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**Research Article**

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# 3 $\alpha$ -Androstanediol Kinetics in Man

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**ABSTRACT** Kinetics of 5 $\alpha$ -androstane-3 $\alpha$ , 17 $\beta$ -diol (3 $\alpha$ -diol) were studied in man. Clearance rates were determined by both the constant infusion and single injection techniques. Production rates were calculated as the product of clearance rate data and plasma values in the a.m. obtained by a radioimmunoassay specific for 3 $\alpha$ -diol. Mean metabolic clearance rates were 1,776 $\pm$ 492 (SD) liters/day in males and 1,297 $\pm$ 219 (SD) liters/day in females. Metabolic clearance rates by single injection were similar. Calculated production rates are 208 $\pm$ 26 (SD)  $\mu$ g/day in males and 35 $\pm$ 11  $\mu$ g/day in females, which are significantly different. Hepatic extraction of 3 $\alpha$ -diol determined by hepatic vein catheterization during constant infusion was 76% which was greater than expected from information on in vitro binding in plasma. The kinetic data is of interest since 3 $\alpha$ -diol has a calculated inner pool ( $V_i$ ) volume of 12-14 liters, similar to 17 $\beta$ -hydroxyandrost-4-en-3-one (testosterone) and 5 $\alpha$ -androstan-17 $\beta$ -ol-3-one (dihydrotestosterone), but the calculated outer pool ( $V_o$ ) of 33.5 liters is very large as are the metabolic rate and transfer constants. In contrast to testosterone and dihydrotestosterone, 3 $\alpha$ -diol, although bound to sex hormone binding globulin, has a high metabolic clearance of which a large fraction represents extrahepatic (splanchnic) metabolism. A production rate of 3 $\alpha$ -diol similar to dihydrotestosterone together with rather unique kinetic characteristics encourages further investigation of the biological role of this potent androgen.

## INTRODUCTION

Recent studies in vivo of androgens and estrogens indicate that many of these steroid hormones are interconvertible. A number of potent androgens and estrogens in blood are derived in part or entirely by peripheral conversion of secreted precursors from the adrenal cortex or gonad (1-5). The term "prehormone has

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been used to characterize this phenomenon (6). Blood testosterone<sup>1</sup> in the female and prepubertal male is derived from secreted andros-4-ene-3,17-dione (androstenedione). Additionally, there is now evidence that testosterone, although the major circulating androgen, is converted peripherally by target tissue and perhaps other sites to 5 $\alpha$ -androstan-17 $\beta$ -ol-3-one (dihydrotestosterone, DHT) which appears to be an important steroid messenger in sexual target tissue (7-10).

Are there other testosterone conversion products with important target tissue actions? One potential conversion product is 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ -androstanediol, 3 $\alpha$ -diol) which has approximately equal potency as an androgen as testosterone (11-13).

This report describes a kinetic in vivo study of this steroid. Blood concentrations, clearance rates, and production rates were determined and calculations were made suggesting its origin and clearance by splanchnic tissue. The study concludes that this steroid androgen has rather unique characteristics in terms of an unusually high extrasplanchnic metabolism.

## METHODS

*Solvents.* All solvents were spectrograde and purchased from Matheson, Coleman, and Bell, Cincinnati, Ohio.

*Radioactive steroids.* [1,2-<sup>3</sup>H]DHT (sp act: 44 Ci/mmol) and [4-<sup>14</sup>C]DHT (sp act: 56.1 mCi/mmol) were obtained from New England Nuclear (Boston, Mass.) and were further purified in the Bush B<sub>3</sub> paper system (petroleum ether, benzene, methanol, water, 33:17:40,10) as previously described (14). [1,2-<sup>3</sup>H]3 $\alpha$ -diol was made by reducing [1,2-<sup>3</sup>H]3 $\alpha$ -hydroxy-5 $\alpha$ -androstan-17-one (androst-17-one) (sp act: 53 Ci/mmol, New England Nuclear) with 17 $\beta$ -hydroxysteroid dehydrogenase, also as previously described, which was then purified with the Bush A (petroleum ether, methanol, water, 5:4:1) and sequentially by the Bush B<sub>3</sub> paper systems (15). [<sup>14</sup>C]3 $\alpha$ -diol was prepared by sodium borohydride reduction of [4-<sup>14</sup>C]DHT. Approx-

<sup>1</sup>*Nomenclature and abbreviations for the steroids used in this study:* 3 $\alpha$ -androstanediol (3 $\alpha$ -diol), 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol; 3 $\beta$ -androstanediol (3 $\beta$ -diol), 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol; androstenedione, androst-4-ene-3,17-dione; androst-17-one, 3 $\alpha$ -hydroxy-5 $\alpha$ -androstan-17-one; dihydrotestosterone (DHT), 5 $\alpha$ -androstan-17 $\beta$ -ol-3-one; testosterone (T), 17 $\beta$ -hydroxyandrost-4-en-3-one.

mately 3  $\mu\text{Ci}$  of  $[4\text{-}^{14}\text{C}]\text{DHT}$  was taken into a round bottom flask, and taken to dryness. After 0.1 ml of 0.1%  $\text{NaBH}_4$  (Matheson, Coleman, and Bell) methanol solution was added to the flask, the reaction was allowed to proceed for 10 min at room temperature. 1 ml of water was then added to stop the reaction, and the reaction mixture was extracted twice with 10 ml of dichloromethane. The extract was then washed twice with 5 ml of water and taken to dryness.  $[^{14}\text{C}]3\alpha\text{-diol}$  was separated from  $[4\text{-}^{14}\text{C}]\text{DHT}$  by using the Bush B<sub>3</sub> (for 3 h) and the Bush A (for 18 h) systems. The yield of  $[^{14}\text{C}]3\alpha\text{-diol}$  was approximately 10%. DHT, 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ -diol), and testosterone are clearly separated from 3 $\alpha$ -diol by these paper systems (16).

The radiochemical purity of the  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  and  $[^{14}\text{C}]3\alpha\text{-diol}$  was confirmed as follows: Aliquots of both substances were mixed and counted ( $^3\text{H}/^{14}\text{C}$  ratio: 4.24) and then one-half of the remaining mixture was chromatographed on the Bush A system for 18 h ( $^3\text{H}/^{14}\text{C}$  ratio: 4.13). The other half was acetylated and run in the Bush B<sub>3</sub> system for 3 h ( $^3\text{H}/^{14}\text{C}$  ratio: 4.28).

**Constant infusion method.** Normal volunteers, who had given informed consent, (five men and four women) were infused with  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  in order to measure metabolic clearance rates by the constant infusion technique as previously described (3, 16). Patients were kept supine for 1 h and blood samples were drawn for measurement of plasma 3 $\alpha$ -diol concentrations. A single priming dose of 0.3–0.6  $\mu\text{Ci}$  of  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  in saline (5 ml) was injected intravenously at about 8:30 a.m. followed immediately by an infusion of the tracer at a constant rate of 0.025–0.05  $\mu\text{Ci}$  per min (0.382 ml/min) using a constant infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) over 2 h. The blood samples were obtained at 105 and 120 min except T. K. and L. A., whose blood samples were taken at 90, 105, and 120 min. In two additional studies a more prolonged infusion for 4 h was performed as further proof that a steady state was achieved for this steroid by constant infusion. The plasma was separated by centrifugation and then frozen immediately at  $-20^\circ\text{C}$  until the measurements were done.

**Single injection study.** Kinetic studies of both  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  and  $[1,2\text{-}^3\text{H}]\text{DHT}$  for comparison were performed in two male adult volunteers. After a rapid intravenous injection of approximately 10  $\mu\text{Ci}$  of  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  or 6  $\mu\text{Ci}$  of  $[1,2\text{-}^3\text{H}]\text{DHT}$ , 5–20-ml blood samples were obtained from another antecubital vein serially at 5, 10, 20, 30, 40, 60, and 90 min. The plasma was separated and frozen until the measurement was done. After quantitation of each isotope and correction of losses as will be described later, the radioactivities expressed as the percent of injected dose per liter plasma were plotted on a semilogarithmic graph against time following injection.

A conversion ratio (CR)<sup>2</sup> in blood is ordinarily calculated as the ratio of counts per minute  $^3\text{H}$  per liter, corrected for recovery, of product to precursor during constant infusion of labeled precursor. When calculated from single injection data (at 60 min) it must be regarded as only an approximation to steady-state approaches.

**Measurement of hepatic extraction.** Hepatic extraction of 3 $\alpha$ -diol was estimated by direct analysis of the peripheral and hepatic venous blood during a continuous infusion of

labeled steroid in three male patients (volunteers who had given informed consent) undergoing right sided cardiac catheterization via the antecubital vein. The catheterization of these patients was scheduled in order to investigate undiagnosed heart murmurs or cardiac sounds of unknown etiology. Neither patient had any clinical or laboratory evidence of heart failure or liver disease.

A constant infusion of  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  was administered by the same constant infusion technique described earlier. During infusion (20  $\mu\text{Ci}/2$  h) a catheter was inserted into each antecubital vein. One of the catheters was brought up into the hepatic vein where blood samples were collected. The other catheter was used for collecting peripheral blood samples. The hepatic and peripheral blood samples were taken at the same time at 90 and 105 min after the priming injection (2  $\mu\text{Ci}$ ). The actual cardiac study was performed after the hepatic vein sampling. Hepatic extraction (HE) was determined using the Fick principle and determined as follows:

$$\text{HE} = \frac{\begin{array}{c} ^3\text{H cpm/liter} - ^3\text{H cpm/liter} \\ \text{peripheral} \quad \text{hepatic} \end{array}}{\begin{array}{c} ^3\text{H cpm/liter} \\ \text{peripheral} \end{array}} \times 100$$

**Plasma radioactivity measurement.** 10–20 ml of plasma were used to measure  $^3\text{H}$  concentrations in the constant infusion and hepatic extraction studies, and 2–10 ml of plasma were used for the single injection studies.

The plasma samples were mixed with approximately 120 cpm (200 dpm) of  $[^{14}\text{C}]3\alpha\text{-diol}$  or  $[4\text{-}^{14}\text{C}]\text{DHT}$  indicators, and extracted twice with three vol of dichloromethane after alkalization with 0.4–0.6 ml of 4 N NaOH. The extract was washed twice with 5–10 ml of water, and then taken to dryness under vacuum.

In order to purify  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$ , the Bush A system was used for 18 h, and for purification of  $[1,2\text{-}^3\text{H}]\text{DHT}$  the Bush B<sub>3</sub> system was then used for 2 h. The samples corresponding to  $\pm 1.5$  cm from the center of the parallel standards were cut out and eluted into counting vials with 10 ml of methanol by using the syringe technique. The dried samples in scintillation fluid were then counted in a Mark I scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.). The characteristics for counting  $^3\text{H}$  and  $^{14}\text{C}$  were as described previously (3).

**Evidence for radiochemical purity of  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  isolated from plasma during infusion.** 20 ml of pooled plasma was extracted during an infusion after addition of  $[^{14}\text{C}]3\alpha\text{-diol}$  indicator, and chromatographed on the Bush A paper system for 18 h. After purification, two-thirds of the sample was acetylated and run again in the Bush A system for 1½ h. The calculated  $^3\text{H}/^{14}\text{C}$  ratios before and after acetylation were 2.54 and 2.64, respectively.

**Evidence that the steady state in plasma was attained during the constant infusion.** Analysis of radioactivity as the purified  $[1,2\text{-}^3\text{H}]3\alpha\text{-diol}$  which was corrected for recovery from the two plasma specimens obtained at 105 and 120 min after the priming dose infusion did not indicate a trend in values. When the value at 120 min was taken as comparison, the mean value at 105 min was  $98 \pm 1.2$  (SE)%. This shows no significant trend in values and that equilibrium was attained. Additionally, a 4-h infusion was performed in both a male and female subject. The data over this longer period with multiple sampling from 105 to 240 min show no trend in values (Table I). Calculation from

<sup>2</sup> Other abbreviations used in this paper: CR, conversion ratio; MCR, metabolic clearance rate; PR, blood production rate; TBG, sex hormone-binding globulin.

TABLE I  
Metabolic Clearance of 3 $\alpha$ -Diol during Prolonged Infusions

Sub- ject	Sex	Infusion rate	Plasma [1,2- <sup>3</sup> H]3 $\alpha$ -diol				Mean	MCR
			105 min	120 min	180 min	240 min		
		<i>cpm/day</i>						<i>liters/day</i>
R. Z.	Male	$3.97 \times 10^7$	25,000	26,700	27,200	25,900	26,200	1,515
B. H.	Female	$4.80 \times 10^7$	39,500	41,700	39,000	41,200	40,400	1,188

the single injection data predicts that a constant level should be reached by 80 min.

**Estimation of plasma 3 $\alpha$ -diol.** Plasma 3 $\alpha$ -diol concentration was measured by using a specific and sensitive radioimmunoassay developed in our laboratory. This technique involves extraction of 3 $\alpha$ -diol with labeled indicator, purification by paper chromatography (Bush A), location by direct scanning of the indicator, and immunoassay using a relatively specific antibody (15).

**Calculation of metabolic clearance and blood production rates of 3 $\alpha$ -diol by constant infusion method.** Metabolic clearance rates (MCR) were determined as previously described (3). Blood production rate (PR) was calculated individually as the product of MCR and a.m. plasma 3 $\alpha$ -diol concentration.

**Analysis of plasma disappearance curves of radioactive steroids.** When the radioactivities in plasma after purification were plotted on a semilogarithmic graph as percent dose per liter of plasma against time, the plasma disappearance curve of [1,2-<sup>3</sup>H]3 $\alpha$ -diol or [1,2-<sup>3</sup>H]DHT was obtained. The disappearance curve could be expressed as the sum of two exponentials by the following general equation:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where  $C(t)$  is plasma radioactivity expressed as the percent of injected dose per liter at time  $t$ ,  $A$  and  $B$  are the  $y$ -

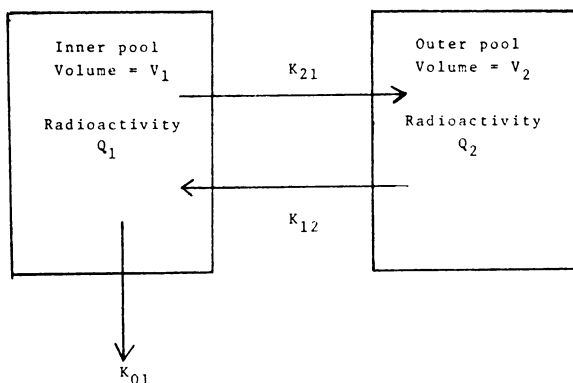


FIGURE 1 Scheme for a two-compartment model applicable to androstenediol dynamics in man. The inner pool (rapidly exchanging pool) is assumed to contain the liver. Metabolism is also assumed to occur in the inner pool and an assumption is made in calculations that steroid concentrations are equal throughout the model. The  $K$  values ( $K_{21}$ ,  $K_{12}$ ,  $K_{01}$ ) are fractional rate constants. The symbol  $Q$  is the total radioactivity in the corresponding compartment.

intercepts of the straight line of the fast and slow components which were made by the usual peeling-off process, and  $\alpha$  and  $\beta$  are their slopes. The metabolic clearance rate was then calculated by determining the area under the disappearance curve.

$$MCR = \frac{\alpha\beta}{A\beta + B\alpha} \quad (2)$$

This type of disappearance curve can be analyzed on the basis of a working two-compartment model if metabolism occurs only in the inner pool as previously proposed by Tait et al. (17, 18). Other models may be as representative but we have chosen the one favored by most of those studying steroid kinetics in vivo (Fig. 1).

The inner pool volume ( $V_1$ ), which is in rapid equilibrium with plasma, is determined by  $1/(A+B)$ . The apparent volume of the outer pool ( $V_2$ ) can be estimated by assuming that the steroid concentrations are the same in both pools in the steady-state situation with the following equations:

$$V_2 = \frac{AB(\alpha - \beta)^2}{(A\beta + B\alpha)^2} V_1 \quad (3)$$

where the model assumes production only in the inner compartment ( $V_1$ ) and

$$V_2 = \frac{A\alpha + B\beta}{A\beta + B\alpha} V_1 \quad (4)$$

in an alternate model where steroid production occurs in the outer compartment ( $V_2$ ).

## RESULTS

**Metabolic clearance rate.** The metabolic clearance rates of 3 $\alpha$ -diol by the constant infusion method were  $1,776 \pm 492$  (SD) liters/day in five normal males and  $1,297 \pm 219$  liters/day in four normal females (Table II). The MCR data from the prolonged infusions are not significantly different from the data obtained from the standard infusions. When corrected for surface area the values are  $914 \pm 148$  liters/day/m<sup>2</sup> in males and  $729 \pm 57$  liters/day/m<sup>2</sup> in females. The MCRs calculated from the single injection study in two normal men were 1,860 and 2,140 liters/day, which was also not significantly different from values obtained by the constant infusion method. The MCR of DHT in the same subjects was 503 and 652 liters/day, which cor-

TABLE II  
Metabolic Clearance and Blood Production Rates of 3 $\alpha$ -Diol in Normal Adults

Sub- ject	Sex	Infused [1,2- <sup>3</sup> H]3 $\alpha$ -diol	Plasma [1,2- <sup>3</sup> H]3 $\alpha$ -diol				MCR	Plasma 3 $\alpha$ -diol concentration	Blood production rate	
			90 min	105 min	120 min	Mean				
		<i>cpm/day</i>		<i>cpm/liter</i>			<i>liter/day</i>	<i>liter/day/m<sup>2</sup></i>	<i>ng/liter</i>	<i><math>\mu</math>g/day</i>
L.	M	4.59 $\times$ 10 <sup>7</sup>		17,500	19,660	18,580	2,470	1,038	91	225
K.	M	4.00 $\times$ 10 <sup>7</sup>	29,300	29,650	32,500	30,500	1,300	793	125	163
G.	M	4.46 $\times$ 10 <sup>7</sup>		27,300	25,600	26,450	1,690	862	123	208
Go.	M	4.19 $\times$ 10 <sup>7</sup>		21,500	19,100	20,300	2,060	1,102	108	222
Le.	M	4.44 $\times$ 10 <sup>7</sup>		31,200	33,990	32,550	1,360	773	163	222
Mean							1,776 $\pm$ 492 (SD)	914 $\pm$ 148		208 $\pm$ 26
Lu.	F	3.91 $\times$ 10 <sup>7</sup>		36,680	36,920	36,800	1,050	645	19	20
B.	F	3.03 $\times$ 10 <sup>7</sup>		21,000	22,000	21,500	1,400	760	32	45
La.	F	3.62 $\times$ 10 <sup>7</sup>	24,200	22,600	23,300	23,300	1,550	770	25	39
H.	F	4.80 $\times$ 10 <sup>7</sup>		39,500	41,700	40,600	1,180	740	31	36
Mean							1,297 $\pm$ 219 (SD)	729 $\pm$ 57 (SD)		35 $\pm$ 11 (SD)

responds to our normal values determined by the constant infusion technique as reported previously (16).

The metabolic clearance rates of 3 $\alpha$ -diol obtained in the three patients used for the hepatic clearance study were 1,990, 1,810, and 1,550 liters/day which is not significantly different from control values.

*Plasma 3 $\alpha$ -diol value.* Plasma 3 $\alpha$ -diol concentrations in the control period were 12.2 $\pm$ 2.2 (SD) ng/100 ml in five males and 2.7 $\pm$ 0.6 ng/100 ml in four females who formed the clearance rate subjects. These values are indistinguishable from normal values reported by us from a much larger group of normal individuals, males 13.7 $\pm$ 4, females 2.0 $\pm$ 0.6 ng/100 ml (15).

*Production rate in blood.* The blood production rates of 3 $\alpha$ -diol calculated in each individual as the product of the MCR and a.m. plasma concentration were 208  $\pm$  26 (SD)  $\mu$ g/day in five normal males and 35 $\pm$ 11 (SD)  $\mu$ g/day in four females (Table II).

*Analysis of kinetic data from single injections of [1,2-<sup>3</sup>H]3 $\alpha$ -diol, DHT, and testosterone.* Various kinetic parameters obtained from the single injection studies are shown in Table IV and Fig. 2. Those data are also graphically illustrated for purposes of interpretation along with previously reported data obtained by us for testosterone (19) in Fig. 3. The data reveal a unique aspect of the metabolism of 3 $\alpha$ -diol. The volume of the inner pool (slower exchangeable pool) V<sub>1</sub> of 3 $\alpha$ -diol is similar to those of testosterone (11.8) and DHT (8.5 liters). The volume of the outer pool (faster exchangeable pool) V<sub>2</sub> is 33.5 liters (70 liters if calculated by Eq. 4), if equal concentration of steroid is assumed throughout. This is in marked contrast to V<sub>2</sub> calculated for DHT or testosterone which is 8 and 12.7 liters, respectively.

The metabolic rate constant (K<sub>21</sub>) and transfer rate

constant (K<sub>21</sub>) are in great contrast to values calculated for DHT and testosterone (Fig. 3).

*Hepatic (splanchnic) and extrahepatic clearance of 3 $\alpha$ -diol.* The hepatic extraction of 3 $\alpha$ -diol determined directly by hepatic vein catheterization was 76.0% in three subjects during continuous infusion of 3 $\alpha$ -diol (Table III).

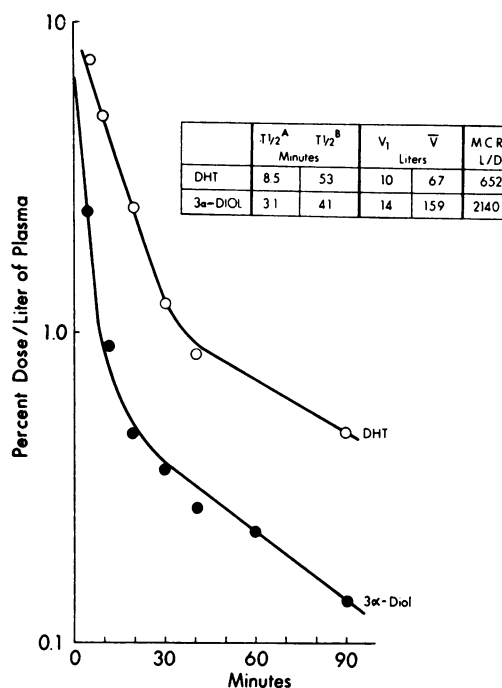


FIGURE 2 Disappearance curves of [1,2-<sup>3</sup>H]DHT and 3 $\alpha$ -diol. Kinetic data on disappearance times, volumes of distribution, and the clearance rates are calculated from the two exponentials which describe the disappearance curves in subject H.

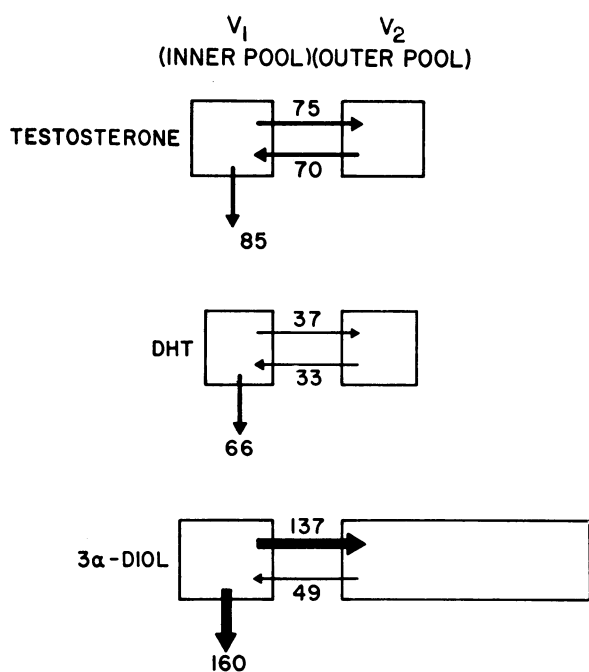


FIGURE 3 Two-compartment models with rate constants calculated from single injection disappearance curves in blood of testosterone (19), DHT, and 3 $\alpha$ -diol in subject H.

Data on the hepatic extraction (HE) allow calculation of hepatic clearance (HC), if one assumes normal hepatic plasma flow (HPF) is 715 liters/day/m<sup>2</sup> plasma and whole blood clearance is 1,300 liters/day/m<sup>2</sup> (20).

$$HC = HE \times HBF$$

$$MCR = HC + \text{extrahepatic clearance}$$

The calculated plasma hepatic clearance of 3 $\alpha$ -diol is 540 (980 whole blood) liters/day/m<sup>2</sup> in three men. Therefore the extrahepatic (splanchnic) clearance of 3 $\alpha$ -diol is 374 liters/day/m<sup>2</sup> plasma or 730 liters/day whole blood. Extrahepatic (splanchnic) clearance is therefore 41% of the overall MCR. Calculated hepatic and extrahepatic clearance of 3 $\alpha$ -diol is illustrated in

comparison with similar previous calculations made for testosterone and DHT (16, 20) in Fig. 4.

## DISCUSSION

The metabolic clearance rate obtained by the constant infusion technique for 3 $\alpha$ -diol was 1,776 $\pm$ 492 liters/day in men and 1,297 $\pm$ 219 in women. The metabolic clearance of 3 $\alpha$ -diol obtained by single injection was in good agreement with the constant infusion data. The only available information on this in the literature is the report by Bird, Choong, Knight, and Clark (21).

Blood production rates of 3 $\alpha$ -diol were calculated as the product of metabolic clearance and plasma concentration in the supine position. The calculated production rates are clearly arbitrary since any possible circadian variation or short-term fluctuations are ignored in our calculation. A single study of 3 $\alpha$ -diol levels throughout the day in a male subject did not reveal any major long-term changes (unpublished data). Our calculations appear justified for comparative purposes since testosterone and DHT production rates have thus far been calculated based upon short-term sampling. With these limitations in mind, the calculated blood production rates of 3 $\alpha$ -diol in men are similar to production rates of DHT, although female production rates are only about one-half that of DHT (16).

Previous studies have indicated that blood DHT in men is derived primarily from the peripheral conversion of testosterone (16). Mahoudeau, Bardin, and Lipsett have also published conversion data for testosterone to 3 $\alpha$ -diol (22). Their data only allowed calculation of the fraction of plasma 3 $\alpha$ -diol arising in testosterone (conversion ratio) since clearance rates of 3 $\alpha$ -diol were not available. The conversion ratio of testosterone (T) to 3 $\alpha$ -diol ( $CR_B^{T-3\alpha}$ ) was 0.013 and that of DHT to 3 $\alpha$ -diol ( $CR_B^{DHT-3\alpha}$ ) was 0.153. In agreement with their data our  $CR_B^{DHT-3\alpha}$  calculated during single injection of [1,2-<sup>3</sup>H]DHT is also 15% at 1 h. The more important conversion rate constant is the transfer constant ( $\rho$ ) value defined as the fraction of a precursor converted to a product per unit time. This can be cal-

TABLE III  
Hepatic Extraction and Metabolic Clearance Rate of 3 $\alpha$ -Diol

Subject	Sex	Infused [1,2- <sup>3</sup> H]3 $\alpha$ -diol <i>cpm/day</i>	Plasma [1,2- <sup>3</sup> H]3 $\alpha$ -diol						Hepatic extraction %	MCR <i>liter/day</i>
			Peripheral vein			Hepatic vein				
			90 min	105 min	Mean	90 min	105 min	Mean		
M.	M	3.23 $\times$ 10 <sup>8</sup>	157,000	168,000	162,500	41,000	30,300	35,600	78.4	1,990
Ba.	M	3.26 $\times$ 10 <sup>8</sup>	174,000	186,700	180,000	41,000	39,000	40,000	77.8	1,810
Z.	M	3.97 $\times$ 10 <sup>7</sup>	26,200	25,000	25,600	6,800	7,600	7,200	72	1,550

culated as:

$$[\rho]^{Pre-Prod} = \frac{MCR^{Prod}}{MCR^{Pre}} \times CR^{Pre-Prod}$$

Thus, with our data on metabolic clearance rates, the  $[\rho]^{T-3\alpha}$  and  $[\rho]^{DHT-3\alpha}$  can be calculated. Calculated values are  $[\rho]^{T-3\alpha} = 0.023$  in men and 0.006 in women while  $[\rho]^{DHT-3\alpha}$  values are 0.43 and 0.22 for men and women, respectively.

The fraction of a product derived from precursor can also be calculated simply as:

Percent product from precursor

$$= \frac{PR^{Pre} \times [\rho]^{Pre-Prod}}{PR^{Prod}} \times 100$$

These calculations suggest that over 70% of blood  $3\alpha$ -diol is therefore derived from testosterone conversion and about 75% of this is derived via DHT. In contrast in females, the calculations suggest that about one-half of blood  $3\alpha$ -diol is from DHT while the contribution to  $3\alpha$ -diol of testosterone is less than 10%. Since androstenedione is a major source in women of DHT it may be a significant prehormone also for  $3\alpha$ -diol.

For many steroids there is a good relationship between steroid-protein binding in blood, hepatic extraction, and metabolic clearance rate. This is particularly true for steroids such as aldosterone and cortisol where extrahepatic metabolism is minimal. However, even with testosterone and DHT the ratio  $MCR^T/MCR^{DHT}$  is similar to the ratio of binding affinities with the sex hormone-binding globulin (TBG) and relative biological activity (11-13). Both the relative biological activity and the binding activity or affinity (23-26) of  $3\alpha$  diol and testosterone are approximately unity.

A recent report suggests significant plasma albumin binding of  $3\alpha$ -diol (27). However, other studies support  $3\alpha$ -diol binding to TBG in human plasma in the presence or absence of albumin.

We were therefore surprised to obtain clearance rates for  $3\alpha$ -diol which are approximately twice that of

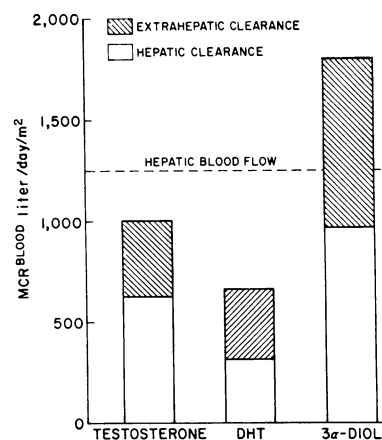


FIGURE 4 Mean metabolic clearance rates of whole blood calculated in liters per day per square meter of testosterone, DHT, and  $3\alpha$ -diol in men. The hatched part of each bar represents calculated extrahepatic (splanchnic) clearance from hepatic extraction data and hepatic blood flow. The magnitude of  $3\alpha$ -diol extrahepatic clearance is apparent in the figure.

testosterone. In order to determine hepatic or splanchnic clearance and extrahepatic clearance, hepatic extractions were determined by direct catheterization of the hepatic vein. The hepatic extractions in three subjects were nearly identical (76%). This contrasts with the hepatic extraction of testosterone which is 44% (28). Therefore, from an in vivo study of liver extraction, the ability to take up  $3\alpha$ -diol is considerably greater than testosterone. Whether this represents less effective in vivo binding in blood for  $3\alpha$ -diol, i.e. albumin binding (27), than for testosterone or whether the liver extracts  $3\alpha$ -diol by additional mechanisms ("free" plus bound) cannot be answered by our studies.

Nevertheless, extrahepatic clearance of  $3\alpha$ -diol is quite high. From calculations of hepatic clearance and the overall metabolic clearance, the calculated extrahepatic clearance is about 730 liters/day/m² whole blood or 374 liters/day/m² plasma. Thus, the extrahepatic clearance of  $3\alpha$ -diol is equal to or greater than

TABLE IV  
Comparison of the Parameters in Kinetics Studies of  $3\alpha$ -Diol and DHT in Normal Men (17, 18)

Subject	Steroid	A	B	$\alpha$	$\beta$	Rate constants			Pool volumes		MCR
						K <sub>21</sub>	K <sub>12</sub>	K <sub>01</sub>	V <sub>1</sub>	V <sub>2</sub>	
		fraction of dose/ liter		Units/day					liter		liters/day
H.	$3\alpha$ -diol	0.067	0.006	322	24.3	137	49	160	13.6	38.6	2,140
	DHT	0.086	0.015	117	18.8	36.5	3.3	66	9.9	10.8	652
W.	$3\alpha$ -diol	0.074	0.009	333	28.5	145	61.5	154	12.0	28.4	1,860
	DHT	0.115	0.028	125	26.3	33.5	45.6	72	7.0	5.1	503

the overall metabolic clearance of either DHT or testosterone (Fig. 4).

Progesterone has somewhat similar characteristics since it is partially bound in plasma (transcortin) and has both a high hepatic and extrahepatic clearance.

With the assumptions inherent in a two-compartment model without metabolism in the outer pool (liver considered to be in the inner pool) and considering equal concentration throughout, 3 $\alpha$ -diol has three interesting characteristics. As shown in Table IV and Fig. 3 the rate of metabolism in the inner pool ( $K_{01} = 160$ ) and the interpool transfer ( $K_{21} = 137$ ) is very high while the calculated volume of the outer pool ( $V_2 = 35$  liters) is also large. Both the information on hepatic and extrahepatic clearance and the compartmental analysis imply a distinct metabolism for 3 $\alpha$ -diol quite different from testosterone or dihydrotestosterone. One interpretation, obviously speculative, is the possibility of high affinity binding of 3 $\alpha$ -diol particularly in extrahepatic tissue. High affinity binding of 3 $\alpha$ -diol is not observed in sexual tissue (29). The role of 3 $\alpha$ -diol as an anabolic messenger is one possibility and 3 $\alpha$ -diol binding in muscle or other tissue should be looked for. In any event this potent androgen and anabolic steroid is produced in amounts in man similar to DHT in the circulation and has unexpectedly unique *in vivo* kinetic characteristics.

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