Formation *in Vivo* of 5α-Dihydrotestosterone by the Canine Epididymis[1] (36881)

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The metabolism *in vitro* of androgenic steroids by epididymal tissue has been studied in various species (1–3). When testosterone (T) was the substrate, significant quantities of 5α-dihydrotestosterone (DHT) were produced (1). It is well known that DHT shows more androgenic effects than T in many systems of bioassay and could thus be more important physiologically than T. Furthermore, evidence has been presented concerning the biological activity of other reduced metabolites of T (4) and it is clear that some of these metabolites are far from inactive (4). Since the physiological significance of an organ can only be determined by experiments *in vivo* and *in vitro* (5) we have devised a method to examine the epididymal metabolism of ^{3}H-T following infusion via the epididymal artery. Data on ^{3}H metabolites of T in epididymal tissue and in epididymal venous blood plasma are presented in the current communication.

Materials and Methods. Experiments were performed on healthy, mongrel, mature, male dogs weighing between 17 and 24 kg. The animals were anesthetized with sodium pentobarbital (30 mg/kg, iv) and a cannula was placed in the left femoral artery.

The initial preparation of the epididymis for infusion via the epididymal artery was as described for the infused testis (6). The epididymal artery was dissected out and a 27 gauge needle, attached to a plastic cannula, was inserted into this artery. The testis was then removed and the epididymis was freed of all testicular tissue. The isolated epididymis was now placed in a metabolic chamber (6) and infused with the dog's oxygenated, arterial blood via the cannula in the epididymal artery. The constant flow of arterial blood was 0.78 ml/min. After a 30 min "warm up" period (6), the experiments started adding ^{3}H-testosterone (sp act 54.8 mCi/μmole), dissolved in 0.9% sodium chloride, to the blood in the epididymal artery at a constant rate of 0.05 μCi/min. The constant rate of 0.9% sodium chloride infusion was 0.21 ml/min. Samples of epididymal venous blood of 30 min duration were collected. At the end of infusion, the epididymis was removed from the metabolic chamber, weighed and homogenized in 50 ml 0.9% sodium chloride. The samples of epididymal venous blood were centrifuged, the blood plasma was removed, the red cells were washed with 0.9% sodium chloride and these washings were added to the plasma samples. All samples were then frozen and kept at −15° until processed for radioactive steroids.

Isolation and quantification of steroids in

TABLE I. ^{3}H-DHT in Epididymal Tissue and Venous Blood Plasma of Epididymides Infused with ^{3}H-T Via the Epididymal Artery.[a]

Organ index	^{3}H-DHT (dpm × 10^3)			
	Plasma sample (min)			Tissue (end of infusion)
	0–30	30–60	60–90	
1	6.5	26.4	36.8	14.2
2	10.4	28.3	26.4	17.5
3	26.1	37.3	37.3	39.7
4	7.8	32.7	39.0	45.8
5	11.2	36.2	46.1	37.4
Av	12.4	32.2	37.1	30.9

[a] Details of the experiment have been given in the material and methods section.

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TABLE II. ^{3}H-DHT in Epididymal Tissue and Venous Blood Plasma.

Organ index	Plasma[a] (ml)	DHT (dpm/ml plasma)[a]	Organ wt (g)	DHT (dpm/g tissue)[b]
1	17.6	2200	3.5	4066
2	17.1	1625	3.6	4855
3	10.5	3744	3.5	11,220
4	16.6	2478	5.0	9165
5	16.2	2997	5.5	6797
Av	15.6	2609	4.2	7221

[a] Data are from 60 to 90 min infusion samples.
[b] Epididymal tissue at the end of infusion (93 min).

blood plasma and tissue. ^{3}H-Testosterone and its ^{3}H-metabolites were isolated and identified as published by our laboratory (7). The purity of steroids isolated by this technique has been published (7).

Results. Following infusion of ^{3}H-testosterone via the epididymal artery, ^{3}H-DHT was isolated from the effluent blood (Table I). The quantity of ^{3}H-DHT produced increased with time during the 90 min infusions and tended to plateau in the latter portion of the experiments. ^{3}H-DHT was also present in the epididymides at the end of infusion with ^{3}H-T via the epididymal artery (Table I).

If the quantities of ^{3}H-DHT present per milliliter of epididymal venous blood plasma during the last period of ^{3}H-T infusion (60–90 min) is compared with that present per gram of epididymal tissue at the end of infusion (Table II), some of the formed ^{3}H-DHT appears to be retained by the epididymal tissue.

Table III depicts ^{3}H-T and metabolites found both in blood plasma and tissue. The blood plasma samples of epididymides infused with ^{3}H-T contained detectable quantities of ^{3}H-DHT and ^{3}H-Δ^4-androstenedione while the epididymal tissue also contained measurable amounts of ^{3}H-androsterone, ^{3}H-androstanediol and a quantity of unidentified radioactivity more polar than androstanediol. The mean ratio of ^{3}H-T to ^{3}H-DHT in the plasma samples ranged from 42 to 75 while in the epididymal tissue this ratio was 3 (Table III).

Discussion. When ^{3}H-T was infused at a constant rate via the epididymal artery, ^{3}H-DHT and ^{3}H-androsterone could be found in the epididymal tissue (Table III). These metabolites of T have also been recorded in slices of epididymides incubated with T (1, 2). Since the epididymis metabolizes T both *in vitro* and *in vivo*, a distinct steroidogenic function must probably be assigned to this organ (5). Current data tend to indicate that DHT may have a direct effect on the epididymis in promoting sperm maturation (8, 9). Therefore, epididymal DHT formation may serve an important function for maintaining reproductive capacity. Moreover, at least in

TABLE III. ^{3}H-Testosterone and Metabolites in Epididymal Venous Blood Plasma and Tissue.[a]

	dpm × 10^{3}[b]						
	T	DHT	Δ^4	Androsterone	3α-diol	Pol. met.	^{3}H-Ratio (T/DHT)
Plasma (min)							
0–30	926 ± 373	12.4 ± 7.9	65.5 ± 47.7	x[c]	x	x	75
30–60	1469 ± 153	32.2 ± 4.6	81.5 ± 34.7	x	x	x	47
60–90	1560 ± 263	37.1 ± 6.3	89.5 ± 53.9	x	x	x	42
Tissue (at end)	97 ± 11.5	30.9 ± 4.9	9.1 ± 1.7	25 ± 4.9	50 ± 7.3	19 ± 3.1	3

[a] Δ^4: Δ^4-androstenedione; 3α-diol: 3α-androstanediol; Pol. met.: polar metabolites, not identified.
[b] Each value represents the mean ± SD from five experiments.
[c] Less than 1000 dpm present in sample.

the canine, testicular DHT (10) may not be the only source for this steroid hormone in the epididymis (Table I). The physiological significance of epididymal androstanediol (Table III) is unexplored and unknown. It is of interest to note that this steroid will maintain secretory activity of prostatic cells grown in a culture medium (4).

It is evident that under our condition of experiment, DHT can be secreted (5) by the epididymis (Table I). The mechanisms underlying such secretion are unclear, though the possibility exists that steroids formed in excess of organ needs can be removed via venous blood (11). Compared to infused ^{3}H-T and biosynthesized ^{3}H-Δ^{4}-androstenedione, a considerable portion of formed ^{3}H-DHT and ^{3}H-androsterone are retained by the epididymal tissue (Tables II and III). Binding of DHT by rabbit epididymal tissue has already been reported (9) and the rat testis may contain an androgen binding protein which is secreted in seminiferous fluid and passes through the epididymis (12). The tissue retention of ^{3}H-DHT recorded in our experiment (Tables II and III) could be due to the presence of a specific androgen binder in the epididymis. Whether this binder is essential for DHT effect on the epididymis (8, 9) remains to be determined.

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