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ANDROGEN METABOLISM IN THE DOG

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SUMMARY

The metabolism of four androgenic compounds, testosterone (T), androstenedione (A), epitestosterone (epi-T) and testosterone glucuronide (T-gl) was studied in the dog. All were predominantly excreted via the biliary route, and since the urinary excretion in intact and biliary fistula dogs was similar, there was an apparent lack of any significant enterohepatic circulation. The metabolism of T was somewhat different from that of A, with indications that the bulk of T is converted to A. All four compounds were preponderantly excreted as glucuronides. Five metabolites of T in bile, i.e., epiandrosterone, eticholanolone and three epimeric androstanediols (5α/3β,17β; 5β/3α,17β and 5β/3β,17β) were identified. The first three compounds were also found to be metabolites of A. Epi-T underwent reduction (5α-androstane-3β,17β-diol) and hydroxylation in ring A and 17-hydroxy oxidation. Radioactivity associated with administered T-gl was eliminated rapidly from the body. Even though the 17α-androgens may be important in canine prostatic physiology, the present study points to the insignificance of the 17α-pathway in the systemic metabolism of T and A.

INTRODUCTION

Studies on androgen metabolism in the dog have been concerned primarily with the secretion of T [1] and its metabolism in a number of tissues [2–12], including those with receptors for T [13, 14]. It appears that the metabolism of T, including that in target organs, of the dog is similar to that of the rat and human, forming preponderantly 5α-metabolites such as 5α-dihydrotestosterone (5α-DHT) and 5α-androstanediols. However, recent reports [13, 14] indicate that the physiologically active androgen in the dog prostate is not 5α-DHT but its 17α,3-hydroxy analog, 5α-androstane-3α,17α-diol, and that the latter compound is bound to specific proteins in the prostatic cytosol and nuclei [13, 14], and enhance prostatic DNA- and RNA-polymerase activities [11, 15]. In contrast, in the rat, not only 5α-DHT, but also other 5α-androstanediols have definite effects on the prostate [16]. Thus, different animals may have different physiologically active androgens controlling prostatic integrity.

On the assumption that different androgens may be metabolized differently in the dog, the present study deals with the fate of injected labeled T, A, epi-T and T-gl in male dogs; the results of analyses of the metabolites of these compounds in bile and urine are also presented.

MATERIALS AND METHODS

Experiments and analyses were carried out according to methods previously described in detail [17]. Labeled steroids were purchased from the New England Nuclear Company (Boston, MA), and their purity was checked by paper chromatography before use. The following combinations of labeled steroids were injected into male dogs to study excretion and conjugation and to identify metabolites: (1) A mixture of 10–20 μCi of [^{3}H]-T (S.A. = 40 Ci/mM) and 3–5 μCi [^{14}C]-A (S.A. = 57 mCi/mM) was injected intravenously into four biliary fistula dogs, Nos. 1–4, and four intact dogs, Nos. 5–8. (2) 20 μCi of [^{3}H]-T and 9 μCi of [^{14}C]-A were separately and simultaneously injected into a renal artery and the portal vein of a biliary fistula dog, No. 9. (3) A mixture of 60 μCi of [^{3}H]-epi-T (S.A. = 58 mCi/mM) and 19 μCi of [^{14}C]-T (S.A. = 58 mCi/mM) was injected intravenously into dog No. 10, a biliary fistular animal. (4) A mixture of 18 μCi of [^{3}H]-T-gl and 6 μCi of [^{14}C]-T was injected intravenously into dog No. 11, a biliary fistula animal. Bile and urine were collected from animals Nos. 1–4, and Nos. 9–11 at intervals through cannulated bile fistulas and urinary catheters, respectively, for six h after injection. Urine and feces were collected from intact dogs Nos. 7 and 8 as indicated in the respective sections of *Results* below. The collected bile and urine samples were eluted through DEAE-Sephadex A-25 columns with System B [17] to resolve the conjugate forms. The latter were then hydrolyzed with β-glucuronidase and the resultant aglycones separated into metabolite groups by Lipidex 5000 column chromatography [17]. The metabolites were further isolated and/or purified on paper or thin layer chromatography. This was followed by final identification by co-crystallization methods. Feces were mixed with distilled water and aliquots were oxidized in a Packard Tri-carb Sample Oxidizer model 306 prior to determination of radioactivity.

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Table 1. Average % recovery of administered dose in bile and urine after i.v. injection of labeled steroid mixtures

	Min after injection	Injected* [^{3}H]-T + [^{14}C]-A		Injected† [^{3}H]-epi-T + [^{14}C]-T		Injected‡ [^{3}H]-T-gl + [^{14}C]-T	
		^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Bile	0–15	0.7 ± 0.9	0.6 ± 0.6	0.0	0.0	2.1	1.9
	15–30	9.6 ± 2.5	7.4 ± 1.6	0.1	0.2	18.8	15.0
	30–60	16.9 ± 5.0	13.9 ± 3.4	4.4	10.6	32.8	23.5
	60–120	13.6 ± 4.7	12.9 ± 4.0	12.9	17.2	17.3	11.9
	120–240	8.2 ± 4.1	8.8 ± 4.4	7.0	5.6	8.2	7.3
	240–360	3.5 ± 1.4	4.5 ± 1.9	10.8	8.6	0.9	1.2
	Total	52.5 ± 11.7	48.0 ± 11.9	34.5	42.2	80.1	60.8
Urine	0–15	0.1 ± 0.1	0.1 ± 0.1	0.0	0.0	6.5	1.5
	15–30	1.0 ± 1.0	1.1 ± 1.1	0.0	0.0	2.5	2.2
	30–60	4.4 ± 3.6	4.9 ± 4.3	1.2	1.7	3.7	4.5
	60–120	7.3 ± 2.9	8.2 ± 3.3	1.4	1.7	3.9	7.5
	120–140	7.5 ± 5.2	8.5 ± 5.9	14.1	12.6	2.5	6.6
	240–360	3.7 ± 3.4	4.6 ± 4.2	12.0	7.3	0.9	2.5
	Total	24.4 ± 14.2	26.5 ± 17.6	28.7	23.3	20.0	24.8

* Mean and standard deviation of 4 animals (Dogs 1–4). † Dog No. 10. ‡ Dog No. 11.

RESULTS

1. *Injection of* [^{3}H]-*T and* [^{14}C]-*A*

a. *Excretion.* [^{3}H]-T (approx. 20 μCi) and [^{14}C]-A (approx. 3 μCi) were injected into four male dogs. Nos. 1–4. The recovery of radioactivity in bile and urine is shown as the average percentage of the administered dose excreted (see Table 1). The excretion rates and pattern of these two steroids were quite similar; the total excretion in bile and urine was 50% and 25%, respectively. The highest rate of excretion was observed in bile between 15 and 120 min after injection. The excretion rates in the four animals ranged as follows: [^{3}H] = 37–66% in bile and 12–25% in urine; ^{14}C = 36–61% in bile and 15–29% in urine. In another experiment, two intact dogs (Nos. 5 and 6) were injected with the labeled steroid mixture as above and the excretion of radioactivity into urine examined. The results of urinary excretion were not significantly different from the bile fistula dogs: [^{3}H]-excretion in 6 h in the two intact dogs was 16.6% and 20.9%, respectively, and [^{14}C]-excretion was 19.8% and 24.8%, respectively, indicating that an enterohepatic circulation of T and A plays only a minor role in the dog. In another experiment, two intact dogs (Nos. 7 and 8) were injected as above in order to ascertain the daily excretion rate into urine and feces. In 7 days (Table 2) the excretion of urine was: [^{3}H] = 23% and 22% in dog Nos. 7 and 8, respectively; nearly 95% of this radioactivity was recovered in the first two days of collection. The fecal excretion in 3 days was: [^{3}H] = 32% and [^{14}C] = 38% from dog No. 7, and [^{3}H] = 21% and [^{14}C] = 27% from dog No. 8. The urinary excretion was not significantly different from that found in urine collected in the experiments described above.

Differently labeled T was simultaneously injected into the renal artery [^{3}H] and portal vein [^{14}C] of a dog (No. 9) to compare renal and biliary excretion of this compound. The [^{14}C] was almost exclusively excreted into the bile (Table 3): 57% in the first hour and a total of 69% after 3 h. The rate of [^{3}H]-excreted into the bile (36% after 3 hours) was not significantly different from that obtained after intravenous injection (compare with dog Nos. 1–4, Table 1). The [^{3}H]-label found in the urine was 12% and the [^{14}C]

Table 2. Percent recovery of radioactivity in urine and feces after intravenous injection of [^{3}H]-testosterone and [^{14}C]-androstenedione into two dogs

Days after injection	Urine						Feces					
	Dog 7			Dog 8			Dog 7			Dog 8		
	^{3}H	^{14}C	Ratio	^{3}H	^{14}H	Ratio	^{3}H	^{14}C	Ratio	^{3}H	^{14}C	Ratio
1	22.4	33.9	4.4	16.6	17.6	6,2	26.4	31.6	5.5	0.4	0.5	4.4
2	0.2	0.2	6.5	4.5	4.7	6.2	5.0	6.0	5.6	20.7	26.7	5.0
3	0.2	0.2	5.3				0.5	0.6	4.8			
4	0.1	0.1	6.4	0.6	0.8	4.6						
5–7	0.1	0.1		0.4	1.3	2.4						
Total	23.0	34.5	4.4	22.1	24.4	5.9	31.9	38.2		21.1	27.2	9.4

Table 3. Percent recovery of radioactivity after simultaneous injection* of [^{3}H]-testosterone into the renal artery and [^{14}C]-testosterone into the portal vein of a male dog

Minutes after injection	Bile			Urine		
	^{3}H	^{14}C	Ratio	^{3}H	^{14}C	Ratio
0–10	0	0	0.7	0.8	1.4	1.3
10–20	1.6	14.8	0.2	1.7	2.8	1.3
20–30	3.7	15.8	0.5	1.5	1.4	2.3
30–40	4.5	11.3	0.9	3.7	2.8	2.8
40–50	5.1	10.4	1.1	3.0	2.0	3.3
50–60	4.5	4.7	2.1	1.3	0.8	3.7
60–80	6.3	5.6	2.5	2.5	1.3	4.1
80–100	3.8	2.5	3.3	2.2	1.1	4.3
100–120	2.6	1.5	3.7	1.5	0.7	4.7
120–150	2.3	1.2	4.2	1.7	0.8	4.8
150–180	1.7	0.7	4.8	1.3	0.5	4.9
Total	36.1	68.5	1.1	21.2	15.6	3.0

* Dog No. 9 was injected with 20 μCi ^{3}H and 9.2 μCi ^{14}C; ^{3}H/^{14}C = 2.2.

was 11% in the first hour, and after three hours the [^{3}H] was 21% and the [^{14}C] was 15%.

The conjugate types in bile and urine obtained from dog Nos. 1–4 following injection of [^{3}H]-T and [^{14}C]-A were separated by DEAE-Sephadex A-25 column chromatography [17]. The patterns were similar in bile and urine. Seven peaks were isolated as shown in Fig. 1 (T metabolites in solid line). The methods used for characterization of these fractions and peaks have been described previously in detail [17]. The first fraction had one peak (B1) and contained uncharged compounds, approximately 40% of which were ether soluble, indicating unconjugated metabolites. The second (B2, B3, B4) and third (B5, B6, B7) fractions were glucuronide conjugates, which were satisfactorily hydrolyzed with β-glucuronidase (82–96%). B2 and B5 (Fig. 1) proved to be glucuronide peaks of polar metabolites; B3 and B6 glucuronides of monohydroxy androgens; and B4 and B7 monoglucuronides of dihydroxy metabolites. Only a small percentage of the radioactivity was excreted as uncharged compounds (B1) in bile and slightly more (6%) in the urine. The majority (90–97%) of the metabolites were excreted in bile and urine as glucuronides. More [^{3}H] than [^{14}C]-appeared in the second fraction, whereas more [^{14}C] than [^{3}H] appeared in third fraction. No noticeable change with time was found in the glucuronide fractions. The tendency for excretion of more [^{3}H] than [^{14}C] in the second fraction and *vice versa* in the third fraction continued with time. The preferential excretion of a label with time was more noticeable in the second fraction, especially B4. The isotope ratio [^{3}H/^{14}C] of B4 decreased with time because the rate of excretion of [^{3}H] in that peak decreased, while [^{14}C] excretion remained relatively constant. The isotope ratios in the third fraction remained relatively constant. In urine, a larger percentage of the excreted radioactivity consisted of polar metabolites (B2 and B5) when compared to bile.

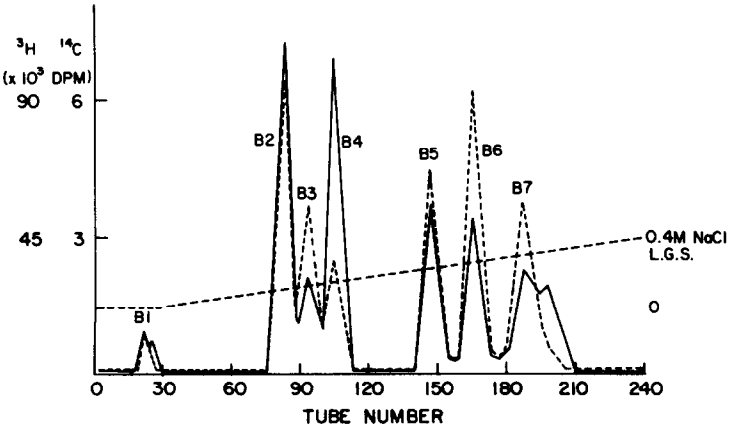

Fig. 1. The typical DEAE-Sephadex A-25 column elution pattern of androgen conjugates in dog bile and urine collected following intravenous injection of [^{3}H]-testosterone (---) and [^{14}C]-4-ene-androstenedione (——). The fractions are designated as indicated on Fig., B1 is uncharged, B2 to B7 are glucosiduronates.

Table 4. Percent radioactivity recovered in bile and urine after i.v. injection of [^{3}H]-testosterone and [^{14}C]-androstenedione into 2 dogs

	Dog 1						Dog 2					
	Bile			Urine			Bile			Urine		
	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C
Etiocholanolone	4.7	6.3	5.4	1.0	1.6	4.6	3.8	4.5	10.0	0.5	1.0	6.6
Epi-androsterone	4.9	7.1	5.0	2.1	3.9	4.2	8.4	6.6	6.6	0.6	1.4	5.6
5α-Androstane-3β, 17β-diol	12.3	9.4	9.6	3.3	5.2	4.6	8.4	14.6	8.0	0.7	1.3	7.1
5β-Androstane-3α, 17β-diol	14.6	*	—	2.1	*	—	12.9	*	—	0.9	*	—
5β-Androstane-3β, 17β-diol	*	*	—	*	*	—	0.9	*	—	*	*	—
Polar metabolites	11.5	12.2	6.9	11.5	12.1	6.9	21.3	22.3	12.5	7.9	7.7	13.4

* Not found.

b. *Aglycone identification.* The peaks of the second and third fractions were hydrolyzed with β-glucuronidase and the resulting aglycones isolated with Lipidex 5000 column chromatography. The separation pattern was presented previously [17]. (Each isolated peak was further separated or purified on paper and thin layer chromatography, and the final identification was made by recrystallization with standard carriers). The identified metabolites are shown in Table 4, results are expressed as a percentage of the administered dose.

Among identified metabolites, the [^{3}H]-labeled 5α- and 5β-androstanediols predominated; almost half of the excreted radioactivity appeared as these compounds. Diols were the major identified metabolites of [^{14}C]-A. The most definite difference in metabolism of [^{3}H]-T and [^{14}C]-A is in 5β-formation, suggesting that 5β-diols are formed only from T. 17-Keto metabolites were obtained both from [^{3}H]-T and [^{14}C]-A, [^{3}H] and [^{14}C]-labeled 5α (epiandrosterone) and 5β (^{3}H and ^{14}C labeled etiocholanolone) compounds being included. Because of the predominance of the ^{14}C label in the 17-keto metabolites, we postulate that T is first converted to A, then metabolized to 17-keto metabolites. Polar unidentified metabolites were formed in considerable amounts.

2. *Epi-T metabolism*

A mixture of [^{3}H]-epi-T and [^{14}C]-T was injected i.v. (dog No. 10, Table 1) and bile and urine collected. Even though the biliary and urinary excretion of radioactivity was rather small in this animal, a significant difference in the excretion rate from that of T and A was observed. In bile, ^{3}H was excreted (34.5%) at a lower rate than ^{14}C (42.2%), but in urine the ^{3}H excretion was higher (28.7%) than that of ^{14}C (23.3%).

The bile collected between 30 and 120 min was fractionated through DEAE-Sephadex columns and the elution pattern in shown in Fig. 2 (T metabolites in solid line). The resolution of ^{3}H peaks was very similar to that of the ^{14}C peaks; however, a few quantative and qualitative differences were seen. [^{3}H]-labeled B2 was smaller than the [^{14}C]-labeled counterpart, whereas [^{3}H]-B3 was very large. There was another peak found between [^{3}H]-B5 and [^{3}H]-B6 which did not have a [^{14}C]-labeled counterpart, and [^{3}H]-B7 was eluted earlier than [^{14}C]-B7.

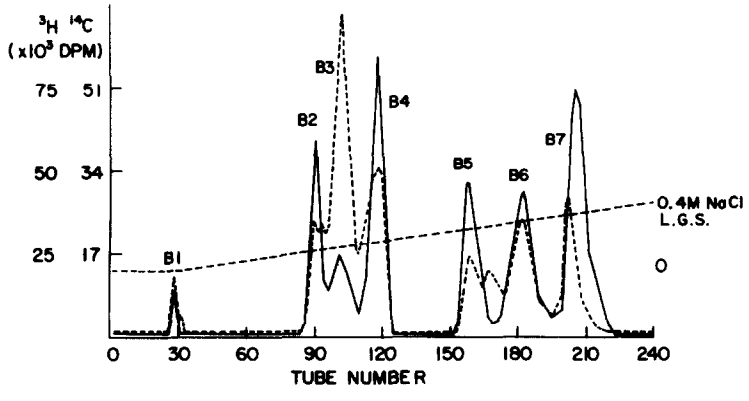

Fig. 2. DEAE-Sephadex A-25 column chromatographic pattern of dog bile collected following injection of [^{3}H]-epitestosterone (---) and [^{14}C]-testosterone (——). B1 is uncharged fraction and B2 to B7 are monoglucosiduronates.

Table 5. Biliary metabolites identified in 30–120 min bile collection following intravenous injection of [^{3}H]-epi-testosterone

Metabolites	% Distribution
Epi-androsterone	8.5
Etiocholanolone	6.3
Epi-testosterone	2.5
5α-Androstane-3β,17α-diol	16.9
5α-Androstane-3β,17β-diol	5.0
Unidentified diols	
Fraction II—Peak B3	1.2
Fraction II—Peak B6	11.5
Polar metabolites (Unidentified)	41.4

The satisfactory enzymatic hydrolysis with β-glucuronidase (92–93%), control extraction (2–3%) and enzyme inhibition extraction (13–30%) studies (see Methods) confirmed that the second (B2-B4) and third (B5-B7) ^{3}H fractions are glucosiduronate conjugates. Almost twice as much ^{3}H appeared in the second fraction (62%) as in the third (31%). The elution pattern of T metabolites (in this case ^{14}C labeled) was not significantly different from that of previous experiments.

The aglycones resulting from enzyme hydrolysis of the second and third fractions above, were next separated into metabolite groups by column chromatography on Lipidex 5000. The pattern of the second fraction showed a remarkable difference between ^{3}H and ^{14}C, suggesting different metabolism of T and epi-T in the dog. Table 5 presents the metabolites identified in bile collected from 30 to 120 min, the quantity of each compound being expressed as a percentage of the total radioactivity in the 30 to 120 min bile collection. In the 17-keto-steroid group, epi-androsterone (8.5%) and etiocholanolone (6.3%) were identified. It is probable that these metabolites were formed through androstenedione. Even though T-gl was not detected following the injection of labeled T or A (see above), epi-T-gl was found in small quantity (2.5%). Two androstanediols, 5α-androstane-3β,17α-diol (16.9%) and 5α-androstane-3β,17β-diol (5.0%), were identified, but no 5β-diols were detected. The most polar dihydroxy metabolite peak obtained from the Lipidex column may be the 5β-androstane-3α,17α-diol, since t.l.c. mobility seemed to be similar to that reported in the literature [18]. Polar unidentified metabolites constituted 41.4% of the radioactivity in that bile collection. Crystallization to constant specific activity was used to identify the metabolites obtained in this part of the study.

The urine collected between 120 and 240 min was also applied to DEAE-Sephadex column. The conjugate pattern was the same as that obtained from the 30 to 120 min bile sample, but quantative differences were observed: glucosiduronates of polar metabolites (B1 and B4) were found in larger amounts and those of dihydroxy metabolites in smaller amounts, respectively, than in bile.

3. *T-gl metabolism*

It has been reported that in the human [19] and the rat [20] T-gl undergoes further 5β-metabolism without prior cleavage of the glucuronide moiety. In order to study the metabolism in the dog (No. 11), [^{3}H]-T-gl and [^{14}C]-T were injected i.v. and the biliary metabolites analyzed.

The excretion of radioactivity shown in Table 1 indicates that ^{3}H was excreted extensively into the

Table 6. Testosterone glucuronide metabolites identified in 30–120 min bile collection following intravenous injection of [^{3}H]-testosterone glucuronide

Metabolites	% Distribution
Androsterone	4.5
Etiocholanolone	1.5
5β-Androstane-3β,17β-diol	10.8
5β-Androstane-3α,17β-diol	55.2
Unidentified monohydroxy metabolite in Fraction III	2.2
Polar unidentified metabolites	17.5

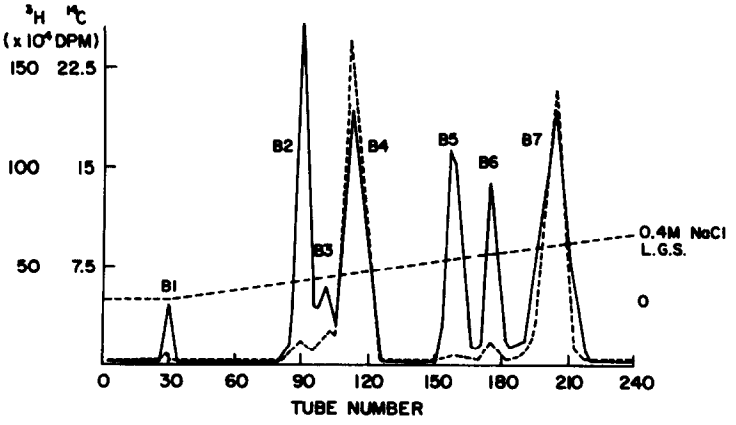

Fig. 3. Dog bile obtained following intravenous injection of [^{3}H]-testosterone glucuronide (---) and [^{14}C]-testosterone (——), was fractionated by DEAE-Sephadex A-25 column chromatography. B1 is uncharged compound and B2 to B7 are glucosiduronates.

bile (80% in 6 h) and that the total elimination in bile and urine was almost 100% in 6 h. Compared with ^{14}C excretion, the conjugated [^{3}H]-labeled steroid is eliminated rapidly from the body. Another interesting finding is that ^{3}H excretion is significantly higher in the first urine collection (0–15 min, 6.5% recovery), suggesting rapid filtration of the conjugates without absorption or metabolism by the kidney.

The separation pattern of [^{3}H]-labeled conjugates shown in Fig. 3 is of interest when compared with that of the [^{14}C]-labeled conjugates. As with the administration of T or A, ^{3}H was also eluted as two fractions (second and third fractions, the first uncharged fractions being absent), the second fraction contained 60.2% of the radioactivity applied to the column and the third fraction contained 35.7% of the radioactivity. These fractions were readily hydrolyzed (94–95%) with β-glucuronidase. However, it is of interest that the ^{3}H label is preponderantly distributed in peaks B4 and B7, which in our experience indicates dihydroxy metabolites.

Upon Lipidex fractionation of hydrolysate of either the second or third conjugate fractions of Fig. 3, four major peaks were obtained. After identification of metabolites, the quantitative relation of metabolic conversion is expressed in Table 6 as percent of radioactivity in bile collected at 30–120 min. Two 5β-diols, 5β-androstane-3α,17β-diol (55%) and 5β-androstane-3β,17β-diol (11%), were the major metabolites of [^{3}H]-T-gl. The urine sample obtained during the same period was eluted through a DEAE-Sephadex column, and a pattern almost identical to bile was obtained, suggesting that the same metabolites were excreted. The urine collected early (0–15 min) contained a significantly large amount of ^{3}H; T-gl was identified as the major metabolite with 70% recovery in this time period.

DISCUSSION

The salient features obtained in the present study on the metabolism of 4 androgenic compounds (T, A, epi-T and T-gl) in the dog were:

1. The significance of the biliary route as a means of excretion of the conjugated metabolites of all 4 compounds. 2. Apparent lack of a significant enterohepatic circulation for any of the compounds. 3. Glucuronidation as the almost exclusive means of conjugation of all metabolites. 4. Somewhat different metabolism of T vs that of A, with indications that the bulk of T is converted to A in the dog. 5. The insignificance of the 17α-pathway in the metabolism of T and A. 6. Difference between the metabolism of epi-T vs that of T and A. 7. Further metabolic conversion of T-gl.

T and A are endogenously biosynthesized in the testis of the dog [1]; epi-T has not been identified as a testicular secretion, but its metabolism is of interest since 17α-androstanediol had been shown to be a physiologically active androgen in the dog prostate, if not *the* active androgen in that organ [11, 14, 15, 21]. The metabolism of T-gl was studied since it has been observed in humans and rats [19, 20].

The injected labeled androgens were mostly excreted into bile, as shown in Table 1. Following the administration of a mixture of T and A, the rate and pattern of excretion of the two steroids were very similar, approximately 50% of the injected label being found in bile and 25% in urine during 6 h. It is known that the excretory routes of steroids differ in animal species, as Kirdani *et al.* have shown [22] after the administration of estriol into eight different animal species. In the case of androgens, the major excretory route in the human [23] and baboon [24] is urinary, whereas in the rat [20, 25], rabbit [26] and cat [27] it is biliary (>60%). The recovery of substantial radioactivity in feces (Table 2) after intravenous injection and similar urinary excretion in intact animals and those with biliary fistulas suggests a lack of a significant enterohepatic circulation for androgens, as has been shown for estriol [22], in the dog. The same results were obtained by other workers in the rat [25].

The initial high percent excretion of radioactivity in urine following administration of T-gl (6.5% in 0–15 min) is probably due to the direct passage of unmetabolized T-gl through the kidney (even though the conjugating moiety may be hydrolyzed and reconjugated). This direct passage of T-gl was not observed in bile, indicating that the conjugated steroid is not simply transferred through the hepatic cells from blood into the bile. On the other hand, T injected directly into the renal artery was not characterized by a different excretion pattern from that of peripherally injected T, except for slightly greater elimination into the urine. T was quickly excreted in large amounts into the bile when it was injected into the portal vein. All of these results indicate that androgen metabolites are excreted predominantly in the bile in dogs.

Glucosiduronates were the only conjugates detected in bile and urine. Even though sulfates and/or diconjugates were not found in our experiments, a few reports indicate the existence of these conjugates in the dog. Harri *et al.*[3] isolated sulfates of androgens in *in vitro* incubation studies of canine intestine and a similar conjugate formation was reported by Tamm *et al.*[2] using *in vitro* canine liver perfusions. Martin *et al.*[29] indicated that less than 5% of the excreted androgens in the beagle were conjugated as sulfates in the bile. We did not observe sulfates, since these conjugates were present in such minor quantities that they probably could not be isolated with our DEAE-Sephadex column chromatography system.

Even though the percent glucuronide excretion in bile did not vary with time, the aglycones with which the glucuronide moiety was conjugated did change; glucuronides of dihydroxy compounds decreased with

time, whereas those of the polar compounds increased.

We have identified five metabolites of T in the bile and three of A. The three aglycones of A were also among the five of T, i.e. epiandrosterone, etiocholanolone and 5α-androstane-3β,17β-diol. However, these three compounds were formed from A in a higher percentage than from T, suggesting that T may first be metabolized to A. The 5β-diols were found only as metabolites of T, a point of distinction between the metabolism of T and A. 5β-Androstane-3β,17β-diol was identified as a minor metabolite. This 5β metabolism of T has been studied by Baulieu *et al.*[19] in human subjects and also demonstrated in the rat by Matsui *et al.*[20]. To further elucidate the metabolism of T in the dog, labeled T-gl was injected and the results show more than 65% of the metabolites of T-gl were 5β-diols, with 5β-androstane-3α,17β-diol being excreted in about five times greater quantity than 5β-androstane-3β,17β-diol. The metabolism of T-gl in the dog indicates that 5β-diols are derived from T following an initial T-gl conjugation. The metabolites of T identified in our study correlate with the data obtained from *in vitro* and *in vivo* studies of T metabolism in canine intestine by Harri *et al.*[3], in which they identified 3α- and 3β-hydroxy-5α-androstane-17-ones (the latter predominated), 5α-androstane-3β,17β-diol and 5β-androstane-3α,17β-diol; 5α/3β and 5β/3α metabolite formation from T and A was a major feature of the metabolism in the dog [3].

Epi-T metabolism is somewhat different from that of T and A. The major metabolites were those resulting from reduction (5α-androstane-3β,17β-diol) and hydroxylation of ring A with the 17α-hydroxy being oxidized. However, that 17-oxidation did take place is indicated by the significant amounts of epiandrosterone, etiocholanolone and 5α-androstane-3β,17β-diol found in the bile. 5α-Androstane-3β,17α-diol is directly metabolized with 5α-reduction and 3β-hydroxylation and this conversion was largest in quantity. Another peak (B6, 11.5%) was *tentatively* identified also as a 17α-metabolite, since no ^{14}C-peak of T or A metabolites coincided with the [^{3}H]-labeled peak. It is of interest that a small quantity of epi-T-gl was identified; no T-gl was found in bile or urine following the administration of T, A and T-gl. No 5β metabolite from epi-T was detected. In the beagle bile 5α-androstane-3β,17α-diol was identified, indicating that epi-T may be formed endogenously in dogs [29]. In the study of epi-T metabolism in the human [18], the major urinary metabolite identified was unmetabolized epi-T with a minor quantity of other metabolites; it was suggested, on the basis of the limited metabolism in the A ring, that the hepatic 5α- and 5β-reductases utilize epi-T as substrate to a very limited extent. Thus, the metabolism of epi-T is more active in the dog than in the human.

The metabolic conversion of T-gl was established in the dog, as described above. In contrast to some excretion into the urine the direct excretion of T-gl into bile was not found, indicating T-gl is first metabolized within the liver cells before being excreted into the bile.

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