.3	12.7	18.4
Etiocholanolone	2.6	3.6	2.6	0.6	0.7	3.4	3.3	4.3
Testosterone				0.07				
Epi-testosterone	2.1	3.2	2.3	0.2	0.2	2.9	2.3	3.4
5α-Androstane-3α, 17β-diol	0.5	0.7	2.5	0.1	0.2	2.8	0.6	0.9
5α-Androstane-3α, 17α-diol	1.4	2.3	2.2	1.1	1.5	2.7	2.6	3.8
5β-Androstane-3α, 17β-diol	5.8	0.8	23.3	2.5	0.3	23.2	8.3	1.2
Polar, unidentified	19.9	22.1	3.2	4.3	3.2	4.7	24.3	25.3

Ratio injected = 3.5.

Table 4. Percent recovery of identified metabolites in glucuronide fraction of urine and bile from the male baboon

	Urine			Bile			Total urine + bile	
Metabolites	[^{3}H]	[^{14}C]	Ratio	[^{3}H]	[^{14}C]	Ratio	[^{3}H]	[^{14}C]
Androsterone	22.6	34.7	4.2	3.5	5.2	4.3	26.1	40.0
Etiocholanolone	1.5	1.8	5.4	1.0	0.8	7.8	2.5	2.6
Testosterone	0.6			0.2	0.02	64.0	0.8	0.02
Epi-testosterone	0.5	0.9	4.0	0.1	0.1	5.4	0.6	1.0
5α-Androstane-3α, 17β-diol	1.5	0.9	10.1	0.3	0.2	9.7	1.9	1.2
5α-Androstane-3α, 17α-diol	1.7	2.1	5.3	0.6	0.7	6.0	2.4	2.8
5β-Androstane-3α, 17β-diol	12.2	1.8	42.4	7.9	1.0	48.5	20.0	2.8
Polar, unidentified	12.4	12.7	6.2	4.9	2.9	10.8	17.3	15.6

[^{3}H/^{14}C]-ratio injected = 6.4.

and male baboon, respectively. This conversion from [^{14}C]-A was small; 1% and 3% in the female and male baboon, respectively. Large amounts were listed as unidentified polar metabolites, ^{3}H = 24% and ^{14}C = 25%, in the female baboon and ^{3}H = 17% and ^{14}C = 16% in the male baboon. The ^{3}H/^{14}C ratio was slightly lower in urine and higher in bile than that of the injected mixture.

The aglycones solvolyzed from the sulfate fractions were separated either on paper or thin layer chromatography. The peaks of radioactive metabolites were obtained coincidentally with the following unlabeled standards: androsterone, epi-androsterone, T, 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol. Polar metabolites were present largely in F-III. Further identification and quantitation of metabolites were not performed.

4. *Human subject*

[^{3}H]-T and [^{14}C]-A were injected i.v. into a 59 year old normal man. Urine was collected at intervals for 8 h and the collected samples analyzed as above.

Urinary excretion patterns and rates were similar to those of the baboons (Fig. 1). The total excretion was 61.76% for ^{3}H and 60.41% for ^{14}C in 8 hours, mainly excreted in the initial 4 h. The excretion patterns and rates of ^{3}H and ^{14}C were not significantly different.

The conjugate pattern in the human urine was the same as that of the baboon (Fig. 6). Three fractions, i.e., uncharged, glucosiduronates and sulfates, were isolated; diconjugates were not found. The uncharged fraction constituted 1.38% for ^{3}H and 2.6% for ^{14}C of the injected dose and almost 80% of this was ether extractable. Glucosiduronates constituted 55% of ^{3}H and 53.15% of ^{14}C of the administered dose. One difference between the human and baboon was observed: The second peak of glucosiduronate fraction eluted after DEAE-Sephadex column chromatography (Figs 3 and 6) was smaller in the human than in the baboon urine. Sulfates were found as 5.2% of ^{3}H and 6.8% of ^{14}C of the injected radioactivity. In the human urine, glucosiduronates descreased with time while sulfates increased (Table 5).

Table 5. Change of conjugate pattern with time of collection in human urine

	Percent of injected dose					
	Uncharged		Glucosiduronates		Sulfates	
Time (h)	[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]
0–1	0.1	0.1	19.9	19.5	0.7	0.8
1–2	0.2	0.3	11.0	10.7	0.3	0.3
2–4	0.3	0.4	12.8	11.5	0.9	1.4
4–6	0.2	0.2	6.6	6.5	0.6	0.8
6–8	0.3	0.3	4.4	4.8	0.5	0.5
Total	1.1	1.3	54.7	53.0	3.2	4.0

The aglycone metabolites resulting form the hydrolysis of the glucosiduronate fraction were separated by Lipidex 5000 chromatography. As shown in Fig. 7, the pattern was similar to that of baboon bile and urine (Fig. 5). However, quantitative differences were observed; two mono-hydroxy metabolite peaks (P2 and P3) were larger and dihydroxy metabolites, especially P6, smaller than those in baboon bile and urine. Quantitatively, androsterone and etiocholanolone were the major metabolites identified (Table 6)

Table 6. Metabolites identified in the glucosiduronate fraction of human urine

Metabolites	[^{3}H]	[^{14}C]	Ratio
Androsterone	15.7	17.9	2.9
Etiocholanolone	22.7	21.9	3.4
Testosterone	0.7	0.1	15.9
Epi-testosterone	0.2	0.05*	14.5
5α-Androstane-3α, 17β-diol	0.6	0.3	6.2
5β-Androstane-3α, 17β-diol	2.0	0.9	7.4
Polar, unidentified	11.0	9.8	3.7

[^{3}H/^{14}C]-ratio injected = 3.3. * Not reliable.

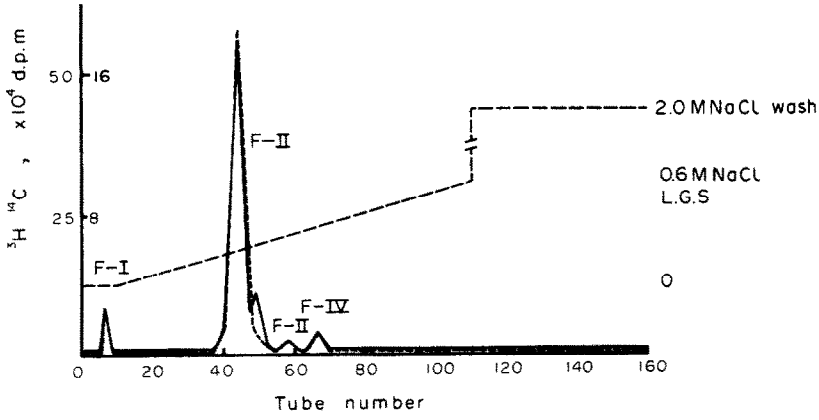

Fig. 6. DEAE-Sephadex A-25 column chromatographic fractionation of androgen conjugates in normal male human (59 years old) urine following intravenous injection of [^{3}H]-testosterone and [^{14}C]-androstenedione. Three conjugate types were obtained: F-I, uncharged; F-II, glucosiduronates; F-III and F-IV, sulfates.

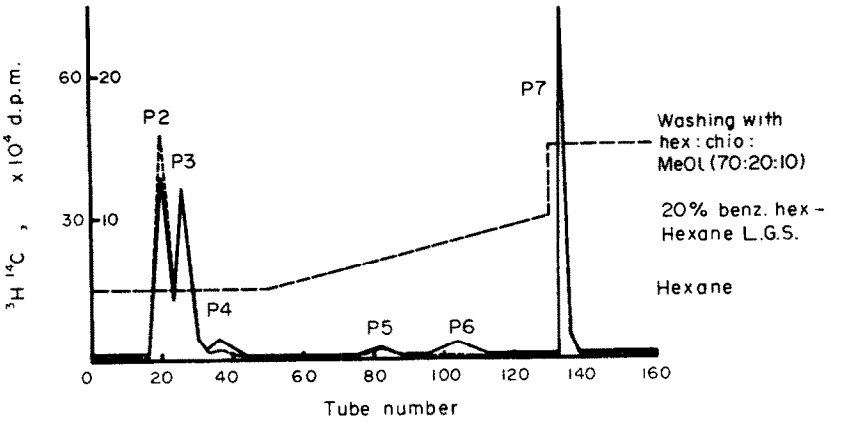

Fig. 7. Lipidex 5000 column chromatographic separation pattern of androgen metabolites in glucosiduronate fraction of human urine (59 year old normal male), collected following intravenous injection of [^{3}H]-testosterone (solid line) and [^{14}C]-4-ene-androstenedione (dotted line). The following metabolites were identified: P2, androsterone; P3, etiocholanolone; P4, testosterone and epi-testosterone; P5, 5α-androstane-3α,17β-diol; P6, 5β-androstane-3α,17α-diol; P7, unidentified.

and almost 40% of the administered dose was recovered as these metabolites with etiocholanolone exceeding androsterone. The conversion to diols (5α-androstane-3α,17β-diol and 5β-androstane-3α,17α-diol) was small compared with that found in the baboon. T-glucosiduronate (T-gl) was formed mostly from T; 0.77% of ^{3}H and 0.16% of ^{14}C. 5α-Androstane-3α,17α-diol was not identified in the human urine. Polar unidentified metabolites constituted 11% of ^{3}H and 10% of ^{14}C of the administered dose.

DISCUSSION

The use of sub-human primates, primarily the baboon, as surrogates for the human in various metabolic studies, including that of steroid hormones, necessitates a comparison of metabolic patterns in these two species. The present study was undertaken in order to ascertain the metabolic fate of T and A in the baboon and human. The results indicate that, except for minor differences (mostly quantitative), the metabolic conversion and conjugation in the baboon, as well as the rates of excretion, are similar in these two species. The present study on the human corroborates earlier results previously reported from this laboratory[5], particularly since, at that time, we were able to use subjects with T-tube bile drainage.

T and A were injected in the present study since these are the major androgens secreted by the human testes[6]. The preponderant excretion of radioactivity associated with T and A was observed in human urine, as reported previously[5, 7]. The excretion of radioactivity in the baboon was similar to that of the human; both excreted about 60% of the administered dose in urine and extensive excretion was observed in the initial 4 h. One distinction between baboon and human urinary excretion was that the excretion of [^{3}H]-T was smaller in the baboon than that of [^{14}C]-androsterone. The biliary excretion in the baboon was about one third of the urinary excretion and the radioactivity was excreted largely in the first two hours. The excretion of [^{3}H]-T was higher in bile than that of [^{14}C]-androsterone.

Previous work from this laboratory[5] showed that on the average nearly 80% of the label was recovered in the urine in 24 h after administration of [^{14}C]-T, while a little over 10% was excreted in bile in subjects with T-tubes. Less than 10% of the injected dose was excreted in the feces of non-fistula subjects, whereas in T-tube patients, 0.1–2% was excreted. Thus, the excretory routes and rates of excretion of T appear to be very similar in the human and baboon.

Considering the high urinary excretion in the baboon and human, it could generally be said that phylogenetically higher mammals excrete more androgens and their metabolites in the urine, whereas lower mammals such as the rat[8], rabbit[9], cat[10] and dog[11] excrete more androgens in the bile.

Glucosiduronates were the preponderant urinary and biliary conjugates in baboon and human urine and bile in the present work and in previously reported studies[5], with sulfates being second. The presence of diconjugates (sulfoglucosiduronates and disulfates) has been reported in rat bile following administration of labeled T and T-gl[8]. Disulfates have been separated from human urine[12, 20] and bile[13] and quantitated. In the previous study from this laboratory[11], diconjugates in dog bile and urine could not be found, but in another study[14] a small amount of sulfates (<5%) was shown in dog bile. A comprehensive study of urinary steroid excretion and conjugation by the baboon[15] demonstrated the presence of disulfates in this species, in which 3 metabolites were identified from a total of a very large number of steroids. The present study indicated the excretion of diconjugates in baboon urine and bile, and their identification was tentatively carried out using enzyme hydrolysis and a solvolytic procedure; furthermore, radioactivity associated with glucosiduronates decreased with time, especially in

bile, whereas excretion of sulfates and diconjugates increased. This suggests that conjugation occurs rapidly after the injection, but that the rate of excretion varies, depending on the type of conjugate, e.g., glucuronidation occurring at a faster rate than sulfation or diconjugation. A report on *in vivo* perfusion studies[16] with human liver pointed out that the perfused labeled T is conjugated quickly either with glucosiduronate or sulfate; at one minute after the injection, 65% of the radioactivity in the hepatic vein was conjugated, and after 40 minutes, the ratios of radioactivity in the free, sulfated and glucosiduronated fractions were 2:3:4. The renal clearance of the glucosiduronates is known to be much higher than that of sulfates[17]. These observations are corroborated by the results of our studies in baboons.

In baboon urine and bile, androsterone and etiocholanolone were identified, and the former occurred in largest quantity. The androsterone/etiocholanolone ratio was 3.8 for ^{3}H, 4.3 for ^{14}C in the female and 10.3 for ^{3}H and 15.2 for ^{14}C in the male baboon. Analysis of baboon urine has shown that the endogenous androsterone/etiocholanolone ratio is 5–23[15]; and thus the conversion to androsterone predominates in this animal. These 17-ketosteroids are known to be the major metabolites in the human. More than 60% of the recovered radioactivity was found in these two metabolites, and the androsterone/etiocholanolone ratio was 0.7. Other studies with radioactive[18, 19] or endogenous steroids[20] have shown that the androsterone/etiocholanolone ratio in human urine ranges from 0.7–1.9. Thus, the predominance of androsterone formation in the baboon is a special feature of that animal which differs from the human.

T and epi-T were excreted in small quantity as glucosiduronates in both urine and bile. T-gl was mostly derived from [^{3}H]-T, but epi-T both from [^{3}H]-T and [^{14}C]-androsterone. The conversion to epi-T is higher from [^{14}C]-androsterone than [^{3}H]-T, suggesting that A is a direct precursor of epi-T, as was reported by Brooks *et al.*[21].

Two 5α-diols and one 5β-diol were identified in baboon urine and bile in the present study. It is of interest that there is greater conversion of [^{3}H]-T and [^{14}C]-A to 5α-androstane-3α,17α-diol than to 5α-androstane-3α,17β-diol. Since this 17α-diol had a higher [$^{3}H/^{14}C$] ratio than the injected sample, it was probably derived from [^{3}H]-T through A. There is a report[13] that this diol is excreted as a disulfate in human bile. Recent studies with dog prostate[22–24] indicate that 5α-androstane-3α,17α-diol is an active androgen in this species; however, the physiological activity of this diol has not been evaluated in the human and baboon. The urinary conversion from [^{3}H]-T and [^{14}C]-A to 5α-androstane-3α,17β-diol in the baboon is very similar to that of the human in our study and others[18].

After androsterone, 5β-androstane-3α,17β-diol was quantitatively the second largest metabolite in the baboon, and its derivation from [^{3}H]-T being 6–7 times higher than from [^{14}C]-A, and was the most pronounced difference observed between the metabolism of T and A. This 5β-diol is postulated to be metabolized through the prior formation of T-gl, as had been shown in the human[25], rat[8] and dog[3], using labeled T-gl as a precursor. Total 5β-diol derivation in the baboon was 8.4% in the female and 20.1% in the male baboon from [^{3}H]-T; whereas, in the human it was 2.0% in this study and 2–5% in others[25]. Thus, another difference in T metabolism between the baboon and human is the abundant formation of 5β-diol in the former. The quantitation of metabolites showed that 5α- or 5β-reduction in combination with 3α-hydroxylation is a preponderant metabolic pathway in the baboon.

Even though polar metabolites in the glucosiduronate fraction were not studied in the present work, it can be speculated from their chromatographic behavior on thin layer, that the baboon excretes more complicated polar metabolites than the human. Aglycones in the sulfate fraction were not identified, but some tentatively identified (androsterone, epi-androsterone, T and 5α-androstane-3α,17β-diol) are found in the sulfate fraction of baboon urine[15], human urine[12] and bile[13]. The metabolic identification in diconjugate fractions is of interest but difficult because of the small amount of radioactivity associated with and poor hydrolysis of these conjugates.

It was stated by Setchell *et al.*[15] in their study of urinary excretion and conjugation of corticosteroids that the baboon is a suitable animal model for the study of human corticosteroid metabolism. In a previous study we demonstrated that the metabolic fate of estriol was similar in baboon and man[26]. Our present study on androgen metabolism has indicated important similarities and minor differences between the baboon and human. For the investigator who is aware of these essential similarities and some of the differences, it appears that the baboon could serve as a suitable surrogate for the human in the study of steroid metabolism.

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