

Testosterone and Androstenedione Blood Production Rates in Normal Women and Women with Idiopathic Hirsutism or Polycystic Ovaries *

C. WAYNE BARDIN † AND MORTIMER B. LIPSETT

(From the Endocrinology Branch, National Cancer Institute, Bethesda, Md.)

Summary. The average plasma testosterone concentration of women with either hirsutism or polycystic ovaries and hirsutism was higher ($p < 0.01$) than that of normal women although the ranges overlapped. Testosterone blood production rates averaged 830 ± 120 SE and $1,180 \pm 310$ SE μg per day in the two groups of hirsute women and 230 ± 33 SE μg per day in normal women. The ranges did not overlap.

The testosterone metabolic clearance rates of hirsute women ($1,090 \pm 140$ SE L per day) and of men ($1,240 \pm 136$ SE L per day) were significantly higher than those of normal women (590 ± 44 SE L per day). These differences persisted when the metabolic clearance rates were corrected for surface area. We suggest that testosterone metabolic clearance rates vary directly with some function of testosterone production.

The mean plasma androstenedione levels (2.8 ± 0.35 SE and 2.8 ± 0.30 SE μg per L) and production rates ($6,060 \pm 450$ SE and $7,360 \pm 345$ SE μg per day) of the women with hirsutism or polycystic ovaries, respectively, were significantly higher than those of normal women (1.5 ± 0.22 SE μg per L; $3,300 \pm 830$ SE μg per day). The androstenedione metabolic clearance rates were the same in each group. Plasma androstenedione was the precursor of 49% of plasma testosterone in normal women and of 26% of plasma testosterone in hirsute women. Thus, 74% of the plasma testosterone in these subjects must have been either secreted or derived from a precursor that did not enter the plasma androstenedione pool.

Introduction

Study of virilization and hirsutism received new impetus with the introduction of accurate methods for measuring plasma testosterone¹ levels. In several series of hirsute women (1-6), the average

plasma testosterone concentration was high, but in each series some of the subjects had normal levels. The role of plasma androstenedione has not been evaluated in hirsute women, and there is no comprehensive study of androgen production rates in such patients. We have therefore measured testosterone and androstenedione plasma levels and production rates in normal women and in women with idiopathic hirsutism and hirsutism associated with polycystic ovaries. Using the model developed by Horton and Tait (7), we have estimated the contribution of androstenedione production to the testosterone production rate and have shown that the excess testosterone is secreted as well as produced from plasma androstenedione. Our finding that the blood testosterone production

* Submitted for publication November 28, 1966; accepted February 2, 1967.

A preliminary report of this work was presented at the Forty-eighth Meeting of the Endocrine Society, June 20, 1966.

† Address requests for reprints to Dr. C. Wayne Bardin, Endocrinology Branch, National Cancer Institute, Bethesda, Md. 20014.

¹ The following trivial names have been used: testosterone, 17 β -hydroxy-4-androsten-3-one; androstenedione, 4-androsten-3,17-dione; dehydroepiandrosterone, 3 β -hydroxy-5-androsten-17-one.

TABLE I
Clinical summary

Sub- ject	Age	Weight	Height	Body surface	Hirsut- ism*	Menses†	Ovula- tory	Ovarian size	Urinary 17-keto- steroids
	<i>years</i>	<i>kg</i>	<i>cm</i>	<i>m²</i>				<i>cm</i>	<i>mg/day</i>
Ia. Normal ovulatory women									
1	22	59	158	1.59		N	Yes	N	
2	33	50	164	1.52		N	Yes	N	
3	21	40	165	1.39		N	Yes	N	
4	26	55	170	1.62		N	Yes	N	
5	33	55	159	1.54		N	Yes	N	
Ib. Anovulatory women									
6	28	61	158	1.61		N	No	N	
7	22	68	156	1.67		N	No	N	12
8	64	61	165	1.66		A	No	N	6
9	35	100	157	2.00		A	No	N	8
II. Women with idiopathic hirsutism									
10	24	85	163	1.89	4+	N	Yes	N	18
11	17	54	163	1.56	2+‡	N	Yes	N	11
12	18	60	165	1.65	3+	N	Yes	N	11
13	23	66	172	1.77	2+	N	Yes	N	13
14	31	49	153	1.43	3+	O	No	3 × 2 × 3	6
15	20	92	165	1.97	1+	O	No	3 × 2 × 2	6
16	31	72	165	1.78	1+	O	No	N	20
17	25	97	165	2.01	2+	A	No	4 × 2 × 2	12
III. Women with polycystic ovaries									
18	33	81	164	1.86	3+‡§	O	No	4 × 6 × 4	8
19	35	117	164	2.18	3+	O	No	4 × 3 × 3	14
20	29	65	175	1.78	2+	N	Yes	6 × 6 × 4	23
21	25	81	172	1.93	1+	O	No	3 × 2 × 2	9
22	35	101	162	2.02	3+‡	O	No	4 × 2 × 2	10

* Facial hirsutism was graded 1+ for each portion of the face involved (side burns, upper lip, chin) and 4+ if the entire beard area was involved. All subjects had a heavy growth of hair on trunk and extremities.

† Abbreviations: N = normal; O = oligomenorrhea; A = amenorrhea.

‡ Slight temporal balding.

§ Clitoromegaly.

|| Patient studied after wedge resection of ovaries.

rate was high in each hirsute woman is the only consistent physiologic difference between normal and hirsute women that has been observed.

Methods

Subjects. The normal subjects volunteered for these studies. Group Ia was composed of five women with normal ovulatory menses. Group Ib contained four non-hirsute subjects: one was receiving oral contraceptives² (Patient 6); one had received oral contraceptives² 5 months previously and was now anovulatory (Patient 7); one had carcinoma of the cervix and was postmenopausal (Patient 8); and one patient had secondary idiopathic amenorrhea (Patient 9). Group II comprised eight women with complaints of hirsutism. In four of them the ovaries were assumed to be normal on the basis of regular ovulatory menses. In three of the four patients with irregular anovulatory cycles in whom culdoscopy

was performed (Patients 14, 15, 17) the ovaries were grossly and histologically normal. Group III contained five hirsute patients with histologically characteristic polycystic ovaries. When groups II and III are combined, they will be referred to as the hirsute subjects. These clinical data are summarized in Table I. Testosterone metabolic clearance rates were determined in six normal men.

All subjects used in this study had normal 24-hour urinary 17-hydroxycorticoid excretion that was suppressed by 2 mg of dexamethasone daily for 3 days. Ovulation was diagnosed either by endometrial biopsy or urinary pregnanediol excretion. Thyroid and liver function tests were normal in all subjects. Urinary steroids measured were as follows: 17-ketosteroids (8), 17-hydroxycorticoids (9), and pregnanediol (10).

Reagents. Solvents were analytical grade and were distilled before use. Androstenedione-1,2-³H (5 c per mmole), androstenedione-4-¹⁴C (50 mc per mmole), testosterone-7 α -³H (10 c per mmole), and testosterone-4-¹⁴C (50 mc per mmole) were obtained commercially³

² Norethynodrel, 5 mg per day, and mestranol, 0.075 mg per day.

³ New England Nuclear Corp., Boston, Mass.

TABLE II
Chromatographic systems

System	Support	Solvents
BM ₁	Silica gel	Benzene:methanol (9:1)
BE ₄	Silica gel	Benzene:ethyl acetate (6:4)
BE ₂	Silica gel	Benzene:ethyl acetate (8:2)
Bush A ₂	Paper	Ligroin:methanol:water (10:7:3)

and further purified by thin layer chromatography in systems BM and BE₄ (Table II). To test for purity of the labeled steroids, we added a portion of each to 200 μ g of authentic steroid and carried through the procedure outlined in Figure 1. The specific activity was estimated by measuring the mass of steroid by absorption at 240 m μ in ethanol and radioactivity by liquid scintillation spectrometry. The specific activity of each steroid remained constant after successive chromatography as the acetate, free alcohol, and 17-ketone.

Chromatography. Thin layer chromatography was performed on 20- \times 20-cm glass plates coated with 25 mm of Brinkmann Porter silica gel GH₂₅₄. Whatman 3MM was used for paper chromatography. The chromatography

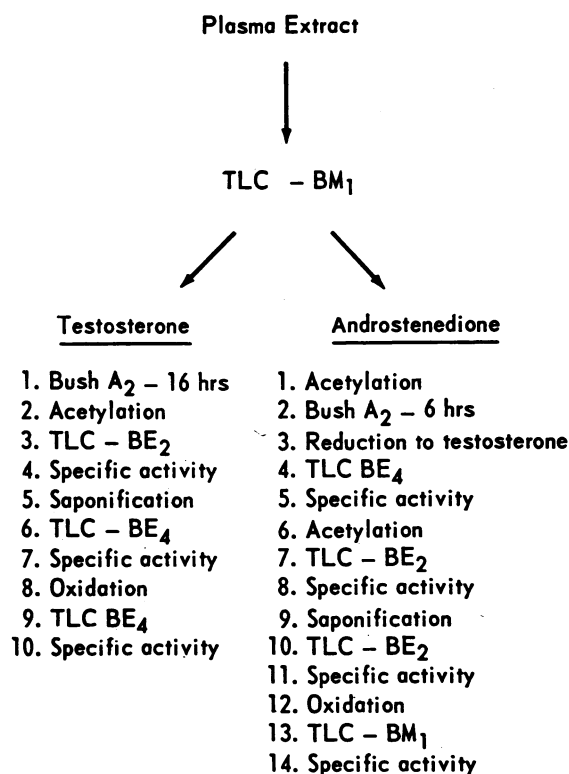


FIG. 1. PURIFICATION OF PLASMA EXTRACTS FOR ESTIMATION OF ISOTOPIC TESTOSTERONE AND ANDROSTENEDIONE. TLC = thin layer chromatography. Steps 12 and 13 were performed on 9 samples and produced no change in specific activity.

systems are listed in Table II. Gas-liquid chromatography and collection of the samples for radioactivity measurements were performed as described previously (11).

Plasma steroids. Forty ml of heparinized blood was obtained for plasma steroid levels immediately before determination of the metabolic clearance rates. Plasma testosterone and androstenedione concentration was measured by a modification (12) of the double isotope technique reported from this laboratory (11). Plasma testosterone and androstenedione samples of 0.007 μ g can be measured with theoretical precisions of 35% and 22%, respectively. The method blanks for 20 ml of plasma with an over-all recovery of 20% were 0.010 μ g per 100 ml for testosterone and 0.0025 μ g per 100 ml for androstenedione and have been subtracted from the reported plasma levels.

Metabolic clearance rates. The metabolic clearance rates of testosterone (MCR^T) and of androstenedione (MCR^A) were measured by the technique of Horton and Tait (7), and their abbreviations are used. All subjects were studied under basal conditions. Approximately 10 to 15 μ g of androstenedione-³H and 0.25 to 0.5 μ g of testosterone-¹⁴C were injected intravenously in 20 ml of 5% ethanol in saline as a priming dose, and starting 30 minutes later, double these quantities were given as a constant infusion for 90 minutes in 170 ml of 5% ethanol in saline. With the infusion of androstenedione-³H and testosterone-¹⁴C at a disintegrations per minute ratio of 30:1, sufficient ³H and ¹⁴C counts were present in 20 ml of plasma to determine the metabolic clearance of both steroids and the conversion ratio of androstenedione to testosterone but not the conversion ratio of testosterone to androstenedione. In one patient (No. 8) the infusion was extended for 12 hours. In two additional subjects (Patients 3 and 17), the isotopes were reversed, and 7 μ g of androstenedione-4-¹⁴C and 2.5 μ g of testosterone-7 α -³H were given as a priming dose, and after 30 minutes twice these quantities were infused over 90 minutes. These data are presented in Table III.

The radioactive steroids were infused with a Bowman infusion pump equipped with a siliconized latex pump tube and Teflon tubing. The latex pump tubing was replaced after every fifth infusion. The tubing effluent was monitored at frequent intervals, generally three to five times during the infusion, and the actual rate of infusion of labeled steroids agreed closely with the calculated rate in each instance. The MCR was calculated from the rate of infusion of the isotope divided by the concentration of isotope in the specific steroid per volume of plasma.

Isotopic testosterone and androstenedione concentrations were determined by reverse isotope dilution on 40 ml of heparinized blood obtained at 50, 70, and 86 minutes of the infusion. The plasma was separated within 30 minutes, and 200 μ g each of testosterone and androstenedione was added to the plasma. The plasma samples were extracted twice with 2 vol of ether:chloroform (3:1) after the addition of 1 ml of 1 N NaOH per 20 ml of plasma. The extracts were washed with water,

TABLE III
Infusion rates and plasma concentration of isotopic steroids

Subject	Infusion		Plasma*		Subject	Infusion		Plasma*	
	Andros- tenedione- ³ H	Testos- terone- ¹⁴ C	Andros- tenedione- ³ H	Testos- terone- ¹⁴ C		Andros- tenedione- ³ H	Testos- terone- ¹⁴ C	Andros- tenedione- ³ H	Testos- terone- ¹⁴ C
	dpm/hr × 10 ⁷	dpm/hr × 10 ⁶	dpm/L × 10 ⁵	dpm/L × 10 ⁴		dpm/hr × 10 ⁷	dpm/hr × 10 ⁶	dpm/L × 10 ⁵	dpm/L × 10 ⁴
1	4.31	1.41	6.9	6.40	12	5.38	1.69	5.43	6.05
2	5.99	1.31	4.78	5.23	13	4.74	1.46	5.55	3.14
3†	1.82	6.80	1.93	21.4	14	4.39	1.44	7.06	5.87
4	4.21	1.42	5.96	6.45	15	5.38	1.69	5.08	2.51
5	4.08	1.59	4.78	7.05	16	4.00	1.59	4.46	3.75
6	2.68	1.17	2.76	7.67	17†	1.82	6.80	1.68	9.31
7	5.04	2.35	4.00	6.00	18	4.85	2.60	3.10	3.94
8		0.434		1.73	19	5.85	1.32	4.18	3.00
9		18.0		34.0	20	4.70	1.79	3.32	5.26
10	4.75	2.51	3.83	6.06	21	4.90	1.55	6.29	4.98
11	2.50	1.45	2.78	3.72	22	4.76	1.63	4.83	2.94

* Mean of the three values obtained at 50, 70, and 86 minutes of the infusion.

† Androstenedione-¹⁴C and testosterone-³H were infused in these patients.

dried, and purified by chromatography and derivative formation as outlined in Figure 1. Oxidation was carried out with 0.2% chromic acid in glacial acetic acid for 10 minutes and reduction with freshly prepared 2% potassium borohydride in water for 20 seconds. Acetylation was performed with acetic anhydride in pyridine and saponification with 0.15 N NaOH in 80% methanol under N₂ overnight. After chromatography of each derivative (Figure 1) the specific activity of both plasma steroids was determined as outlined above. The plasma concentrations of isotopes in testosterone and androstenedione were calculated from the recovery of added unlabeled steroid.

Evidence that the infused steroids reached equilibrium during the 90-minute infusion was provided by the constant level of radioactive testosterone and androstenedione observed in the plasma samples at 50, 70, and 86 minutes. In one patient (No. 8), continuing the infusion for 12 hours produced no change in the observed radioactive testosterone levels. In each subject the mean plasma concentrations for both testosterone and androstenedione were calculated, and the per cent deviation of each sample from the mean was determined. After infusion of testosterone-¹⁴C and androstenedione-³H the deviation of individual samples from the mean was $\pm 1.1\%$ for ¹⁴C in testosterone, $\pm 2\%$ for ³H in androstenedione, and $\pm 6\%$ for ³H in testosterone. These analyses demonstrated no significant trend in the plasma isotopic steroid levels.

³H/¹⁴C of urinary testosterone glucuronoside. After each infusion, urine was collected for 2 days and pooled. One-tenth of the pool from each of six subjects was extracted with 2 vol of ether and then hydrolyzed with beef liver glucuronidase (Ketodase). The freed steroids were extracted with 6 vol of dichloromethane after addition of 50 μ g of testosterone. The extracts were washed with 0.1 N NaOH and water and then partitioned between heptane and 80% ethanol. The heptane was discarded; the ethanol concentration was reduced to 20%,

and the testosterone was extracted with 8 vol of carbon tetrachloride. These extracts were purified as in Figure 1. One-third of the sample was counted at steps 4 and 10, and the ³H/¹⁴C of the remaining one-third was determined on the effluent after gas-liquid chromatography. The ³H/¹⁴C ratios were the same at step 10 and after gas-liquid chromatography.

Radioactivity measurement. Counting was performed with a Packard Tri-Carb liquid scintillation spectrometer, model 4322, operating at 25% efficiency for ³H and at 50% efficiency for ¹⁴C. Discriminator and gain settings were such that less than 0.1% of the ³H was counted in the ¹⁴C channel, and 13% of the ¹⁴C was counted in the ³H channel. With a single isotope, sufficient counts were accumulated to give standard errors of less than 2%. When two isotopes were measured, the samples were counted four times for 50 minutes, and the standard errors for ³H and ¹⁴C were less than 2%. The standard error of the ³H/¹⁴C ratio of urinary testosterone glucuronoside was less than 4%.

Calculations. The symbols and calculations for the several parameters of the testosterone-androstenedione system are those of Horton and Tait (7). The superscripts indicate the steroid, the subscripts the compartment. The symbol z refers to the isotope in testosterone and x to the isotope in androstenedione. The formulas used are as follows:

$$MCR^T = R_z^T/z^T \quad \text{and} \quad MCR^A = R_x^A/x^A,$$

where R_z^T is the disintegrations per minute of testosterone infused per unit time and z^T is the concentration of isotope in testosterone in disintegrations per minute per unit volume of plasma. The androstenedione expressions are analogous.

$$P_B^T = MCR^T \times i^T \quad \text{and} \quad P_B^A = MCR^A \times i^A,$$

where P_B^T is the blood testosterone production rate and i^T is the blood testosterone concentration.

$$C_{BB}^{AT} = x^T/x^A,$$

TABLE IV
Concentrations, production rates, and interconversion of plasma testosterone and androstenedione*

Subject	Plasma testosterone	MCR ^T	P _B ^T	Plasma andros- tenedione	MCR ^A	P _B ^A	x ^T /x ^A	[ρ] _{BB} ^{AT}	$\frac{[\rho]_{BB}^{AT}P_B^A}{P_B^T}$
	μg/L	L/day	μg/day	μg/L	L/day	μg/day			
Ia. Normal ovulatory women									
1	0.25	530	130	1.1	1,500	1,700	0.093	0.033	0.45
2	0.36	600	220	2.1	3,000	6,300	0.16	0.032	0.93
3	0.36	760	260	0.9	2,120	1,890	0.11	0.039	0.28
4	0.39	530	210	1.7	1,700	2,900	0.095	0.030	0.41
5	0.62	540	330	1.8	2,050	3,700	0.12	0.032	0.35
Mean	0.40	590	230	1.5	2,070	3,300	0.12	0.033	0.49
SD	±0.13	±98	±73	±0.50	±577	±1,860	±0.025	±0.003	±0.29
SE	±0.06	±44	±33	±0.22	±260	±830	±0.011	±0.001	±0.11
Ib. Anovulatory women									
6	0.53	370	200	1.6	2,330	3,700	0.25	0.040	0.76
7	1.4	940	1,320	2.4	3,020	7,250	0.085	0.026	0.15
8	0.32	600	190	0.9					
9	0.90	1,270	1,140						
II. Women with idiopathic hirsutism									
10	0.90	990	890	1.9	2,980	5,510	0.058	0.019	0.12
11	1.08	920	990	3.5	2,250	7,700	0.096	0.040	0.31
12	1.01	670	680	2.3	2,380	5,430	0.084	0.024	0.19
13	0.71	1,100	780	2.2	2,050	4,520	0.045	0.024	0.14
14	0.99	590	580	5.1	1,490	7,600	0.092	0.036	0.47
15	0.35	1,610	560	1.8	2,520	4,540	0.040	0.026	0.21
16	0.54	1,020	550	2.9	2,150	6,100	0.075	0.036	0.40
17	0.90	1,750	1,580	2.7	2,610	7,040	0.068	0.046	0.20
Mean	0.81	1,080	830	2.8	2,300	6,060	0.070	0.031	0.26
SD	±0.75	±410	±340	±0.98	±440	±1,280	±0.017	±0.009	±0.13
SE	±0.09	±145	±120	±0.35	±160	±450	±0.006	±0.003	±0.04
III. Women with polycystic ovaries									
18	0.51	1,580	810	2.2	3,720	8,100	0.065	0.028	0.28
19	1.21	1,060	1,280	2.3	3,310	7,600	0.120	0.039	0.23
20	0.70	820	570	1.9	3,400	6,700	0.170	0.041	0.46
21	0.82	750	620	3.4	1,870	6,400	0.054	0.022	0.22
22	1.98	1,330	2,640	3.4	2,360	8,000	0.053	0.030	0.09
Mean	1.04	1,110	1,180	2.8	2,930	7,360	0.092	0.032	0.26
SD	±0.61	±350	±700	±0.7	±775	±770	±0.052	±0.008	±0.13
SE	±0.27	±155	±310	±0.3	±350	±345	±0.023	±0.003	±0.06

* MCR^T and MCR^A = metabolic clearance rate of testosterone and androstenedione; P_B^T and P_B^A = blood production rate of testosterone and androstenedione; x^T/x^A = conversion ratio of labeled testosterone to androstenedione; [ρ]_{BB}^{AT} = fraction of blood androstenedione pool converted to blood testosterone pool.

where C_{BB}^{AT} is the conversion ratio of androstenedione to testosterone in blood.

$$[\rho]_{BB}^{AT} = (MCR^T/MCR^A) \times (x^T/x^A),$$

where [ρ]_{BB}^{AT} is the fraction of the blood androstenedione pool converted to the blood testosterone pool.

$$([\rho]_{BB}^{AT} \times P_B^A)/P_B^T = (x^T/x^A) \times (i^A/i^T),$$

where the expression is the fraction of the blood testosterone production that is derived from blood androstenedione production. A similar expression can be derived for androstenedione production from testosterone. The correction factor, $1 - [\rho]_{BB}^{AT} \times [\rho]_{BB}^{TA}$, for the amount of precursor originating from product has been ignored,

since the product of the two [ρ] values is approximately 0.03.

Errors of the method. The errors of the rates of radioactive steroid infusions determined from the counting errors and from the observed variability of pump tubing effluent were ±4% for R_Z^T and 5% for R_X^A. The theoretical errors of the radioactive steroid levels in plasma calculated from counting errors and specific activity determinations were ±4%, ±4%, and ±11% for these determinations (±3.0, ±4.4, and ±9.9%). The errors of the calculated values were as follows: MCR^T, ±5.7%; MCR^A, ±6.0%; P_B^T, ±35%; P_B^A, ±18%; x^T/x^A, ±11.8%; [ρ]_{BB}^{AT}, ±14.4%. The larger error of P_B^T was calculated for plasma testosterone levels in normal women.

This error is 21% when the plasma testosterone level is in the range of 0.1 μg per 100 ml.

Results

Plasma steroids. The mean plasma testosterone concentration of 0.40 μg per L in the five normal ovulatory women (Table IV) was the same as that of 20 normal women previously reported from this laboratory (0.37 ± 0.02 SE μg per L; range, 0.20 to 0.70 μg per L) (12). The mean plasma levels in women with idiopathic hirsutism (0.81 ± 0.09 SE μg per L) and with polycystic ovaries (1.04 ± 0.27 SE μg per L) were higher than the normal levels ($p < 0.01$). Despite this difference four of the 13 hirsute women had plasma testosterone levels in the normal range.

The mean plasma androstenedione level in the five normal women (1.5 ± 0.22 SE μg per L) was not different from the mean of 20 normal women previously reported (1.67 ± 0.09 SE μg per L; range, 0.9 to 2.1 μg per L) (12). The androstenedione levels in both hirsute groups were significantly greater than those of normal women ($p < 0.05$). As with the testosterone levels, the ranges overlapped.

Metabolic clearance rates. The primary data from all the studies are summarized in Table III. Adequate amounts of tritium and ^{14}C were present in each steroid, and the counting ratios were satisfactory. The mean MCR^T of normal women measured by the constant infusion technique was significantly less than the means of the subjects with

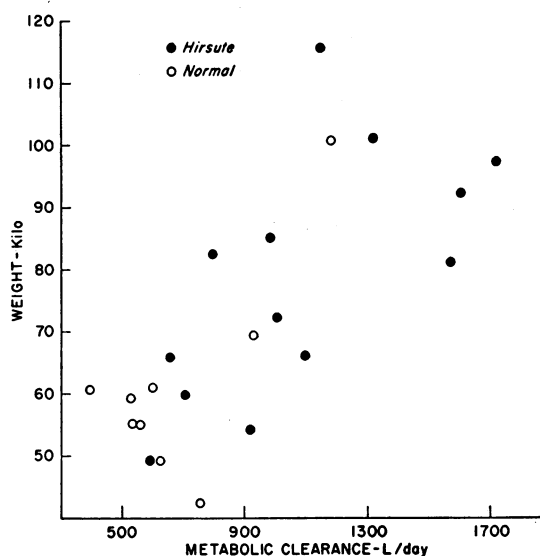


FIG. 2. CORRELATION OF THE TESTOSTERONE METABOLIC CLEARANCE RATE (MCR^T) WITH BODY WEIGHT. The r equals 0.79 with 95% confidence limits of 0.5 to 0.9.

idiopathic hirsutism or polycystic ovaries ($p < 0.01$) (Table IV). Because the higher clearance rates occurred in heavier women, we examined MCR^T as a function of body weight (Figure 2) and found the r to be 0.79 with 95% confidence limits of 0.5 to 0.9. In view of these findings and since the calculated metabolic clearance rate depends not only on rate of steroid metabolism but on its volume of distribution, it is appropriate to correct MCR^T for body size before comparing it among groups. We have elected to express

TABLE V
The metabolic clearance rates of testosterone and androstenedione

Subjects No.	Surface area	MCR^T	MCR^T/m^2	MCR_T/m^2 $\log \text{PB}^T$	MCR^A	MCR^A/m^2
	m^2	L/day			L/day	
Normal women						
5	$1.53 \pm 0.04^*$	590 ± 44	390 ± 40	161 ± 15	$2,070 \pm 260$	$1,360 \pm 185$
Idiopathic hirsutism						
8	$1.75 \pm 0.15^\dagger$	$1,080 \pm 145^\dagger$	$600 \pm 60^\dagger$	202 ± 18	$2,300 \pm 160$	$1,240 \pm 53$
Polycystic ovaries						
5	$1.95 \pm 0.07^\dagger$	$1,110 \pm 155^\dagger$	568 ± 84	185 ± 26	$2,930 \pm 350$	$1,550 \pm 96$
All hirsute women						
13	$1.83 \pm 0.06^\dagger$	$1,090 \pm 140^\dagger$	$588 \pm 47^\dagger$	196 ± 12	$2,540 \pm 178$	$1,360 \pm 65$
Normal men						
6	$1.98 \pm 0.12^\dagger$	$1,240 \pm 136^\dagger$	$627 \pm 60^\dagger$	162 ± 16		

* Mean \pm standard error.

† Significantly different from normal women ($p < 0.01$).

‡ Significantly different from normal women ($p < 0.05$).

MCR^T as a function of surface area. Even with this correction, MCR^T per square meter was higher in the hirsute patients than in the normal women ($p < 0.05$) (Table V). Calculations based on body weight rather than surface area gave similar results. These data suggested that some factor in addition to body size influenced the MCR^T of hirsute women.

We therefore examined the relationship between MCR^T and several parameters of testosterone metabolism. The logarithm of the blood testosterone production rate ($\log P_B^T$) was correlated positively with MCR^T ($r = 0.65$) and with MCR^T per square meter ($r = 0.55$) with rather wide confidence limits. When the MCR^T was corrected for surface area and $\log P_B^T$, then the resulting expression MCR^T per square meter per $\log P_B^T$ was the same for all groups (Table V).

Since we found that the MCR^T of men (Table V) was twice that of women, we calculated the effects of correction for surface area and testosterone production rate on MCR^T . When the MCR^T was corrected for surface area, the MCR^T per square meter of men remained significantly greater than that of women. When the expression MCR^T per square meter per $\log P_B^T$ was calculated, however, the values were the same in men and women.

The MCR^T of the women in group Ib was consistent with that of the other groups with the correction factors just outlined. Of interest were Patients 7 and 9, who had high plasma testosterone levels and MCR^T . When the MCR^T was corrected by surface area and $\log P_B^T$, the MCR^T per square meter per $\log P_B^T$ was the same as in the other groups.

The MCR^A and MCR^A per square meter are summarized in Table V. Although there was no significant difference between the MCR^A of normal and hirsute women at the 0.05 level, the MCR^A of the obese women was greater than that of the normal women. The variances of the means of the MCR^A for each group were reduced markedly by correction for surface area.

Blood production rates. The blood production rates of testosterone and androstenedione from Table IV are summarized in Figures 3 and 4, respectively. Both P_B^T and P_B^T per square meter were two to four times greater in the hirsute groups than in the normal. The ranges did not overlap although some hirsute patients had plasma

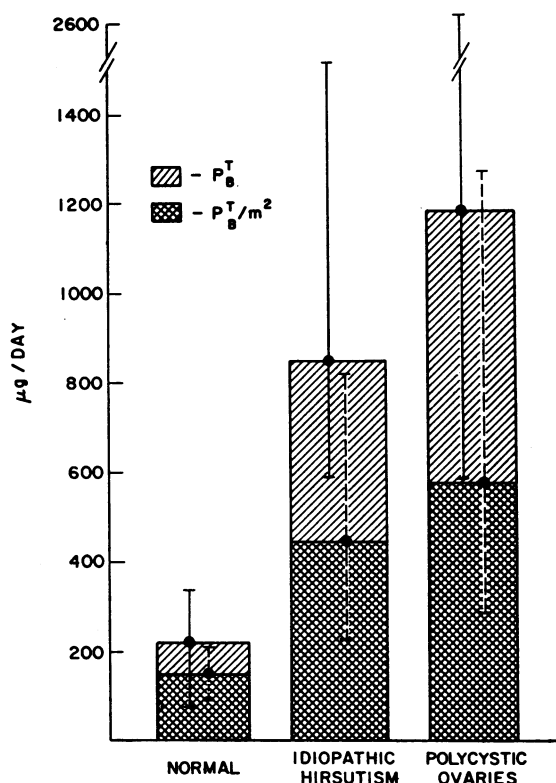


FIG. 3. TESTOSTERONE BLOOD PRODUCTION RATES (P_B^T) AND BLOOD PRODUCTION RATES PER SQUARE METER OF BODY SURFACE (P_B^T/m^2) IN NORMAL AND HIRSUTE WOMEN. Mean and range.

testosterone levels within the normal range. Similarly, P_B^A and P_B^A per square meter were twice as great in the hirsute women.

Conversion ratios, $[\rho]$ values, and fraction of P_B^T from P_B^A . The conversion ratios of labeled androstenedione to testosterone, x^T/x^A , during the infusion of radioactive androstenedione are summarized in Table IV. Ratios of x^T/x^A were the same in the normal and polycystic ovary groups, but x^T/x^A was significantly lower in the normal than in the idiopathic hirsutism group ($p < 0.01$). Despite this, the fraction of the blood androstenedione converted to blood testosterone, $[\rho]_{BB}^{AT}$, was the same in the three groups, the means ranging from 0.031 to 0.033. Since P_B^A was greater in the hirsute subjects, the amount of blood testosterone produced from blood androstenedione ($[\rho]_{BB}^{AT} \times P_B^A$) was higher. It is apparent that since the fractional conversion rate, $[\rho]_{BB}^{AT}$, was the same among all groups, the per cent of blood testosterone derived from blood

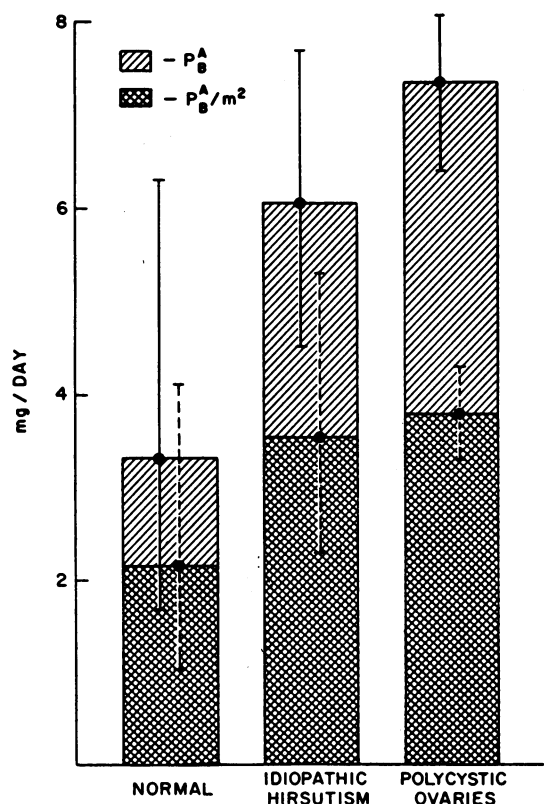


FIG. 4. ANDROSTENEDIONE BLOOD PRODUCTION RATES (P_B^A) AND BLOOD PRODUCTION RATES PER SQUARE METER OF BODY SURFACE (P_B^A/m^2) IN NORMAL AND HIRSUTE WOMEN. Mean and range.

androstenedione is directly proportional to P_B^A/P_B^T . In normal subjects, this ratio was 14.3, and 49% of blood testosterone was derived from blood androstenedione. In the idiopathic hirsutism and polycystic ovary groups, P_B^A/P_B^T were 7.3 and 6.2, respectively, so that a smaller per cent of blood

testosterone originated from blood androstenedione (Table IV). Thus the hirsute subjects must have had either a greater secretion of testosterone or a larger synthesis from some precursor that did not enter the plasma androstenedione pool.

Comparison of $^3H/^{14}C$ ratios of plasma and testosterone and urinary testosterone glucuronide. It has been shown (7, 13) that only a small fraction of testosterone synthesized from blood androstenedione reaches the blood testosterone pool, and it was found that the fractional conversion of blood androstenedione to urinary testosterone glucuronoside ($[\rho]_{BU}^{AT}$) was greater than $[\rho]_{BB}^{AT}$. The large differences between the $^3H/^{14}C$ ratios of plasma testosterone and urinary testosterone glucuronoside (Table VI) re-emphasize this. The ratios of the $^3H/^{14}C$ of testosterone glucuronoside to the $^3H/^{14}C$ of plasma testosterone were not the same in all patients (column 6, Table VI). This demonstrates that $[\rho]_{BB}^{AT}$ has no constant relationship to $[\rho]_{BU}^{AT}$. This is in accord with the suggestion (7) that most of the synthesis of plasma testosterone from androstenedione takes place in peripheral tissues other than the liver.

Discussion

Testosterone. The development of methods for the measurement of plasma testosterone levels promised solutions to several problems associated with virilization and hirsutism. Indeed, to our knowledge, whenever plasma testosterone has been determined in virilized women, the levels have been high. However, the promise of a relationship between plasma testosterone and hirsutism

TABLE VI
Isotopic ratios in urinary testosterone glucuronoside and free plasma testosterone after the infusion of androstenedione- 3H and testosterone- ^{14}C

(1) Subject no.	(2) Diagnosis	(3) $^3H/^{14}C$ of isotopes given	(4) $^3H/^{14}C$ of urinary testosterone glucuronoside	(5) $^3H/^{14}C$ of plasma testosterone	(6) Ratio of column 4 divided by ratio of column 5
<i>dpm</i>					
3	Normal	25.0	10.9	0.84	13
5	Normal	28.2	10.9	0.79	14
12	Idiopathic hirsutism	32.8	10.2	0.79	13
15	Idiopathic hirsutism	32.8	15.9	0.78	20
20	Polycystic ovary	31.9	20.1	1.12	18
22	Polycystic ovary	32.1	7.25	0.89	8

has proved illusory. In several studies of hirsute women, plasma testosterone levels ranged from normal to high (1-6). Our data are similar to those previously reported; that is, although the mean plasma testosterone level differed significantly between the normal and hirsute groups, not all the patients had abnormal testosterone levels.

In contrast to the normal levels of plasma testosterone reported in various studies in hirsute subjects, testosterone production rates were higher than normal in each hirsute patient. This somewhat unexpected finding made us consider that some aspects of testosterone production or clearance in addition to plasma levels may be of significance in the development of hirsutism. The recent reports of testosterone metabolism by skin (14) may be pertinent to the clearance of testosterone, but meaningful estimates of the fraction of testosterone metabolized by skin cannot be made as yet.

The thesis has been advanced that hirsutism may be a manifestation of increased end-organ sensitivity to normal androgen levels (15). This was based on the obvious differences in the rate of development of hirsutism by women receiving testosterone. This thesis was supported further by the occurrence of normal plasma testosterone levels in some hirsute women. Our findings of a uniformly increased testosterone production rate do not support the hypothesis that differences in end-organ sensitivity are the sole determinants of hirsutism. Although this is a relatively small series, it is the only one in which a consistent biochemical difference was observed in hirsutism.

It should not be inferred, however, that an increased testosterone production rate will be necessarily associated with hirsutism. End-organ sensitivity may be the determinant in these instances. Two of our subjects, Patients 7 and 9, had high testosterone production rates but no hirsutism.

These results differ from those previously reported from our laboratory (5) when testosterone production rates were being measured by isotope dilution into urinary metabolites. The reasons for the overestimates of testosterone production rates by this method have been discussed fully (13, 16). The differing results emphasize the importance of using the metabolic clearance tech-

nique for meaningful assessments of testosterone production.

The extrapolation of testosterone production rates obtained by the metabolic clearance technique to estimates of 24-hour production rates should be cautious. Our patients were studied in the basal state before arising in the morning. Under these conditions, the testosterone metabolic clearance technique would be maximal; it has been shown to decrease by as much as 35% upon standing (17). Changes in metabolic clearance could thus affect plasma testosterone levels. Further, circadian variations in plasma testosterone levels in women have not been examined, and significant changes in these levels would alter the calculated 24-hour production rate. These considerations emphasize that all patients should be studied under similar metabolic conditions if comparisons are to be made.

Androstenedione. Although androstenedione has been recognized as an androgen, its quantitative importance as a precursor of plasma testosterone in women was demonstrated only recently (7). Mahesh and Greenblatt (18) reported high androstenedione levels in ovarian vein blood from women with polycystic ovaries. These studies make it imperative that any examination of androgen overproduction include an analysis of androstenedione production and conversion to testosterone.

Plasma androstenedione levels and production rates in patients with hirsutism or polycystic ovaries were about twice those of normal women. This excess androstenedione may be of ovarian origin in women with polycystic ovaries (18), but its source is unknown in patients with idiopathic hirsutism. Suppression of the adrenal cortex in a large group of hirsute women permitted Jayle, Scholler, Mauvais-Jarvis, and Métay (19) to distinguish between adrenal cortical and ovarian hirsutism. The more recent demonstration (20) that adrenal suppression markedly decreases urinary testosterone excretion in most hirsute women points to the role of the adrenal cortex in the secretion of testosterone or its precursors.

Horton and Tait (7) found that 60% of the plasma testosterone of normal women was derived from plasma androstenedione. In our control group 49% of the testosterone was so derived, which is in reasonable agreement with the former investigators.

However, the fractional conversion of androstenedione to testosterone, $[\rho]_{BB}^{AT}$, was lower than that observed by Horton and Tait (7). This discrepancy is due to the lower MCR^T observed in our study. It should be noted that this difference in the MCR^T affects only the calculation of P_B^T and $[\rho]_{BB}^{AT}$. The $[\rho]_{BB}^{AT}$ was the same in all of our subjects. Therefore, the higher testosterone production rate of the hirsute patient cannot be due to increased rate of synthesis of testosterone from plasma androstenedione in the liver or other peripheral tissue. In the hirsute patients, 26% of the plasma testosterone was derived from plasma androstenedione. Thus 74% of the 830 μg of plasma testosterone in women with idiopathic hirsutism and 1,180 μg in subjects with polycystic ovaries must have been either secreted or derived from precursors that did not enter the plasma androstenedione pool.

Testosterone secretion from the polycystic ovary has been demonstrated by several investigators (3, 6, 18). In addition, the adrenal cortex has been shown to secrete testosterone in hirsute women (4).

The question of other precursors has not been examined thoroughly as yet. The secretion of possible precursors such as dehydroepiandrosterone, its sulfate, and 5-androsten-3 β ,17 β -diol has been reported (21). We have estimated that as much as 50 μg per day of blood testosterone production could be derived from the secretion of 10 mg per day of dehydroepiandrosterone (22). Tait and Horton (23) have discussed fully the question of testosterone precursors other than androstenedione and concluded that their quantitative importance is small. If these considerations are correct, then the increased testosterone production rates in hirsutism are due, in large part, to testosterone secretion. Differential suppression of the adrenal cortex and ovary will be necessary to assess the relative roles of each gland in the secretion of testosterone and androstenedione. In preliminary studies in patients with hirsutism, we have observed that dexamethasone caused a significant reduction of plasma testosterone in five of nine patients and of plasma androstenedione in all nine patients.

Metabolic clearance rates and methodology. The concept (24) and methods (25) of measuring

metabolic clearances have been clearly outlined by Tait and his co-workers. In a study of three men and two women (7), the testosterone metabolic clearance rates were reported to be the same, although those of the women were less than those of the men. Hudson, Dulmanis, Coghlan, and Wintour (26) reported that metabolic clearance rates of testosterone of men and women were the same, but the details of the infusion technique were not given. Southren, Tochimoto, Carmody, and Isurugi (27), using the single injection technique, found that men had a greater testosterone metabolic clearance than women and in a subsequent abstract (28) stated that the clearance rate by constant infusion in men was twice that in women. Our studies are in accord with these findings.

The reasons for the discrepancies among the groups are not immediately apparent, but several important features of this study should be emphasized.

1) Teflon tubing was used for each infusion, and the rate of infusion of isotope entering the patient was checked by monitoring the infusion at a stopcock behind the infusion needle. Levin, Friedrich, and Labotsky (29) have noted that polyethylene tubing may adsorb testosterone and androstenedione.

2) The purification of the plasma steroids was extensive in this study, each sample being purified by derivative formation and chromatography until specific activities and $^3\text{H}/^{14}\text{C}$ ratios agreed within 5% on the last two and generally three measurements.

3) In spite of the marked differences in MCR^T among the groups studied, the MCR^A was the same in each group and agreed closely with those reported by Horton and Tait (7). This is good evidence against systematic errors in the method for measuring testosterone metabolic clearance rates.

Therefore, after failing to find methodologic errors, we have accepted as fact that testosterone metabolic clearance rates are higher in men than in women and that they are higher in women with hirsutism or polycystic ovaries than in normal women. The possible reasons for this are of some interest.

Since the MCR is obtained by dividing the rate of infusion of the radioactive steroid by the plasma

radioactivity of that steroid, the MCR will depend on the volume of distribution and the turnover rate. In the simplest case, the one compartment system, $MCR = V\gamma$ where γ is the turnover rate (30). Because of this, to compare the actual clearance of steroids from the plasma, one must correct the MCR by some factor proportional to volume of distribution. We have used the calculated surface area for this correction. Even after this correction, however, the MCR per square meter was higher in men and in the hirsute women than in normal women.

The linear regression of MCR^T per square meter with the $\log P_B^T$ is demonstrated by the fact that the function MCR^T per square meter per $\log P_B^T$ was the same for each group. This correlation suggested the typical dose-response curve characteristic of most biologic assays where the response is linear only when plotted against the logarithm of the dose.

The explanation of this phenomenon can be only speculative at present. There are many studies demonstrating that hepatic enzyme systems capable of metabolizing androgens are altered by testosterone. Recently, the presence of a plasma testosterone-binding protein has been reported (31), and it was suggested that its level was higher in women than in men. This testosterone-binding protein was also observed to increase in pregnancy. Whether the presence of such a binding protein would alter testosterone metabolic clearance rates would depend on the binding affinity and capacity of the protein in relation to the plasma testosterone level. If significant amounts of testosterone were bound, the decrease in metabolic clearance rate would be analogous to the slowed cortisol turnover rates seen in women with increased levels of cortisol-binding globulin. It is of note that the patient with the lowest testosterone metabolic clearance in this study was taking norethynodrel, 5 mg per day, and mestranol, 0.075 mg per day. Our data could thus be interpreted as being consistent with a depression of a testosterone-binding protein by increased testosterone production.

Acknowledgments

We gratefully acknowledge the technical assistance of David Collins and Amel French.

References

1. Forchielli, E., G. Sorcini, M. S. Nightingale, N. Brust, R. I. Dorfman, W. H. Perloff, and G. Jacobson. Testosterone in human plasma. *Analyt. Biochem.* 1963, 5, 416.
2. Hudson, B., J. Coghlan, A. Dulmanis, M. Wintour, and I. Ekkel. The estimation of testosterone in biological fluids. 1. Testosterone in plasma. *Aust. J. exp. Biol. med. Sci.* 1963, 41, 235.
3. Dignam, W. J., R. J. Pion, E. J. Lamb, and H. H. Simmer. Plasma androgens in women. II. Patients with polycystic ovaries and hirsutism. *Acta endocr. (Kbh.)* 1964, 45, 254.
4. Burger, H. G., J. R. Kent, and A. E. Kellie. Determination of testosterone in human peripheral and adrenal venous plasma. *J. clin. Endocr.* 1964, 24, 432.
5. Korenman, S. G., M. A. Kirschner, and M. B. Lipsett. Testosterone production in normal and virilized women and in women with the Stein-Leventhal syndrome or idiopathic hirsutism. *J. clin. Endocr.* 1965, 25, 798.
6. Lloyd, C. W., J. Lobotsky, E. J. Segre, T. Kobayashi, M. L. Taymor, and R. E. Batt. Plasma testosterone and urinary 17-ketosteroids in women with hirsutism and polycystic ovaries. *J. clin. Endocr.* 1966, 26, 314.
7. Horton, R., and J. F. Tait. Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. *J. clin. Invest.* 1966, 45, 301.
8. Peterson, R. S., and C. E. Pierce. Methodology of urinary 17-ketosteroids in *Lipids and the Steroid Hormones in Clinical Medicine*, F. W. Saunders and F. W. Saunders, Jr., Eds. Philadelphia, J. B. Lippincott, 1960, p. 147.
9. Wilson, H., and M. B. Lipsett. Use of periodate oxidation in the clinical analysis of urine corticoids. *Analyt. Biochem.* 1963, 5, 217.
10. Kirschner, M. A., and M. B. Lipsett. The analysis of urinary steroids using gas-liquid chromatography. *Steroids* 1964, 3, 277.
11. Kirschner, M. A., M. B. Lipsett, and D. R. Collins. Plasma ketosteroids and testosterone in man: a study of the pituitary-testicular axis. *J. clin. Invest.* 1965, 44, 657.
12. Bardin, C. W., and M. B. Lipsett. Estimation of testosterone and androstenedione in human peripheral plasma. *Steroids* 1967, 9, 71.
13. Korenman, S. G., and M. B. Lipsett. Is testosterone glucuronoside uniquely derived from plasma testosterone? *J. clin. Invest.* 1964, 43, 2125.
14. Rongone, E. L. Testosterone metabolism by human male mammary skin. *Steroids* 1966, 7, 489.
15. Kennedy, B. J., and I. T. Nathanson. Effect of intensive sex steroid hormone therapy in advanced breast cancer. *J. Amer. med. Ass.* 1953, 152, 1135.

16. Tait, J. F., and R. Horton. Some theoretical considerations on the significance of the discrepancy in urinary and blood production rate estimates of steroid hormones, particularly in those of testosterone in young women. *Steroids* 1964, **4**, 365.
17. Lipsett, M. B., H. Wilson, M. A. Kirschner, S. G. Korenman, L. M. Fishman, G. A. Sarfaty, and C. W. Bardin. Studies on Leydig cell physiology and pathology: secretion and metabolism of testosterone. *Recent Progr. Hormone Res.* 1966, **22**, 245.
18. Mahesh, V. B., and R. B. Greenblatt. Steroid secretions of the normal and polycystic ovary. *Recent Progr. Hormone Res.* 1964, **20**, 341.
19. Jayle, M. F., R. Scholler, P. Mauvais-Jarvis, and S. Méta. Excretion des stéroïdes chez des femmes présentant un virilisme pileux associé à des troubles du cycle menstruel. *Acta Endocr. (Kbh.)* 1961, **36**, 375.
20. Nichols, T., C. A. Nugent, and F. H. Tyler. Glucocorticoid suppression of urinary testosterone excretion in patients with idiopathic hirsutism. *J. clin. Endocr.* 1966, **26**, 79.
21. Wieland, R. G., C. De Courcy, R. P. Levy, A. P. Zala, and H. Hirschmann. $C_{19}O_2$ steroids and some of their precursors in blood from normal human adrenals. *J. clin. Invest.* 1965, **44**, 159.
22. Korenman, S. G., and M. B. Lipsett. Direct peripheral conversion of dehydroepiandrosterone to testosterone glucuronide. *Steroids* 1965, **5**, 509.
23. Tait, J. F., and R. Horton. The *in vivo* estimation of blood production and interconversion rates of androstenedione and testosterone and the calculation of their secretion rates *in* *Steroid Dynamics*, G. Pincus, T. Nakao, and J. F. Tait, Eds. New York, Academic Press, 1966, p. 393.
24. Tait, J. F. Review: the use of isotopic steroids for the measurement of production rates *in vivo*. *J. clin. Endocr.* 1963, **23**, 1285.
25. Tait, J. F., B. Little, S. A. S. Tait, and C. Flood. The metabolic clearance rate of aldosterone in pregnant and nonpregnant subjects estimated by both single-injection and constant-infusion methods. *J. clin. Invest.* 1962, **41**, 2093.
26. Hudson, B., A. Dulmanis, J. P. Coghlan, and M. Wintour. The measurement of androgen production in normal subjects. *Aust. Ann. Med.* 1966, **15**, 236.
27. Southren, A. L., S. Tochimoto, N. C. Carmody, and K. Isurugi. Plasma production rates of testosterone in normal adult men and women and in patients with the syndrome of feminizing testes. *J. clin. Endocr.* 1965, **25**, 1441.
28. Southren, A. L., G. G. Gordon, and S. Tochimoto. Plasma production rates of testosterone in normal men and women; a physiological study using both a single injection and constant infusion technique. *J. clin. Invest.* 1966, **45**, 1075.
29. Levin, J., E. H. Friedrich, and J. Lobotsky. Steroid adsorption with polyethylene tubing. *J. clin. Endocr.* 1965, **25**, 1519.
30. Tait, J. F., and S. Burstein. *In vivo* studies of steroid dynamics in man *in* *The Hormones*, G. Pincus, K. V. Thimann, and E. B. Astwood, Eds. New York, Academic Press, 1964, vol. 5, p. 441.
31. Pearlman, W. H., and O. Crépy. Steroid-protein interaction with particular reference to testosterone-binding by human serum. *J. biol. Chem.* 1967, **242**, 182.