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QUANTIFICATION OF ENDOGENOUS TESTOSTERONE AND DIHYDROTESTOSTERONE AND THEIR POSSIBLE INTRACELLULAR DETERMINANTS IN VARIOUS TISSUES OF THE MALE GUINEA PIG

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SUMMARY

Radioimmunoassay techniques have been employed to measure the endogenous concentration of testosterone (T) and dihydrotestosterone (DHT) within the plasma, seminal vesicle epithelium (SVE), seminal vesicle muscle (SVM), prostate gland (P), and other tissues of mature male guinea pigs. Additional studies have been performed comparing both the *in vivo* and *in vitro* distribution and metabolism of tritiated testosterone within these same tissues. The endogenous concentration of T and DHT within the sex accessory organs and plasma was determined to be as follows: SVE: (DHT = 12.31 ± 0.57, T = 3.29 ± 0.44 pg/mg), SVM: (DHT = 4.37 ± 0.56, T = 4.33 ± 0.37 pg/mg), P: (DHT = 7.19 ± 1.34, T = 3.83 ± 0.51 pg/mg), and plasma: (DHT = 1.8 ± 0.13, T = 4.41 ± 0.31 pg/mg). Further studies revealed that, with the exception of steroidogenic tissues (adrenal and testis), a good correlation appears to exist between a tissue's capacity to form and retain [^{3}H]-DHT either *in vivo* or *in vitro*, and the endogenous content of DHT quantified within. This study demonstrates the selective capacity of the sex accessory gland epithelium as well as the sex accessory gland muscle to concentrate endogenous androgen from plasma, and that this capacity to do so appears to be determined, at least in part, by the selective ability to form and retain DHT.

INTRODUCTION

Investigations concerning the mechanism by which circulating androgens exert their physiological influence within the male sex accessory organs have been extensive and these results have been thoroughly reviewed [1–4]. In this regard, both *in vivo* and *in vitro* studies utilizing the substrate [^{3}H]-testosterone, have demonstrated that radioactive androgens are selectively accumulated by male sex accessory tissues when compared to non-sex accessory tissues. Within the male sex accessory tissues, testosterone (T) is rapidly converted into dihydrotestosterone (DHT) as well as other androgens, and in turn DHT is selectively bound to cytoplasmic and nuclear proteins. Although the relative ratios of various intracellular androgen metabolites have been shown to vary according to the available concentrations of hydrogen donors or acceptors [5, 6], DHT is normally the major metabolite of testosterone extracted from sex accessory tissues which have been previously exposed to tritiated testosterone either *in vivo* or *in vitro*. That non-sex accessory organs contain little [^{3}H]-DHT following systemic injection of [^{3}H]-T, is most likely related to rather low concentrations of 5α-reductase and/or specific androphilic molecules within these tissues.

Within the male sex accessory organs, most audioradiographic studies concerned with androgen localization have indicated that the epithelial component of the organ contains the highest concentration of tritium [7, 8]. Supporting this work, efforts in the authors' laboratory revealed that upon intraperitoneal injection of [^{3}H]-T to male guinea pigs, the epithelium of the seminal vesicle contained the highest concentration of [^{3}H]-DHT, in relation to other tissues examined, and upon *in vitro* exposure of a variety of tissues to [^{3}H]-T, this same tissue appeared to have the greatest 5α-reductase activity [9].

Even with the vast amount of information that has accumulated concerning androgen metabolism and binding within male sex accessory tissues, the exact intracellular endogenous concentration of T and DHT within many male sex accessory tissues and other peripheral tissues has not been analyzed. Hence the significance of a tissue's 5α-reductase activity and/or intracellular androgen binding capacity, relative to the intracellular concentration of T and DHT is not yet appreciated.

The following trivial names are used in place of chemical names.
Androstandiol—5α-androstane-3α, 17β diol and 5α-androstane-3β, 17β diol. Androstanedione—5α-androstane-3,17, dione. Androstenedione—androst-4-ene-3,17-dione. Cortisol—11β, 17,21-trihydroxypregn-4-ene-3,20-dione. Dihydrotestosterone—5α-androstane-17β-ol-3-one. Estradiol—estra-1,3,5 (10)-triene-3,17β-diol. Estriol—estra-1,3,5 (10)-triene-3,16α, 17β-diol. Testosterone—17β-hydroxyandrost-4-en-3-one.

The purpose of the present study was to determine by RIA the exact endogenous concentration of T and DHT within the sex accessory organs, steroidogenic organs such as adrenal and testis, and in other peripheral non-sex accessory tissues not particularly dependent upon circulating testosterone for normal growth and function. In addition, the relative *in vitro* 5α-reductase activity and the *in vivo* formation and retention of [^{3}H]-DHT have been investigated within each tissue in an attempt to correlate these processes with the endogenous concentration of DHT quantified within each tissue. Particular attention was directed at the guinea pig seminal vesicle which can be separated into its epithelial and fibromuscular components allowing individual analysis of the two different and important sex accessory organ tissue components. A preliminary abstract of the work has previously been published [10].

EXPERIMENTAL

Animals. Mature male guinea pigs (body weight of approximately 700 g) were housed in the university animal quarters for at least one week prior to use. Animals were fed a standard laboratory diet and water *ad libitum.* All animals were sacrificed by a sharp blow to the head. In studies utilizing separated epithelium and muscle preparations of the seminal vesicle and ileum, the separation was performed as previously described [11]. For castrations, scrotal orchiectomies were performed under ether anesthesia.

Radioimmunoassay of testosterone (T) and dihydrotestosterone (DHT). Quantification of plasma levels of both T and DHT were performed by methods described by New England Nuclear Corporation (NEN) [12]. Intracellular endogenous androgen levels were quantified by first homogenizing 40–60 mg. of tissue in 1.5 ml of sterile-distilled water with a ground glass homogenizer. Both plasma (0.5–1.0 ml) and tissue homogenates (1.5 ml) were first extracted in 10.0 ml of glass-distilled methylene chloride by vortexing each sample for one minute. Samples were then centrifuged at 1,000 *g* for 15 min and the aqueous phase was then removed by aspiration. Each organic solvent–tissue mixture was then repeatedly vortexed with 1.0 ml of 0.1 N NaOH, 0.1 N acetic acid, and finally 1.0 ml of sterile distilled water. After the rinse with each solution, the aqueous phase was removed by aspiration. The methylene chloride extract was then dried in a vacuum oven and concentrated in the tips of the extraction tubes by repetitive reconstitution and drying of the samples with decreasing vol. of methanol (5.0 ml, 2.5 ml, 1.0 ml, 0.4 ml, 0.25 ml). The extracts were separated into T and DHT fractions by Sephadex LH-20 column chromatography utilizing 2,2,4-trimethylpentane–benzene–methanol (90:5:5, by vol.) as the eluting solvent. The column eluates were then concentrated at the tips of the collection tubes by repetitive reconstitutions and dryings as previously described. A departure from the published procedure of NEN was taken in that 10 ml disposable glass pipettes with a tight fitting glass bead at the tip were used to support the Sephadex columns. The cross-contamination of T and DHT within their respective fractions following Sephadex separation was approximately 10%. Recovery of tritiated T or DHT following extraction and separation was 65–70%. These analyses of cross-contamination and recovery were performed with each experiment and utilized radiochemically pure steroid as evidenced by migration with authentic non-radioactive standards in two separate thin layer chromatographic systems [9].

Assay of both T and DHT steroid fractions was done utilizing commercially prepared (NEN), desicated, lypholyzed rabbit antisera specific for T and DHT. Although the advertised specificity of the antibody indicated a 100% cross reactivity between T and DHT, assay for each steroid required separate antibody dilutions in the authors' laboratory due to differences in displacement characteristics of radioactive hormone displayed by non-radioactive standard preparations of both T and DHT (Fig. 1). In these studies, 50 pg of non-radioactive DHT was equivalent to 18 pg of non-radioactive T in standard curve preparations in which tritiated T was utilized, whereas, 50 pg of non-radioactive T was equivalent to 110 pg of non-radioactive DHT in a standard curve preparation in which tritiated DHT was utilized. To adjust the standard curves such that most unknown samples could be read in the middle portion (50% displacement of tritiated steroid from antibody) of the standard curves, DHT assays were performed with antibody preparations reconstituted to 3.5 mls of assay buffer, whereas, T assays were performed with the antibody preparations reconstituted to 5.0 mls with assay buffer. Cross-reactivity of the NEN testo-

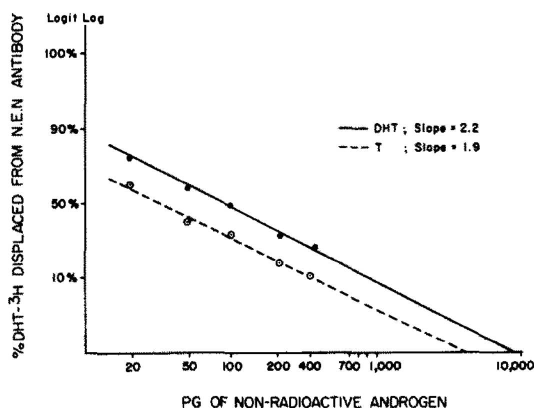

Fig. 1. Comparison of displacement characteristics of radioactive androgen from the NEN-androgen specific antibody by preparations of cold standard testosterone (T) or dihydrotestosterone (DHT). Studies were performed by comparing the relative capacity of either cold T or DHT to displace either radioactive T or DHT from their respective standard curves. The example shown is the displacement of tritiated-DHT (6,000 c.p.m.) from the antibody by either cold standard preparations of T or DHT. Antibody was diluted with 3.5 ml of assay buffer.

sterone antibody was less than one percent with steroids such as; estradiol, estriol, androstandiol, progesterone, and cortisol as determined by the authors. A description of the cross-reactivity of other steroids with the antibody was furnished by NEN [12]. With each assay, pure sterile-distilled water blanks, internal non-radioactive standards of T and DHT, and plasma from immature female rats were analyzed. Water blanks were determined by performing a complete extraction, separation, and analysis procedure on 1.0 ml of sterile glass distilled water. Without exception, water blanks produced readings which were normally equivalent to 2.0 pg or less. Internal T and DHT standards were performed by the addition of 200 or 400 pg of either T and/or DHT to water or tissue homogenates and then subjecting the samples to the complete assay procedure. The readings obtained for the exogenous steroid, which accounted for extraction efficiency, cross-contamination of the steroids, and the presence of endogenous hormone were then compared to theoretical values. The quantification of endogenous T and DHT in the variety of tissues examined was appropriately influenced by the exogenous addition of known quantities of non-radioactive T and DHT. Internal standard values were in the same relationship to theoretical values regardless of whether the analyses were performed in water or in the various tissues. Specifically, internal standard studies resulted in values which were 93–95% of the expected values for both 200 and 400 pg standards. Analysis of plasma obtained from immature female rats revealed low levels of T (0.118 ± 0.004 pg/ml) and DHT (0.047 ± 0.006 pg/ml), when compared to intact male plasma values. Fluorocil precipitation of samples removed in excess of 90% of assayable material. Fluorocil precipitation of a variety of male tissue and plasma samples prior to assay was performed by vortexing plasma and tissue homogenates for 1.0 min in the presence of a solution containing 80 μg/ml of fluorocil and subsequent centrifugation of samples at 1000 g for 10 min. The supernatants of these samples were then extracted, separated, and analyzed along with aliquots of the same homogenate which were diluted to an equivalent concentration with water. Results of these particular experiments revealed that prior fluorocil precipitation removed 94–99% of endogenous or exogenous steroid present within each sample. In preliminary assays of plasma and tissues, varying vols of reconstituted column eluates were assayed to establish approximate aliquots of unknown material which displaced the equivalent of 50–100 pg of either T or DHT, representing displacements in the mid-range (50% displacement range) of the standard curve. Intra-assay variation, based on multiple analyses of the same sample within a given assay was in the range of 4–7%. The inter-assay variation and biological variation are reflected in the standard error of the mean as all values represent the mean $\pm$ S.E.M. of numerous separate determinations in at least two different assays.

In vivo *distribution and metabolism of* [^{3}*H*]-*testosterone.* Assessment of *in vivo* distribution and metabolism of [^{3}H]-T was performed by injecting [1,2-^{3}H]-testosterone (300 μCi/kg; 2.6 μg/kg) intravenously in 0.5 ml isotonic saline–ethanol (9:1, v/v) via the inferior vena cava through an abdominal incision in animals anesthetized with pentobarbital (35 mg/kg). Animals were sacrificed at 15, 30, and 60 min intervals following tritiated hormone administration. Tissues were rapidly excised, rinsed in Krebs–Ringer bicarbonate buffer (KRB), blotted, wrapped in aluminum foil, and frozen in liquid nitrogen. The seminal vesicle and ileum were separated into the epithelial and muscular components prior to freezing. Steroid extraction, chromatography, and identification procedures for the tissue's steroid metabolites in this laboratory have been previously described [9].

In vitro *five-α-reductase activity.* The *in vitro* incubation of tissue samples were performed at 37° using a KRB buffer (pH 7.4) kept under an atmosphere of 95% O_2, 5% CO_2. Tissues were chopped into 0.5 mM cubes on a MacIlwain Tissue Chopper, blotted, and 40–60 mg pre-weighed samples were transferred to the incubation flasks which contained 2.0 mls of 1.0×10^{-8} M [1,2-^{3}H]-testosterone. Reactions were terminated and steroids were extracted by the addition of 2.0 mls of ice-cold ether–chloroform (1:2, v/v) and vortexing for two separate 15–20 second periods. Samples were then centrifuged (1000 g for 10 min); such a procedure removed in excess of 95% of the steroid from the aqueous phase. An aliquot of the aqueous phase was assessed for radioactivity and the remainder was removed by aspiration. An aliquot of the organic solvent phase was then subjected to t.l.c. procedures to separate the various radiosteroids [9].

Protein determinations were performed according to the method of Lowry *et al.* [14] and DNA determinations were performed according to Schneider[15].

MATERIALS

All animals were purchased from Hilltop Laboratories (Scottsdale, PA). All radioactive steroids as well as the steroid antibody were purchased from New England Nuclear Corporation. The purity of the [1,2-^{3}H]-testosterone (40–60 Ci/mmol) and [1,2-^{3}H]-dihydrotestosterone (40–60 Ci/mmol) was routinely determined, and when repurification was necessary, the Sephadex column chromatography procedure outlined by New England Nuclear was utilized.

All glassware used in the RIA procedure was of the disposable type as purchased from Kimble Co. All organic solvents used in the RIA were glass distilled and were supplied by Burdick and Jackson Co. The water used was a sterile distilled product of Travenol.

Non-glass distilled organic solvents and the Norit-A (Charcoal) were supplied by Fisher Scientific Co. Sephadex LH-20 was purchased from Pharmacia Fine

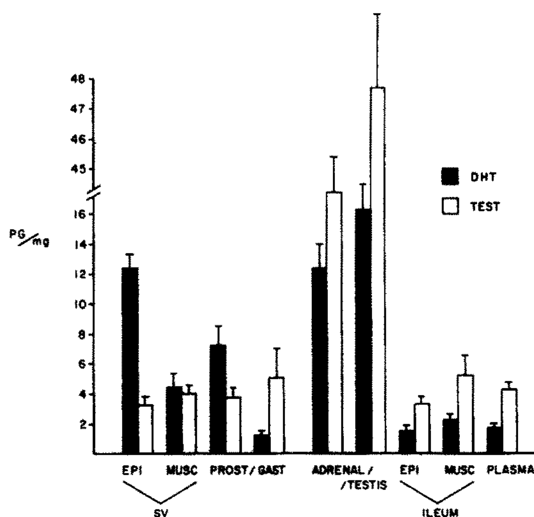

Fig. 2. Quantification of endogenous testosterone (T) and dihydrotestosterone (DHT) concentration in the plasma and various tissues of the male guinea pig. Values represent the mean ± S.E.M. of 6 or more values and are expressed as pg of endogenous steroid per mg tissue wet weight. The following abbreviations and combinations are used as follows: SV/seminal vesicle; Epi/Epithelium; Musc/Muscle; Prost/Prostate; Gast/Gastrocnemius Muscle.

Chemicals. All non-radioactive steroids and other dry chemicals were products of Sigma Chemical Co. or their suppliers. The commercially prepared t.l.c. plates (Silica Gel G) were supplied by Brinkman Corp.

RESULTS

Endogenous concentration of testosterone (T) and dihydrotestosterone (DHT)

Of all tissues in which endogenous androgens were quantified (Fig. 2) the guinea pig testis contained the highest concentration of both T (T = 47.8 ± 6.6 pg/mg) and DHT (DHT = 16.3 ± 1.6 pg/mg). The endogenous concentration of T within the testis was approximately 10 times the level quantified within plasma (T = 4.4 ± 0.3 pg/mg), while DHT in the testis was approximately 16 times as concentrated as that quantified within plasma (DHT = 1.18 ± 0.13 pg/mg). The adrenal gland also possessed levels of endogenous androgen (T = 17.6 ± 2.6 pg/mg; DHT = 12.3 ± 1.3 pg/mg) in concentrations greatly exceeding plasma levels.

Within the sex accessory tissues, the epithelium of the seminal vesicle contained the highest concentration of endogenous androgen (Fig. 2), with DHT being present in approximately 4 times the concentration of T (DHT = 12.3 ± 0.6 pg/mg; T = 3.3 ± 0.4 pg/mg). The seminal vesicle muscle in comparison contained relatively equal concentrations of both androgens (DHT = 4.4 ± 0.6 pg/mg; T = 4.0 ± 0.4 pg/mg). The concentration of endogenous DHT within the epithelium of the seminal vesicle exceeded that in the muscle by approximately three fold. The guinea pig prostate which was analyzed as a composite of both epithelial and fibromuscular tissue, contained DHT in concentration that was intermediate to that of the seminal vesicle epithelium and muscle (DHT = 7.2 ± 1.3 pg/ml; T = 3.8 ± 0.5 pg/ml). Endogenous levels of T within the prostate and the seminal vesicle tissues were not significantly different from those levels that were quantified within the plasma. The non-steroidogenic, non-sex accessory tissues such as the gastrocnemius muscle or separated preparations of epithelium and muscle from the ileum, possessed endogenous levels of endogenous T and DHT which were not significantly different from androgen levels quantified in the plasma.

Comparison of endogenous androgens within each tissue in terms of wet weight or protein did not appreciably alter the relative levels of these steroids (Table 1). However, the relative concentration of endogenous T and DHT was increased in the seminal vesicle muscle when the data was expressed in terms of DNA content due to the relatively lower levels of DNA in the seminal vesicle muscle (Table 1).

Effect of castration on sex accessory organ androgen concentration

Castration of male guinea pigs for twenty-four hours resulted in significant depletion of endogenous levels of both T and DHT from the plasma and all sex accessory tissues (Table 2). Although castration caused a significant depletion of endogenous androgen content in these tissues, they all exhibited significant retention of DHT when compared to plasma. In relation to plasma, the seminal vesicle epithelium and prostate demonstrated greater retention of T and DHT than the seminal vesicle muscle. Twenty-four hour castration did not significantly alter either tissue protein concentration or DNA content (table 3).

In vivo *distribution and metabolism of [^3H]-testosterone*

The intravenous injection of [^{3}H]-T (300 μCi/kg; 2.6 μg/kg) via the inferior vena cava was performed in order to assess the *in vivo* distribution and metabolism of this steroid within various tissues of the male guinea pig. Animals were sacrificed and tissues were excised and assessed for radioactive androgens at 15, 30 and 60 min post injection. In Fig. 3, the concentrations of [^{3}H]-T, [^{3}H]-DHT and [^{3}H]-androstandiol in different tissues at 15 min post-injection are depicted. Of all tissues examined the sex accessory organs contained the highest concentration of [^{3}H]-DHT. Within the sex accessory organs, the seminal vesicle epithelium contained the greatest concentration of [^{3}H]-DHT, followed in order by the prostate and seminal vesicle muscle. This general difference in [^{3}H]-DHT levels between the epithelium and muscle of male sex accessory organs and differences between these tissues and a variety of others was generally consistent throughout the 60 min time course (table 4). As also shown in table 4, it can be noted that of the ether–chloroform soluble radioac-

Table 1. Comparison of the endogenous concentrations of testosterone and dihydrotestosterone in various tissues of the male guinea pig

	Endogenous Dihydrotestosterone	Endogenous Testosterone
	pg Steroid/mg Tissue	
Seminal vesicle epithelium	12.26 ± 0.57	3.29 ± 0.44
Seminal vesicle muscle	4.37 ± 0.56	4.03 ± 0.32
Prostate	7.19 ± 1.34	3.83 ± 0.51
Adrenal	12.32 ± 1.30	17.60 ± 2.57
Testis	16.25 ± 1.63	47.74 ± 6.59
Gastrocnemius	1.27 ± 0.17	5.04 ± 1.99
Ileum epithelium	1.66 ± 0.14	3.20 ± 0.40
Ileum muscle	2.14 ± 0.26	5.14 ± 1.40
Plasma	1.18 ± 0.13	4.41 ± 0.31
	(n = 15)	
	pg Steroid/mg Protein	
Seminal vesicle epithelium	91.49 ± 4.6	24.56 ± 3.3
Seminal vesicle muscle	36.12 ± 4.3	33.51 ± 3.1
Prostate	48.91 ± 8.8	26.05 ± 3.5
Adrenal	66.96 ± 7.1	96.70 ± 14.0
Testis	180.56 ± 18.1	530.89 ± 73.4
Gastrocnemius	8.45 ± 1.1	32.72 ± 12.7
Ileum epithelium	13.39 ± 1.8	25.80 ± 7.0
Ileum muscle	16.59 ± 1.1	39.84 ± 10.9
	(n = 6)	
	pg Steroid/μg DNA	
Seminal vesicle epithelium	1.12 ± 0.05	0.30 ± 0.04
Seminal vesicle muscle	2.29 ± 0.29	2.11 ± 0.19
Prostate	1.46 ± 0.27	0.78 ± 0.10
Adrenal	2.25 ± 0.24	3.22 ± 0.47
Testis	3.72 ± 0.37	10.93 ± 0.16
Gastrocnemius	0.86 ± 0.12	3.43 ± 1.35
Ileum epithelium	0.25 ± 0.03	0.48 ± 0.04
Ileum muscle	0.25 ± 0.03	0.59 ± 0.16
	(n = 6)	

Data for steroid concentrations are expressed on the basis of wet weight, protein concentration and DNA concentration. Values represent the mean ± S.E.M. of at least 6 observations (number in parentheses).

tivity, [^{3}H]-DHT was the major intracellular androgen recovered from the sex accessory organs. In contrast, with the exception of the liver, testosterone was the major androgen recovered from the non-sex accessory tissues. The liver contained principally the oxidized androgens, androstenedione and androstandione.

In vitro [^{3}H]-testosterone metabolism in various tissues of the male guinea pig

In vitro incubation of various tissues from the male guinea pig with [^{3}H]-T (table 5) for 15 min revealed marked differences in T assimilation among the tissues similar to those observed in the previous *in vivo* studies. Almost without exception, the qualitative dif-

Table 2. Effect of twenty-four hour castration on endogenous levels of testosterone (T) and dihydrotestosterone (DHT) in plasma and sex accessory organs of the male guinea pig

	Pg Steroid/mg Tissue	
	Intacts	Castrates
Seminal vesicle epithelium		
T	3.29 ± 0.44	0.96 ± 0.10*
DHT	12.26 ± 0.57	1.32 ± 0.04*†
Seminal vesicle muscle		
T	4.03 ± 0.37	0.04 ± 0.002*
DHT	4.37 ± 0.56	0.48 ± 0.001*†
Prostate		
T	3.83 ± 0.51	0.48 ± 0.21*
DHT	7.12 ± 1.31	1.03 ± 0.40*†
Plasma		
T	4.41 0.31	0.04 0.01*
DHT	1.18 0.13	0.12 0.04*

* Statistically different from intacts ($P < 0.01$) using Student's t-test. † Statistically different from castrate plasma ($P < 0.05$) using Student's t-test. Values represent the mean ± S.E.M. of at least 6 separate determinations.

Table 3. Effect of twenty-four hour castration on protein concentration and DNA concentration in various tissues of the male guinea pig

	Mg Protein/mg Tissue	
	Intacts	24 h Castrates
Seminal vesicle epithelium	134 ± 6.7	119 ± 6.1
Seminal vesicle muscle	121 ± 4.2	127 ± 7.4
Prostate	147 ± 1.2	161 ± 5.8
Adrenal	154 ± 5.1	157 ± 3.9
Testis	184 ± 8.7	—
Gastrocnemius	90 ± 0.7	182 ± 2.4
Ileum epithelium	124 ± 2.3	126 ± 5.6
Ileum muscle	129 ± 8.3	119 ± 5.2
	μg DNA/mg Tissue	
	Intacts	24 h Castrates
Seminal vesicle epithelium	10.98 ± 1.59	11.04 ± 0.60
Seminal vesicle muscle	1.91 ± 0.19	2.42 ± 0.43
Prostate	4.91 ± 0.32	3.70 ± 0.18
Adrenal	1.47 ± 0.12	1.24 ± 0.11
Testis	5.47 ± 0.88	—
Gastrocnemius	4.32 ± 0.26	4.05 ± 0.33
Ileum epithelium	6.69 ± 1.33	8.86 ± 0.91
Ileum muscle	8.72 ± 1.18	7.12 ± 0.62

Values are represented as mean ± S.E.M. of at least 8 observations.

ferences of [^{3}H]-T assimilation seen between tissues *in vivo* were similar *in vitro* (tables 4 and 5). Once again the seminal vesicle epithelium exhibited a greater capacity to convert [^{3}H]-T into [^{3}H]-DHT than any other sex accessory tissue. In non-sex accessory tissues, the gastrocnemius muscle exhibited a significant capacity to accumulate testosterone, but very little capacity to form [^{3}H]-DHT (table 5). Other non-sex accessory organs exhibited little uptake or metabolism of [^{3}H]-T to [^{3}H]-DHT with the exception of hepatic tissue. In these *in vitro* experiments, oxidized metabolites ([^{3}H]-androstenedione and [^{3}H]-androstanedione) comprised less than 10% of the total radioactivity in all tissues with the exception of the liver.

DISCUSSION

Quantification of plasma androgen levels within many male species has been previously reported including guinea pig [16, 17], rat and mouse [18, 19], dog [20, 21], and human [22, 23]. These studies noted differences in testosterone and DHT concentrations among the numerous species, and in addition, various diurnal patterns within species. Plasma testosterone levels quantified in the male guinea pig in this study were well within the range of previous reports, but DHT levels had not been previously reported. Plasma DHT levels reported herein, are slightly higher than those reported in the male rat [24]. These differences in plasma DHT levels between rat and guinea pig

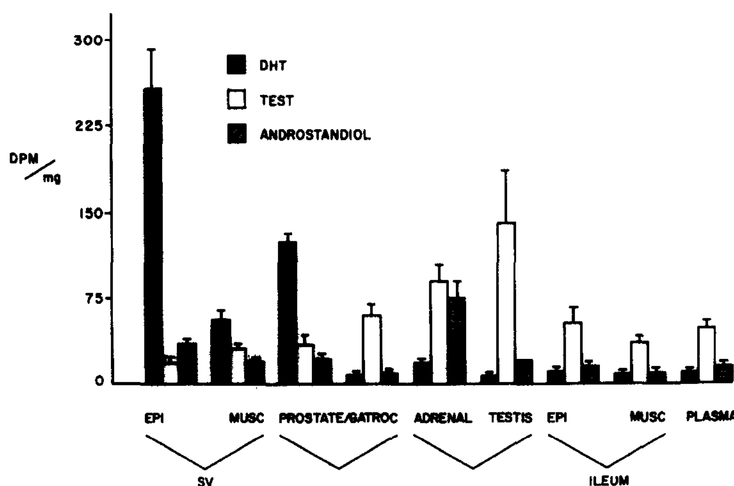

Fig. 3. The concentration of certain radioactive androgens in various tissues 15 min following the intravenous injection of [^{3}H]-testosterone (300 μCi/Kg). Values represent the mean ± S.E.M. of at least 8 observations. The following abbreviations or combinations are used as follows: SV/seminal vesicle; Epi/Epithelium; Mus/Muscle; Gastroc/Gastrocnemius Muscle.

Table 4. The *in vivo* distribution and metabolism of [1,2-^{3}H]-testosterone by various tissues of the male guinea pig

	Radioactive Androgen	15 Min	30 Min	60 Min
Seminal vesicle epithelium	Total tritium	344 ± 36	372 ± 49	212 ± 27
	Dihydrotestosterone	259 ± 44	225 ± 63	144 ± 21
	Testosterone	20 ± 7	23 ± 4	8 ± 2
	Androstandiol	38 ± 5	59 ± 19	33 ± 6
	Androstenedione and androstanedione	27 ± 6	65 ± 18	27 ± 6
Seminal vesicle muscle	Total tritium	126 ± 14	142 ± 17	90 ± 14
	Dihydrotestosterone	57 ± 7	66 ± 8	36 ± 3
	Testosterone	31 ± 5	24 ± 6	20 ± 6
	Androstandiol	20 ± 3	18 ± 6	28 ± 6
	Androstenedione and androstanedione	18 ± 5	32 ± 8	14 ± 3
Prostate	Total tritium	220 ± 27	199 ± 29	135 ± 15
	Dihydrotestosterone	124 ± 6	102 ± 27	72 ± 9
	Testosterone	36 ± 9	28 ± 3	16 ± 3
	Androstandiol	24 ± 2	28 ± 4	18 ± 3
	Androstenedione and androstanedione	36 ± 3	41 ± 6	29 ± 4
Gastrocnemius muscle	Total tritium	89 ± 10	99 ± 20	117 ± 19
	Dihydrotestosterone	6 ± 1	3 ± 1	4 ± 1
	Testosterone	63 ± 10	60 ± 18	84 ± 16
	Androstandiol	8 ± 2	5 ± 1	8 ± 2
	Androstenedione and androstanedione	12 ± 3	31 ± 7	21 ± 2
Adrenal	Total tritium	254 ± 29	185 ± 19	137 ± 28
	Dihydrotestosterone	18 ± 3	17 ± 4	10 ± 1
	Testosterone	90 ± 17	64 ± 13	30 ± 5
	Androstandiol	76 ± 15	50 ± 8	42 ± 8
	Androstenedione and androstanedione	70 ± 18	54 ± 17	31 ± 7
Testis	Total tritium	387 ± 41	306 ± 37	185 ± 59
	Dihydrotestosterone	7 ± 1	16 ± 3	7 ± 1
	Testosterone	143 ± 57	206 ± 20	143 ± 57
	Androstandiol	22 ± 5	56 ± 18	22 ± 5
	Androstenedione and androstanedione	29 ± 7	28 ± 7	13 ± 4
Liver	Total tritium	3451 ± 357	4281 ± 847	4305 ± 622
	Dihydrotestosterone	37 ± 7	11 ± 1	11 ± 3
	Testosterone	99 ± 21	123 ± 58	350 ± 17
	Androstandiol	58 ± 9	32 ± 13	45 ± 10
	Androstenedione and androstanedione	3256 ± 319	4115 ± 775	4222 ± 594
Ileum epithelium	Total tritium	184 ± 19	198 ± 27	112 ± 14
	Dihydrotestosterone	12 ± 3	16 ± 4	8 ± 2
	Testosterone	56 ± 14	46 ± 8	22 ± 4
	Androstandiol	14 ± 2	17 ± 3	15 ± 5
	Androstenedione and androstanedione	102 ± 13	119 ± 36	67 ± 9
Ileum muscle	Total tritium	179 ± 19	148 ± 13	117 ± 9
	Dihydrotestosterone	9 ± 3	9 ± 4	10 ± 2
	Testosterone	37 ± 5	50 ± 9	28 ± 7
	Androstandiol	10 ± 2	7 ± 2	14 ± 3
	Androstenedione and androstanedione	121 ± 39	82 ± 31	65 ± 16
Plasma	Total tritium	62 ± 1	52 ± 1	32 ± 1
	Dihydrotestosterone	—	—	—
	Testosterone	45 ± 1	35 ± 1	22 ± 1
	Androstandiol	—	—	—
	Androstenedione and androstanedione	—	—	—

Animals were injected intravenously with [1,2-^{3}H]-testosterone (300 μCi/kg) and sacrificed at 15, 30, and 60 min intervals. Values represent the mean ± S.E. of 8 or more values and data are expressed as d.p.m.'s/mg tissue wet weight. Dash denotes nondetectable levels of radioactivity.

appear to be accurate due to the fact that quantification of DHT levels in rat plasma in the authors' laboratory (unpublished observation) recorded levels of DHT similar to those previously reported for rat plasma. Twenty-four hour castration depleted nearly all endogenous androgen (table 3) from the plasma similar to previous reports in the male rat [24].

The finding that steroidogenic tissues such as the

Table 5. *In vitro* metabolism of [1,2-^{3}H]-testosterone in various tissues of the male guinea pig

		d.p.m./mg 15 Min
Seminal vesicle epithelium	Androstandiol	428 ± 58
	Testosterone	44 ± 10
	Dihydrotestosterone	3888 ± 175
	Androstenedione	69 ± 7
	Androstanedione	54 ± 8
Seminal vesicle muscle	Androstandiol	298 ± 12
	Testosterone	681 ± 96
	Dihydrotestosterone	2315 ± 6
	Androstenedione	74 ± 6
	Androstanedione	28 ± 3
Prostate	Androstandiol	322 ± 31
	Testosterone	529 ± 156
	Dihydrotestosterone	2896 ± 12
	Androstenedione	192 ± 12
	Androstanedione	196 ± 4
Gastrocnemius muscle	Androstandiol	145 ± 12
	Testosterone	2195 ± 102
	Dihydrotestosterone	184 ± 48
	Androstenedione	47 ± 12
	Androstanedione	39 ± 6
Liver	Androstandiol	139 ± 6
	Testosterone	232 ± 22
	Dihydrotestosterone	155 ± 10
	Androstenedione	2726 ± 25
	Androstanedione	124 ± 3
Ileum epithelium	Androstandiol	7 ± 1
	Testosterone	40 ± 6
	Dihydrotestosterone	20 ± 3
	Androstenedione	—
	Androstanedione	—
Ileum muscle	Androstandiol	4 ± 2
	Testosterone	45 ± 4
	Dihydrotestosterone	21 ± 6
	Androstenedione	1 ± 0
	Androstanedione	4 ± 1

Tissues were incubated for 15 min with [1,2-^{3}H]-testosterone (1.0×10^{-8} M) in KRB buffer. Values represent the mean ± S.E. of 8 or more values and are expressed as d.p.m./mg tissue wet weight.

testis and adrenal possess the highest intracellular concentration of both testosterone and DHT (Fig. 2) is not particularly surprising. Previous studies measuring testosterone and DHT levels within the testis [25], testis fluid [19, 26], and epididymal fluid and tissue [19] in male rats have demonstrated significant concentrations of both androgens which are in excess of those quantified within plasma. Other studies have identified testicular DHT production in the rat [27, 28], rabbit [29] and dog [30]. In the present studies, the guinea pig adrenal also possessed high levels of endogenous intracellular testosterone and DHT, although less than quantified within the testis. The relative level of these two steroids within the adrenal were approximately equal (Fig. 2). Similar to the testis, levels of adrenal testosterone and DHT were in excess of those quantified within plasma. Endogenous levels of testosterone and DHT within the adrenal had not been previously reported. Recently, *in vitro* production of testosterone by rat adrenal tissue has been demonstrated [24], however, DHT formation was not studied. In the present studies, the *in vivo* metabolism of testosterone to DHT within the testis and adrenal were relatively low compared to male sex accessory tissues (table 4). Therefore, even though 5α-reductase activity is low in steroidogenic tissues in relation to sex accessory tissues, its activity, coupled with an extremely high concentration of the substrate, testosterone, may be sufficient to account for the high endogenous concentration of DHT observed within both the testis and adrenal. Cytoplasmic binding characteristics in the testis were not determined in this study, but specific, high affinity androgen binding substance(s) with an affinity for DHT in excess of testosterone, has been reported in the rat testis [32]. Preliminary Scatchard plot analysis in the authors' laboratory studies of DHT binding in the adrenal cytosol in the adrenal cytoplasm revealed a low affinity, non-specific binding capacity for [^{3}H]-DHT [10].

Of those tissues which are dependent upon adequate concentrations of circulating plasma androgen as their source of intracellular androgen, only the male sex accessory organs possessed levels of endogenous androgen in significant concentrations greater than those found in plasma. Previous studies have shown that male sex accessory organs contain high concentrations of DHT [19, 21, 32–34]. In this study, the guinea pig sex accessory tissues possessed DHT in concentrations greater than plasma, while endogenous levels of testosterone within the sex accessory tissues did not vary significantly from plasma (Fig. 2). Since DHT is concentrated in both epithelium and muscle of the guinea pig sex accessory organs, this suggests that specific intracellular processes are involved within these tissues which allow for the selective accumulation of DHT. In this regard, the male sex accessory tissues exhibited the greatest capacity to form and retain [^{3}H]-DHT of all tissues examined both *in vivo* and *in vitro* (tables 4 and 5). Although not shown, preliminary investigations in the authors' laboratory revealed that the male sex accessory tissues also exhibited the greatest number of high affinity receptor sites for [^{3}H]-DHT of all tissues examined [10]. These studies also revealed that the affinity of the sex accessory organ cytoplasmic androphiles for testosterone is greater than affinities observed in other non-sex accessory tissues. This would suggest that testosterone should also accumulate in the sex accessory tissues in relation to plasma. However, direct measurement of testosterone levels indicates that this is not the case and suggests that the major portion of plasma testosterone, upon entry into the sex accessory tissue cell, is rapidly shunted to DHT and therefore is not available for binding and accumulation in significant intracellular concentrations.

Non-sex accessory tissues such as the gastrocnemius muscle, or separated preparations of epithelium and muscle for the guinea pig ileum possessed endogenous androgens in concentration which were essentially equivalent to those quantified in plasma (Fig. 2). In addition to exhibiting low endogenous androgen concentrations, the non-sex accessory tissues also exhibited low 5α-reductase activity, and low retention of [^{3}H]-DHT.

In summary, with the exception of steroidogenic tissues which synthesize androgen, this study demonstrates a selective capacity of the male sex accessory tissues to concentrate endogenous androgens, particularly DHT, compared to non-sex accessory tissues. Hence, a non-steroidogenic tissue's inherent capacity, or lack thereof, to form or retain DHT appears to be an important determinant of the endogenous intracellular DHT content quantified within. Based on these various parameters of androgen sensitivity the present studies are in general agreement with the concept that the epithelium of the male sex accessory organs is the most androgen sensitive component of the gland. However, the fibromuscular stroma of the male sex accessory organs is indeed different from non-sex accessory organ fibromuscular tissue with respect to the endogenous levels of DHT and the intracellular factors contributing to it. Therefore, the question of the role of intracellular androgen in the fibromuscular stroma of the sex accessory organs appears to be of importance. Possibly, function of the sex accessory organ stroma is regulated in part by androgen, as well as other steroid sex hormones. Certainly a complete appreciation of the contribution of the stroma to sex accessory organ function, and factors influencing both epithelial and stromal function must await future experimentation. Given the observation of the importance of the mesenchyme for support and induction of normal epithelial differentiation [36] and the consideration that certain neoplastic proliferations of the prostate may be consequential to initial stromal influences [*cf.* 35], the factors influencing epithelial function, fibromuscular stroma function, as well as their interaction together in normal and abnormal growth of the prostate should be given appropriate attention in future investigations.

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