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ESTROPHILIC MOLECULES IN THE MALE GUINEA PIG

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SUMMARY

Following the intravenous administration of [^{3}H]-estradiol (300 μCi/kg) to mature male guinea pigs, the sex-accessory tissues (seminal vesicle muscle, seminal vesicle epithelium and prostate gland) and kidney incorporated greater concentrations of the native steroid than the liver, ileum, skeletal muscle or plasma. The seminal vesicle muscle was generally able to concentrate the greatest amount of [^{3}H]-estradiol. With a series of *in vitro* experiments, it was determined that these high concentrations of radioactivity associated with the sex-accessory tissues were not the result of active transport systems. However, it appears as if high affinity, steroid specific cytosolic estradiol binding molecules may serve as the intracellular determinants for the tissue selective distribution and retention of [^{3}H]-estradiol *in vivo*. Using Scatchard plot analyses, it was found that the seminal vesicle muscle possessed both the greatest concentration of estradiol binding sites and the highest equilibrium association constant. The cytosolic binding activities of the seminal vesicle epithelium, prostate gland and kidney were less than that of the seminal vesicle muscle but in great excess of the liver, skeletal muscle or plasma. These findings suggest that estradiol may potentially be a significant contributor to the function of sex-accessory tissue smooth muscle.

INTRODUCTION

While the investigations of estrogen action in various male species have laid the foundation for the estrogenic therapy of prostatic disease, numerous experimental observations have also focused attention to the idea that the endogenous estrogens in the male sex accessory organs may be significant contributors to the normal as well as the neoplastic growth of these organs. Specifically, estrogens have been quantified in the plasma of a variety of male species [*cf.* 1] and in the canine prostate[2]. Estrogen treatment of males can induce growth of the sex accessory organ fibromuscular tissue and can, under certain conditions, synergize with androgen in these organs [*cf.* 1, 3, 4]. A variety of studies have demonstrated a tissue specific localization of estrogen in the male sex accessory organs[5–8] and also have identified estrophilic molecules in these organs [9–11]. Furthermore, using combinations of estradiol and 3α,17β-androstandiol, investigators have been able to induce growths in canine prostate glands comparable to spontaneous benign prostatic hyperplasia[12]. In addition, investigators have also reported elevated levels of androgens and estrogens in hyperplastic prostate glands[2, 13, 14].

In an effort to expand upon the current knowledge of estrogen action in the male sex accessory organs, several facets of the intracellular fate of the hormone were examined in these organs as well as other tissues of the mature male guinea pig. Included in this investigation were (1) the *in vivo* distribution and metabolism of estradiol (2) the *in vitro* characterization of estradiol uptake and metabolism, and (3) the characterization and quantification of cytosolic binding of estradiol. Throughout this particular study, emphasis was placed on the comparative characteristics of estrogen dynamics in the epithelial and fibromuscular tissues of the guinea pig sex accessory organs, since the seminal vesicle of this species may be surgically separated into its two components for separate analysis.

EXPERIMENTAL

In vivo *distribution, metabolism and retention of* 17β[^{3}*H*]*-estradiol.* Guinea pigs were anesthetized by an intra-peritoneal injection of pentobarbital (35 mg/kg). The abdomens of the anesthetized animals were opened and the animals were given the injections of 17β[^{3}H]-estradiol (300 μCi/kg) in a vehicle of saline–ethanol (10:1, v/v) into the inferior vena cava. At various post-injection times, spanning 7 min to 4 h, the animals were sacrificed and blood and various tissues were rapidly removed. The blood was centrifuged and the resulting plasma was frozen. The

The following trivial names are used in place of IUPAC designated names: Androstandiol—5α-androstane-3α,17β-diol or 5α-androstane-3β,17β-diol. Androstanedione—5α-androstane-3,17-dione. Androstenedione—4-androstene-3,17-dione. Cortisol—11β-17,21-trihydroxy-4-pregnene-3,20 dione. Dihydrotestosterone—17β-hydroxy-5α-androstan-3-one. Estradiol—1,3,5(10)-estratriene-3,17β-diol. Estriol—1,3,5(10)-estratriene-3,16α,17β-triol. Estrone—3-hydroxy-1,3,5(10)-estratrien-17-one. Progesterone—4-pregnene-3,20-dione. Testosterone-17β-hydroxy-4-androsten-3-one.

tissues were rinsed, blotted on moist paper towels, wrapped in aluminium foils, frozen in liquid nitrogen and stored at minus 20°C until the time of homogenization. The ileum and seminal vesicles were slit longitudinally, rinsed, separated into their epithelial and muscular components with a blunt scalpel and blotted prior to freezing[15].

Homogenization of each sample was performed at 0–4°C in 2 ml of distilled water using a Polytron homogenizer. The homogenizer was rinsed twice with 3 ml of chloroform–ether (2:1, v/v) and these rinses were added to the aqueous homogenates. Steroid samples were extracted into chloroform–ether by vortexing the samples for 1 min and allowing them to stand overnight at 0–4°C. The following day the samples were centrifuged to separate the aqueous and organic phases. Following centrifugation, a 100 μl aliquot of the aqueous phase of each sample was taken for assessment of water soluble radioactivity and a four ml aliquot of the organic phase was dried, reconstituted in 0.5 ml of chloroform, and spotted on commercially prepared thin-layer chromatograms (Silica Gel G) along with non-radioactive estradiol, estrone and estriol. The thin layer plates were developed in ether–chloroform (3:7, v/v). The validity of this separation technique was confirmed by utilizing duplicate plates that were developed in benzene–ethanol (140:60, v/v). Following visualization with iodine vapors, areas of the thin layer chromatogram corresponding to estradiol ($R_f = 0.54$) estrone ($R_f = 0.81$) and estriol ($R_f = 0.08$) were scraped into scintillation vials and assessed for radioactivity by liquid scintillation spectrometry. Another 20 μl of the reconstituted sample which was applied to the top of the thin-layer chromatogram in an area not exposed to the solvent was scraped into scintillation vials and used as a measure of total chloroform–ether soluble radioactivity. The usual radioactivity recovery obtained with the combined procedures of homogenization, extraction and chromatography was 80%.

With the spectrum of tissues sampled and using the estimated total weights of such tissues in the guinea pig[16] it was determined that 90% of the radioactivity injected into the animal was accountable.

In vitro *characterization and quantification of estrogen uptake and metabolism.* Guinea pigs were killed by a blow at the base of the skull and tissue samples were rapidly removed. Each pre-weighed tissue piece (50–70 mg) was incubated in 1.0 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing various molar concentrations of steroid and/or metabolic inhibitor. In certain experiments, the seminal vesicle muscle and prostate gland were chopped on a McIlwain tissue chopper into 0.5 mm squares prior to incubation. The tissues were incubated in a Dubnoff metabolic shaker incubator in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. To ascertain the degree of non-specific absorption of the steroids to the tissues, tissues were incubated for five s in the labeled steroid–buffer mixture, blotted on moist paper towels, wrapped in aluminum foil and frozen in liquid nitrogen. The levels of 17β[^{3}H]-estradiol associated with the tissue after this brief exposure to the steroid is referred to as a blank value to zero time value. Following the *in vitro* exposure of the tissue to the steroid for durations up to and including 60 min, the tissues were blotted on moist paper towels, wrapped in aluminum foils and frozen in liquid nitrogen. Hereafter, the quantification of steroid uptake and metabolism in the tissues is identical to that previously outlined. Incubation media samples were, with the exception of the homogenization step, assessed for tritiated estrogen content in the same manner as the tissue samples.

Oxygen consumption by the various tissues was assessed using a Yellow Springs Oxygen Monitor. After incubation for thirty minutes in Robinson's Phosphate Buffer in the presence or absence of a metabolic inhibitor, the oxygen consumption was monitored over a 5 min period.

Cytosol binding. Guinea pigs were killed by a blow at the base of the skull and tissues were rapidly removed. Tissues were homogenized (20% w/v) in Tris buffer (pH 7.4) at 0–4°C, with 10–15 strokes of a conical Teflon–glass homogenizer. Samples were then subjected to centrifugation at 2000 *g* for 10 min followed by centrifugation of the supernatant fraction at 100,000 *g* for one h. The final supernatant fraction was designated the cytosol. Four-tenths milliliter of this supernatant (2–4 mg) was usually taken for incubation. In an attempt to obtain approximately equal protein concentration for all tissues, the liver and pancreas cytosols were diluted 2 fold prior to incubation. In defining the optimal conditions for binding, these samples were incubated in 2.3×10^{-10} M [^{3}H]-estradiol-17β for varying time periods (30 min to 6 h), at varying temperatures (0–37°C) and at varying protein concentrations (0.4 mg–4 mg). To insure stability of the pH of the Tris buffers with changing temperatures, it was necessary to add 70 μl of an alkaline solution (0.3 g Tris alkali/100 ml water) to samples incubated at 20 degrees while 85 μl of this alkaline solution were added to samples incubated in 37°C. To maintain equal osmolarities in all samples, 77 μl of a concentrated Tris solution, pH 7.4 (0.38 g Tris/100 ml water), were added to samples incubated at zero and 10°C.

Following the incubations, free and protein-bound tritiated steroid was separated using dextran-coated charcoal (0.0025 g of dextran T-70, and 0.25 g of Norit A in 100 ml saline). Samples were vortexed immediately after the addition of 1.0 ml of the charcoal solution and left to stand for 5 min. The samples were then centrifuged at 2000 *g* for 5 min, after which the supernatant fraction was decanted and assessed for bound 17β[^{3}H]-estradiol. The dextran-coated charcoal solutions were prepared fresh daily and were stirred immediately and continuously following preparation. This particular separation procedure resulted

in precipitation of 95–98% of the free steroid at 2, 5 and 10 min of exposure to charcoal as evidenced by dextran-coated charcoal precipitation of standard amounts of tritiated estradiol dissolved in the buffer. There was no statistically significant precipitation of cytosolic proteins in that protein concentrations were the same before and 2, 5 and 10 min after charcoal precipitation. Over this same time period, the amount of saturable hormone binding remained constant.

In determining the relative contributions of specific (saturable) and nonspecific (nonsaturable) binding to the total amount of estrogen binding, companion assays were done in which samples were incubated with tritiated estradiol and a 100 fold excess concentration of non-radioactive estradiol. For Scatchard plot analyses[17], various molar concentrations of 17β[^{3}H]-estradiol (2.3×10^{-10} M–1.0×10^{-9} M) were incubated alone or in the presence of a 100 fold excess of unlabeled estradiol. That binding which was not abolished in the presence of the unlabeled hormone was used to determine the non-specific or non-saturable binding. In this regard the ratios of nonspecifically bound hormone to free (unbound hormone) were calculated in the assays in which both the labeled and unlabeled estradiol were present. This ratio was found to be constant, within a tissue, for all the hormone concentrations ranging from 2.3×10^{-10}–1.0×10^{-9} M. To calculate the non-specific binding in samples containing only radioactive hormone, the free hormone in these samples was multiplied by the pre-determined ratio for non-specific binding in samples containing both radioactive hormone and a 100 fold excess of non-radioactive hormone. The specific binding of the hormone in samples containing only radioactive hormone was then determined by subtracting this calculated value for nonspecific binding from the total amount of hormone bound. This procedure may be summarized in the equations below where R denotes radioactive hormone, and NR denotes the nonradioactive estradiol.

$$\text{Specifically Bound}_R = \text{Total Bound}_R - \text{Nonspecifically Bound}_R$$

$$\text{Nonspecifically Bound}_R = \text{Free}_R \times \frac{\text{Bound}_R + NR}{\text{Free} + NR}.$$

Scatchard plots of the data to obtain specific binding constants and sites per mg cytosol protein were done by plotting specific bound hormone divided by free hormone against specifically bound hormone. The slope of the line and X intercept values were generated from at least five separate points using linear regression analysis. All lines for specific binding that were generated had correlation coefficients of 0.90–0.99 and were representative only of a single class of binding sites.

Protein determinations on cytosols were performed according to the method of Lowry *et al.*[18].

Statistics. All statistical analyses were performed using the Student's t-test or analyses of variance matched to Duncan's multiple range test[20].

MATERIALS

Mature male guinea pigs (body weight 600–800 g) were purchased from Hilltop Laboratories (Scottsdale, PA) and were housed in the university animal quarters for at least one week prior to experimentation. The animals were fed a standard laboratory chow and water *ad libitum*. The radioactive steroids were purchased from New England Nuclear Corporation. The purity of 17β, 2,4,6,7-[^{3}H]-estradiol (91 Ci/mol) was assessed routinely with the thin-layer chromatography procedure and when steroid impurity exceeded 5–10% the hormone was repurified according to the methods of Butcher *et al.* (1974), [19]. The Sigma Chemical Company supplied the non-radioactive steroids as well as the EDTA and Tris buffer mixtures. Fisher Laboratories provided the sodium cyanide and the Norit A charcoal. The ouabain was supplied by the Aldrich Supply House. Silica Gel G chromatograms were purchased from the Brinkman Company while the Pharmacia Chemical Company supplied the Sephadex LH-20 and Dextran T-70. Organic solvents were purchased from either the Burdick and Jackson or Fisher Chemical Companies. All water used was deionized glass-distilled.

RESULTS

Distribution of tritiated estrogens following intravenous radiosteroid administration

The distribution and metabolism of [^{3}H]-estradiol-17β at various post-injection times, following the intravenous administration of the steroid, is recorded in Table 1. In general, the radioactivity in all the tissues and the plasma decreased with time. From 7 to 30 min, the total chloroform–ether soluble radioactivity associated with the sex-accessory tissues (*e.g.* seminal vesicle muscle, seminal vesicle epithelium, and prostate gland), ileum and testes was intermediate between the high concentrations of radiosteroid in the kidney and pancreas and the very low concentration of total chloroform–ether soluble radioactivity in the liver and gastrocnemius muscle. By 4 h post-injection time, however, the greatest concentrations of total radioactivity were associated with the kidney, ileum epithelium and sex-accessory tissues. Despite the initial high steroid concentrations associated with the pancreas and testes, these tissues retained only negligible concentrations of labeled hormone.

In examination of the individual steroids comprising the total chloroform ether soluble radioactivity, it was found that the liver, kidney, pancreas and ileum all contained measurable concentrations of tritiated estrone and estriol as well as the labeled estradiol. The total chloroform–ether soluble radioactivity associated with the sex-accessory tissues, testes and

Table 1. The distribution of tritiated estrogens following intravenous injection of 17β[^{3}H]-estradiol (300 μCi/kg). Radioactive estradiol was injected into the inferior vena cava and at varying times post-injection, the animals were sacrificed and tissues were assessed for radioactivity. Data are expressed as d.p.m. radiosteroid/mg or d.p.m. radiosteroid/μl

	7 Min	15 Min	30 Min	60 Min	2 h	4 h
Seminal vesicle epithelium						
Total chloroform–ether soluble radioactivity	1441 ± 205*	725 ± 85	473 ± 58‡	306 ± 38‡	155 ± 29‡	180 ± 36
1. Estradiol	1390 ± 107†	690 ± 70	439 ± 58	297 ± 13	147 ± 27	169 ± 36
2. Estrone	27 ± 3	—	16 ± 1	—	—	—
3. Estriol	—§	—	—	—	—	—
Aqueous fraction	63 ± 16	24 ± 3	21 ± 6	12 ± 2	8 ± 2	30 ± 11
Seminal vesicle muscle						
Total chloroform–ether soluble radioactivity	792 ± 143	628 ± 29	609 ± 66	443 ± 40	243 ± 31	230 ± 36
1. Estradiol	764 ± 62	600 ± 37	590 ± 71	399 ± 51	237 ± 29	222 ± 39
2. Estrone	—	—	—	—	—	—
3. Estriol	—	—	—	—	—	—
Aqueous fraction	64 ± 16	39 ± 3	35 ± 4	24 ± 3	8 ± 3	9 ± 2
Prostate gland						
Total chloroform–ether soluble radioactivity	450 ± 55	350 ± 36	410 ± 34	250 ± 23	76 ± 7	106 ± 12
1. Estradiol	427 ± 31	320 ± 42	370 ± 58	231 ± 16	72 ± 2	99 ± 10
2. Estrone	—	—	—	—	—	—
3. Estriol	—	—	—	—	—	—
Aqueous fraction	71 ± 7	43 ± 6	35 ± 7	37 ± 13	20 ± 3	12 ± 3
Testes						
Total chloroform–ether soluble radioactivity	1008 ± 92	615 ± 68	589 ± 30	291 ± 48	46 ± 8	35 ± 3
1. Estriadiol	949 ± 81	584 ± 71	556 ± 62	282 ± 30	42 ± 5	29 ± 4
2. Estrone	21 ± 4	—	—	—	—	—
3. Estriol	—	—	—	—	—	—
Aqueous fraction	16 ± 2	14 ± 1	14 ± 2	11 ± 1	9 ± 2	5 ± 3
Gastrocnemius						
Total chloroform–ether soluble radioactivity	54 ± 19	98 ± 15	200 ± 19	109 ± 1	20 ± 9	12 ± 2
1. Estradiol	49 ± 6	90 ± 9	186 ± 30	91 ± 4	16 ± 5	11 ± 2
2. Estrone	—	—	—	—	—	—
3. Estriol	—	—	—	—	—	—
Aqueous fraction	12 ± 5	11 ± 2	12 ± 3	10 ± 2	8 ± 3	22 ± 15
Liver						
Total chloroform–ether soluble radioactivity	224 ± 29	137 ± 7	122 ± 17	79 ± 15	46 ± 2	52 ± 9
1. Estradiol	77 ± 8	34 ± 5	8 ± 2	10 ± 3	9 ± 3	11 ± 4
2. Estrone	104 ± 12	61 ± 16	31 ± 7	50 ± 18	8 ± 1	14 ± 5
3. Estriol	25 ± 9	11 ± 3	13 ± 2	12 ± 5	26 ± 2	25 ± 2
Aqueous fraction	392 ± 64	171 ± 13	106 ± 11	60 ± 6	18 ± 4	20 ± 5
Kidney						
Total chloroform–ether soluble radioactivity	1600 ± 298	1065 ± 76	965 ± 132	467 ± 35	210 ± 22	248 ± 57
1. Estradiol	679 ± 84	430 ± 43	373 ± 43	316 ± 32	212 ± 40	200 ± 46
2. Estrone	431 ± 154	223 ± 41	143 ± 9	121 ± 32	—	43 ± 12
3. Estriol	36 ± 10	27 ± 5	18 ± 5	7 ± 2	—	—
Aqueous fraction	1427 ± 553	3421 ± 624	2815 ± 204	1041 ± 133	450 ± 263	367 ± 32
Pancreas						
Total chloroform–ether soluble radioactivity	2612 ± 552	3586 ± 591	3584 ± 826	941 ± 150	120 ± 34	66 ± 16
1. Estradiol	470 ± 59	379 ± 39	401 ± 59	333 ± 28	33 ± 11	42 ± 13
2. Estrone	483 ± 120	1508 ± 395	1952 ± 344	292 ± 60	78 ± 26	20 ± 3
3. Estriol	19 ± 3	22 ± 1	15 ± 5	20 ± 4	—	—
Aqueous fraction	54 ± 5	57 ± 11	47 ± 8	34 ± 5	29 ± 5	34 ± 8
Ileum epithelium						
Total chloroform–ether soluble radioactivity		286 ± 47		99 ± 11	453 ± 120	155 ± 48
1. Estradiol		228 ± 61		40 ± 4	40 ± 2	23 ± 4
2. Estrone		44 ± 14		86 ± 33	360 ± 97	40 ± 13
3. Estriol				5 ± 2	25 ± 10	29 ± 13
Aqueous fraction		21 ± 6		353 ± 146	81 ± 20	282 ± 40

Table 1 (continued)

Ileum muscle					
Total chloroform–ether soluble radioactivity	343 ± 60		114 ± 11	178 ± 48	64 ± 12
1. Estradiol	337 ± 53		66 ± 6	43 ± 7	34 ± 6
2. Estrone	49 ± 8		30 ± 2	130 ± 42	27 ± 5
3. Estriol	—		3 ± 1	7 ± 4	5 ± 2
Aqueous fraction	39 ± 4		55 ± 15	51 ± 15	125 ± 72
Plasma					
Total chloroform–ether soluble radioactivity	64 ± 2	20 ± 1	14 ± 3	—	—
1. Estradiol	49 ± 2	13 ± 3	12 ± 2	—	—
2. Estrone	—	—	—	—	—
3. Estriol	—	—	—	—	—
Aqueous fraction	—	—	—	—	—

* Mean tissue d.p.m./mg ± S.E.M. of total chloroform–ether soluble radioactivity. † Mean tissue d.p.m./mg ± S.E.M. of individual estrogens. ‡ Significantly different ($P < 0.05$) than seminal vesicle muscle. § Non-detectable radiosteroid concentrations. n = At least six observations.

skeletal muscle consisted mainly (greater than 90%) of [^{3}H]-estradiol-17β.

A comparison of the levels of [^{3}H]-estradiol-17β recovered from 7 min to 4 h post-injection, reveals that the sex-accessory tissues generally contained relatively high concentrations of the estradiol. At 2 h post-injection (Fig. 1), there was a clear demarcation of the relatively high [^{3}H]-estradiol-17β concentrations associated with the kidney and seminal vesicle epithelium and muscle from that recovered in all other tissues. Interestingly, within the sex-accessory tissues, the seminal vesicle epithelium contained the highest concentration of [^{3}H]-estradiol-17β at 7 and 15 min post-injection, but at all subsequent times (*e.g.* 30, 60, 120 and 240 min) the muscle of the seminal vesicle contained the greatest concentration of [^{3}H]-estradiol-17β. These differences between the seminal vesicle muscle and epithelium were statistically different at 30, 60 and 120 min post-injection. In general, the prostate gland always contained less tritriated estradiol than both components of the seminal vesicle.

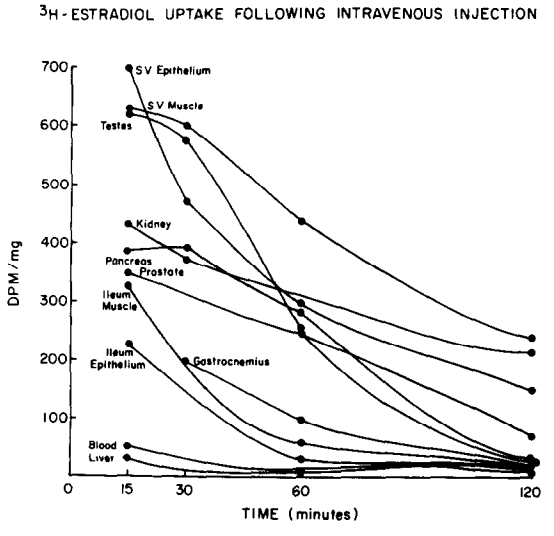

Fig. 1. Distribution of tritiated estradiol following intravenous administration. At various intervals following the intravenous injection of [^{3}H]-estradiol (300 μCi/kg) tissues were excised and assessed for radioactivity. Values for this figure were obtained from the data in Table 1.

In vitro *characterization of* 17β[^{3}H]*-estradiol uptake*

To more fully determine the characteristics of [^{3}H]-estradiol-17β uptake, distribution and metabolism in various tissues of the male guinea pig, a series of *in vitro* experiments were performed using [^{3}H]-estradiol. Although not shown, with these studies it was found that similar to the *in vivo* distribution study, the liver, kidney and pancreas all oxidized the tritiated estradiol to labeled estrone while the sex-accessory tissues and testes demonstrated essentially no metabolic alteration of the steroid to labeled estrone or estriol. Among the sex-accessory tissues the rate of uptake of the tritiated steroid was linear up to and including ten minutes of incubation time (Table 2). The zero minute (5 s incubation) or blank values were relatively high and constituted a significant percentage of the steroid incorporation values associated with the longer incubation periods. Subtracting these blank values from the values obtained with longer incubation periods, demonstrated that the differences in the initial rates for [^{3}H]-estradiol uptake among various tissues were rather small although the rate of estradiol uptake in the seminal vesicle muscle (92 d.p.m./mg/min) was slightly greater than that of the seminal vesicle epithelium (73 d.p.m./mg/min) or prostate gland (80 d.p.m./mg/min). Following 10 min of *in vitro* incubation, these rates of estradiol uptake allowed for concentration of the hormone in these tissues at least five fold in relation to the incubation medium. Correction of the data for these zero minute steroid incorporation values seems justified in that they appear to represent only non-specific steroid adsorption to the tissues as 5 s incubations carried out with a variety of labeled compounds including 5.5×10^{-10} M

Table 2. Summary of the radioactivity recovered from the seminal vesicle and prostate gland following *in vitro* incubations with [^{3}H]-estradiol (5.5×10^{-10} M)

	0 Min	2 Min	5 Min	7 Min	10 Min	15 Min
Seminal vesicle epithelium	666 ± 23*	904 ± 80	1075 ± 14	1179 ± 120	1350 ± 148	1464 ± 399
Seminal vesicle muscle	306 ± 25	544 ± 51	767 ± 42	952 ± 49	1215 ± 163	1223 ± 71
Prostate gland	325 ± 46	458 ± 31	709 ± 30	888 ± 17	991 ± 97	1031 ± 46

Tissue slices (0.5 mm) were incubated *in vitro* in Krebs–Ringer bicarbonate buffer at 37°C. At the end of the designated time periods tissue and incubation media were separated and assessed for radioactivity. Data are expressed as d.p.m. [^{3}H]-estradiol/mg. n = at least 5 observations. * = Mean ± S.E.M.

estriol, estradiol, progesterone, dihydrotestosterone, testosterone and desoxycorticosterone all resulted in approximately the same absorption of tritiated material to the tissues.

Studies of the influence of temperature (not shown) on the *in vitro* uptake of [^{3}H]-estradiol indicated that increasing temperatures had a positive influence on steroid uptake with maximal uptake occurring at 37°C. For example, when the seminal vesicle muscle was incubated with 5.5 ± 10^{-10}M [^{3}H]-estradiol, the uptake (mmol $\times 10^{-8}$/g ± S.E.M.) at 0°, 20° and 37°C was 0.20 ± 0.02, 0.47 ± 0.07 and 0.59 ± 0.08 respectively. Similar temperature-related rate increases were also observed with the seminal vesicle epithelium and prostate gland. Testing the rate of [^{3}H]-estradiol uptake at 37°C over the molar concentration range of 5.5×10^{-10} to 1.0×10^{-5} demonstrated that the uptake systems for estradiol in these sex-accessory tissues had affinities approaching infinity (Table 3, Fig. 2). Such studies were also performed at 0°C and 20°C and a similar lack of saturation in estradiol uptake by the sex-accessory tissues was observed (not shown). Evaluation of the *in vitro* uptake of [^{3}H]-estradiol by the seminal vesicle tissues in the presence of sodium cyanide (1×10^{-3} M) revealed that this metabolic inhibitor had no significant effect on the radiosteroid concentrations associated with these tissues (Table 4). The chemical effectiveness of the cyanide was confirmed with experiments which demonstrated that this metabolic inhibitor decreased ($P < 0.05$) oxygen consumption by these tissues to 60% of control levels. In light of the experimental evidence generated from these *in vitro* uptake experiments, it does not appear likely that the abilities of the sex-accessory tissues to concentrate estradiol in relation to the plasma was the result of an active transport system for the steroid across the cell membranes.

In pursuit of the hypothesis that the somewhat tissue-selective *in vivo* distribution of [^{3}H]-estradiol may be closely related to the activity of steroid and tissue specific estrophilic molecules, experiments were performed to characterize and quantify cytosolic estrophiles in the various tissues. As a prelude to the Scatchard plot analyses of cytosolic estradiol binding activity, the optimal experimental conditions for binding with respect to temperature and incubation periods were determined.

Table 5 compares the sex-accessory tissues cytosolic [^{3}H]-estradiol binding at various temperatures and for varying incubation periods. For both the seminal vesicle muscle and epithelium, cytosolic binding was temperature dependent with maximal binding at 20°C. In contrast to the seminal vesicle cell types, cytosolic binding by the prostate gland appeared essentially temperature independent from 0–20°C. The binding of estradiol in all samples was decreased at 28°C and abolished by incubation of samples at 37°C (not shown). For all the sex-accessory tissues, at all temperatures examined, equilibrium of the

Table 3. The 17β[^{3}H]-estradiol recovered from the guinea pig seminal vesicle and prostate gland following seven-minute *in vitro* incubations in various molar concentrations of 17β[^{3}H]-estradiol

Media-molar concentration	Epithelium	Muscle	Prostate
5.5×10^{-10} M	0.50 ± 0.08*	0.59 ± 0.08	0.52 ± 0.06
9.2×10^{-10} M	0.80 ± 0.04	1.11 ± 0.14	1.01 ± 0.08
2.75×10^{-9} M	2.75 ± 0.30	3.02 ± 0.22	3.00 ± 0.28
5.50×10^{-9} M	7.00 ± 0.97	6.20 ± 0.68	6.00 ± 0.74
1.00×10^{-8} M	18.00 ± 1.12	10.40 ± 0.80	
5.00×10^{-8} M	98.20 ± 8.00	60.00 ± 4.80	
1.00×10^{-7} M	160.00 ± 4.00	120.00 ± 9.00	
1.00×10^{-6} M	1,400.00 ± 200.00	970.00 ± 80.00	
1.00×10^{-5} M	12,500.00 ± 1,000.00	9,600.00 ± 1,000.00	

* Mean ± S.E.M. n = 5 observations. Tissue slices (50 mg) were incubated *in vitro* in Krebs-Ringer bicarbonate buffer at 37°C. Tissues and incubation media were separated and assessed for radioactivity. Data are expressed as mmol radiosteroid 10^{-8}/g of tissue wet weight.

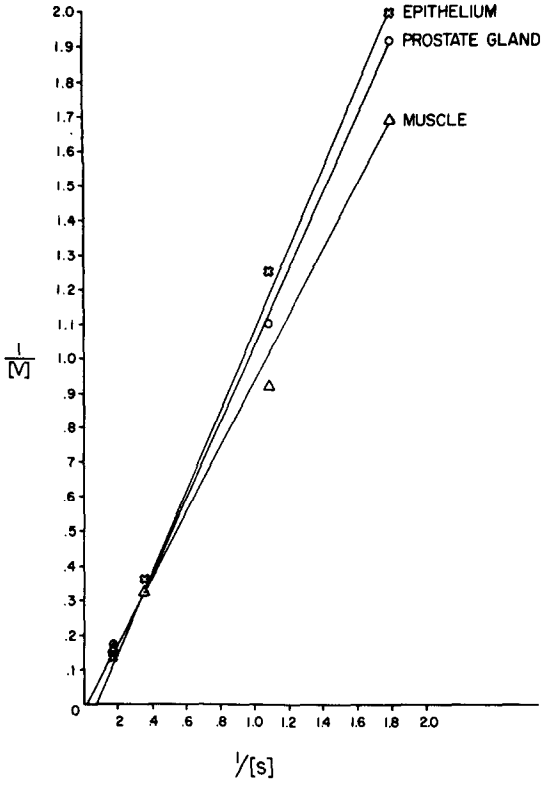

Fig. 2. Double reciprocal plot analyses of estradiol uptake by the seminal vesicle tissues and prostate gland. Uptake velocities (mmol of radiosteroid $\times 10^{-8}$/g/7 min) were calculated from the data in Table 3. Substrates used in this study ranged from 5.5×10^{-10} M to 5.5×10^{-9} M.

estradiol with the binding components of the cytosol was achieved with four hours of incubation, and although not shown, equilibrium of the steroid with the estrophilic molecules was also achieved in all other tissues examined with four hours of incubation. With all of the cytosols examined, there was no metabolic alteration of the radiosteroid following 4 h of incubation at 0°C. Based on this data, Scatchard plot analyses were generally carried out by incubation of the cytosol for four h at both 0 and 20°C.

The molar concentration of [^{3}H]-estradiol-17β employed in the Scatchard plot analyses ranged from 2.3×10^{-10} to 1.0×10^{-9}. Protein dependency for estradiol binding was determined by incubating cytosolic aliquots containing various protein concentrations (all brought to a constant volume of 0.4 ml) for four hours with 2.3×10^{-10} M [^{3}H]-estradiol. Figures 3 and 4 representatively depict the relationship of tritiated estradiol binding to the cytosols of seminal vesicle muscle, seminal vesicle epithelium, prostate gland, kidney, liver and pancreas as a function of the cytosolic protein concentration. In general, steroid binding was linear up to and including 4–5 mg of cytosolic protein. For all tissues 0.4 ml of cytosols, generated from 20% w/v homogenization of tissues (with a subsequent two fold dilution of liver and pancreas cytosol), usually contained protein concen-

Table 5. Comparisons of the cytosolic binding of [^{3}H]-estradiol by the guinea pig seminal vesicle and prostate gland at different temperatures and incubation intervals

	Muscle	Epithelium	Prostate gland
0°			
1 h	5.14 ± 0.10*	1.10 ± 0.20	1.84 ± 1.36
2 h	6.00 ± 0.40	1.30 ± 0.14	2.20 ± 0.37
4 h	7.00 ± 0.52	1.56 ± 0.27	2.00 ± 0.19
6 h	6.78 ± 0.89	1.47 ± 0.20	
10°			
1 h	9.20 ± 0.53		2.20 ± 0.18
2 h	12.40 ± 0.39		1.84 ± 0.22
4 h	12.87 ± 1.40		2.20 ± 0.26
6 h	12.64 ± 2.03		
20°			
1 h	13.97 ± 1.43	2.94 ± 0.11	1.84 ± 0.19
2 h	16.54 ± 1.68	6.30 ± 1.04	2.04 ± 0.30
4 h	17.27 ± 1.51	6.30 ± 0.99	1.84 ± 0.19
6 h	16.17 ± 2.00	5.88 ± 0.87	1.84 ± 0.25
28°			
1 h	7.61 ± 0.69	2.88 ± 0.32	1.02 ± 0.16
2 h	10.87 ± 0.97	2.94 ± 0.26	1.02 ± 0.10
4 h	10.94 ± 2.10	3.06 ± 0.41	1.16 ± 0.14
6 h	9.66 ± 1.64	3.06 ± 0.42	1.10 ± 0.19

* Mean ± S.E.M. n = at least five determinations. Cytosol samples were incubated with 2.3×10^{-10} M [^{3}H]-estradiol for 1–6 h at 0–37°C. Data are expressed as fmol [^{3}H]-estradiol bound/mg cytosolic protein. Incubation of all samples at 37°C abolished cytosolic binding.

Table 4. The *in vitro* effect of sodium cyanide on the *in vitro* uptake of [^{3}H]-estradiol by the guinea pig seminal vesicle tissues

	Tissue to incubation medium concentration ratio (d.p.m./mg divided by d.p.m./μl)	
	Control	Cyanide
Muscle	6.62 ± 0.28*	6.58 ± 0.20
Epithelium	5.50 ± 0.36	5.54 ± 0.40

* Mean ± S.E.M. n = five observations. Tissues were pre-incubated in Krebs–Ringer bicarbonate buffer at 37°C either in the presence or absence of 1×10^{-3} M sodium cyanide. At the end of thirty minutes, the radiosteroid (5.5×10^{-10} M) was added and samples incubated for 7 min after which the tissues and media were separated and assessed for radioactivity.

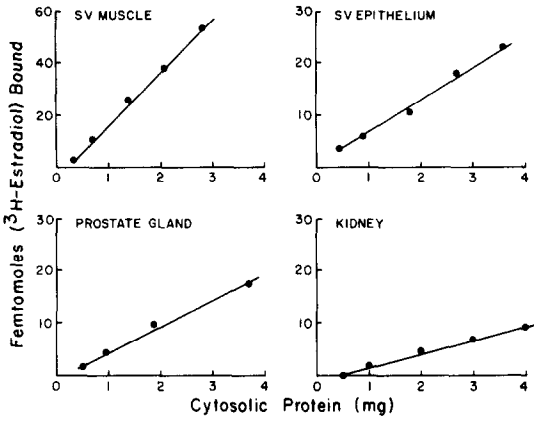

Fig. 3. Representative presentation of the fmol of [^{3}H]-estradiol bound when cytosols of varying protein concentrations were incubated with 2.3×10^{-10} M radiosteroid. Cytosols of varying protein concentrations, all brought to a constant volume of 0.4 mls, were incubated for four h at 20°C with the radiosteroid. At the end of the incubation, free and protein bound steroid were separated with the dextran-charcoal suspension.

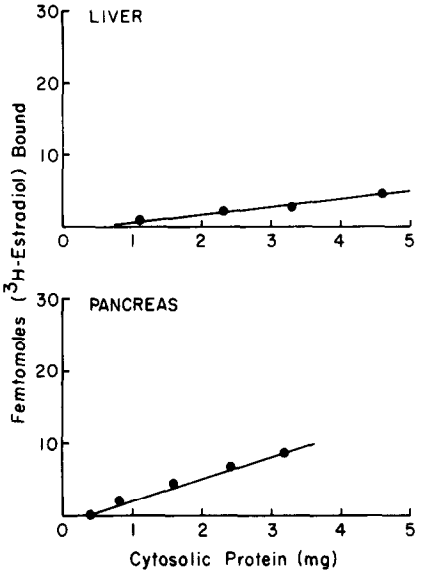

Fig. 4. Representative presentation of the fmol of [^{3}H]-estradiol bound when cytosols of varying protein concentrations were incubated with 2.3×10^{-10} M radiosteroid. Cytosols of varying protein concentrations, all brought to a constant vol. of 0.4 mls, were incubated for four h at 20°C with the radiosteroid. At the end of the incubation, free and protein bound steroid were separated with the dextran-charcoal suspension.

trations within the range of 2.5–4 mg protein. For this reason, 0.4 ml of cytosol generated from a 20% w/v homogenization was routinely employed for the Scatchard plot analyses.

Table 6 depicts the association constants (K_a) and binding sites for [^{3}H]-estradiol calculated for the various tissues when incubations were carried out at 0°C. There was a sharp demarcation of the sex-accessory tissue and testes specific association constants from those associated with all other tissues. Among the sex-accessory tissues, the seminal vesicle muscle possessed the greatest association constant. Of the high affinity binding sites determined for various tissues at 0°C, the greatest concentrations were found in the seminal vesicle muscle and prostate gland. The seminal vesicle epithelium and testes possessed only moderate receptor concentrations.

In comparing the specific association constants at 0° and 20°C (Tables 6 and 7), it was found that the increased temperatures resulted in at least a four fold elevation in the K_a values for estradiol binding in the kidney, seminal vesicle epithelium and seminal vesicle muscle. At 20°C, the seminal vesicle muscle clearly possessed the highest specific association constant followed in order by the seminal vesicle epithelium, kidney, testes and prostate gland. Representative Scatchard plots for these 20°C experiments are shown in Fig. 5. At 20°C it was observed that for every tissue examined, the number of specific binding

Table 6. Comparisons of the association constants (K_a values) and binding sites derived from Scatchard plot analyses (0°C) for cytosolic estradiol binding by various tissues

	Association constant (K_a) $\times 10^9$L/mol)	Binding sites (mol $\times 10^{-14}$/mg protein)
Seminal vesicle epithelium	1.94 ± 0.2*	0.76 ± 0.06
Seminal vesicle muscle	4.11 ± 0.68	2.30 ± 0.30
Prostate gland	3.06 ± 0.39	1.44 ± 0.20
Testes	2.99 ± 0.49	0.29 ± 0.05
Kidney	0.60 ± 0.02	0.60 ± 0.08
Liver	non-detectable	non-detectable
Pancreas	non-detectable	non-detectable
Gastrocnemius	non-detectable	non-detectable

* Mean ± S.E.M. n = at least four observations. Cytosols were incubated with concentrations of [^{3}H]-estradiol ranging from 2.3×10^{-10} M to 1.0×10^{-9} M either in the presence or absence of a 100 fold excess of unlabeled estradiol. Following four hour incubations, free and protein bound steroid were separated with the dextran–charcoal suspension. Specific or saturable binding was calculated as outlined in the Experimental section.

Table 7. Comparisons of the association constants (K_a values) and binding sites derived from Scatchard plot analyses (20°C) for cytosolic estradiol binding by various tissues

	Association constant (K_a) $\times 10^9$ L/mol	Binding sites (mol $\times 10^{-14}$/mg protein)
Seminal vesicle epithelium	8.58 ± 1.30*	0.69 ± 0.10
Seminal vesicle muscle	24.63 ± 4.10	2.08 ± 0.34
Prostate gland	2.62 ± 0.31	1.80 ± 0.21
Testes	3.22 ± 0.38	0.27 ± 0.04
Kidney	4.90 ± 0.27	0.47 ± 0.04
Liver	non-detectable	non-detectable
Pancreas	non-detectable	non-detectable
Gastrocnemius	non-detectable	non-detectable

* Mean ± S.E.M. n = at least four observations. Cytosols were incubated with concentrations of [^{3}H]-estradiol ranging from 2.3×10^{-10} M to 1.0×10^{-9} M either in the presence or absence of a 100 fold excess of unlabeled estradiol. Following four hour incubations, free and protein bound steroid were separated with the dextran-charcoal suspension. Specific or saturable binding was calculated as outlined in the Experimental section.

sites did not differ from that recorded at 0°C (Tables 6 and 7).

DISCUSSION

The distribution of radiolabeled estrogens following systemic administration has been examined in a variety of male species. In the rat, Mangan *et al.*[7] reported that one hour after the intraperitoneal administration of ^{3}H-diethylstilbestrol the greatest concentration of radioactivity was associated with the prostate gland. Tveter[21] found that following the intra-muscular administration of [^{3}H]-estradiol-17β into three month old rats, the radioactivity associated with the prostate gland was intermediate between the relatively high tritium concentrations in the liver and pituitary gland and the rather negligible levels in the skeletal muscle and blood. In contrast to these two studies, Eisenfeld and Axelrod[22] demonstrated that following the intravenous administration of [^{3}H]-estradiol-17β, the steroid concentration within the rat prostate gland and epididymus was quite small in comparison with those tritium concentrations recovered from the adrenal gland, anterior pituitary and fat. In the male rabbit, Promislow and coworkers[23] reported that after the intravenous ad-

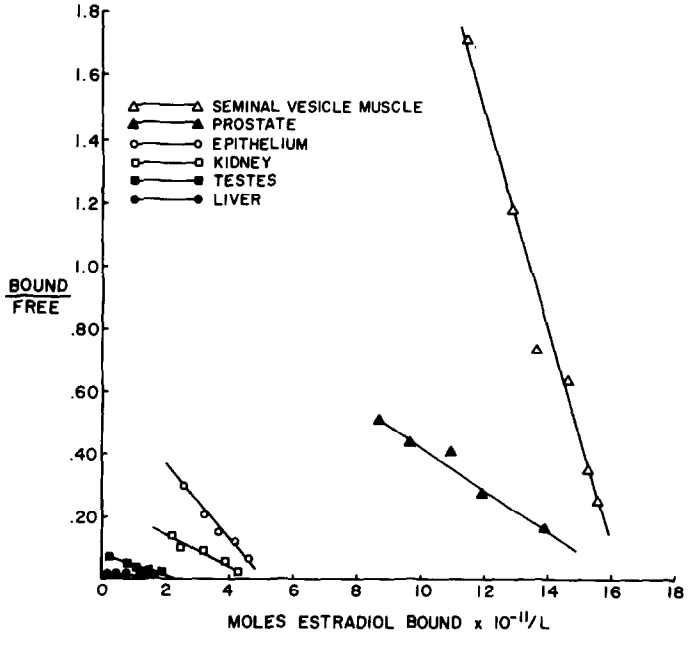

Fig. 5. Representative Scatchard plot analyses (20°C) of the high-affinity cytosolic binding of estradiol by various tissues of the male guinea pig. Experiments were performed at 20°C as outlined in the Experimental section. Lines represent specific or saturable binding and the mean ± S.E.M. for multiple Scatchard plot determinations of affinity and binding sites are summarized in Table 7.

ministration of [^{3}H]-diethylstilbestrol, the total radioactivity in the prostate gland was less than that in the liver, bladder and serum. When human prostatic cancer patients were administered radiolabeled stilbestrol diphosphate, greater steroid concentrations were recovered from the prostate gland than from the testes, muscle or fat[24, 25]. Using the male dog, Varkarakis *et al.*[6] reported that one hour after the intravenous injection of [^{3}H]-estriol, the concentration of total radioactivity associated with the prostate gland was twice that associated with the blood, skeletal muscle and testes. Also in the dog, Kirdani *et al.*[5] found that 30 min after the intravenous administration of [^{3}H]-estriol, the pancreas, followed in order by the liver and kidney, concentrated the greatest amounts of total radioactivity. The concentration of tritium associated with the prostate gland was less than that in the kidney but greater than that in the skeletal muscle, testes, thyroid gland and plasma. It can be difficult to interpret and compare these results of estrogen distribution studies in the rat, rabbit, human and dog in that only total radioactivity was assessed. Comparing only total radioactivity concentrations in organs such as the kidney, liver, pancreas and sex-accessory tissues may obscure important metabolic differences among these organs. Furthermore, it is valuable to separately analyze the epithelium and muscle of male sex-accessory tissues with respect to steroid hormone dynamics in that important differences may exist in the way these two cell lines assimilate estrogens and androgens. For example, investigations by Schwartz and Mawhinney[26] demonstrated that following the intravenous administration of [^{3}H]-testosterone to male guinea pigs, the seminal vesicle epithelium always contained a much greater concentration of [^{3}H]-dihydrotestosterone than the seminal vesicle muscle. In the present study (Table 1, Fig. 1), the seminal vesicle usually possessed a significantly greater concentration of [^{3}H]-estradiol than the seminal vesicle epithelium. If sex-accessory organ epithelium differs from the stroma in estrogen assimilation, then differences recorded to date in different species with respect to sex-accessory gland estrogen uptake and retention may relate in part to differences in the relative amounts of epithelium and fibromuscular stroma contained within the different glands.

In the present study the individual estrogens composing the total chloroform–ether soluble radioactivity were determined and the epithelial and fibromuscular components of the seminal vesicle were individually assessed. Unfortunately, only intact prostate glands could be analyzed in that the procedures to separate this gland into its two components have not been developed. It was found that in the male guinea pig, the distribution and retention of ^{3}H-estradiol was tissue selective. In general, the greatest concentrations of the radiosteroid were associated with the sex-accessory tissues and kidney. Among the sex-accessory tissues, the seminal vesicle muscle generally possessed the greatest ability to concentrate the [^{3}H]-estradiol.

With the use of *in vitro* incubations it was determined that the high concentration of [^{3}H]-estradiol associated with the sex-accessory tissues following intravenous administration was not due to the presence of active transport systems for the steroid across the cell membranes. Interestingly, in the female rat [^{3}H]-estradiol was found to be concentrated by the uterus and vagina following both intravenous administration and *in vitro* incubations [*cf.* 27], but this ability of the rat uterine strips to concentrate estradiol *in vitro* could not be attributed to a membrane located active transport system[28].

A very viable alternative explanation for the somewhat tissue-specific retention of [^{3}H]-estradiol following the intravenous administration of the hormone was provided by the analyses of cytosolic binding (Tables 5–7). Scatchard plot analyses, carried out at the optimal temperature (20°C) revealed that the relative activities of high affinity cytosolic estrophiles generally correlated with the distribution and retention of the injected [^{3}H]-estradiol.

It is interesting that in contrast to the K_a values for estradiol binding in the seminal vesicle epithelium, seminal vesicle muscle and prostate gland at 0°C, there was such a wide divergence in the 20°C specific association constants calculated for the different sex-accessory tissues. In comparing the 0° K_a values with those calculated for 20°C, there was a six-fold increase for the seminal vesicle muscle and a four-fold increase for the seminal vesicle epithelium. No change in the association constants was obtained with the prostate gland at the different temperatures. Possibly the tissue-selective enhancement of estradiol affinity for the estradiol receptors at 20°C may be due to varying concentrations of endogenous dihydrotestosterone released from binding sites. Competition by dihydrotestosterone with estradiol for the estradiol receptor sites may diminish or even mask a temperature-related increase in affinity of the estradiol for the estradiol receptors in the seminal vesicle epithelium and prostate gland. Regardless of differences in their affinities for estradiol, similar types of estrophiles appear to exist in the sex-accessory tissues in that the steroid specificity for these molecules (estradiol > estrone > dihydrotestosterone and testosterone) has been shown to be consistent in the seminal vesicle epithelium, seminal vesicle muscle and prostate gland[29].

Studies in this laboratory of androgen distribution and binding in the guinea pig have provided interesting contrasts to the present analyses of estrogen dynamics[26, 29]. Injected testosterone localized in the sex accessory tissues primarily in the form of dihydrotestosterone; the seminal vesicle epithelium contained the highest concentrations of the androgen followed in order by the prostate gland, seminal vesicle muscle, and non-sex-accessory tissues. Androgen binding was identified in both epithelium and muscle, with andro-

philic activity in the epithelium greatly exceeding that of the muscle. The temperature optimum for androgen binding was 0°C. Studies of steroid specificity of androgen binding in both these cell types have demonstrated the order of competitor activity for androgen binding to be dihydrotestosterone > testosterone > estradiol and progesterone > estrone.

Cytosol estrophilic and androphilic molecules in the male guinea pig sex-accessory organs appear to be distinct entities, differing in their steroid specificity and in their relative tissue distribution. Androgen binding molecules predominate in the seminal vesicle epithelium while in the seminal vesicle muscle the population of estrogen receptors greatly exceeds that of the androgen receptors. These findings to date suggest that estrogens may be or at least have the potential to be significant contributors to the function of male sex-accessory organs, particularly the smooth muscle of the organs.

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