Blood Levels and Production Rate of 17-Hydroxypregnenolone in Man

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ABSTRACT A reliable radio-ligand assay has been developed for the measurement of 17-hydroxypregnenolone in human peripheral vein plasma. The mean plasma concentration of 17-hydroxypregnenolone was, in men, 1.9 mμg/ml; and in women, 3.5 mμg/ml. These means were not significantly different from each other, and the levels were the same in the follicular and luteal phases of the menstrual cycle. In women, the adrenal cortex was the source of the 17-hydroxypregnenolone; in men, 40% was produced by the testis. Since the metabolic clearance rate was about 2000 liters/24 hr production rate estimates were 4–7 mg/24 hr.

The conversion of blood 17-hydroxypregnenolone to blood 17-hydroxyprogesterone and dehydroepiandrosterone was measured. This varied from 5 to 20%. Thus, in women during the follicular phase, 17-hydroxyprogesterone derived from blood 17-hydroxypregnenolone could be the major fraction of the 17-hydroxyprogesterone production rate. Blood 17-hydroxypregnenolone is a minor precursor of blood dehydroepiandrosterone.

INTRODUCTION

17-Hydroxypregnenolone[1] is an intermediate in the biosynthesis of steroid hormones secreted by the gonads and adrenal cortex. It has been identified in the adrenal venous effluent (1) and in peripheral blood (2). Because the peripheral levels are low, there has been no estimate of its plasma level. Further, its role as a potential precursor of such plasma steroids as dehydroepiandrosterone and 17-hydroxyprogesterone has not been determined. We have therefore developed a method for quantifying plasma concentrations of 17-hydroxypregnenolone and have examined several aspects of its metabolism.

METHODS

Materials

Reagents and chemicals. Organic solvents were analytical reagent grade and were not purified further. Absolute ether (Mallinckrodt Chemical Works) was used from freshly opened 1 lb. cans. Ethylenediaminetetraacetate (EDTA) was obtained as the disodium salt. β-Mercaptoethanol was obtained from Eastman Organic Chemicals, Rochester, N. Y., and stored at 4°C. Glycerol was spectrograde. Sigma's nicotinamide adenine dinucleotide (NAD^+) grade III was used. Crystalline bovine serum albumin was obtained from Armour Pharmaceutical Co. Deionized water was used in preparing enzyme media and buffers. All vessels used in the enzyme preparation were washed with a solution containing 2.0 g EDTA and 1.35 g $NaHCO_3$ per liter (pH 7.0).

Precoated thin-layer plates (silica gel, F254, E. Merck A.G., Darmstadt, Germany) were washed by ascending chromatography with methanol and ether for 12 hr each before use.

17-Hydroxypregnenolone (Mann Research Labs) recrystallized twice from ethanol and twice from acetone had a mp of 272–274°C.

17-Hydroxypregnenolone-7α-^3H (Sa 10 Ci/mmole) obtained from New England Nuclear was purified by thin-layer chromatography (TLC). After TLC a trace amount of the labeled steroid was added to authentic 17-hydroxypregnenolone and recrystallized to determine constancy of specific activity. Once radiochemical purity was demonstrated a stock solution of 1 μCi/20 μl in absolute ethanol was prepared and stored at − 16°C.

Pseudomonas testosteroni as lyophilized powder was generously supplied by Dr. P. Talalay of Johns Hopkins University. This material was sealed in a polyethylene bottle and stored in a desiccator at − 16°C.

Enzyme preparation. 0.25 g of the lyophilized powder of *Pseudomonas testosteroni* was placed in a small cellu-

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[1] The following abbreviations and trivial names have been used: 17-hydroxypregnenolone = 3β,17α-dihydroxypregn-5-en-20-one; 17-hydroxyprogesterone = 17α-hydroxypregn-4-ene-3,20-dione; dehydroepiandrosterone = 3β-hydroxyandrost-5-en-17-one; pregnenetriol = pregn-5-ene-3β,17α,20α-triol; dexamethesone = 9α-fluoro-16α-methyl-11β,17α,21-trixydroxypregn-1,4-diene-3,20-dione; fluoxymesterone = 9α-fluoro-17α-methyl-11β, 17β-dihydroxyandrost-4-en-3-one; and progesterone = pregn-4-ene-3,20-dione.

lose nitrate tube and mixed with 3.5 ml of the enzyme medium. This consisted of 0.05 M potassium phosphate, 0.003 M EDTA, 0.05% β-mercaptoethanol, and 20% glycerol at pH 7.0 (3). The mixture was then sonicated for 15 min at 0°C (4) and centrifuged at 20,000 *g* for 60 min. The supernatant was decanted into a screw top glass tube to which glycerol was added to make a 50% solution. This preparation was stored at −16°C. The enzymes in this crude preparation were found to be stable for at least 1 yr and are still in use.

Enzyme solution. An aliquot of the crude enzyme preparation was diluted 1 : 50 with 1% crystalline bovine albumin in the enzyme medium (3). This dilute enzyme solution stored at 4°C was found to be stable for at least 2 months.

Pyrophosphate buffer plus NAD⁺ solution. The solution used in the enzyme reaction consisted of 10 μmoles sodium pyrophosphate and 0.5 μmoles NAD^+ per 0.5 ml (pH 8.9). This mixture made in 100-ml amounts and stored at 4°C was stable for at least 1 month.

Methods

Briefly, 17-hydroxypregnenolone was measured by competitive protein binding after conversion of the steroid to 17-hydroxyprogesterone. Following extraction from plasma, 17-hydroxypregnenolone was separated from 17-hydroxyprogesterone by TLC, converted enzymatically to 17-hydroxyprogesterone, and then measured as described earlier by us (5).

Method in detail

Plasma extraction. To 3–5 ml of heparinized plasma were added 1/20 volume of 1 N NaOH and 1000 dpm (0.01 mμg) of 17-hydroxypregnenolone-^{3}H. After mixing, the sample was extracted twice with 5 volumes of ether. The ether extracts were combined, washed with 1/20 volume of 1% acetic acid and distilled water, dried over sodium sulfate, and evaporated under air.

Chromatography. Using methanol : ether (1 : 1) the residue was spotted on a thin-layer plate and placed in a tank saturated with the solvent system benzene : methanol (9 : 1). The plate was developed twice to the top. Parallel standards of 17-hydroxypregnenolone were located by streaking with a solution of sulfuric acid and absolute ethanol (2 : 1, v : v). An area 6 mm above and below the center of the 17-hydroxypregnenolone markers was scraped off (total area 2 cm²). The loosened silica was sucked into a disposable pipet which was tightly plugged with a small wad of glass wool previously washed with methanol and ether. The samples were eluted with 1.5 ml of acetone into 13-ml glass-stoppered, conical, centrifuge tubes and the solvent was evaporated under air.

Enzyme conversion. This reaction was carried out at room temperature. To each centrifuge tube, 0.5 ml of the NAD^+ pyrophosphate buffer solution was added and the tubes agitated briefly. 20 μl of the dilute enzyme solution was then added to each tube. The tubes were agitated briefly and allowed to stand for 10 min. After the reaction period, the samples were extracted once with 10 ml of ether, the water phase removed with a disposable pipet, the ether dried over sodium sulfate, and the samples centrifuged for 5 min. The ether was then poured into a centrifuge tube, evaporated under air, and the residues transferred to thin-layer plates. From this point, the method is the same as that described for 17-hydroxyprogesterone (5). The average conversion of 17-hydroxypregnenolone to 17-hydroxyprogesterone was 92.1 ±5.0 (SD) (n = 18).

Metabolic clearance rate (MCR) and conversion ratios. The MCR of 17-hydroxypregnenolone was measured by the constant infusion technique (6) as described previously (7). All studies were begun between 8 and 9 a.m. with subjects recumbent and fasting. ½ hr after a priming dose of 17α-hydroxypregnenolone-^{3}H, an infusion of the labeled steroid was started and blood samples collected at 80, 100, and 120 min. The blood was centrifuged immediately and the plasma separated. To each plasma sample were added 200 μg of recrystallized 17-hydroxypregnenolone, dehydroepiandrosterone, and 17-hydroxyprogesterone, and 400 cpm of

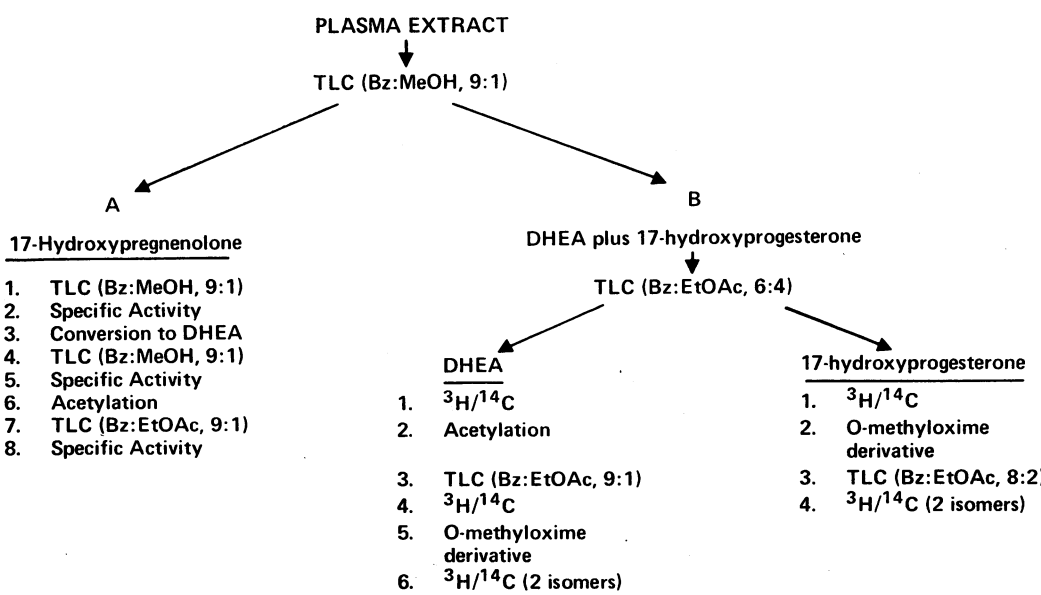

FIGURE 1 Flow sheet for measurement of 17-hydroxypregnenolone-^{3}H levels in plasma for metabolic clearance rates.

17-hydroxyprogesterone-^{14}C and dehydroepiandrosterone-^{14}C. The samples were then extracted with 5 volumes of ether and processed according to Fig. 1.

17-Hydroxypregnenolone was measured by the method of Oertel and Eik-Nes (8) using a Gilford 2000 spectrophotometer with 1.4-ml quartz cuvettes. Acetylation was performed by overnight incubation in acetic anhydride-pyridine. 17-Hydroxypregnenolone was oxidized to dehydroepiandrosterone by sodium periodate after reduction with sodium borohydride (9). The *o*-methyloxime derivative was made according to Fales and Luukkainen (10).

Evaluation of the 17-hydroxypregnenolone assay

Specificity. The steroids listed in Table I were added to steroid-free plasma in a concentration of at least 20 mμg/ml. None of the steroids displaced label in the binding assay, i.e., the result was equivalent to a plasma blank. It is to be particularly noted that the separation of 17-hydroxypregnenolone from 17-hydroxyprogesterone is complete in the first chromatography since an equivalent of 50 mμg/ml of the latter gave results indistinguishable from a blank value. Further, when 17-hydroxyprogesterone-^{14}C was added to plasma, no ^{14}C was detected in the 17-hydroxypregnanolone area after TLC. Two steroids that were not checked for possible interference, 3β, 21-dihydroxy-5-pregnen-20-one and 3β-17, 21-trihydroxy-5-pregnen-20-one, were potential contaminants because they would be converted by the enzymes to 11-deoxycorticosterone and 11-deoxycortisol respectively, both of which bind well to CBG (14). However, the subsequent acetylation separates these steroids from 17-hydroxyprogesterone as shown previously (5).

Sensitivity. The method blank was evaluated by assaying 5-ml samples of distilled water. The mean 17-hydroxypregnenolone level was 0.04 mμg ±0.04 (SD) (n = 19). This value was independent of the volume of water used.

The plasma blank was determined using steroid-free plasma obtained from an orchiectomized, adrenalectomized man. 2.5 ml of this plasma had a 17-hydroxypregnenolone level of 0.1 mμg ±0.12 (SD) (n = 6) and 5 ml had a level of 0.14 mμg ±0.09 (SD) (n = 6). Thus there is additional substance in plasma that apparently displaces label from CBG in the assay. We have not examined other steroid-free plasmas. Assuming that the plasma blank represents material other than 17-hydroxypregnenolone, its contribution of about 0.03 mμg/ml is negligible relative to physiologic ranges of the steroid.

TABLE I

Steroids Added to Steroid-Free Plasma

Pregn-4-ene-3, 20-dione	21-Hydroxypregn-4-ene-3, 20-dione
11β-Hydroxypregn-4-ene-3, 20-dione	17α21-Dihydroxypregn-4-ene-3, 20-dione
6β-Hydroxypregn-4-ene-3, 20-dione	11β, 17α, 21-Trihydroxy-pregn-4-ene-3, 20-dione
17β-Hydroxypregn-4-ene-3, 20-dione	3β-Hydroxypregn-5-ene-20-one
11β, 17α-Dihydroxypregn-4-ene-3, 20-dione	17β-Hydroxy-androst-4-ene-3-one
Pregn-4-ene-3, 11, 20-trione	Androst-4-ene-3, 17-dione
17β-Hydroxypregn-4-ene-3, 11, 20-trione	11β-Hydroxyandrost-4-ene-3, 17-dione
20α-Hydroxypregn-4-en-3-one	3β-Hydroxyandrost-5-en-17-one
20β-Hydroxypregn-4-en-3-one	Estra-1, 3, 5(10)-triene-3, 17β-diol

Accuracy. When known amounts of 17-hydroxypregnenolone equivalent to 2–8 mμg/ml were added to steroid-free plasma, recovery averaged 89% with a coefficient of variation for the entire range of 8%. The reasons for recoveries of less than 100% are not known but this experience is similar to that reported for the determination of 17-hydroxyprogesterone in plasma (5). It appears that the process of extraction and purification of 17-hydroxypregnenolone and 17-hydroxyprogesterone introduced substances that inhibit binding of the steroid added for recovery.

Precision. The intra-assay coefficient of variation evaluated by doing multiple determinations of the sample plasma sample was found to be 8% (n = 9). Precision in these assays depends almost entirely on using a plasma aliquot that will yield an amount of 17-hydroxypregnenolone that will fall within the useful range of the standard curve. Our curves covered the range of 0–3 mμg and had good precision between 80% and 20% bound. We therefore used the portion of the curve between 0.25 and 1.5 mμg. The inter-assay coefficient of variation for levels less than 3 mμg/ml was 20% (n = 13) and 9% for levels greater than 3 mμg/ml (n = 9).

RESULTS

Plasma concentration. The basal plasma levels of 17-hydroxypregnenolone in men and women were measured in specimens obtained between 7 and 9 a.m. The mean level in men, 1.9 mμg/ml, was lower than the mean level in women, 3.5 mμg/ml (Fig. 2). Because the variances in the two groups were different, the data were analyzed using logarithmic transformation. The logarithms of the means then did not differ significantly (men, 1.6; women 2.7). There was no significant difference between the 17-hydroxypregnenolone levels of women in the follicular and luteal phases of the menstrual cycle.

Adrenal cortical suppression was performed in 12 men using dexamethasone, 1 mg orally at midnight. The 17-hydroxypregnenolone level fell to 40% of control values, whereas cortisol levels reached 10% of control values (Fig. 3). By contrast, when dexamethasone was given to women, there was complete suppression of 17-hydroxypregnenolone secretion (Fig. 3). These data suggested a testicular contribution to the secretion rate.[2] Therefore, fluoxymesterone, a synthetic androgen, was given to eight normal men in a dose of 40 mg daily for 3 days to suppress Leydig cell function. This resulted in a 40% fall in 17-hydroxypregnenolone levels (Fig. 3).

In two normal men, 17-hydroxypregnenolone was

[2] The term secretion rate is used to indicate the rate of entry of a steroid into the plasma as a result of secretion from an endocrine gland. Production rate signifies the total rate of entry of the steroid from all sources into the plasma compartment.

measured every 6 hr for 2 days. The circadian rhythm was marked and paralleled that for cortisol. Additional studies (Fig. 4) showed a consistent fall in 17-hydroxypregnenolone plasma levels from 8 a.m. to 5 p.m.

Metabolic clearance rate and conversion ratio. The primary data appear in Table II. The rates of infusion of isotope, monitored through a three-way stopcork at the infusion needle, remained constant. We have elected to average in the two discordant data (P.B., 163; C.S., 141) for 17-hydroxypregnenolone-^{3}H in plasma since we could find no source of error. The ^{3}H/^{14}C ratios of the products, dehydroepiandrosterone and 17-hydroxyprogesterone, were constant.

From these data, MCR's, conversion ratios (^{3}H [product]/(^{3}H [precursor]), and blood production rates were calculated (Table III). A "mean" plasma level, 69% of the 8 a.m. value, was used to obtain the production rates. The MCR's varied from 1400 to 2700 liters/24 hr, but this spread was decreased when they were expressed as a function of surface area. The blood production rates ranged between 2 and 8.5 mg/24 hr. The conversion ratios, dehydroepiandrosterone/17-hydroxypregnenolone and 17-hydroxyprogesterone/17-hydroxypregnenolone were 4–20%. In the two cases with the highest conversion ratios, the final dehydroepiandrosterone derivative was recovered from the counting vials, saponified, chromatographed by TLC, and the ^{3}H/^{14}C ratio measured again. The ratio was unchanged. Since the metabolic clearance rates of all three steroids are approximately the same, these conversion ratios are equivalent to the transfer factors.

DISCUSSION

The finding of pregnenetriol in the urine of normal men and women (11) and the identification of its probable plasma precursor, 17-hydroxypregnenolone, in the

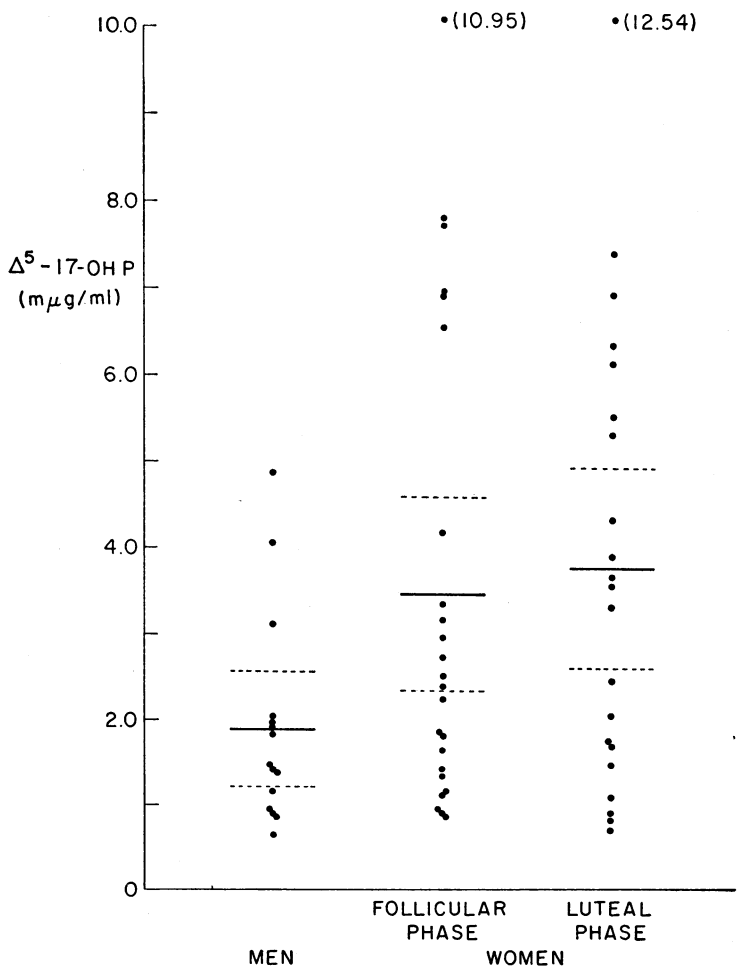

FIGURE 2 Plasma concentrations of 17-hydroxypregnenolone (Δ^5-17-OHP), means ±2 SE.

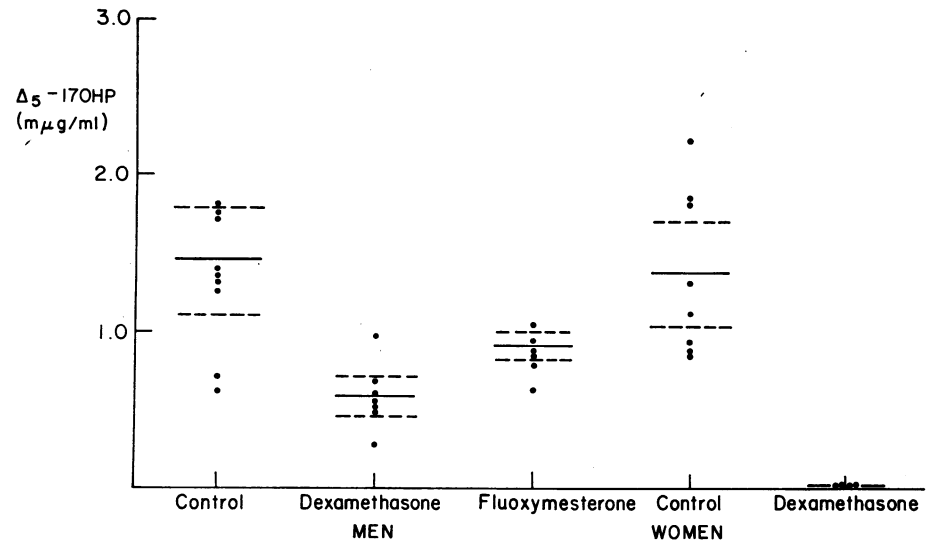

FIGURE 3 Effects of dexamethasone and fluoxymestrone on plasma 17-hydroxypregnenolone levels in normal men and women, means ±2 SE.

adrenal venous effluent (1) established the secretion of 17-hydroxypregnenolone. Its low concentration in plasma precluded attempts of accurate measurement. This can be estimated however using the production rate data obtained from the specific activity of urinary pregnentriol after infusing labeled 17-hydroxypregnenolone and the metabolic clearance rate. Thus, with production rates of 4–10 mg/24 hr (12, 13) and MCR's of about 2000 liters/24 hr, the plasma level would be 2–5 mμg/ml. Using the assay described here, mean plasma levels varied between 1.9 and 3.5 mμg/ml. The agreement between these data allows a further inference, namely, that plasma 17-hydroxypregnenolone is the single important precursor of urinary pregnenetriol. Had some other precursor of urinary pregnenetriol such as 17-hydroxypregnenolone sulfate been metabolized to urinary pregnenetriol by a route other than through plasma 17-hydroxypregnenolone, then the estimated urinary production rate would be falsely high and the plasma levels would not agree.

Our initial impression was that the plasma 17-hydroxypregnenolone concentration was higher in women than in men. Estimation of the data of Fig. 2 revealed that the variance was much greater for the female population; thus a normal distribution about the mean could not be assumed. After logarithmic transformation of the data, the means did not differ significantly. The several high values noted in women were not artefactual; each was repeated at least once and the value confirmed in a separate assay. We are unable to determine from these data whether there is more than one population of women with respect to 17-hydroxypregnenolone levels or whether there is only a greater variability of secretion among women. It is apparent though from many other studies that plasma cortisol concentrations (14, 15) do not show a similar degree of variability.

TABLE II

Rates of Infusion and Plasma Tritium in 17-Hydroxypregnenolone, Dehydroepiandrosterone, and 17-Hydroxyprogesterone

Times of sampling . . .	Rate of infusion*			17-Hydroxypregnenolone			Dehydroepi-androsterone			17-Hydroxy-progesterone		
	80′	100′	120′	80′	100′	120′	80′	100′	120′	80′	100′	120′
	cpm/min × 10^{-3}			*cpm/liter* × 10^{-3}			*cpm/liter* × 10^{-3}			*cpm/liter* × 10^{-3}		
P. B. ♀ 24 yr	153.1	157.5	154.4	101.5	96.2	163.0	4.5	5.5	4.6	3.9	6.5	4.7
C. S. ♀ 25 yr	170.3	168.2	165.9	91.6	141.0	90.0	3.6	5.6	3.8	4.0	3.9	3.9
C. H. ♀ 23 yr	186.3	193.0	185.4	26.0	32.4	31.5	6.8	5.8	6.2	3.4	3.2	3.3
K. C. ♂ 22 yr	242.7	244.1	247.7	85.7	53.3	68.7	11.7	12.4	12.8	—	—	—

* Sampled at the infusion needle.

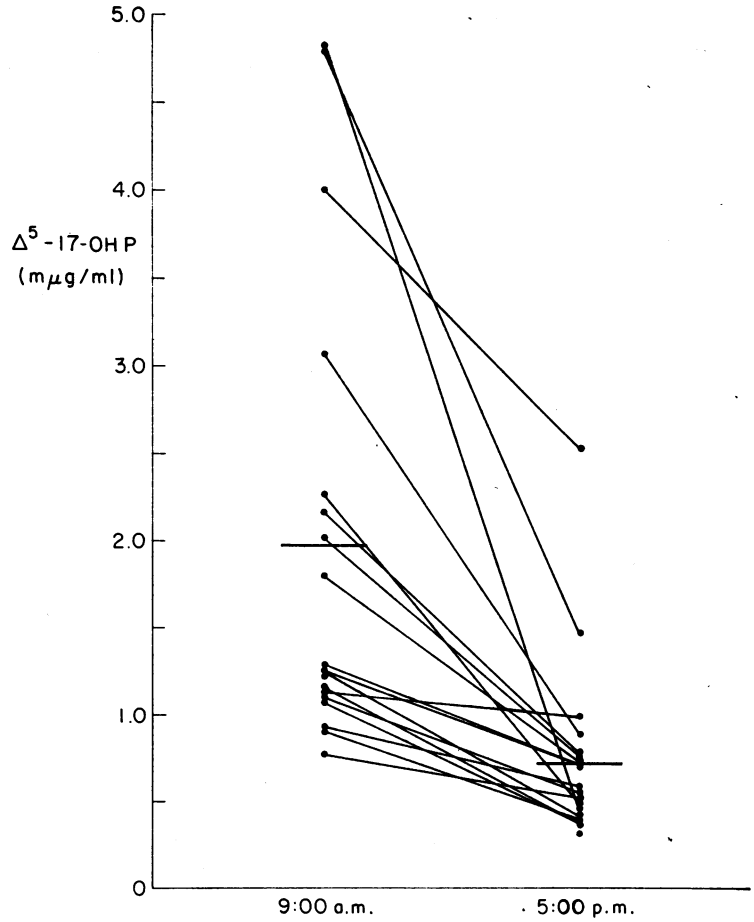

FIGURE 4 Plasma concentrations of 17-hydroxypregnenolone at 8:00 a.m. and 5:00 p.m. in six normal men. Each subject was studied three times.

The origin of plasma 17-hydroxypregnenolone is apparent from the data of Fig. 3. In women, almost all of the steroid must have been secreted by the adrenal cortex since dexamethasone reduced the amount in plasma by more than 90%. Since follicular and luteal phase concentrations were equal, it is unlikely that the corpus luteum secretes significant amounts of 17-hydroxypregnenolone.

In men, the testis makes an important contribution to plasma 17-hydroxypregnenolone. The suppression of 17-hydroxypregnenolone levels by fluoxymesterone may be assumed to reflect suppression of LH secretion and consequent decreased Leydig cell function. Thus about one-third of the estimated 17-hydroxypregnenolone production rate must result from testicular secretion. With knowledge of this fact, it is now pertinent to compare again the adrenal cortical secretion of 17-hydroxypregnenolone between the sexes. Since the mean plasma level is lower in men than in women and since the testis contributes one-third of this in men, the adrenal cortex of women must secrete more 17-hydroxypregnenolone than that of men per unit body surface. To our knowl-

TABLE III

17-Hydroxypregnenolone Plasma Levels, Metabolic Clearance Rates, Blood Production Rates, and Conversion Ratios to Dehydroepiandrosterone and 17-Hydroxyprogesterone

	P. B.	C. S.	C. H.	K. L.
Plasma level, *mμg/ml*	7.0	2.2	2.9	4.6
Calculated mean plasma level*	4.8	1.5	2.1	3.2
Metabolic clearance rate				
liters/24 hr	1650	1350	2170	2670
liters/24 hr/m²	1030	840	1360	1400
Blood production rate‡, *mg/24 hr*	7.9	2.0	4.6	8.5
Conversion ratio in blood				
Dehydroepiandrosterone	4.3	4.0	20.6	18.4
17-Hydroxyprogesterone	4.5	3.8	11.1	—

* 69% of the 8:00 a.m. value.
‡ Product of the MCR and calculated mean plasma level.

edge, this is the first evidence that an adrenal secretory product is higher in women than in men.

As 17-hydroxypregnenolone is secreted primarily by the adrenal cortex, a diurnal variation would be expected. This was in fact found and the magnitude of the variation was similar to that for cortisol.

We have previously examined the blood levels and production rates (16) of the related steroid 17-hydroxyprogesterone (17-OHP), and it is of some interest to compare them. In men, 90% of 17-OHP was secreted by the testis, whereas only 40% of 17-hydroxypregnenolone was secreted by the testis. Both steroids showed a marked diurnal variation; however, the changes in plasma concentration of 17-OHP were independent of ACTH secretion (16). In women, the corpus luteum was an important source of 17-OHP; by contrast little 17-hydroxypregnenolone was secreted by the corpus luteum. In men, the production rate of 17-OHP was about 2.0 mg/24 hr; the estimated testicular secretion of 17-hydroxypregnenolone was about one-third of 4 mg or 1.3 mg/24 hr.

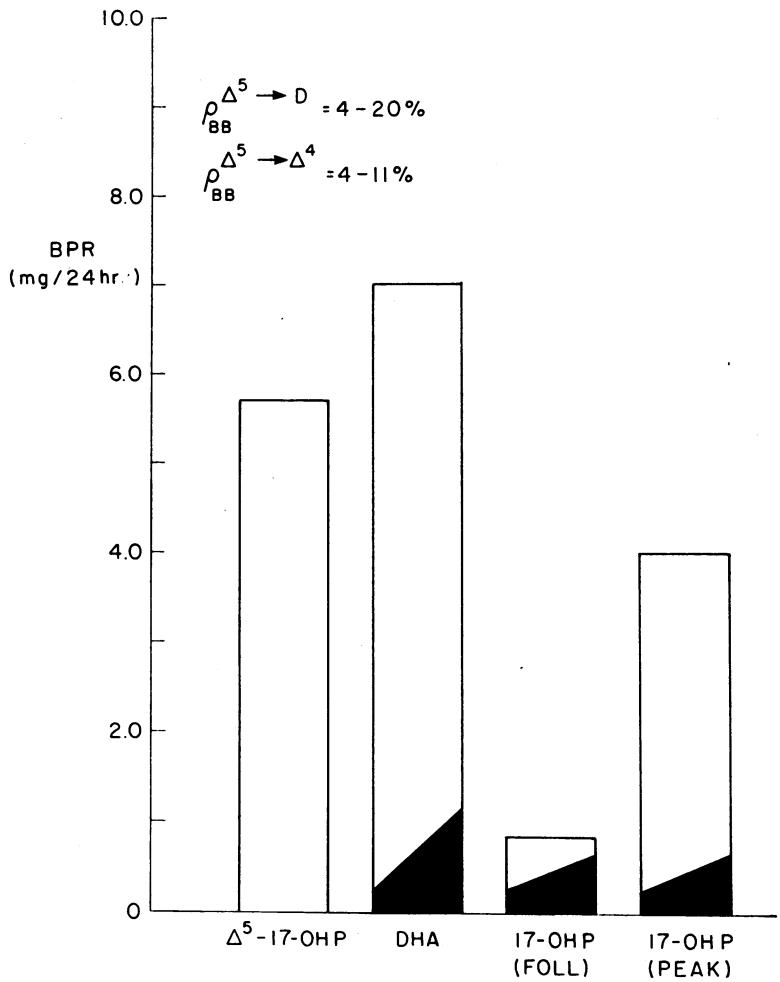

FIGURE 5 Contribution of 17-hydroxypregnenolone as a plasma precursor of dehydroepiandrosterone and 17-hydroxyprogesterone. The height of each bar gives the normal blood production rate (BPR) of 17-hydroxypregnenolone (first bar, labeled Δ^5-17-OHP), dehydroepiandrosterone (DHA), and of 17-hydroxyprogesterone (17-OHP) during the follicular phase and at its peak level coincident with ovulation. Shaded areas represent the upper and lower limits of that fraction of the production rates accounted for by conversion from 17-hydroxypregnenolone.
$\rho_{BB}^{\Delta 5 \rightarrow D}$ = transfer factor, 17-hydroxypregnenolone to dehydroepiandrosterone in blood.
$\rho_{BB}^{\Delta 5 \rightarrow \Delta 4}$ = transfer factor, 17-hydroxypregnenolone to 17-hydroxyprogesterone in blood.

Since it has been shown that the liver has the capacity to metabolize Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids (17) and that this occurs in man (18), the role of 17-hydroxypregnenolone as a precursor of 17-OHP was examined. Conversion ratios and transfer factors in three patients were 3.8, 4.5, and 11%. Thus, conversion of 17-hydroxypregnenolone under some circumstances could account for an important fraction of 17-OHP production (Fig. 5). In women in the follicular phase, this could vary from 20 to almost 100% of the 17-OHP production rate. During the luteal phase however, the production rate of 17-OHP increases greatly, whereas that of 17-hydroxypregnenolone remains unchanged; therefore, the fractional contribution of 17-hydroxypregnenolone is considerably smaller.

Still another steroid transformation presumably occurring outside the endocrine glands is the metabolism of 17α-hydroxy-C_{20}-ketosteroids to 17-ketosteroids. Thus, 17-OHP infused intravenously was converted in low yield in man to urinary testosterone (19). We found a surprisingly high rate of conversion of 17-hydroxypregnenolone to dehydroepiandrosterone ranging from 4 to 20%. The two highest conversion ratios were examined further by recovering dehydroepiandrosterone acetate from the counting solution, saponifying it, and measuring the $^3H/^{14}C$ ratio after rechromatography. There was no change in $^3H/^{14}C$ ratio. Using a previous estimate of the plasma dehydroepiandrosterone production rate (20), it can be seen (Fig. 5) that the higher blood production rates of 17-hydroxypregnenolone would account for 1–2 of the 7 mg/24 hr of the estimated dehydroepiandrosterone production rate.

Our estimates of 17-hydroxypregnenolone production rate are based on a "mean" plasma level of 0.69 of the 8 a.m. value. This fraction was obtained from the single study of two men whose plasma level was measured every 6 hr for 2 days. It is admittedly a rough approximation, but the marked diurnal changes necessitate some estimate of this sort in order to extrapolate results in the morning to 24-hr production rates. It has been shown that 10% of labeled 17-hydroxypregnenolone given intravenously to normal adults appears in the urine as pregnenetriol (12, 21). The excretion rate of pregnenetriol has been estimated at about 0.5 mg/24 hr (22). These data yield a 24 hr production rate of about 5 mg/24 hr, in good agreement with our average blood production rate of 5.7 mg/24 hr.

Since 17-hydroxypregnenolone, progesterone, and 17-OHP are intermediates in the biosynthesis of the active gonadal steroids, testosterone and estradiol, and of cortisol as well, we have considered whether the study of plasma levels of these steroids can contribute to an understanding of steroid biosynthesis within the gland. They all have similar clearance rates, so the plasma after stimulation of a gland will reflect secretion. From our previous studies of the production rates of the precursors of testosterone for example, testicular secretion rates may be estimated as follows: progesterone, < 0.2–0.3 mg/24 hr, 17-hydroxypregnenolone, 1.3 mg/24 hr; 17-OHP, 1.8 mg/24 hr; and testosterone, 7 mg/24 hr. We would propose as an hypothesis that the secretion rate of these biosynthetic intermediates resulting from a "leaky" gland (23) reflects the importance of the biosynthetic pathway. Thus, if pregnenolone is converted to 17-OHP via 17-hydroxypregnenolone rather than via progesterone, then 17-hydroxypregnenolone secretion will be higher than progesterone secretion. Since 17-hydroxypregnenolone is secreted at higher rates than progesterone by testis and adrenal cortex, we would therefore assume that biosynthesis of testosterone and cortisol from pregnenolone proceeds through 17-hydroxypregnenolone rather than via progesterone. Similarly the synthesis of estradiol during the time just before follicular rupture is characterized by high 17-hydroxypregnenolone and 17-OHP secretion rates and low progesterone secretion rates. It is apparent that alternate hypotheses may also be proposed; the important consideration is that methods are available for testing these hypotheses.

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