

Estradiol and Testosterone Secretion by Human, Simian, and Canine Testes, in Males with Hypogonadism and in Male Pseudohermaphrodites with the Feminizing Testes Syndrome

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ABSTRACT The role of the human testis in the production of 17β -estradiol (E_2) was investigated by determining the concentration of E_2 and testosterone in peripheral and spermatic vein plasma samples. Specimens were obtained from eight normal men, three men with hypogonadism, and two patients with the incomplete form of the feminizing testes syndrome. For comparison, similar studies were performed in four monkeys, 10 mongrel dogs, and 4 additional dogs who were given 1000 IU of human chorionic gonadotropin/day for 5 days. Plasma E_2 was measured by radioimmunoassay utilizing sheep anti- E_2 serum preceded by ether extraction and thin layer chromatographic separation of plasma steroids. Procedural blanks, which were subtracted from all reported values were 14.1 ± 0.74 (SEM) pg for deionized water and 13.1 ± 0.66 pg for charcoal-adsorbed pooled male plasma. Pooled male and pooled female control plasmas averaged 17 ± 0.71 pg/ml and 95 ± 6.9 pg/ml, respectively; individual adult male specimens ranged between 8 and 28 with a mean of 18 ± 1.4 pg/ml. In the eight normal men, the mean peripheral vein E_2 concentration was 20 ± 1.6 pg/ml,

while the spermatic vein concentration was 50 times as great, 1049 ± 57 pg/ml. All three patients with testicular abnormalities had low spermatic vein E_2 concentrations (160, 280, and 416 pg/ml). Lesser E_2 gradients were found across the simian (3-fold) and canine (approximately 12-fold) testes. Testicular testosterone gradients (human 110-, simian 10-, and canine 77-fold) were greater than the E_2 gradients in all three species. In four dogs, HCG treatment elicited a 6-fold increase in peripheral and a 9-fold increase in spermatic vein testosterone concentrations; however, peripheral and spermatic vein E_2 concentrations did not differ from control values. Spermatic vein E_2 concentrations were > 4600 and 2210 pg/ml (post-HCG) in two patients with the incomplete form of the feminizing testes syndrome. Postorchietomy, peripheral E_2 and testosterone concentrations fell precipitously in both patients, confirming the major contribution of the testes, in this syndrome, to circulating E_2 and testosterone. These studies provide direct evidence that the human testis secretes estradiol.

INTRODUCTION

The role of the human testis in estrogen production has been a controversial subject for many years. As early as the late 1920's, estrogen was found in normal male urine by bioassay (1-4). Steinach and Kun in 1937 (5) and later West, Damast, Sarro, and Pearson (6), demonstrated that parenteral testosterone could be converted to estrogens. Leach, Maddock, Tokuyama, Paulsen, and Nelson (7) and others (8-11) showed that HCG increased urinary estrogen excretion while

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castration produced a fall. With advances in chemical and tracer methodology, E₂ was isolated from human testes (12), E₂ synthesis from acetate was demonstrated in testicular homogenates (13), and radiolabeled androgens were shown to be estrogen precursors in blood (14, 15) and urine (16-18).

Quantitative studies of the source of estrogens in men indicated that peripheral conversion could not account for all of estrone (E₁) production. However, Brenner, Hutchinson, Siiteri, and MacDonald (19) concluded that all of E₂ production could be accounted for by peripheral conversion while Longcope, Kato, and Horton (15) stated that at least 50% of E₂ production was derived from peripheral conversion. Direct proof of the secretion of a hormone requires the demonstration of a greater concentration in the venous effluent of the gland than in the peripheral blood. Testicular-estrogen gradients have been demonstrated in six male pseudohermaphrodites with the syndrome of feminizing testes (20-22) and in one male with gynecomastia (23). However, the secretion of estrogens by normal testes had not been established before the simultaneous reports by Leonard, Flocks, and Korenman (24) and from our laboratory (25) at the 53rd meeting of the Endocrine Society.

The purpose of this study was to determine whether the normal human testis secretes E₂ by measuring E₂ in peripheral and spermatic venous samples in men. For comparison, results of similar studies in monkeys, dogs, and in patients with primary hypogonadism and in male pseudohermaphrodites with the incomplete feminizing testes syndrome are also presented.

METHODS

Subjects. Peripheral and spermatic venous blood samples were drawn from eight normal men (23-61 yr old) during an elective herniorrhaphy (seven subjects) or varicocelectomy (one subject) under spinal anesthesia. The spermatic venous samples were obtained by carefully inserting a 21 gauge scalp vein needle into a large venule of the spermatic plexus, close to the external inguinal ring. Paired specimens were also obtained from two subjects with unilateral testicular atrophy, one subject with oligospermia and a varicocele, and two patients with the incomplete form of the feminizing testes syndrome. Both patients with "partial" feminizing testes had an XY karyotype, female body habitus including breast development, minimal (L. B.) to modest virilization (C. R.), and inguinal testes and epididymis with Leydig cell hyperplasia and defective spermatogenesis. L. B. (age 13 yr, 5 months) was given daily intramuscular injections of 1000 IU of human chorionic gonadotropin (HCG)¹ for the 4 days before her orchiectomy. Informed consent was obtained from all subjects in this study.

¹ *Abbreviations used in this paper:* Δ⁴A, androstendione; DHEA, dehydroepiandrosterone; FSH, follicle-stimulating hormone; HCG, human chorionic gonadotropin; LH, luteinizing hormone.

Femoral arterial and spermatic venous blood samples were obtained from 10 mongrel dogs while under barbiturate anesthesia. An additional four mongrel dogs were given 1000 IU of HCG (A.P.L., Ayerst) intramuscularly/day for 5 days with peripheral venous specimens drawn before treatment.² Femoral arterial and spermatic venous samples post-HCG were obtained under control conditions. Paired specimens were also obtained from four adult male monkeys.

The plasma concentrations of LH and FSH were determined by a double-antibody radioimmunoassay, as previously described utilizing the highly purified NIH pituitary standards LER 960 for luteinizing hormone (LH) and LER 869 for follicle-stimulating hormone (FSH) (26, 27).³ Plasma testosterone concentrations were determined by competitive protein-binding analysis (28).

17β-Estradiol radioimmunoassay. 2,4,6,7-³H-17β-Estradiol (SA 95 Ci/mole) obtained from New England Nuclear Corporation, Boston, Mass., was used without further purification. Radiochemical homogeneity (>95%) of the ³H-E₂ was frequently verified by thin-layer chromatography (Gelman ITLC-SA:benzene:ethyl acetate, 4:1). Rivanol-treated and bovine serum albumin-adsorbed sheep antiserum to 17β-estradiol-17 succinyl-BSA was kindly supplied by Dr. Raymond Vande Wiele of Columbia University. 1-ml portions of a 1:10,000 dilution of this antiserum were stored at -20°C and were diluted with 0.1 M phosphate buffer to a final concentration of 1:500,000 immediately before each assay. After the addition of 1000 cpm of ³H-E₂ to monitor procedural losses, 0.1-2.0 ml plasma samples were treated with 1/10th volume 1 N NaOH and then extracted twice with 5-10 ml of freshly distilled, anesthetic grade anhydrous diethyl ether. The combined ether extracts were evaporated under air at 37°C and then submitted to thin-layer chromatography (Gelman ITLC-SA) in a 4:1, benzene:ethyl acetate system after a preliminary development with benzene alone (R_f = 0.77); 17β-estradiol (R_f = 0.61); testosterone (R_f = 0.45). The 2 × 2 cm E₂ areas were localized by comparison with a "cold" E₂ marker and placed in 2.0 ml deionized water before reextraction twice with 5 ml diethyl ether. The combined ether extracts were evaporated to dryness in 12 × 75 mm disposable, methanol-washed assay tubes. The residues were dissolved in 1.0 ml spectrograde MeOH, and 0.2 ml was removed for monitoring recovery (average recovery = 61 ± 1% SEM) and the remaining 0.8 ml was evaporated to dryness and submitted to radioimmunoassay. All of the spermatic vein samples were processed utilizing two or more different plasma portions.

A 10 point standard curve was prepared in triplicate using 5-300 pg of authentic estradiol (Cal-Biochem). Unknowns and standards were dissolved in 0.6 ml of 0.1 M phosphate buffer followed by the sequential addition of 0.1 ml portions of 0.1% Knox gelatin,⁴ 1/500,000 antibody solution and ³H-E₂ (12,000 cpm/0.1 ml phosphate buffer). Control tubes without antibody were run in all assays. After the addition of ³H-E₂, the tubes were swirled for 5 sec

² This study was performed with the assistance of Dr. F. Ganong and Mr. Roy Shackelford of the Department of Physiology, University of California San Francisco under U. S. Public Health Service Grant No. AM-06074.

³ Conversion factors to LER 907 in this assay system are: 1 ng LH (LER 960) = 40 ng LER 907; and 1 ng FSH (LER 869) = 100 ng LER 907.

⁴ Kindly suggested by Dr. Julie Hotchkiss and Dr. E. Knobil of the Department of Physiology, University of Pittsburgh.

and incubated for 2 hr at room temperature. Bound and free E_2 were separated by the addition of 0.1 ml of dextran-coated charcoal (0.5% Norit A, 0.05% dextran-80 in phosphate buffer). After the addition of charcoal, the contents were vigorously mixed, the tubes placed in an ice bath for 1 hr, and then centrifuged for 10 min at 3000 RPM and 4°C. The supernatants, containing the bound fraction, were decanted into glass scintillation vials and counted for 20 min in a liquid scintillation counter (Packard TriCarb model 3375) using 10 ml of a modified Bray's solution. Counting efficiency was 30%. Absolute binding varied between 40 and 50%; in the control tubes, $2.7 \pm 0.2\%$ SEM of the total $^3H-E_2$ remained in the supernatant.

The reproducibility of the standard curve, plotted as B/B_0 versus picograms of estradiol, is illustrated in Fig. 1. When plotted semilogarithmically, a linear relationship was obtained between 15 and 200 pg (Fig. 2). Cross-reactivity of E_1 was 30-60% while the cross-reactivity of testosterone was insignificant ($< 0.001\%$).

Blank and control values. 2 ml deionized water and charcoal-adsorbed plasma specimens were processed completely for each assay and averaged 14.1 ± 0.74 and 13.1 ± 0.66 pg, respectively. In an attempt to determine the source of the methodologic blank, a reagent blank (water/ether and methanol) was incorporated into each assay tube in eight assays. This yielded a residual plasma blank of 8.3 ± 1.1 pg, presumably secondary to the chromatographic step. Procedural blanks were always subtracted before the calculation of the reported values. Pooled male and pooled female plasma samples averaged 17 ± 0.71 pg/ml and 95 ± 6.9 pg/ml, respectively, while individual adult male specimens ranged between 8 and 28 with a mean of 18 ± 1.4 pg/ml. Interassay coefficient of variation was 24% for the pooled male plasma and 17.7% for the pooled female plasma. The results of the addition of 25-200 pg of "cold" E_2 to 2.0 ml charcoal-adsorbed plasma aliquots are graphically illustrated in Fig. 3.

RESULTS

Normal and hypogonadal men. Table I lists the results in eight normal males (23 to 61 yr old) and three young men (22-28 yr old) with testicular pathology.

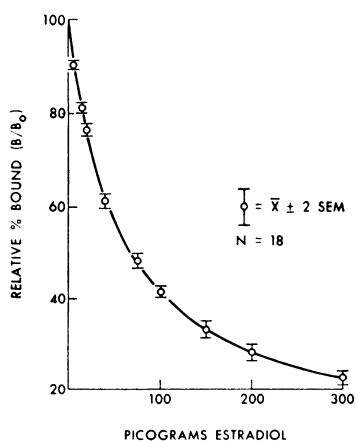


FIGURE 1 Composite standard curve (18 assays) for the 17β -estradiol radioimmunoassay plotted as relative per cent bound, B/B_0 , vs. picograms of 17β -estradiol.

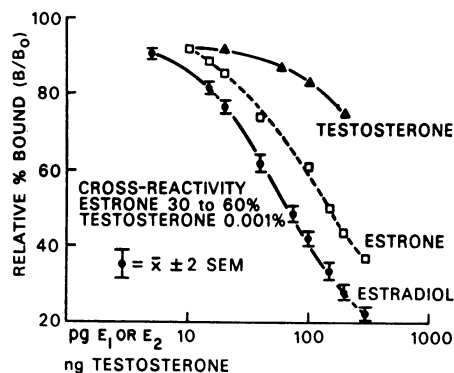


FIGURE 2 Cross-reactivity of estrone (30 to 60%) and testosterone ($< 0.001\%$) in the 17β -estradiol radioimmunoassay. Estrone and estradiol are plotted on the abscissa as picograms, testosterone as nanograms (10^9 pg).

The mean peripheral plasma E_2 concentration in this group of normal male subjects was 20 ± 1.6 pg/ml, while the mean concentration in spermatic vein plasma was 50 times as great, 1049 ± 57 pg/ml. The mean peripheral testosterone concentration, 5.97 ± 0.52 ng/ml, agreed well with our previously reported normal male values, 6.25 ± 0.27 ng/ml (28). The mean spermatic vein testosterone concentration was 712 ± 79 ng/ml, a 110-fold testicular gradient for testosterone. The three patients with testicular abnormalities (patients I, J, and K), two with unilateral atrophy, and one with oligospermia, all had normal peripheral E_2 values, but low concentrations of spermatic vein E_2 . Two of the three (I and K) had low spermatic vein testosterone concentrations, although their peripheral testosterone values were in the normal male range.

Feminizing testes syndrome. The results of our studies in two patients with the incomplete form of the feminizing testes syndrome are illustrated in Fig. 4. Testicular gradients of dehydroepiandrosterone (DHEA) and androstendione (Δ^4A) were also determined in patient C.R. and three control subjects. C.R., age 16 yr, 2 months, had normal adult male values in peripheral blood for DHEA, Δ^4A , and E_2 and a peripheral testos-

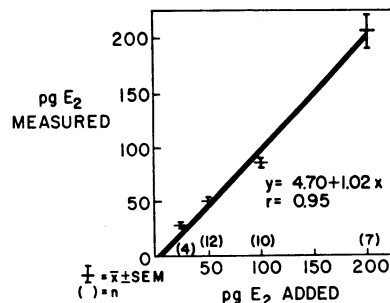


FIGURE 3 Calculated recovery of authentic 17β -estradiol added to 2.0 ml of charcoal-adsorbed pooled male plasma.

TABLE I
17 β -Estradiol and Testosterone Gradients across the Human Testis

Subject	Age	Clinical and/or pathological diagnosis	17 β -Estradiol		Testosterone	
			Peripheral vein	Spermatic vein	Peripheral vein	Spermatic vein
			pg/ml		ng/ml	
A	23	Varicocele	21	1235	5.43	350
B	24	Herniorrhaphy	17	990		
C	24	Herniorrhaphy	24	950	5.35	843
D	42	Herniorrhaphy	27	970	7.92	832
E	49	Herniorrhaphy	18	790	5.06	511
F	49	Herniorrhaphy	16	1260	3.27*	695
G	53	Herniorrhaphy	13	1030		821
H	61	Herniorrhaphy	22	1165	6.10	933
Mean Concn (\pm SEM)			20 \pm 1.6	1049 \pm 57	5.97 \pm 0.52	712 \pm 79
I	22	Oligospermia varicocele	28	160	5.18	99.8
J	28	Unilateral "atrophic" testis	24	280	6.81	95.3
K	26	Unilateral inguinal testis with tubular hyalinization	23	416	2.95	996

* Specimen drawn from same arm as intravenous infusion (value excluded from calculation of the mean).

terone concentration (3.37 ng/ml) found in advanced puberty in boys. Spermatic vein concentrations of DHEA, 120 ng/ml, and Δ^4 A, 53.2 ng/ml, were twice normal (three normals: mean DHEA 64.3 \pm 7.1 ng/ml SD; mean Δ^4 A 22.3 \pm 13.6 ng/ml SD). Spermatic vein testosterone was high normal; however, the spermatic vein E_2 was greater than 4600 pg/ml, at least four times normal. Preoperatively, LH was elevated at 8.5 ng/ml and rose to 14.4 ng/ml postoperatively. In contrast, the concentration of plasma FSH was normal (2.2 ng/ml) preoperatively, but rose strikingly to 54 ng/ml 8 days after a bilateral orchietomy.

L.B. had similar control values for testosterone and E_2 . After 4 days of HCG treatment, a significant rise in testosterone and a probable rise in E_2 were noted in peripheral blood. After treatment with HCG, spermatic vein testosterone concentration was in the high normal range, whereas spermatic vein E_2 was 2200 pg/ml, approximately twice the normal value for adult males. After orchietomy, peripheral DHEA and Δ^4 A in C.R. dropped from 6.9 to 2.3 ng/ml and 1.3 to 0.5 ng/ml, respectively, while testosterone and E_2 were virtually undetectable in both patients.

Monkeys. Four monkeys (Table II) had peripheral E_2 concentrations similar to those in normal men (< 7–33 pg/ml), but their spermatic vein E_2 concentrations averaged only 50 pg/ml. Monkey 4 had a remarkably

high spermatic vein testosterone value (81.4 ng/ml), while his peripheral testosterone concentration was similar to the other animals. With the inclusion of monkey 4, the mean simian testicular gradients for E_2 and testosterone were only 3- and 10-fold, respectively.

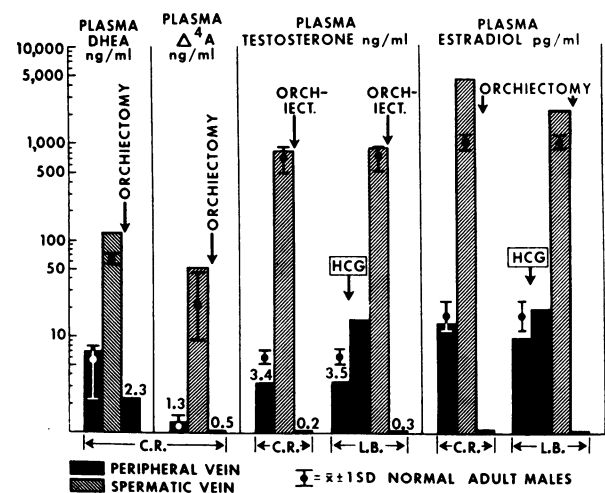


FIGURE 4 Testicular steroid gradients and the response to orchietomy in two patients with the "incomplete" testicular feminization syndrome: C. R. 16 yr, 2 months and L. B. 13 yr, 5 months old. 1000 IU HCG/day were given intramuscularly for 4 days to L. B. before orchietomy.

TABLE II
17β-Estradiol and Testosterone Gradients across the Simian Testis

Monkey No.	Wt	17β-Estradiol		Testosterone	
		Periph- eral vein	Spermat- ic vein	Peripheral vein	Spermat- ic vein
	kg	pg/ml		ng/ml	
1	12.8	<7	41	<3.0	8.53
2	12.6	33	43		11.5
3	7.5	12	37	3.66	15.9
4	6.8	15	80	2.86	81.4
Mean Concn (±SEM)		17 ±5.7	50 ±10	3.17 ±0.25	29.3 ±17

Control and HCG-treated dogs. Table III lists the results in 10 control and 4 HCG-treated mongrel dogs. Peripheral plasma E₂ was detectable in only five animals while spermatic vein E₂ ranged from 31 to 460 pg/ml in the control animals and from 19 to 120 pg/ml in the HCG-treated group. Similarly, peripheral and spermatic vein testosterone concentrations ranged widely in this heterogeneous group of mongrel dogs. A 77-fold testosterone and approximately a 12-fold E₂ gradient were found across the canine testes. HCG treatment elicited

TABLE III
17β-Estradiol and Testosterone Gradients across the Canine Testis

Dog No.	17β-Estradiol		Testosterone		
	Femor- al artery	Spermat- ic vein	Femor- al artery	Spermat- ic vein	
	pg/ml		ng/ml		
1	12	460	2.07	182	
2	8	32	0.50	8.33	
3	<7	47	1.88	88.5	
4	<7	30			
5	<7	63			
6	<7	31	2.27	140	
7	7	135	4.28	577	
8	<7	45	1.22	27.0	
9			1.95	44.9	
10	12	53	0.71	81.4	
Mean Concn (±SEM)		8.2 ±0.7	99.6 ±46	1.86 ±0.42	144 ±65
	Pre/ Post- HCG‡		Pre/ Post- HCG‡	Post- HCG‡	
11	12/8	43	2.91/ 5.25	656	
12	14/<7	56	1.00/ 3.64	2149	
13	<7/18	120	1.14/11.23	2258	
14	<7/<7	19	0.52/10.89	474	
Mean Concn (±SEM)		60 ±22	1.39 ±0.52/7.75 ±1.9	1384 ±475	

* All <7 pg/ml values were treated as 7 pg/ml.

‡ 1000 IU HCG/day for 5 days.

a 6-fold increase in peripheral and a 9-fold increase in spermatic vein testosterone; however, peripheral and spermatic vein plasma E₂ concentrations did not differ from the controls.

DISCUSSION

Testicular E₂ and testosterone gradients. In agreement with the previous indirect estimates of Fishman et al. (14) and Longcope et al. (15), the data presented clearly demonstrate that the human testis secretes estradiol; the mean spermatic vein concentration of plasma E₂ was 1049 pg/ml, 50 times as great as in peripheral plasma. Remarkably similar values were simultaneously reported by Leonard et al. who used celite chromatography and a uterine cytosol-binding assay (24). The testicular testosterone gradient of 706 ng/ml in normal men agrees well with the recent report by Laatikainen, Laitinen, and Vihko (29) and falls within the wide range of values previously reported (30-32). Comparison of the results in man with two other mammalian species reveals that the simian and canine testes also secrete E₂, but in smaller amounts. In all three species, testicular testosterone gradients were greater than the E₂ gradients.

The peripheral E₂ and testicular E₂ gradients in this group of mongrel dogs must be regarded as approximate values only since most dogs had undetectable (< 7 pg/ml) peripheral E₂ levels. This finding agrees well with previous reports indicating either low or absent estrogen excretion in dogs (33). Siegel, Forchielli, Dorfman, Brodey, and Prier, using a fluorometric technique, found a small testicular E₂ gradient in three out of four normal dogs, but the reported spermatic vein concentrations were much higher (average of five dogs: 470 pg/ml), attributable to the apparent relative insensitivity of their technique (34). The lack of a significant rise in peripheral or spermatic vein E₂ concentrations in the HCG-treated dog may merely reflect our current inability to precisely define low basal values or the marked heterogeneity of values found in this small group of mongrel dogs. Since HCG-treated normal men exhibit an increase in peripheral E₂ concentrations,⁵ there may be a species difference either in peripheral aromatization of androgens or testicular responsiveness to HCG.

Feminizing testes syndrome. The concentration of plasma E₂ in testicular venous plasma has been reported in five patients with the complete form (20, 21) and one patient with the incomplete form of the feminizing testes syndrome (22). (The reported values range from 3,100 to 20,000 pg/ml). The results in our two patients, obtained by the more sensitive and specific radioimmuno-assay technique, confirm that the testes in this syndrome secrete significant amounts of E₂. The

⁵ Weinstein, R., and M. M. Grumbach. Unpublished data.

elevated testicular E_2 , DHEA, and Δ^4A values, and the high normal spermatic vein testosterone concentrations in C.R. were most likely secondary to the intense LH stimulation (plasma LH 8.5 ng/ml) preoperatively. HCG treatment in L.B. (studied at 13 yr and 5 months of age) induced a marked rise in peripheral testosterone and a probable increase in peripheral E_2 . These data and the precipitous fall in peripheral E_2 and testosterone after orchiectomy demonstrate the major contribution of the testes, in this syndrome, to circulating E_2 and testosterone. The normal peripheral vein E_2 values, despite markedly elevated spermatic vein concentrations, may be secondary to decreased peripheral conversion of testosterone to E_2 , as reported by French et al. (22) or to an increased metabolic clearance rate.

The discordance between the plasma FSH and LH values, as reflected by the elevated LH and normal FSH concentrations preoperatively in C.R., is similar to data presented by Faïman and Winter (35). These findings, in light of the known end-organ androgen unresponsiveness which seems to include impaired testosterone suppression of LH secretion, suggest that E_2 is the principal sex steroid controlling gonadotropin secretion in these patients and are further evidence for separate feedback mechanisms for LH and FSH.

Estimation of testicular E_2 secretion. The determination of the testicular E_2 gradient alone does not permit a direct calculation of testicular E_2 secretion. However, based upon the mean testosterone blood production rate of 7 mg/day for normal adult males (36) and upon the experimentally determined testosterone and estradiol gradients, the testicular E_2 secretion is estimated at 10 μ g/day. A comparison of the reported production rates of E_2 (approximately 40 μ g/day) (15, 19) with our estimate of testicular E_2 secretion indicates that testicular secretion may account for approximately one-fourth of E_2 production in normal men.

No attempt was made in this study to determine the testicular site of aromatization of C19 precursors to estradiol, whether this occurs primarily in the Leydig cells, the seminiferous tubules, or in both compartments. Such studies would enhance our understanding of the origin and physiologic significance of E_2 in men. The observations reported herein direct attention to the possible role of E_2 arising from the testis as well as from conversion from precursor steroids in peripheral tissues, in the regulation of FSH secretion in the male.

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