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Origin of Estrogen in Normal Men and in Women with Testicular Feminization*

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ABSTRACT. The purpose of this study was to quantify the various sources of estrone (E_1) and 17β-estradiol (E_2) production in normal men and in women with testicular feminization. The mean production rate of E_1 in four young adult men was 58 μg/24 h, while that of E_2 was 44 μg/24 h. In these men, E_1 production could be accounted for totally by extraglandular formation through 1) aromatization of plasma androstenedione, 2) conversion of E_2 which was formed from the aromatization of plasma testosterone, and 3) conversion of secreted E_2. In these men, only 12 μg or less of E_2 production could not be accounted for by extraglandular formation from plasma C_{19} precursors, and is presumed to have arisen by testicular secretion.

In six women with testicular feminization, the mean production rate of E_1 was 99 μg/24 h, while that of E_2 was 77 μg/24 h. The amount of E_2 production that arose by glandular secretion could be computed in four of these women and was considerably greater than that found in the young adult men. In these women with testicular feminization, an average of 44 μg/24 h E_2 could not be accounted for by extraglandular formation and is presumed to have arisen by testicular secretion.

The mean plasma production rate of testosterone in the normal men was 5.7 mg/24 h, while that in the women with testicular feminization was 8.3 mg/24 h. However, the range of plasma production rates of testosterone in the women with testicular feminization was large (1.3–17.0 mg/24 h). (*J. Clin Endocrinol Metab* **49:** 905, 1979)

ESTROGEN production in normal adult men is known to arise by extraglandular formation from plasma prehormones and by testicular secretion (1–11). Quantitative studies, however, of the relative contribution of extraglandular estrogen formation, compared to testicular secretion, to total estrone (E_1) and 17β-estradiol (E_2) production in normal men and in phenotypic or genotypic males who are feminized are sparse. The purpose of this study was to quantify the amount of estrogen produced by extraglandular formation and testicular secretion in normal men and to compare these values with those found in women with testicular feminization.

For the purpose of quantifying the sources of origin of estrogen in normal young adult men and in women with testicular feminization, we considered three possible mechanisms of E_1 and E_2 formation: 1) glandular secretion, 2) extraglandular formation by the aromatization of plasma C_{19} steroids, and 3)extraglandular formation from secreted estrogen, *i.e.* E_1 formation from secreted E_2, and E_2 formation from secreted E_1. Since presently it is not feasible to quantify glandular secretion directly in intact human subjects, the present study was undertaken to 1) quantify daily E_1 and E_2 production rates and 2) determine the portions of E_1 and E_2 production which was formed from plasma C_{19} or C_{18} prehormones. Based on the results of earlier studies (6, 9, 12, 13), we assumed that if the rates of E_1 and E_2 production exceeded the amount which could be attributed to extraglandular formation, the difference arose from estrogen secretion by the gonads, the adrenals, or both.

In earlier studies in nonpregnant subjects (14–17), it was found that plasma dehydroisoandrosterone and dehydroisoandrosterone sulfate are poor estrogen precursors, except in subjects with choriocarcinoma (16, 18), and further, the small amount of these substances which are converted to estrogen proceed, in part, through the sequence plasma dehydroisoandrosterone → plasma androstenedione(A) → E_1 (17). Accordingly, of the potential plasma C_{19} precursors of E_1 and E_2 in normal adult men and in women with testicular feminization, plasma A and testosterone (T) are the quantitatively important prehormones of extraglandular E_1 and E_2 formation.

Materials and Methods

Subjects

The subjects of this study were four normal adult men, 26–35 yr of age, who were ambulatory and pursuing their usual activities, except during the period of isotope-labeled steroid

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tracer infusions, and six healthy women with testicular feminization, aged 14–44 yr. Each of the women with testicular feminization had complete absence of virilization of the external genitalia, diminished sexual hair, a short blind-ending vagina, and absence of the uterus; each woman had a 46 XY karyotype and was sex chromatin negative. Other pertinent clinical data for each of the women with testicular feminization are presented in Table 1. Interestingly, each of the six women had a sister with testicular feminization; indeed, subjects A and B were sisters, as were subjects C and D. In each woman with testicular feminization, bilateral orchiectomy was performed after the studies were completed, and the histology of the testes was as follows: 1) small seminiferous tubules with thickened basement membranes lined by many Sertoli cells, 2) no spermatogenesis, and 3) fibrosis and Leydig cell hyperplasia of the interstitial tissue. The epididymis, when described (subjects A and E), contained poorly developed ductal structures lined by low columnar epithelium. The rudimentary vas deferens was partially dissected free and separately examined in subject E; it was found to contain no ductal epithelium and to consist of smooth muscle only. The location and size of the testes in each subject are presented in Table 1.

Measurement of the plasma production rates of A and T (^{p}PR-A and ^{p}PR-T) and the transfer constants of interconversion of plasma A and T ($[\rho]_{BB}^{AT}$ and $[\rho]_{BB}^{TA}$)[1]

In the four normal men and in subject B with testicular feminization, a tracer dose of [7α-^{3}H]A plus [4-^{14}C]T in 500 ml 5% glucose containing 5% ethanol was infused iv through Teflon tubing at a constant rate for approximately 4 h. Blood samples were collected approximately 5, 35, and 65 min before the end of the tracer infusion. Each plasma sample was divided into two portions. From one portion, A and T were isolated, and the ^{3}H to ^{14}C ratio of each was determined as described below. To the other portion of each plasma sample, a known quantity of [^{14}C]A and [^{3}H]T was added, after which A and T were isolated. From the ^{3}H to ^{14}C ratios of A and T from the paired plasma samples, the concentrations of plasma [^{3}H]A and [^{14}C]T were computed. $[\rho]_{BB}^{AT}$ and $[\rho]_{BB}^{TA}$ were computed as described previously by Horton and Tait (19).

In subjects A, C, D, E, and F with testicular feminization, a tracer dose of [^{14}C]T plus [^{3}H]E_2 was infused preoperatively, *i.e.* before castration, for 4 h. Urine was collected for 3–4 days from the beginning of the infusion. Blood samples were collected during the infusion, as described above, and to each plasma sample [^{3}H]T and [^{3}H]A were added as internal recovery standards. From the ^{3}H to ^{14}C ratios of the isolated plasma A and T, the concentrations of [^{14}C]A and [^{14}C]T were computed. From the ^{3}H to ^{14}C ratio of urinary E_2, the transfer constant of conversion of plasma T to E_2 ($[\rho]_{BU}^{TE_2}$) was calculated.

In subjects A, E, and F with testicular feminization, a tracer dose of [^{14}C]A and [^{3}H]E_1 was infused in a separate study. Blood samples were obtained during these infusions as described above. Plasma samples were processed as described, and urine was collected for 72–96 h. From the plasma samples, the concentrations of isotope-labeled A and T were determined. From the ^{3}H to ^{14}C ratio of urinary E_1, $[\rho]_{BU}^{AE_1}$ was computed.

Measurement of the daily production rates of E_1 and E_2 (PR-E_1 and PR-E_2) and the transfer constant of conversion of plasma E_2 through E_1 ($[\rho]_{BU}^{E_2E_1}$) and of plasma estrone through E_2 ($[\rho]_{BU}^{E_1E_2}$)

A tracer dose of [4-^{14}C]E_2 and [6,7-^{3}H]E_1 in 10 ml normal saline containing ethanol (10%, vol/vol) was administered iv as a single bolus to the four normal men and to subjects A, B, E, and F with testicular feminization. After the administration of these tracers, urine was collected for 72 h. After β-glucuronidase treatment of the pooled 72-h urine specimens, E_1, E_2, and estriol (E_3) were isolated as previously described (1, 20). From the specific activity of urinary [^{3}H]E_1 (disintegrations per min/μg) and the amount of radioactivity in the injected tracer ([6,7-^{3}H]E_1), PR-E_1 (micrograms per 24 h) was calculated. From the relationship of the ^{3}H to ^{14}C ratios of urinary E_1 and E_2 to those of the injected tracers, $[\rho]_{BU}^{E_1E_2}$ and $[\rho]_{BU}^{E_2E_1}$ were computed (21). From the specific activities of urinary E_1 and E_2 and the amount of radioactive tracers infused, the E_1-independent rate of formation of E_2 and the E_2-independent rate of formation of E_1 were computed by the method described by Gurpide *et al.* (21). The E_1-independent rate of formation of E_2 is equal to secreted E_2 plus E_2 formed directly from plasma T. The E_2-independent formation of E_1 is equal to secreted E_1 plus E_1 formed from plasma A. These data were used in computing the sources of E_2 and E_1 in the subjects of this study (see Table 8 and Fig. 1).

Measurement of the transfer constant of conversion of plasma A through E_1 ($[\rho]_{BU}^{AE_1}$) and plasma T through E_2 ($[\rho]4_{BU}^{TE_2}$)

After the infusion of a tracer dose of [6,7-^{3}H]E_1 plus [4-^{14}C]A, urine was collected for 72 h. From the pooled urine sample, E_1, E_2, and E_3 were isolated, and their ^{3}H to ^{14}C ratios were determined. As previously described (1), the fraction of infused [^{14}C]A metabolized in a manner similar to that of the infused [^{3}H]E_1 was calculated, *i.e.* $[\rho]_{BU}^{AE_1}$ = the ^{3}H to ^{14}C ratio of the injected tracers divided by the ^{3}H to ^{14}C ratio of urinary E_1.

It is important to note that the fraction of plasma A converted to E_1, as computed in this manner, represents that fraction of plasma A aromatized in the tissue sites of conversion and does not denote the fraction of plasma A recovered as the urinary estrogen metabolite. We are of the view that this is the most accurate means of measuring the transfer constants of conversion of plasma C_{19} prehormones to their respective C_{18} product hormones. We have adopted this view since extremely long infusion times are required to achieve steady state conditions between the rate of infusion of ^{14}C-labeled C_{19} prehormones and ^{3}H-labeled C_{18} product hormones and constancy of the ^{3}H to ^{14}C ratio of C_{18} product hormones in blood (22). Moreover, when such steady state is achieved, the amount of radiolabeled C_{18} hormone in plasma available for establishing radiochemical homogeneity is exceedingly small. Importantly, if infusions of sufficient amounts of radiolabeled [^{3}H]E_1 and [^{14}C]A are infused for sufficient time to achieve steady state conditions, the ^{3}H to

[1] The following abbreviations are used: E3 = estriol; [ρ], transfer constant of conversion; $[\rho]_{BB}$, transfer constant of conversion computed from plasma values; $[\rho]_{BU}$, transfer constant of conversion computed from urine values.

TABLE 1. Clinical findings in subjects with testicular feminization

Patient	Age at time of study (yr)	Ht (cm)	Breast development	Location of testes	Size of testes (cm)
A	15	174[a]	Large	Intraabdominal	Right 4 × 2 × 2 Left 6 × 3 × 2
B	18	179	Large	Intraabdominal	Right 5.0 × 2.5 Left 7.0 × 4.5
C	14	175[b]	Large	Inguinal	Right 4 × 2.5 Left 4 × 2
D	17	175	Large	Right intraabdominal (reduced from inguinal location at herniorrhaphy at age 6); left inguinal	Right 5 × 2.5 Left 3 × 2.5
E	22	175	Small	Intraabdominal	Right 2.5 × 1.5 × 1.5 Left 2.8 × 2.1 × 1.1
F	44	173	Medium	Intraabdominal	Right 4.5 × 1.2 Left 3.2 × 1.3

[a] At age 18 (time of gonadectomy).
[b] Adult height.

^{14}C ratio of E_1 in blood and that in urine are similar, if not identical (22). Thus, it can be concluded that all of the C_{18} product hormone derived from plasma C_{19} precursor enters the blood without further metabolism in the tissue sites of aromatization. Only one exception to this generalization has been described, that being found in an unusual case of heterosexual precocity in a boy with massive extraglandular aromatization of plasma C_{19} steroids (23). In this boy, there was sulfurylation of the E_1 formed in the tissue sites of aromatization before entry of the C_{18} product into the circulation.

Kelly and colleagues (24, 25) found that the ^3H to ^{14}C ratios of urinary metabolites of E_1 after the administration of [^{3}H]E_1 and [^{14}C]A differed in some subjects. Based on these findings, these investigators questioned the validity of the physiological model assumed in such studies. We believe that such findings resulted from incomplete urine collections. Recently, it was shown that 1) the rate of excretion of radiolabeled E_1 metabolites was inversely related to body weight, 2) the rate of E_1 excretion was faster than that of E_3, and 3) after the infusion of [^{3}H]E_1 and [^{14}C]A, the rate of excretion of [^{3}H]E_1 metabolites was faster than that of [^{14}C]E_1 metabolites derived from [^{14}C]A (22). When urine collections were complete and of sufficient duration to ensure complete excretion of radiolabeled metabolites, the ^3H to ^{14}C ratios of E_1 metabolites in urine were very similar. Taken together, these findings are supportive of the view that all E_1 derived from plasma A enters the plasma as E_1, and therefore, $[\rho]^{AE_1}_{BU^1} = [\rho]^{AE_1}_{BB^1}$.

The fraction of plasma A converted to E_2 via the sequence plasma A → plasma T → E_2 → E_1 is quantitatively insignificant, since the product of $[\rho]^{AT}_{BB} \times [\rho]^{TE_2}_{BU}$ is very small. The amount of E_2 derived from conversion of plasma T was calculated from the ^3H to ^{14}C ratio of urinary E_2 after the iv administration of [^{3}H]E_2 plus [^{14}C]T to the four normal men. However, in this measurement, the amount of E_2 formed from plasma T via the indirect sequence plasma T → plasma A → E_1 → E_2 is quantitatively important compared to the direct conversion of plasma T to E_2. For this reason, the fraction of plasma T converted directly to E_2 ($^d[\rho]^{TE_2}_{BU}$) was computed by subtracting that fraction of E_2 derived from plasma T via the indirect pathway, *i.e.* plasma T → plasma A → E_1 → E_2.

Measurement of the concentration of isotope-labeled A and T in plasma

Appropriate isotope-labeled internal standards were added to portions of each plasma sample, which were then extracted with 2 vol methylene chloride. These extracts were purified by gradient elution chromatography using celite, thin layer chromatography, and crystallization, methods which have been described in detail (12, 22, 23).

Measurement of the plasma concentrations of A and T

Plasma levels of A and T were determined by RIA (26) or by a double isotope dilution technique described by Siiteri *et al.* (27). Measurements of plasma E_1 and E_2 were not performed systematically in these subjects, since some of the women with testicular feminization were studied before the development of RIA procedures for plasma estrogens.

Urinary metabolites

The 72- to 96-h urine specimens were adjusted to pH 5.0, acetate buffer was added, and the acidified buffered urine was incubated with β-glucuronidase for 72 h at 37 C. The liberated steroids were extracted with ethyl acetate, and the urinary estrogens were purified by gradient elution chromatography with celite, thin layer chromatography, acetylation, and crystallization (14, 16). One portion of E_1, E_2, and E_3 was acetylated with nonradiolabeled acetic anhydride, and the other portion was acetylated with tritium-labeled acetic anhydride. From the

^3H to ^{14}C ratios of the acetates prepared with nonradioactive acetic anhydride, the fractional conversion of plasma A or T to their respective estrogen product hormones was calculated, as described above. Additionally, from the difference in the ^3H to ^{14}C ratio of the two acetates and the specific activity of acetic anhydride, the specific activities of the urinary metabolites with respect to both tritium and carbon-14 were calculated (20).

Results

PR-E1 and PR-E2

After the iv administration of [^{3}H]E_1 plus [^{14}C]E_2 to four normal young adult men and to four of the women with testicular feminization, the specific activities of urinary E_1, E_2, and E_3 were determined. These data are presented in Table 2. From the specific activity of urinary [^{3}H]E_1, the PR-E_1 was calculated and ranged from 48–65 μg/24 h in the normal men. The PR-E_2 was calculated from the specific activity of urinary [^{14}C]E_2 and ranged from 35–48 μg/24 h in the four normal men (Table 2). In the women with testicular feminization, the PR-E_2 ranged from 53–121 μg/24 h (Table 2), while PR-E_1 varied from 29–167 μg/24 h. In four subjects, these values were measured after the simultaneous infusion of [^{3}H]E_1 and [^{14}C]E_2 from the specific activities of urinary metabolites; in two subjects, the PR-E_2 was computed from the specific activity of urinary [^{3}H]E_2 after the infusion of [^{3}H]E_2 and [^{14}C]T.

It should be emphasized that PR-E_2 is constituted in part by contribution from E_1 and, likewise, PR-E_1 arises in part from E_2 conversion. Accordingly, the two PRs are not additive in the sense of representing total estrogen production.

Also from this study, $[\rho]_{BU}^{E_1E_2}$ and $[\rho]_{BU}^{E_1E_2}$ were calculated in each subject, and these values are also presented in Table 2. In agreement with other studies (21), approximately 50% of E_1 suffered its metabolic fate via E_2, whereas approximately 90% of E_2 was metabolized via E_1 in these subjects. It should be noted that these measurements do not represent the fractional conversion of plasma E_1 to plasma E_2 or plasma E_2 to plasma E_1, which are much smaller. Rather, these values were computed from urinary data and constitute a reflection of total metabolism regardless of whether the product entered blood before further metabolism, *e.g.* conjugation.

Availability of plasma A and T for conversion to estrogen

The results of the studies in which the plasma PRs and the fractional interconversion of plasma A and T were measured are presented in Table 3. The MCRs of plasma T (MCR-T) in the four normal men ranged from 940–1144 liters/24 h (mean, 1046 liters/24 h). These values are similar to those previously found for normal men by other investigators (19). The MCR of plasma A ranged from 2004–2464 liters/24 h (mean, 2187 liters/24 h).

TABLE 2. PR-E_1 and PR-E_2

Subject	SAs of urinary metabolites (dpm × 10^{-3}/μg)									$[\rho]_{BU}^{E_2E_1}$	$[\rho]_{BU}^{E_1E_2}$	PR-E_1[b]	PR-E_2[b]
	^{3}H:^{14}C ratios[a]			E_1		E_2		E_3					
	E_1	E_2	E_3	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C				
Normal males													
1	10.0	5.2		108.0	10.8	79.6	15.3			0.98	0.53	65	47
2	11.0	4.7	9.9	114.0	10.4	74.0	15.7	75.9	7.7	0.90	0.48	62	46
3	10.6	5.1	9.6	147.0	13.9	105.0	20.7	130.0	13.6	0.93	0.52	48	35
4	10.7	4.3	9.8	122.0	11.4	64.2	14.9	124.0	12.6	0.92	0.44	58	48
Mean										0.93	0.49	58	44
Testicular feminization													
A	9.6	4.1	9.9	107.0	11.1	36.6	8.9	92.7	9.3	1.02	0.42	66	80
B	9.0	3.7	9.9	42.2	4.7	32.7	8.7	47.9	4.9	1.09	0.37	167	83
C[c]						4.2							64
D[c]				5.9		5.9		5.1					122
E	11.8	8.7	9.9	245.0	20.8	118.0	13.6	105.0	10.6	0.83	0.88	29	53
F	9.2	4.5	9.2	78.9	8.6	55.3	12.4	64.6	7.0	1.07	0.45	89	58
Mean										1.00	0.53	95	77

[a] For ease of comparison, the ^{3}H to ^{14}C ratios and specific activities (for subjects 1–4 and A, B, E, and F) have been adjusted to an injected tracer dose of 21.1 × 10^6 dpm [^{3}H]E_1 and 2.15 × 10^6 dpm [^{14}C]E_2.

[b] Total PRs (micrograms per 24 h) are calculated from the specific activity of the appropriate metabolite relative to the isotope of the infused tracer specific to that metabolite, *i.e.* ^{3}H for E_2 and ^{14}C for E_2.

[c] In these two subjects (C and D), the PR-E_2 was computed from the specific activity of urinary [^{3}H]E_2 after the infusion of [^{14}C]T and [^{3}H]E_2 (2.15 × 10^6 dpm). These two subjects did not receive an infusion of [^{14}C]E_2 and [^{3}H]E_1, and thus, the $[\rho]_{BU}^{E_2E_1}$ and $[\rho]_{BU}^{E_1E_2}$ in these women were not measured.

TABLE 3. Measurement of the plasma PRs and interconversion of A and T

Subject	Injected tracer dose (dpm × 10^{-6})		Time of infusion (min)	T						A						MCR (liters/24 h)[b]		Plasma					
				Carbon-14[a]			Tritium[a]			Carbon-14[a]			Tritium[a]										
	T	A		1[e]	2	3	1	2	3	1	2	3	1	2	3	T	A	$[\rho]^{TA}_{BB}$	$[\rho]^{AT}_{BB}$	cA[c]	cT[c]	PR-A[d]	PR-T[d]
Normal males																							
1[f]	104.7	144.8	180	716	736	744	53	53	55	41	41	38	473	480	457	1,144	2,464	0.118	0.053	1.16	6.42	2,850	7,340
2[f]	104.7	144.8	170	914	948	968	47	52	58	30	35	39	535	589	628	940	2,100	0.082	0.040	2.02	5.83	4,232	5,480
3[f]	104.7	144.8	250	607	587	552	49	58	64	16	19	20	382	417	447	1,036	2,004	0.060	0.071	1.01	4.84	2,024	5,014
4[f]	94.8	145.6	173	733	748	742	37	37	36	18	18	19	557	558	553	1,065	2,180	0.051	0.032	1.23	4.82	2,688	5,428
Mean																1,046	2,187	0.078	0.049	1.36	5.48	2,950	5,740
Testicular feminization																							
A[g]	48.2		235	713		717				19.3		19.5				413					3.24		1,338
A[g]		75.5	260	32	36	35				194	186	173					2,276	0.15	0.034	1.30		2,959	
B[f]	111.9	137.9	175	1,171	1,300	1,261	61	60	64	36	35	38	413	366	390	740	2,912	0.12	0.040	2.54	8.23	7,390	6,090
C[g]	53.7		280	313	313	318										872					17.20		15,200
D[g]	71.6		254	407	466	489										892				5.16	19.06		17,000
E[g]	114.6		240	1,211	1,061	1,521				39.1	47.2	50.5				544					4.96		2,698
E[g]		104.8	170	53.2	55.5	56.9				638	663	583					1,414	0.10	0.04	1.29		1,824	
F[g]	63.2		227	380	390	386				9.5	9.3	9.2				1,040					6.98		7,259
F[g]		75.5	275	24	24.4	24.2				150	149	152					2,630	0.06	0.06	1.19		3,130	
Mean																750	2,308	0.11	0.04	2.30	9.95	3,826	8,264

[a] Expressed as disintegrations per min × 10^{-3}/liter plasma.
[b] MCR calculated from the mean plasma concentration of isotope-labeled steroid.
[c] Expressed as micrograms per liter. cA, Concentration of androstendione in plasma; cT, concentration of testosterone in plasma.
[d] Expressed as micrograms per 24 h.
[e] Plasma sample number.
[f] In these subjects, [^{14}C]T and [^{3}H]A tracers were infused simultaneously.
[g] In these subjects, [^{14}C]T and/or [^{14}C]A were infused in separate studies.

These values are also similar to those found by others (19). $[\rho]^{TA}_{BB}$ in these subjects ranged from 0.051–0.118 (mean, 0.078). $[\rho]^{AT}_{BB}$ ranged from 0.032–0.071 (mean, 0.049). These values are also similar to those reported by other investigators (19).

In the six women with testicular feminization, the MCR-T ranged from 413–1040 liters/24 h (mean, 750 liters/24 h), a value significantly less than that found in the normal men. The MCR-A was measured in four of these women and the mean value was 2308 liters/24 h, ranging from 1414–2912 liters/24 h. The range of MCR-A in these women was large, but the mean of these values is similar to that of the normal men. $[\rho]^{TA}_{BB}$ and $[\rho]^{AT}_{BB}$ in women with testicular feminization were similar to those of the normal men.

The plasma concentrations of A and T were determined in multiple 10 to 20-ml samples of plasma obtained from each subject at various times of the day or from a pool of plasma which was prepared from blood samples obtained at various times of the day (Table 3). From the product of the plasma concentrations of A and T and their respective MCRs, ^{P}PR-A and ^{P}PR-T were computed. The ^{P}PR-A in the normal men ranged from 2.0–4.2 mg/24 h (mean, 2.95 mg/24 h). The ^{P}PR-T in these men ranged from 5.0–7.3 mg/24 h (mean, 5.7 mg/24 h). The values for ^{P}PR-A and ^{P}PR-T in the normal men of this study were similar to those reported for normal men by others (19).

The mean ^{P}PR-T in the six women with testicular feminization was 8.3 mg/24 h, a value slightly greater than the mean ^{P}PR-T in the normal men. However, the ^{P}PR-T in these women varied from 1.3–17.0 mg/24 h. The mean ^{P}PR-A in four of these women was 3.8 mg/24 h, ranging from 1.8–7.4, values somewhat greater than those of the normal men.

$[\rho]^{AE_1}_{BU}$

After the simultaneous administration of [^{14}C]A and [^{3}H]E_1, the ^{3}H to ^{14}C ratios of urinary E_1, E_2, and E_3 were determined after purification of these metabolites. These data are presented in Table 4. Previously, we have found that the ^{3}H to ^{14}C ratios of multiple urinary metabolites of E_1 are very similar after the simultaneous injection of [^{3}H]E_1 and [^{14}C]A (1, 22). This finding is supportive of the view that the plasma [^{14}C]A converted to estrogen suffers a metabolic fate similar to that of the iv administered internal standard [^{3}H]E_1 and that the estrogen formed in the tissue sites of aromatization of plasma A is principally if not exclusively E_1. From the relationship of the ^{3}H to ^{14}C ratio of the injected tracers to that of the ^{3}H to ^{14}C ratio of isolated urinary E_1 (glucuronoside), the extent of conversion of plasma A to E_1 can be calculated. In these studies, the extent of conversion of plasma A to E_1 in the normal men varied from 0.013–0.017 (mean, 0.016).

In three of the women with testicular feminization, $[\rho]^{AE_1}_{BU}$ was measured and found to range from 0.012–0.014 (mean, 0.013; Table 4). These values are similar to those found in normal young men and women (1).

The extent of conversion of plasma T to E_2

The ^{3}H to ^{14}C ratios of the urinary metabolites after the simultaneous injection of [^{3}H]E_2 and [^{14}C]T are presented in Table 5. It is evident that the ^{3}H to ^{14}C ratio of

TABLE 4. $[\rho]^{AE_1}_{BU}$

Subjects	^{3}H:^{14}C ratio of urinary metabolites[a]			$[\rho]^{AE_1}_{BU}$
	E_1	E_2	E_3	
Normal males				
1	25.5	25.2	25.6	0.015
2	28.0	29.0	30.0	0.013
3	22.7	24.8	25.4	0.017
4	22.2	22.0	23.6	0.017
Mean				0.016
Testicular feminization				
A	28.5	27.0	31.1	0.013
E	32.1	29.9	33.0	0.012
F	27.1	27.6		0.014
Mean				0.013

[a] For ease of comparison, the ^{3}H to ^{14}C ratios have been adjusted to a common tracer dose ratio: ^{3}H to ^{14}C, 0.37; [^{3}H]E_1, 2 μCi; and [^{14}C]A, 5.3 μCi.

TABLE 5. $[\rho]_{BU^2}$

Subjects	^{3}H:^{14}C ratios of urinary metabolites[a]			Total $[\rho]^{TE_2}_{BU^2}$	Direct $[\rho]^{TE_2}_{BU^2}$
	E_1	E_2	E_3		
Normal males					
1	18.9	22.7	18.9	0.0044	0.0035
2	29.0	35.5	28.5	0.0028	0.0023
3	20.4	26.2	21.7	0.0038	0.0033
4	20.6	26.9	20.7	0.0037	0.0034
Mean				0.0037	0.0031
Testicular feminization					
A	31.1	43.1	34.3	0.0023	0.0015
C	68.0	75.7	62.2	0.0013	
D	43.7	40.2		0.0025	
E	43.5	43.2	41.3	0.0023	0.0014
F	43.6	55.0	42.0	0.0018	0.0010
Mean				0.0020	0.0013

[a] For ease of comparison, all ^{3}H to ^{14}C ratios have been adjusted to a common ^{3}H to ^{14}C tracer dose ratio of 0.10.

E_2 was higher than those of E_1 and E_3 in each subject studied. This was to be expected, since the tritium-labeled tracer was E_2 and the carbon-14-labeled tracer T has access to E_1 by conversion from E_2 plus the indirect pathway via plasma A to E_1, as described earlier. The [^{14}C]E_1 derived from plasma [^{14}C]T via plasma A lowers the ^{3}H to ^{14}C ratios of E_1 and E_3 relative to E_2 since $[\rho]_{BU}^{E_1E_2}$ is only about 0.5 (21). On the basis of the extent of $[\rho]_{BB}^{TA}$ and $[\rho]_{BU}^{AE_1}$ and the fraction of E_1 metabolized via E_2 ($[\rho]_{BU}^{E_1E_2}$), the fraction of plasma T giving rise to E_2 via the indirect pathway can be calculated. In this way, the expected difference in the ^{3}H to ^{14}C ratios of E_1 and E_2 after [^{3}H]E_2 and [^{14}C]T infusion may be computed and compared to the observed difference. The expected difference in ratios computed for these subjects was 16%, whereas the observed difference was 19%. This close correlation between computed and observed differences in ^{3}H to ^{14}C ratios provides evidence that plasma T is converted to E_2 directly and indirectly via the plasma A $\rightarrow E_1 \rightarrow E_2$ pathway. The conversion of plasma T to E_2 directly can be computed by subtracting that fraction which reached E_2 via the indirect pathway from the total conversion of plasma T to E_2. The direct fractional conversion of plasma T to E_2 in the normal men ranged from 0.0023–0.0035 (mean, 0.0031).

Total $[\rho]_{BU}^{TE_2}$ was measured in six women with testicular feminization and was found to range from 0.0013–0.0025 (mean, 0.0020); [d]$[\rho]_{BU}^{TE_2}$ was estimated in three subjects and was found to range from 0.0010–0.0015. These values are similar to those found in normal women but significantly less than those found in the normal young men of this study.

The ^{3}H to ^{14}C ratios of urinary estrogen metabolites after the simultaneous administration of [^{14}C]T and [^{3}H]E_1 in two young adult men

To substantiate the validity of the hypothesis stated above relative to the dual pathway for the conversion of plasma T to E_2, two of the normal men (subjects 3 and 4) were given a tracer dose of [^{14}C]T plus [^{3}H]E_1. If plasma T were converted exclusively to E_1, the ^{3}H to ^{14}C ratios of all urinary E_1 metabolites would be identical. However, as is evident from the results presented in Table 6, the ^{3}H to ^{14}C ratios of E_2 were less than those of E_1 and E_3, which were similar in these two subjects. These results are also consistent with the conclusion that plasma T is converted to E_2 through direct conversion to E_2 and via plasma A, which subsequently is converted to E_1 and thence to E_2.

Origin of estrogen in normal young adult men and in women with testicular feminization

The PR-E_1 and PR-E_2 computed for the women with testicular feminization in each of the several studies are presented in Table 7. By computing the amount of E_1 derived from plasma A, the amount of E_2 derived from plasma T directly and, in turn, the amount of E_1 derived from E_2 which arose from plasma T, and the amount of E_2 derived from plasma A via E_1, the rate of E_1 production from the utilization of plasma C_{19} precursors and the rate of production of E_2 from plasma C_{19} precursors can be computed and compared with the independently measured total PR-E_1 and PR-E_2 in these subjects. These calculations and comparisons are presented in Table 8 and summarized in schematic form in Fig. 1. For example, in subject 1 (normal man), [p]PR-A was 2.85 mg/24 h, 1.5% of which was converted to E_1, yielding 41 μg/24 h. In the same subject, 7.34 mg plasma T were produced daily, 0.35% of which was converted directly to E_2, yielding 24

TABLE 6. The ^{3}H to ^{14}C ratios of urinary estrogen metabolites after the iv administration of [4-^{14}C]T and [6,7-^{3}H]E_1

Subjects	^{3}H:^{14}C ratios of urinary metabolites		
	E_1	E_2	E_3
Normal men			
3	25	20	26
4	19	16	22

Doses of injected tracers: [^{14}C]T, 18.5 μCi and [^{3}H]E_1, 2 μCi; ^{3}H to ^{14}C ratio, 0.108.

TABLE 7. PR-E_1 and PR-E_2 in women with testicular feminization

Subject	Study (tracers infused)	PR (μg/24 h)		Independent formation rate (μg/24 h)[a]	
		E_1	E_2	E_1	E_2
A	[^{3}H]E_1 + [^{14}C]E_2	77	80	0	92
	[^{3}H]E_1 + [^{14}C]A	90			
B	[^{3}H]E_1 + [^{14}C]E_2	167	83	129	34.5
	[^{3}H]E_2 + [^{14}C]T	153			
C	[^{3}H]E_2 + [^{14}C]T		64		
D	[^{3}H]E_2 + [^{14}C]T		122		
E	[^{3}H]E_1 + [^{14}C]E_2	27	49	0	103
	[^{3}H]E_1 + [^{14}C]A	57			
	[^{3}H]E_2 + [^{14}C]T	54[b]	81		
F	[^{3}H]E_1 + [^{14}C]E_2	89	58	53	34
	[^{3}H]E_1 + [^{14}C]A	132	68[b]		
	[^{3}H]E_2 + [^{14}C]T	114[b]	65		

[a] E_2-independent E_1 formation and E_1-independent E_2 formation rates were computed from the specific activities of urinary E_1 and E_2 and the amount of radioactivity infused as [^{3}H]E_1 and [^{14}C]E_2 according to the equation presented by Gurpide *et al.* (13).

[b] PR-E_1 estimated from the specific activity of urinary E_1 when the infused tracer was E_2 or PR-E_2 estimated from the specific activity of urinary E_2 when the infused tracer was E_1, *i.e.* PR-E_2 = R'[^{3}H]E_1 × $[\rho]_{BU}^{E_1E_2}$ ÷ the specific activity of urinary E_2.

TABLE 8. Origin of E_1 and E_2 in young men and in women with testicular feminization

Subject	E_1					E_2				
	PR-E_{1_A}	PR-$E_{1_{E_{2_T}}}$	PR-$E_{1_{E_{2_A}}}$	PR-E_{1_s}[a]	Measured PR-E_1	PR-E_{2_T}	PR-$E_{2_{E_{1_A}}}$	PR-$E_{2_{E_{1_L}}}$	PR-E_{2_s}[a]	Measured PR-E_2
Normal Males										
1	41	24	1	0	65	24	22	0	1	47
2	42	11	8	0	62	12	25	0	9	46
3	33	15	2	0	48	16	17	0	2	35
4	43	16	11	0	58	17	19	0	12	48
Testicular feminization										
A	36	2	63	0	90	2	15	0	63	80
B[b]	91	8	35	23	167	8	34	7	34	83
E	21	8	36	0	46	4	18	0	43	65
F	41	7	34	28	110	7	18	15	34	68

PR-E_{1_A}, PR-E_1 derived from the utilization of plasma A; PR-$E_{1_{E_{2_T}}}$, PR-E_1 derived from E_2 produced from the aromatization of plasma T; PR-$E_{1_{E_{2_A}}}$, PR-E_2 derived from the conversion of secreted E_2; PR-E_{1_s}, secretory rate of E_1; PR-E_{2_T}, PR-E_2 derived from the aromatization of plasma T; PR-$E_{2_{E_{1_A}}}$, PR-E_2 derived from E_1 which was formed from the aromatization of plasma A; PR-$E_{2_{E_{1_L}}}$, PR-E_2 derived from the conversion of secreted E_1; PR-E_{2_s}, secretion rate of E_2.

Values are expressed as micrograms per 24 h.

[a] The secretion of E_2 and E_1 was computed as the difference in the measured PR and that calculated to have arisen by extraglandular formation, except in subject F in whom the best fit of the data is presented after computation of E_1-independent E_2 formation and E_2-independent E_1 formation rates using the equation presented by Gurpide *et al.* (13).

[b] The values in subject B are estimates based upon the assumption that the $[\rho]^{AE}_{BU}$ value in this subject (not measured) was the same as that of her sister (subject A) and the same as that of the mean of the women with testicular feminization (*viz.* 0.013).

μg E_2 from plasma T daily. In turn, 98% or 24 μg/24 h E_2 derived from plasma T suffered its metabolic fate via E_1. Accordingly, in this subject, 65 μg E_1/day were produced from plasma A and T. In the same man, 24 μg E_2 were formed daily directly from plasma T. In addition, 54% of the 41 μg E_1 derived from plasma A each day suffered its metabolic fate via E_2. In this subject, the daily E_2 production from plasma C_{19} precursors was comprised of 24 μg E_2 derived from the direct conversion of plasma T to E_2 and 22 μg E_2 derived from plasma A via E_1, a total of 46 μg/24 h. If PR-E_1 and PR-E_2 from the plasma prehormones were compared with total PR-E_1 and PR-E_2, which were measured independently from the specific activity of urinary E_1 (glucuronoside) and E_2 (glucuronoside), these values were very similar. Specifically, in subject 1, all E_1 and 98% of E_2 production could be accounted for from the extraglandular aromatization of plasma C_{19} precursors. If one takes into account the amount of E_1 formed from secreted E_2, then in each of the normal men, the total daily production of E_1 was more than accounted for (101–121%) by the utilization of plasma prehormones. In the normal men, 75–98% of E_2 production could be accounted for by the utilization of plasma T and A.

In the women with testicular feminization, the various sources of E_1 and E_2 could be estimated in four of the subjects (Table 8). In two of the four, all E_1 formation could be accounted for by extraglandular formation through 1) the aromatization of plasma A, 2) plasma T through the sequence plasma T $\rightarrow$ E_2 $\rightarrow$ E_1, and 3) through the conversion of secreted E_2. In the other two subjects (B and F), E_1 production exceeded the amount attributable to extraglandular formation. Twenty-three micrograms of E_1 per 24 h in subject B and 28 μg E_1/24 h in subject F are presumed to have arisen by glandular secretion, likely testicular. On the other hand, in all four subjects, considerably more E_2 was produced than could be accounted for by extraglandular formation. In these four women, the mean secretion rate of E_2 was 44 μg/24 h (range, 34–63 μg/24 h). These values were computed by comparing total E_2 production with that computed to have arisen by extraglandular formation. Interestingly, in these women, the rate of secretion of E_2 was much more similar among these four women than was T secretion, which varied widely.

Discussion

It has been known for more than 50 yr that men produce estrogen, and a considerable body of evidence has accrued which is supportive of the view that testicular secretion is the principal source of estrogen production in adult men. However, with the demonstration that the administration of T to men resulted in an increase in urinary estrogens, it became apparent that plasma T

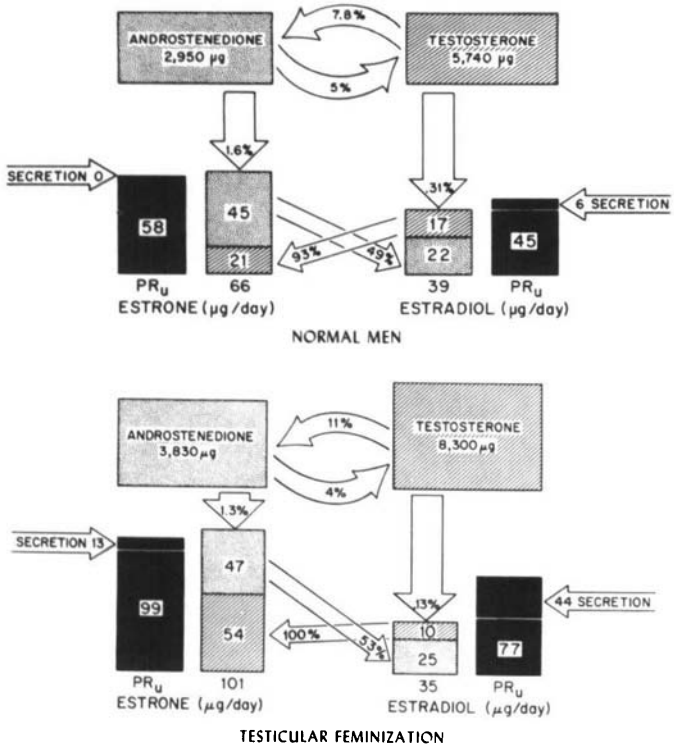

FIG. 1. Summary of the sources of estrogen formation in normal young adult men and in women with testicular feminization. PR-E_1 and PR-E_2 (PR_u) were measured by isotope dilution methods (▬). The estimation of the contribution of plasma precursors A and T to E_1 and E_2 production was calculated from the plasma PR of the precursors and the transfer constants of conversion of precursor to product. The PR_u of E_1 is the average of the values of the subjects of this study measured from isotope dilution techniques and does not equal exactly that computed to have arisen from the utilization of plasma precursors at extraglandular sites. The portion of E_2 production attributed to glandular secretion is the mean excess of the PR_u-E2 over that computed to have arisen by extraglandular formation from plasma precursors.

might give rise to estrogen in tissues other than the testes. By the administration of radioactive T and the isolation thereafter of radioactive estrogens from urine, many workers have demonstrated that the phenomenon of extraglandular estrogen formation represents a potentially important physiological process. It is only in recent years, however, that attempts have been made to quantify the various sources of estrogen production in men, *i.e.* glandular secretion and extraglandular formation from circulating prehormones.

In an early study, Fishman *et al.* (2) concluded that more than two thirds of the estrogen produced in normal men may be secreted by the testes. Longcope *et al.* (3) reached a similar conclusion by the use of constant infusions of isotope-labeled plasma precursors (T and A). These investigators estimated that 70–80% of E_1 and up to 50% of E_2 arose from sources other than the aromatization of plasma C_{19} precursors. In preliminary studies (4, 6), we obtained evidence that estrogen formation in young adult men could be accounted for principally by the peripheral conversion of plasma precursors. Lipsett reached a similar conclusion employing similar methodology (5).

In the present study, E_1 formation was more than accounted for by the aromatization of plasma A and T plus the conversion of secreted E_2 to E_1 in the normal men. Thus, it is concluded that very little E_1 is secreted by the normal adult human testis. This finding is in fundamental agreement with that of Weinstein *et al.* (11). While these investigators found higher levels of E_1 in spermatic venous blood than in peripheral venous blood, they concluded, on the basis of the concentration of T and E_1 in spermatic vein plasma and an assumed testicular T secretion of 7 mg/24 h, that testicular secretion of E_1 was no more than 2.5 μg/24 h.

However, in the normal men of this study, E_2 production could not be accounted for totally by extraglandular formation. In these men, from 0–12 μg E_2 formation/24 h were computed to have arisen by mechanisms other than the aromatization of plasma A and T and are presumed to have arisen by glandular secretion, probably from the testes.

Several groups have attempted to estimate the rate of secretion of estrogens by the testes in normal men by measuring the concentration of estrogens in spermatic and peripheral venous plasma. In a most careful study, Kelch *et al.* (7) demonstrated significant gradients for E_2 across the testis; nevertheless, these investigators concluded that testicular secretion of E_2 contributed no more than 25% of total E_2 production in normal men. On the basis of a comparison of the concentration of E_2 and T in spermatic vein blood and assuming a T secretion of 7 r ϙ/24 h, Kelch *et al.* estimated testicular E_2 secretion to be no more than 10 μg/24 h. If they had assumed a T secretion of 5.7 mg/24 h (the mean of the normal men of this study), the estimated testicular secretion of E_2 would have been 8 μg/24 h. The mean secretion of E_2 calculated for the normal men in the present study (6 μg/24 h) was nearly identical to that estimated by Kelch and associates.

The experimental design employed in the present study permits the utilization of a noninvasive method to estimate the kinetics of androgen-estrogen production and a quantitative evaluation of the various sources of origin of estrogen in normal and abnormal endocrine states. The principal disadvantage of this method resides in the fact that the concentrations of the plasma C_{19} prehormones fluctuate during the day. In the present study, for example, the calculated PR-E_1 derived from plasma precursors exceeded that independently computed for total E_1 production in six of eight subjects studied, and the calculated mean PR-E_1 from plasma precursors exceeded the mean total PR-E_1 (Table 8). If the PRs of plasma precursors are computed from single

measurements of plasma concentrations, the daily rates of production may be over- or underestimated. In the present study, the magnitude of this problem was lessened but not eliminated by averaging the plasma concentrations of A and T in multiple blood samples obtained at frequent intervals throughout the day.

In the women with testicular feminization, PR-E_1 (99 μg/24 h) and PR-E2 (77 μg/24 h) were approximately twice those found in the normal young men. The difference was principally accounted for by the secretion (presumably testicular) of E_2. Whereas the testicular secretion of E_2 in the normal men was 12 μg/24 h or less, in the women with testicular feminization, the mean E_2 secretion was approximately 44 μg/24 h. The extensive metabolism of secreted E_2 through E_1 was principally responsible for the increased E_1 production in these women. However, evidence was obtained for the direct secretion of E_1 in two of the women with testicular feminization.

French *et al.* (28) in a study of a 25-yr-old woman with incomplete testicular feminization (minimal clitoral hypertrophy) found testicular vein concentrations of E_2 which were 25 times those found in peripheral blood, while testicular vein E_1 was somewhat less than peripheral levels. A 16-yr-old woman with incomplete testicular feminization, studied by Kelch *et al.* (7), had a spermatic vein E_2 concentration which was 4 times that of normal men. Thus, the finding in these two studies of E_2 secretion by the testes in subjects with testicular feminization is in keeping with the demonstration in the present study of secretion of E_2 in women with testicular feminization.

It is interesting to note that the rate of E_2 secretion in women with testicular feminization did not vary greatly in the four subjects in whom secretion could be estimated. On the other hand, in these four women, the secretion rate of T varied from 1.3–8.3 mg/24 h. In subject A, with the lowest ^{P}PR-T, it might be assumed that she had not completed the pubertal process, since she was 15 yr old at the time of study. The ^{P}PR-T in her 18-yr-old sister (subject B) was 6.1 mg/24 h. However, in the 14-yr-old subject C, the ^{P}PR-T was 15.2 mg/24 h, while that of her 17-yr-old sister (subject D) was 17.0 mg/24 h. The fact that T secretion (and plasma T levels) can vary so widely among women with typical complete testicular feminization has not been recognized previously. Indeed, if it were not for the strong genetic and clinical evidence, the diagnosis in two of the affected women would be suspect and difficult to separate from hereditary defects in T biosynthesis. The reason(s) for this variability in T secretory rates is unknown. On the one hand, it could be the passive consequence of variable damage to the cryptorchid testes; on the other, it may imply some more fundamental difference in the manifestation of the mutant gene in affected individuals.

The mean PR-T in four adult siblings with testicular feminization studied by Judd *et al.* (29) was 6738 ± 774 μg/24 h, a value not significantly greater than that of the five normal men they studied (5300 ± 45 μg/24 h). In these same subjects, the mean plasma T concentration of 10.6 ± 1.3 μg/liter was significantly greater than that found in normal men.

The MCR-T was less in the women with testicular feminization than in the young men. This may be attributable to one or both of two metabolic events. If the increased MCR-T which is observed with increasing production of T (30) were blunted in these women as a result of their androgen insensitivity, then a low MCR-T would be expected. Alternatively, the higher production of estrogen may have given rise to increased T-binding globulin and a resultant decreased in MCR-T. Judd *et al.* also observed that the MCR-T is lower in women with testicular feminization than in normal men (29).

$[\rho]^{TE_2}_{BU}$ in these six women with testicular feminization was significantly less than that found in normal men.

The results of the present studies in normal men and subjects with complete testicular feminization together with our previous findings using similar techniques in subjects with the Reifenstein syndrome (31) and the syndrome of incomplete testicular feminization (32) make it possible to deduce the mechanism by which feminization occurs in these disorders. Each of these disorders results in male pseudohermaphroditism in which 46 XY males are resistant (to a varying degree) to androgens and, as a result, differentiate partially or completely as phenotypic females. In each, the androgen resistance results in a high mean plasma level of LH, an elevated secretion of estrogen by the testis, and feminizing signs, particularly breast enlargement, at the expected time of puberty. Of particular interest is the fact that these disorders constitute a spectrum of phenotypes. The Reifenstein syndrome subjects are men with severe hypospadias who develop gynecomastia after puberty. Subjects with incomplete testicular feminization (possibly a Reifenstein syndrome variant) are phenotypic females who develop both feminizing and virilizing signs at puberty. Subjects with complete testicular feminization develop pure feminine characteristics. It is also of note that in each of these disorders, the androgen resistance is believed to be the result of a defect in the X-linked cytoplasmic androgen-binding protein that mediates androgen action in the normal male. In the usual woman with complete testicular feminization, the binding protein is completely absent, whereas in the other two, the protein is detectable but deficient (33). In all of these disorders, E_1 and E_2 production rates are elevated, as is estrogen secretion by the testis, presumably as the result of elevated gonadotropin secretion. It is striking, however, that in these disorders, there is no direct relation-

ship between measured estrogen secretion and the degree of feminization. Two phenotypic men with the Reifenstein syndrome had PR-E_2 of 225 and 200 μg/24 h and testicular secretion rates of E_2 of 179 and 142 μg/24 h (31), much greater than the observed PR-E_2 of 53–121 μg/24 h in the six patients with complete testicular feminization. The PR-E_2 in one subject with the incomplete form of testicular feminization was intermediate (138 μg/24 h) (32), but the ^{P}PR-T in the three groups overlapped. On the other hand, in otherwise normal men with androgen insensitivity as the cause of infertility (34), E_2 secretion was intermediate between that found in normal men and that found in women with testicular feminization. We conclude, therefore, that feminization in men with androgen resistance is dependent upon increased E_2 production after puberty, but that the degree of feminization is influenced by the severity of the androgen resistance. As we formulate it, an effective ratio of E_2 to androgen at some cellular level must be the rate-limiting factor that determines the degree of feminization.

From the results of these studies and others, it is apparent that extraglandularly produced E_2 and/or E_1 derived from their respective plasma precursors can stimulate male breast growth. The latter situation may arise from a variety of causes, including deficient T production as a result of injury, disease, or inhibition of synthesis by drug treatments such as spironolactone. At the same time, increased conversion of plasma A and T to their respective estrogen product hormones has been observed in aging (35), obesity (9, 25, 36, 37), hyperthyroidism (38), and liver disease (9, 39). Since estrogen production in men arises principally from the extraglandular aromatization of plasma precursors, it is apparent that alterations in androgen-estrogen physiological manifestations may occur as the consequence of several different metabolic aberrations which result in altered estrogen to T production ratios. These latter conditions include increased testicular secretion of estrogen, increased conversion of plasma precursors to estrogens, decreased T secretion, or a combination of these metabolic aberrations.

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