Metabolic Clearance Rate and Blood Production Rate of Testosterone and Dihydrotestosterone in Normal Subjects, during Pregnancy, and in Hyperthyroidism

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ABSTRACT The metabolic clearance rate (MCR) and blood production rate (BP) of testosterone (T) and dihydrotestosterone (DHT), the conversion of plasma testosterone to plasma dihydrotestosterone, and the renal clearance of androstenedione, testosterone, and dihydrotestosterone have been studied in man. In eight normal men, the MCR^T (516±108 [SD] liters/m²/day) was significantly greater than the MCR^{DHT} (391±71 [SD] liters/m²/day). In seven females, the MCR^T (304±53 [SD] liters/m²/day) was also greater than the MCR^{DHT} (209±45 [SD] liters/m²/day) and both values were less than their respective values in men ($P < 0.001$). In men the conversion of testosterone into dihydrotestosterone at 2.8±0.3% (SD) was greater than that found in females, 1.56±0.5% (SD) ($P < 0.001$). In five pregnant females the MCR^T (192±36 [SD] liters/m²/day), the MCR^{DHT} (89±30 [SD] liters/m²/day) and the conversion of testosterone into dihydrotestosterone (0.72±0.15%) (SD) were significantly less than the values found in nonpregnant women. In five females with hyperthyroidism, the MCR for testosterone and dihydrotestosterone were similar to those observed in pregnant females, but the conversion of testosterone into dihydrotestosterone (2.78±1.7%) (SD) was greater, and similar to that found in men. In men the production of dihydrotestosterone was 0.39±0.1 (SD) mg/day, 50% being derived from the transformation of plasma testosterone. In women the production of DHT was 0.05±0.028 (SD) mg/day, only 10% coming from testosterone. During pregnancy, the production of testosterone and dihydrotestosterone are similar to that in normal women. In three patients with testicular feminization syndrome (an adult with hyperthyroidism and two children) these two MCRs were greatly reduced compared to the normal females, but the conversion of testosterone into dihydrotestosterone was in the limits of normal male range

In the normal subjects the renal clearance of androstenedione was greater than that of testosterone and dihydrotestosterone. Less than 20% of the dihydrotestosterone and less than 10% of the androstenedione in the urine is derived from the plasma dihydrotestosterone and androstenedione.

INTRODUCTION

During the past few years increased attention has been focused on dihydrotestosterone (DHT)[1] as the result of the discovery that testosterone (T) is converted mainly into dihydrotestosterone at the site of its target tissues (1–2) and the fact that part of the biological actions of testosterone could be produced by dihydrotestosterone (3). More recently, it has been shown that the plasma concentration of dihydrotestosterone is higher in males than in females, and in both sexes its concentration is far lower than testosterone (4–6). At present our knowledge of production, origin and metabolism of dihydrotestosterone is small.

This paper presents a study of the metabolic clearance rate (MCR) of dihydrotestosterone, the conversion of testosterone into dihydrotestosterone, and the production

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[1] *Trivial names and abbreviations used in this paper:* androstenedione (Δ), 4-androstene-3,17-dione; BP, blood production rate; dehydroepiandrosterone (DHA), 3 β-hydroxy-5-androstene-17-one; dihydrotestosterone (DHT), 5 α-androstan-17 β-ol-3-one; MCR, metabolic clearance rate; TeBG, testosterone-estradiol binding protein; testosterone (T), 17 β-hydroxy-4-androstene-3-one; TLC, thin-layer chromatography.

of dihydrotestosterone in normal subjects and during pregnancy. The majority of these parameters were also measured in five women with hyperthyroidism and in three cases of testicular feminization syndrome. The renal clearances of androstenedione, testosterone, and dihydrotestosterone were measured in normal subjects of both sexes.

METHODS

Subjects

15 normal persons, eight men (subjects 1–8) and seven women (subjects 9–15), aged 22–36 yr were studied. Five women were studied during the first trimester of pregnancy (8–11 wk) on the day of a therapeutic abortion for medico-social reasons (subjects 16–20). Five females with hyperthyroidism (subjects 21–25) were studied before giving any treatment. Three patients with a testicular feminization syndrome were studied. The first (subject 26), was 60 yr old and had developed hyperthyroidism 10 months previously. The other two were children aged 4 and 1 yr; one of them (subject 28) had been studied under basal conditions (first study) and 15 days after treatment with diethylstilbestrol, 2.5 mg/day.

Steroids

Testosterone-4-^{14}C (SA 50 mCi/mmole), dihydrotestosterone-4-^{14}C (SA 50 mCi/mmole), androstenedione-1,2-^{3}H (SA 42 Ci/mmole) (Δ), and dihydrotestosterone-1,2-^{3}H (SA 30 Ci/mmole) were obtained from CEA Saclay, France. All these compounds were purified by paper chromatography using system A (see below) before each infusion. With the exception of the dihydrotestosterone-1,2-^{3}H, the purity was over 98%. The first lot (No. 568-569) of the dihydrotestosterone was more than 98% pure after paper chromatography and was verified by recrystallization. A second lot of this compound (No. 568-270) appeared to be more than 98% pure after chromatography in the system A, as the scanning of the paper after chromatography only demonstrated a single radioactive peak with the same Rf as nonradioactive dihydrotestosterone. However, after studying the MCR in five subjects (three men and two women) and obtaining results far higher than those found previously, the purity of this compound was examined by recrystallization and was found to be only 60%.[2] Subsequently, dihydrotestosterone-1,2-^{3}H was always purified by chromatography in the systems A and B, and the purity verified by recrystallization was over 98%.

Chromatographic systems

The following systems were used:

(A) Paper chromatography: hexane: methanol: water (100: 90: 10).

(B) Alumina thin-layer chromatography (TLC): benzene: ethyl acetate (60: 40).

(C) Silica gel TLC: chloroform: ethanol (98: 2).

(D) Silica gel TLC: benzene: dichloromethane: methanol (150: 150: 6).

(E) Silica gel TLC: hexane: acetone (200: 35).

Gas-liquid chromatography was performed with an F. & M. apparatus model 402 (Hewlett-Packard Co., Avondale Div., Avondale, Pa.) using 2% SE-30 packing (Applied Science Laboratories Inc., State College, Pa.) and an ionization flame detector.

Radioactivity on paper and thin-layer plates was detected by scanning with a windowless flow counter (Packard radiochromatogram scanner, model 7200; Packard Instrument Co., Inc., Downers Grove, Ill.). The radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, model 3003, at a tritium and carbon-14 efficiencies of 38% and 60%, respectively. 16% of the ^{14}C was counted in the tritium channel.

Plasma concentration of nonradioactive testosterone and dihydrotestosterone

The concentration of these steroids was measured by the double isotopic dilution method (6–8).

Infusions. In all subjects but pregnant women the studies were commenced between 8 and 9 a.m. The subject had been lying down for 40–60 min before commencing the test. A blood sample was taken to measure the plasma testosterone and dihydrotestosterone concentration. The priming dose (about a quarter of the total dose of each isotope) was then injected. 20 min later, an i.v. infusion of testosterone-^{14}C and dihydrotestosterone-^{3}H was continued and run at a constant rate for 220 min. 30–40 ml samples of blood were taken at 130, 160, 190, and 220 min after starting the infusion. The subject voided urine 120 min after starting the infusion and once again at the end of the infusion. The urine formed between the 120th and 220th min was collected.

In the pregnant women, the infusion was commenced between 5 and 6 a.m. The duration of the infusion varied between 210 and 310 min. Blood samples were taken at various times after start of infusion: subject 16 at 150, 210, 280, and 310 min; subject 17 at 160, 180, 200, and 210 min; subject 18 at 140, 160, 180, and 210 min; subject 19 at 160, 190, 230, and 260 min; subject 20 at 140, 160, 180, and 210 min.

In three normal males in addition to the infusion of testosterone-^{14}C and dihydrotestosterone-^{3}H another study comprising testosterone-^{14}C and androstenedione-^{3}H was made a few days later. The protocol of this second study was identical to the first.

Analyses of the plasma. 200 μg of testosterone, androstenedione, and dihydrotestosterone were added to each sample of plasma. The plasma was extracted three times with 2 vol of ether. The extracts were then chromatographed in the A system. Testosterone and androstenedione (dihydrotestosterone had the same Rf as androstenedione in this system) were located using a 254 nm ultraviolet light and eluted separately. The dried extracts containing testosterone and the complex androstenedione + dihydrotestosterone were rechromatographed in the B system for 2 hr. In this system, dihydrotestosterone is well separated from androstenedione and etiocholanolone. The testosterone, dihydrotestosterone, and androstenedione spots were eluted separately and the solvent evaporated. The sample containing androstenedione was dissolved in a mixture of pyridine: acetic anhydride (1: 1) and kept at room temperature overnight. The solvent was evaporated and the dry extracts, as well as those of the testosterone and the dihydrotestosterone were chromatographed separately on silical gel TLC in the C system. Each compound was eluted sepa-

[2] A summary of the results obtained with the second lot of impure dihydrotestosterone-1,2-^{3}H has been published (7). The impurity of the dihydrotestosterone explains why the mean MCR^{DHT} was similar to the MCR^{T}. The present paper does not include these false high values.

rately. A portion was used to calculate the yield by gas-liquid chromatography, the remainder used to measure the radioactivity.

Analysis of the urine. After the addition of 200 μg of testosterone, androstenedione, and dihydrotestosterone, the urine was extracted three times with 2 vol of ether. The pooled ether extracts were washed twice with 1/10 of their volume of 0.1 N HCl. The samples were then chromatographed in systems A and B as for the plasma. The dried extracts of testosterone, androstenedione, and dihydrotestosterone were then dissolved in 1 ml of a pyridine: acetic anhydride mixture (1:1) and kept at room temperature overnight. The solvents were evaporated and the dried extracts purified by bidimensional TCL in systems D and E. The testosterone acetate, dihydrotestosterone acetate, and androstenedione were eluted, and after evaporating the solvent, the residue was saponified with 0.5 ml 0.4 M KOH in methanol. Finally the samples were chromatographed in system C. After elution a portion was taken to calculate the yield and the remainder to count the radioactivity.

Measurement of the binding of testosterone and dihydrotestosterone to the plasma proteins

The percentage binding of testosterone and dihydrotestosterone to plasma proteins was measured by equilibrium dialysis, using the method described by Forest, Rivarola, and Migeon (9). The values obtained were not corrected for the 1:5 dilution of the plasma.

Definitions

The conversion ratio is the ratio between the plasma concentration of the radioactive product and the precursor injected, after a constant infusion of the precursor (10, 11). The transfer constant is the percentage of the precursor converted to product in the peripheral blood (10, 11). The conversion ratio of testosterone into androstenedione is represented by Δ-^{14}C/T-^{14}C and that of testosterone into dihydrotestosterone by DHT-^{14}C/T-^{14}C.

The transfer constant of testosterone into dihydrotestosterone is: $(\rho)_{BB}^{T\text{-}DHT} = (MCR^{DHT}/MCR^{T}) \times (DHT\text{-}^{14}C/T\text{-}^{14}C)$.

RESULTS

MCR of testosterone and dihydrotestosterone. In normal subjects equilibrium was reached 120 min after starting the infusion, both for the injected percursors and their metabolites. In pregnancy it appeared that this equilibrium was reached after 140 minutes of continuous infusion. (Table I). However, it is possible that the foetoplacental unit between 8 and 11 wks of pregnancy represents a small compartment in comparison to that of the mother. Thence, even if the foetoplacental unit was not in equilibrium with the maternal compartment, the variations in plasma radioactivity would be very small.

In man the MCR^{T} was greater than the MCR^{DHT} ($P < 0.01$). In normal females, both the MCR^{T} and the MCR^{DHT} are significantly less than the respective values in men ($P < 0.001$), although as in men the MCR^{T} was greater than the MCR^{DHT} ($P < 0.01$). The values for MCR^{T} and MCR^{DHT} in males and MCR^{T} in females are similar to those reported by others (12–14), but the MCR^{DHT} in women was a little higher than that found by Mahoudeau, Bardin, and Lipsett (13).

During pregnancy both the MCR^{T} and MCR^{DHT} were much lower than in normal women (Table I) ($P < 0.01$ and $P < 0.001$, respectively). In hyperthyroid females the MCR^{T} and MCR^{DHT} (Table I) were similar to the values observed in pregnancy and significantly less than in normal women ($P < 0.01$ and $P < 0.001$).

In the patient with testicular feminization syndrome and hyperthyroidism (subject 26) the MCR^{T} and MCR^{DHT} were similar to the women with hyperthyroidism. In the two children with testicular feminization syndrome the MCR^{T} and MCR^{DHT} were both low. However, the MCR^{T} observed in these two children was similar to that found in normal children before puberty (unpublished data). The treatment of one of these patients with diethylstilbestrol for 15 days did not modify the MCRs.

In all the subjects, except one man, the MCR^{T} was greater than the MCR^{DHT}, and in all of them these two clearance rates were significantly correlated (Fig. 1).

Conversion ratios and transfer constant $(\rho)_{BB}^{T\text{-}DHT}$ *(Table II).* In men and in women the mean conversion ratios of testosterone into androstenedione were similar to those of testosterone into dihydrotestosterone. However, the two conversion ratios in men were significantly higher than in women ($P < 0.001$).

During pregnancy the conversion ratio of testosterone into androstenedione tends to increase and that of testosterone into dihydrotestosterone to decrease, but the values were not significantly different from those observed in the normal females. In the women with hyperthyroidism the mean conversion ratios for testosterone into androstenedione were significantly less than in both pregnant and nonpregnant women ($P < 0.01$). On the other hand, the conversion ratio of testosterone into dihydrotestosterone in hyperthyroidism was greater than in the normal, and pregnant women ($P < 0.05$ and $P < 0.01$).

In the three patients with testicular feminization syndrome the conversion ratio of testosterone into androstenedione was low and similar to the hyperthyroid females, although the conversion ratio of testosterone into dihydrotestosterone was in the range for normal males. In patient number 28, treatment with estrogens caused an increase in the conversion ratio of testosterone into androstenedione and a reduction of that for testosterone into dihydrotestosterone.

In the eight normal males average value for $(\rho)_{BB}^{T\text{-}DHT}$ was significantly higher than that found in females ($P < 0.001$). The values reported by Mahoudeau et al. (13) in two normal men (2.9 and 6.6) and in three

TABLE I

Plasma Concentrations of Radioactive Δ, T, and DHT during Constant Infusion of T-^{14}C and DHT-^{3}H

Subject	Surface area	Infusion rate		Mean ±SD of the four samples Δ	T	DHT	MCR^{T}	MCR^{DHT}
		cpm/min		*cpm/ml*	*cpm/ml*	*cpm/ml*	*liter/24 hr per m²*	
16	1.65	^{14}C	58,900	—	272±19	4.6±1.14	188	
		^{3}H	20,960			282±9		64
17	1.61	^{14}C	79,220	6.1±0.63	262±17	5.6±1.71	195	
		^{3}H	25,000			181±21		119
18	1.52	^{14}C	65,700	15±2.15	286±29	3.1±0.27	216	
		^{3}H	50,500			391±22		123
19	1.81	^{14}C	91,500	12.5±1.08	559±38	10±0.74	129	
		^{3}H	96,620			1269±63		60
20	1.51	^{14}C	35,100	4.5±0.18	253±11	2.8±0.46	132	
		^{3}H	120,000			1460±25		79
Mean±SD							172±36	89±30
21	1.53	^{14}C	37,900	2.3±0.25	188±12	—	189	
		^{3}H	49,320			—		
22	1.46	^{14}C	60,650	3.8±0.19	288±13	11.7±1.21	204	
		^{3}H	43,200			514±9		82
23	1.48	^{14}C	91,480	3.6±0.18	541±19	30.2±2.11	164	
		^{3}H	50,100			549±39		89
24	1.50	^{14}C	92,400	3.3±0.20	398±16	43.7±5.12	221	
		^{3}H	26,800			244±19		104
25	1.53	^{14}C	92,800	4.7±0.19	357±10	11.6±0.71	244	
		^{3}H	27,200			243±11		105
Mean±SD							204±30	95±12
26	1.81	^{14}C	75,180	4.7±0.15	364±12	19.8±0.58	163	
		^{3}H	19,070			175±12		87
27	0.68	^{14}C	38,800	7.7±0.26	536±9	16.3±0.26	151	
		^{3}H	33,100			681±22		101
28	0.46							
1st study		^{14}C	32,630	6.2±0.41	473±21	21.6±1.21	212	
		^{3}H	24,000			528±12		140
2nd study		^{14}C	36,260	12.5±0.38	506±14	14±0.51	196	
		^{3}H	23,680			560±9		132
Normal males, mean±SD (8 subjects)							516±108	391±71
Normal females, mean±SD (7 subjects)							304±53	209±45

hirsute females (6.2, 2.0, and 4.2) are greater than our figures. Nevertheless, because of the small number of subjects studied, the dispersion of the values and given that the MCR^{T} and MCR^{DHT} were measured in different experiments, the precise comparison with these authors' data is difficult.

In pregnant women the $(\rho)_{BB}^{T\text{-}DHT}$ is significantly less than in nonpregnant women ($P < 0.01$). In the hyperthyroid females the $(\rho)_{BB}^{T\text{-}DHT}$ values were varied, but the mean was greater than that measured during pregnancy ($P < 0.05$), despite the MCR^{T} and MCR^{DHT} being similar. There appeared to be a relation between the increase of the $(\rho)_{BB}^{T\text{-}DHT}$ and the severity of the hyperthyroidism. The patients (subjects 23 and 24) with very high transfer constants had severe hyperthyroidism (Table I). In the three patients with testicular feminization syndrome the value for the transfer constant was within the limits for normal men. In patient number 28, the treatment with estrogen reduced the transfer constant by 37% despite the unchanged MCR^{T} and MCR^{DHT}.

Transfer constant of androstenedione to dihydrotestosterone. As indicated in the Methods section, three normal men were studied twice. The first test involved an infusion of T-^{14}C and DHT-^{3}H. In the second test Δ-^{3}H (between 250,000 and 290,000 cpm/min) and T-^{14}C were infused constantly for 220 min. The results of these two studies are summarized in Table III. In these

TABLE II

Conversion Ratios for Testosterone to Androstenedione (Δ-^{14}C/T-^{14}C) and to Dihydrotestosterone (Δ-^{14}C/DHT-^{14}C) and Transfer Constant of Testosterone to Dihydrotestosterone ($(\rho)_{BB}^{T\text{-}DHT}$) in Different Physiological and Pathological Conditions

Subject	Δ-^{14}C/T-^{14}C	DHT-^{14}C/T-^{14}C	$(\rho)_{BB}^{T\text{-}DHT}$
			%
Pregnancy			
16	—	0.0169	0.57
17	0.0168	0.0154	0.94
18	0.0524	0.0108	0.61
19	0.0223	0.0178	0.82
20	0.0177	0.0110	0.66
Mean ±SD	0.0273±0.017	0.0144±0.003	0.72±0.15
Hyperthyroidism			
21	0.0122	—	—
22	0.0131	0.0406	1.61
23	0.0066	0.0558	3.01
24	0.0082	0.1097	5.12
25	0.0131	0.0324	1.39
Mean ±SD	0.0106±0.007	0.0596±0.03	2.78±1.7
Testicular feminization			
26	0.0129	0.0543	2.89
27	0.0143	0.0304	2.03
28 (1st study)	0.0131	0.0456	2.96
28 (2nd study)	0.0249	0.0276	1.85
Normal males, mean ±SD (8 subjects)	0.0408±0.011	0.0375±0.018	2.80±0.3
Normal females, mean ±SD (7 subjects)	0.0208±0.006	0.0229±0.007	1.56±0.5

three men the MCR^T and the conversion ratio of testosterone into dihydrotestosterone DHT-^{14}C/T-^{14}C in the two studies were similar. As a consequence we assumed that the other parameters which were calculated in one experiment alone would be similar in the other. The direct transfer constant for androstenedione to dihydrotestosterone ($(\rho)_{BB}^{\Delta DHT} \times 100$ direct) was calculated from the formula (MCR^{DHT}/MCR^{Δ}) × (DHT-^{3}H/Δ-^{3}H), where the MCR^{DHT} was calculated at the time of the first experiment and the MCR^{Δ} and

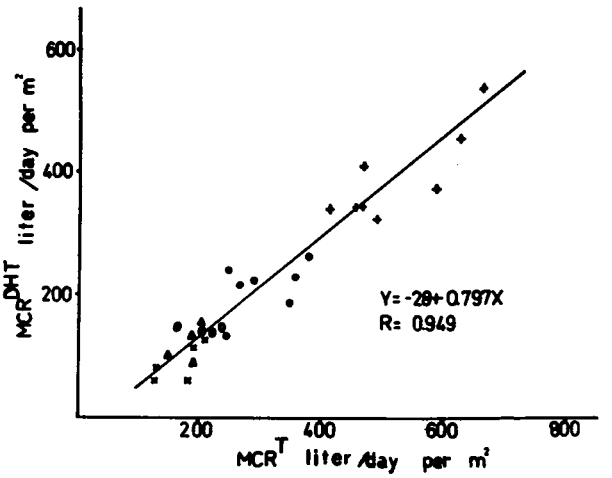

FIGURE 1 Correlation between MCR^T and MCR^{DHT}. + normal males; ● normal females; × pregnancies; ○ hyperthyroidism; ▲ testicular feminization.

DHT-^{3}H/Δ-^{3}H in the second experiment. The indirect transfer constant for androstenedione into dihydrotestosterone ($(\rho)_{BB}^{\Delta\text{-}DHT} \times 100$ indirect) was obtained by multiplying the $(\rho)_{BB}^{\Delta\text{-}T}$ obtained in the second study by the $(\rho)_{BB}^{T\text{-}DHT}$ obtained in the first study. The direct transfer constant in these three subjects was 0.25, 0.39, and 0.32, but the indirect transfer constant was only one-third as large. These results suggest that about 60% of the androstenedione, which is converted into dihydrotestosterone, does not pass through the plasma testosterone pool. Horton and Tait (15) have also described a difference between the direct transfer constant for dehydroepiandrosterone (DHA) into testosterone (0.7%) and the indirect transfer constant $(\rho)_{BB}^{DHA\text{-}A} \times (\rho)_{BB}^{\Delta\text{-}T}$ (0.36%).

Plasma concentration and blood production rates (BP) of testosterone and dihydrotestosterone. The average concentration of testosterone in men is about 12 times greater than dihydrotestosterone; in women

TABLE III

Transfer Constant of Androstenedione to Dihydrotestosterone Calculated from Two Different Studies in the Same Subjects

Subject		MCR^{Δ}	MCR^T	MCR^{DHT}	Δ-^{14}C/T-^{14}C	T-^{3}H/Δ-^{3}H	DHT-^{14}C/T-^{14}C	DHT-^{3}H/Δ-^{3}H	$(\rho)_{BB}^{\Delta\text{-}T}$	$(\rho)_{BB}^{T\text{-}DHT}$	$(\rho)_{BB}^{\Delta\text{-}DHT}$ indirect‡	$(\rho)_{BB}^{\Delta\text{-}DHT}$ direct§
		liter/day per m²							%	%	%	%
1	1st study*		423	342			0.0283			2.30	0.11	0.25
	2nd study	982	462		0.0493	0.1003	0.0257	0.0072	4.73			
3	1st study		657	534	0.0313		0.0298			2.43	0.10	0.39
	2nd study	1199	669		0.0334	0.0829	0.0284	0.0089	4.59			
5	1st study		475	412	0.0342		0.0314			2.73	0.11	0.32
	2nd study	1188	450		0.0282	0.1032	0.0352	0.0093	3.91			

* First study: constant infusion of testosterone-^{14}C and dihydrotestosterone-^{3}H. Second study: constant infusion of testosterone-^{14}C and androstenedione-^{3}H.
‡ $(\rho)_{BB}^{\Delta\text{-}DHT}$ indirect = $(\rho)_{BB}^{\Delta\text{-}T} \times (\rho)_{BB}^{T\text{-}DHT}$.
§ $(\rho)_{BB}^{\Delta\text{-}DHT}$ direct = (MCR^{DHT}/MCR^{Δ}) × (DHT-^{3}H/Δ-^{3}H).

TABLE IV

Plasma Concentrations and Blood Production Rates (BP) of T and DHT and Amount of DHT Coming from T in Pregnancy and Testicular Feminization

Subject	T		DHT		DHT coming from T
	ng/100 ml	*BP, mg/day*	*ng/100 ml*	*BP, mg/day*	*mg/day*
Pregnancy					
16	87	0.27	28	0.029	0.0015
17	88	0.27	38	0.073	0.0025
18	66	0.21	36	0.067	0.0013
19	186	0.43	46	0.050	0.0035
20	84	0.16	37	0.044	0.0011
Mean±SD	102±47	0.27±0.1	37±6	0.052±0.018	0.002±0.001
Testicular feminization					
26	457	1.34	37	0.058	0.038
27	60	0.06	—	—	0.001
28 1st study	32	0.03	8	0.005	0.001
Normal males (8 subjects)					
Mean±SD	709±187	6.48±1.5	56±13	0.39 ±0.10	0.18 ±0.05
Normal females (7 subjects)					
Mean±SD	43±12	0.20±0.08	18±7	0.057±0.028	0.003±0.001

testosterone is only twice the concentration of dihydrotestosterone (Table IV). The concentration of dihydrotestosterone in males is significantly higher than in females ($P < 10^{-7}$). The mean blood production rate of dihydrotestosterone in men was 0.39±0.10 mg/day and in women 0.057±0.028 mg/day, values which are close to those reported by Ito and Horton (12). In men about one-half the dihydrotestosterone production comes from testosterone, but in women only 10% comes from this source.

During the first 3 months of pregnancy the average plasma concentrations of testosterone and dihydrotestosterone are significantly higher than those found in nonpregnant women ($P < 0.01$ and $P < 0.001$). However, the blood production rates of these two steroids in pregnant women are similar to those of normal women (Table IV). As in normal women, less than 10% of the dihydrotestosterone is derived from testosterone during pregnancy.

Renal clearance of radioactive androstenedione, testosterone, and dihydrotestosterone. The values for renal clearance given in Table V are approximate, as the bladder was emptied spontaneously at the 120th min and the 220th min. However, for a given subject, the error in the estimation of the true renal clearance will be similar for the three steroids. On the other hand, this error might explain the variation in the results we obtained.

During the infusion of T-^{14}C and DHT-^{3}H, the renal clearance of androstenedione in men and women was similar (Table V). But the renal clearance of testosterone and dihydrotestosterone (^{14}C and ^{3}H) was greater in males. In both sexes the renal clearance of DHT-^{14}C is several times greater than that of DHT-^{3}H. These results enable it to be estimated that a high proportion (about 95% in males and 80% in females) of the

TABLE V

Renal Clearance (ml/hr) of Δ, T; and DHT at the Steady State

	Constant infusion of T-^{14}C and DHT-^{3}H			
	Δ	T	DHT	
Subject	^{14}C	^{14}C	^{14}C	^{3}H
2 M	360	1.30	3.55	0.75
3 M	515	3.07	47.16	2.03
4 M	—	2.16	26.72	1.08
5 M	385	1.83	28.90	1.48
6 M	316	1.17	—	—
7 M	557	3.81	41.43	2.13
Mean±SD	427±108	2.22±1.34	29.7±17	1.50±0.6
10 F	191	0.75	1.51	0.26
11 F	649	0.95	3.44	0.54
12 F	289	0.79	1.70	0.44
13 F	516	0.80	4.21	0.61
14 F	546	0.98	2.54	0.42
15 F	448	1.54	4.40	1.49
Mean±SD	440±171	0.96±0.3	2.96±1.24	0.63±0.44

	Constant infusion of Δ-^{3}H and T-^{14}C			
	Δ		T	
Subject	^{3}H	^{14}C	^{3}H	^{14}C
1 M	31	378	6.7	2.9
3 M	33	482	7.1	2.2
5 M	30	435	7.8	1.8

urinary dihydrotestosterone is derived from precursors other than the plasma dihydrotestosterone.

During the constant infusion of Δ-^{3}H and T-^{14}C, the renal clearance of Δ-^{3}H was much less than Δ-^{14}C and that of T-^{3}H greater than T-^{14}C. These results enable it to be calculated that only 8% of the urinary androstenedione is immediately derived from the plasma androstenedione, but about 20% of the urinary testosterone comes directly from the plasma testosterone. Furthermore, these results suggest that during the passage of androstenedione and testosterone through the kidney, oxidative pathways are more active than reduction.

Percentage binding of testosterone and dihydrotestosterone. The percentage binding of testosterone to plasma proteins in 30 normal adult men was 93.05 ±1.25% and for dihydrotestosterone 97.43±0.30%; the difference is significant ($P < 0.0001$).[3] The corresponding values in 32 normal women were 95.95 ±1.26% for testosterone and 98.71±0.19% for dihydrotestosterone which differ significantly ($P < 0.001$). The mean percentage binding of testosterone and dihydrotestosterone in men was less than in women ($P < 0.001$ and $P < 0.0001$, respectively). In 34 normal children of both sexes the mean percentage binding of testosterone was 97.08±0.7% and that of dihydrotestosterone 98.96±0.24%, the values found in the boys were similar to those in girls. These values are significantly different from those observed in normal men and women. In the five pregnant women in our study the mean percentage binding of testosterone and dihydrotestosterone were 98.40±0.20 and 99.28±0.19, respectively, which are significantly higher than in normal females ($P < 0.001$ and $P < 0.01$).

In the patient with testicular feminization syndrome, (subject 26) 98.47% of the testosterone and 99.18% of the dihydrotestosterone was bound to protein, values that are within the limits observed in the first trimester of pregnancy. In the child (subject 28) 97.90% of testosterone and 99.01% of dihydrotestosterone were bound. After 15 days treatment with estrogens the percentage binding of testosterone and dihydrotestosterone were 98.58 and 99.38%, respectively.

DISCUSSION

Under physiological conditions the MCR of a steroid depends mainly on its specific binding to the plasma proteins (16). Thus the MCRs of testosterone, estradiol, and cortisol, steroids that are strongly bound to plasma proteins, are lower than the MCRs of androstenedione, estrone, and cortisone which have a weaker binding to plasma proteins (11, 14, 17). The circulating testosterone in the plasma is bound to a specific β-globulin (TeBG, testosterone-estradiol binding protein) (18, 19). The percentage of testosterone bound to plasma proteins is less in men than in women (9, 20); during pregnancy and in hyperthyroidism it is still higher than in nonpregnant women (21, 22). On the other hand, the MCR^{T} is less in women than in men (14) and the values observed in hyperthyroidism and patients treated with estrogens are lower still than in normal women (14, 23, 24, 25). Our results for MCR^{T} in the normal subjects and in women with hyperthyroidism are similar to those previously published (14, 26). During pregnancy the MCR^{T} is also diminished in relation to nonpregnant women.

TeBG has a greater affinity for dihydrotestosterone than for testosterone. Our results showed that the percentage binding of dihydrotestosterone to plasma proteins is greater than that for testosterone and follows the same variations related to different physiological and pathological states. This would lead one to expect that MCR^{DHT} should be less than MCR^{T}. The present results confirm this hypothesis, and furthermore show that the MCR^{DHT} follows the same variations related to sex or different pathological states as the MCR^{T}. It seems that, for these two steroids, there is an inverse relationship between their MCR and the percentage of the steroid bound to proteins.

Our values for Δ-^{14}C/T-^{14}C in males are higher than in females and a little less than those published by Longcope, Kato, and Horton (14) and Gordon, Southren, Tochimoto, Rand, and Olivo (26), but not significantly less. The values for DHT-^{14}C/T-^{14}C in normal subjects published by Ito and Horton (12) showed, as did our results, a significant difference between the values in males 0.09±0.027 and in females 0.051±0.021, but these were about twice as high as our observations. In females with hyperthyroidism the DHT-^{14}C/T-^{14}C ratio is increased, but in pregnancy the opposite was observed. Though in both pregnancy and hyperthyroidism, the MCR^{DHT}/MCR^{T} is similar, the $(\rho)_{BB}^{T\text{-}DHT}$ in hyperthyroidism is significantly greater than in pregnancy. These results show that variations of the $(\rho)_{BB}^{T\text{-}DHT}$ do not always follow the variations of the TeBG level. The thyroid hormones and estrogens have a similar effect on the TeBG (21, 25, 27), but an opposite effect on the 5α-reductase. Thyroid hormones increase the 5α-reductase activity (28); estrogens inhibit this enzyme (29). The results in patient number 28 are in favor of this independent double action of estrogens on the TeBG and 5α-reductase. In this patient the MCR and percentage binding of testosterone and dihydrotestosterone were hardly changed after treatment with estrogen, while the conversion ratio and transfer constant were considerably reduced.

In males the blood production rate of testosterone is

[3] Forest, M. G., and J. Bertrand. 1972. Studies of the protein binding of dihydrotestosterone in human plasma. *Steroids.* **19.**

about 20 times that of dihydrotestosterone and approximately one-half the latter is derived from the peripheral conversion of testosterone. In females the blood production rate of dihydrotestosterone is only one-fourth as large as that of testosterone but less than 10% of the plasma dihydrotestosterone is derived from the plasma testosterone. These results are not in agreement with those reported by Mahoudeau et al. (13). According to these authors nearly all the dihydrotestosterone in men comes from the conversion of testosterone. However, as was pointed out in the Results section these authors only measured $(\rho)_{BB}^{T\text{-}DHT}$ in two men and obtained widely differing values.

From our values of the direct $(\rho)_{BB}^{\Delta\text{-}DHT}$ in males (Table III) the contribution of plasma androstenedione (30) to the production of dihydrotestosterone would be about 10 μg/day (3.000×0.032). If it is accepted that in females the direct $(\rho)_{BB}^{\Delta\text{-}DHT}$ is similar to that in males, the contribution of androstenedione to the production of dihydrotestosterone would also be 10 μg/day which is about 20% of the total production. Here again our results are in disagreement with those of Mahoudeau et al. (13) who concluded that the contribution of plasma androstenedione to the production of dihydrotestosterone would be 60 μg/day, which is the total production. These authors did not measure the $(\rho)_{BB}^{\Delta\text{-}DHT}$ in normal females, but only in three males (2.6 ± 0.9) and three hirsute females (2.2 ± 0.6) from which they extrapolated the results for normal females. Their high values are surprising as the conversion of androstenedione into testosterone is 2.2 ± 0.5% according to Longcope et al. (14) and 3.3 ± 0.3% according to Bardin and Lipsett (31). If one takes this latter value and the mean $(\rho)_{BB}^{T\text{-}DHT}$ given by Mahoudeau et al. (13) as 4%, the indirect $(\rho)_{BB}^{\Delta\text{-}DHT}$ $(\rho)_{BB}^{\Delta\text{-}T} \times (\rho)_{BB}^{T\text{-}DHT} = 4\% \times 3.3\% = 0.13\%$ which is 15 times lower than the direct $(\rho)_{BB}^{\Delta\text{-}DHT}$ given by these authors. These results suggest that about 93% of the plasma androstenedione which is converted into dihydrotestosterone does not pass through the plasma testosterone pool; according to our results this figure would be about 60%.[4]

[4] During the purification of radioactive dihydrotestosterone in the plasma, we had considerable difficulty in separating it from etiocholanolone and isoandrosterone. The Rfs of these three steroids are very close in systems A and C and only in system B did we get a good separation. The Bush A2 and the Mahoudeau et al. I system (13) are very close to our A and C systems. It is possible that in these systems dihydrotestosterone was inadequately separated from etiocholanolone and isoandrosterone. The acetates of dihydrotestosterone, etiocholanolone, and isoandrosterone are more difficult to separate than the free compounds. A contamination of dihydrotestosterone with other metabolites of testosterone and androstenedione could partially explain the similar MCR^{DHT} values found by Mahoudeau and ourselves and the discordance in the values for the $(\rho)_{BB}^{T\text{-}DHT}$ and $(\rho)_{BB}^{\Delta\text{-}DHT}$.

Studies made in vivo and in vitro suggest that the conversion of testosterone into dihydrotestosterone by the target organs is reduced in the testicular feminization syndrome (32–35). In the three patients with this syndrome the over-all conversion of testosterone into dihydrotestosterone was within the limits for males. However, our study does not eliminate the possibility that in certain compartments the transformation of testosterone into dihydrotestosterone might be reduced, provided that these compartments are very small in relation to the whole body. It seems unlikely that a deficiency of 5α-reductase is responsible for the insensitivity to androgens found in patients with testicular feminization syndrome, for Strickland and French (36) and Rosenfield, Lawrence, Liao, and Landau (35) have shown there is also an insensitivity to dihydrotestosterone.

In normal persons estrogens increase the testosterone binding levels and decrease the 5α-reductase activity, while androgens have the opposite effect (20). Patients with the testicular feminization syndrome can be considered as having an excess of estrogens, even though their production rate is normal, due to the absence of the antagonistic effect of androgens. As a consequence one can consider that the two main perturbations of metabolism that have been described in adults with the testicular feminization syndrome (increased testosterone binding levels and reduction of 5α-reductase activity) will be rather the consequence than the cause of the insensitivity to androgens.

ADDENDUM

After the submission of our article for publication Ito and Horton (37) published in detail their results on the metabolism of DHT in normal subjects (five males, four females). The MCR^{DHT}, production of plasma DHT in both sexes, and the $(\rho)_{BB}^{T\text{-}DHT}$ in females are similar to our values, but the $(\rho)_{BB}^{T\text{-}DHT}$ in males was greater (3.9 ± 1% compared with 2.8 ± 0.3%). Their values for the transfer constant of Δ into DHT in three females were 8, 15.7, and 16.3%. However, we recalculated their data and found the mean value was 10 times less than their calculation (1.33% instead of 13.3%). This is still 3–4 times greater than we found in normal males. This discord could be due to $(\rho)_{BB}^{\Delta\text{-}DHT}$ differences in males and females, or because the DHT isolated during the perfusion of Δ was not pure.

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