

Plasma Progesterone and 17-Hydroxyprogesterone in Normal Men and Children with Congenital Adrenal Hyperplasia

CHARLES A. STROTT, TERUYA YOSHIMI, and MORTIMER B. LIPSETT

*From the Endocrinology Branch, National Cancer Institute,
Bethesda, Maryland 20014*

ABSTRACT Plasma 17-hydroxyprogesterone (17-OHP) concentrations in normal men averaged 0.094 $\mu\text{g}/100$ ml. Studies using suppressive doses of androgens and glucocorticoids showed that 90% of the 17-OHP originated from the Leydig cell. The 17-OHP production rate was 1.8 mg/24 hr. Plasma 17-OHP has a marked circadian variation, the 8 p.m. values being only 40% of the 8 a.m. values. Plasma luteinizing hormone measured in the same samples did not vary.

The adrenal cortex has the capacity to synthesize and secrete 17-OHP and progesterone since adrenocorticotrophic hormone (ACTH) caused a fourfold increase in these plasma steroids. In children with congenital adrenal hyperplasia, plasma 17-OHP levels were 50–200 times those of normal men and plasma progesterone was increased 6- to 10-fold over normal men.

INTRODUCTION

Progesterone¹ and 17-hydroxyprogesterone (17-OHP) are intermediates in the biosynthesis of adrenal cortical and gonadal hormones. They are secreted in rela-

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¹The following abbreviations and trivial names are used: progesterone = pregn-4-ene-3,20-dione; 17-hydroxyprogesterone (17-OHP) = 17 α -hydroxypregn-4-ene-3,20-dione; cortisol = 11 β ,17 α ,21-trihydroxypregn-4-ene-3,20-dione; fluoxymesterone = 9 α -fluoro-17 α -methyl-11 β ,17 β -dihydroxyandrost-4-en-3-one; dexamethasone = 9 α -fluoro-16 α -methyl-11 β ,17 α ,21-trihydroxypregn-1,4-diene-3,20-dione; testosterone = 17 β -hydroxyandrost-4-en-3-one; androstenedione = androst-4-ene-3,20-dione; dehydroepiandrosterone = 3 β -hydroxyandrost-5-en-17-one; 17-hydroxypregnenolone = 3 β ,17 α -dihydroxypregn-5-en-20-one; 17-hydroxypregnenolone sulfate = 3-yl-sulfate-17 α -hydroxypregn-5-en-20-one; dehydroepiandrosterone sulfate = 3-yl-sulfate-androst-5-en-20-one; pregnanetriol = 5 β -pregnane-3 α ,17 α ,20 α -triol; pregnanediol = 5 β -pregnane-3 α ,20 α -diol; LH = luteinizing hormone or interstitial cell stimulating hormone; FSH = follicle-stimulating hormone; ACTH = adrenocorticotrophic hormone.

tively small amounts by normal subjects but may have high secretion rates when there are distal enzymatic blocks, such as in congenital adrenal hyperplasia (CAH). To study in greater detail the function of the steroid-producing glands, we have examined the plasma concentration and sources of plasma progesterone and 17-OHP under varying conditions. We shall report here the utility of these measurements for investigating normal Leydig cell function and adrenal cortical function in CAH.

METHODS

Subjects. The normal men and women ranged in age from 20 to 47 yr. When circadian variation was examined, plasma samples were obtained after the subjects had been in the recumbent position for at least 1 hr. Five patients, ranging from 5 to 16 yr of age, with the simple virilizing form of CAH were studied.

Plasma steroids. Cortisol was measured fluorimetrically (1). Plasma 17-OHP was measured by a protein-binding technique (2) that has a coefficient of variation of $\pm 6\%$ at plasma levels as low as 0.020 $\mu\text{g}/100$ ml. Plasma progesterone also measured by a protein-binding method of similar precision (3) was modified so that the free steroid fraction was separated from that fraction bound to protein by adsorption to Florisil (2). Plasma luteinizing hormone (LH) levels in the studies of diurnal variation were estimated by radioimmunoassay (4).² All LH samples were measured in a single assay: the intra-assay coefficient of variation is $\pm 8\%$.

Metabolic clearance rates. The metabolic clearance rates of 17-OHP and progesterone were measured by the constant infusion technique. All studies were begun between 8 and 9 a.m. with the subjects recumbent and fasting. The radioactive steroids were infused and their levels measured as described previously (5). There was no consistent trend of the level of plasma radioactivity in either steroid and the average deviation from the mean was less than 5% for 17-OHP and 7% for progesterone. In one man with localized osteogenic sarcoma, 17-OHP-³H was infused for 10 hr to look for possible diurnal variations in the metabolic clearance rate.

²We wish to thank Dr. Griff T. Ross, Endocrinology Branch, NCI, for the measurement of the plasma LH levels.

RESULTS

Progesterone and 17-OHP in normal adults. The mean plasma concentration of 17-OHP in normal men was 0.094 $\mu\text{g}/100\text{ ml}$, a level significantly higher than that of women in the follicular phase of the menstrual cycle (0.042 $\mu\text{g}/100\text{ ml}$) (Fig. 1). Although the mean 17-OHP level of ovariectomized women was lower than that of women in the follicular phase, the difference was not significant.

Progesterone levels were significantly higher in women than in men (0.041 vs. 0.019 $\mu\text{g}/100\text{ ml}$) (Fig. 1). The mean plasma progesterone level was reduced by ovariectomy but the difference was not significant.

The intraindividual variation of progesterone and 17-OHP was examined by measuring plasma levels at 8 a.m. daily for 6 days in three normal men. The variations from the mean were 52, 38, and 35% for progesterone, and 27, 33, and 18% for 17-OHP.

Of some interest was the finding that the mean 17-OHP level in random plasma samples obtained from women in the luteal phase of the menstrual cycle was four to five times that of women in the follicular phase (Fig. 2).

The implications of this will be discussed elsewhere but it is necessary to be aware of this large change in plasma levels when performing horizontal studies in women.

Since 17-OHP was higher in men than in women who did not have a functioning corpus luteum, it was likely that 17-OHP was secreted by the testis. When a synthetic androgen, fluoxymesterone, was given to normal men at a dose of 40 mg daily for 3 days there was a 90% decrease in plasma 17-OHP (Fig. 3). Dexamethasone in a dosage of 4 mg daily for 4 days, however, did not lower plasma 17-OHP although it produced the anticipated decrease in plasma cortisol concentrations (Fig. 3). When 4000 IU of human chorionic gonadotrophin (HCG) was given for 4 days to three normal men, there was a 2- to 3-fold increase in plasma 17-OHP (Fig. 4). Because of the marked circadian variation in plasma 17-OHP levels (*vide infra*), this effect was more apparent when samples obtained in the late afternoon were compared (Fig. 4). Smaller doses of HCG caused a significant increase in the morning plasma 17-OHP level in three of five normal men.

Because of continuing interest in the circadian rhythm

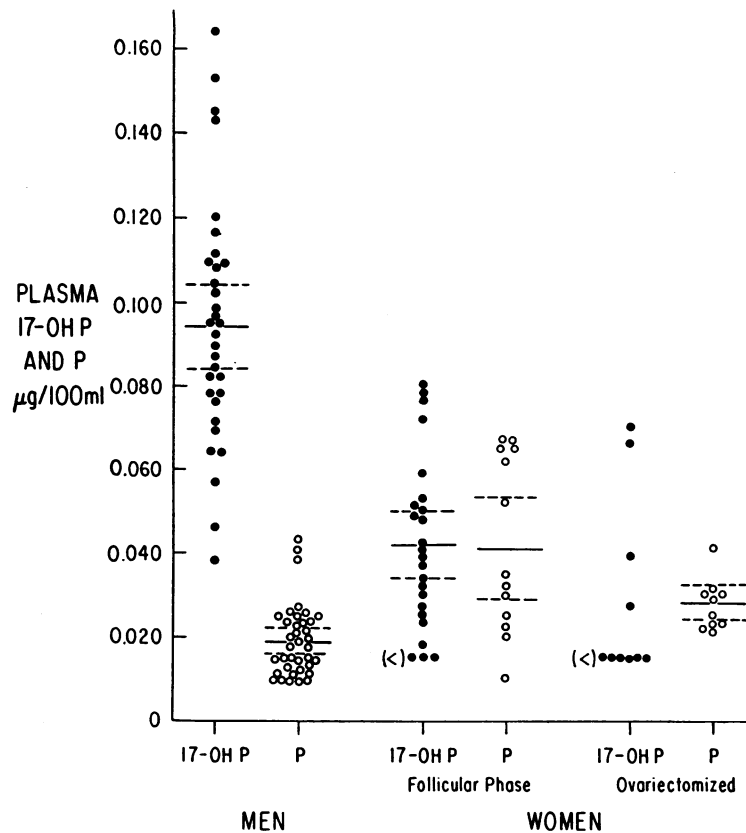


FIGURE 1 Plasma concentration of 17-hydroxyprogesterone (17-OHP) and progesterone (P) in normal men and women. The solid and broken lines for each set of data represent the mean and 95% confidence limits.

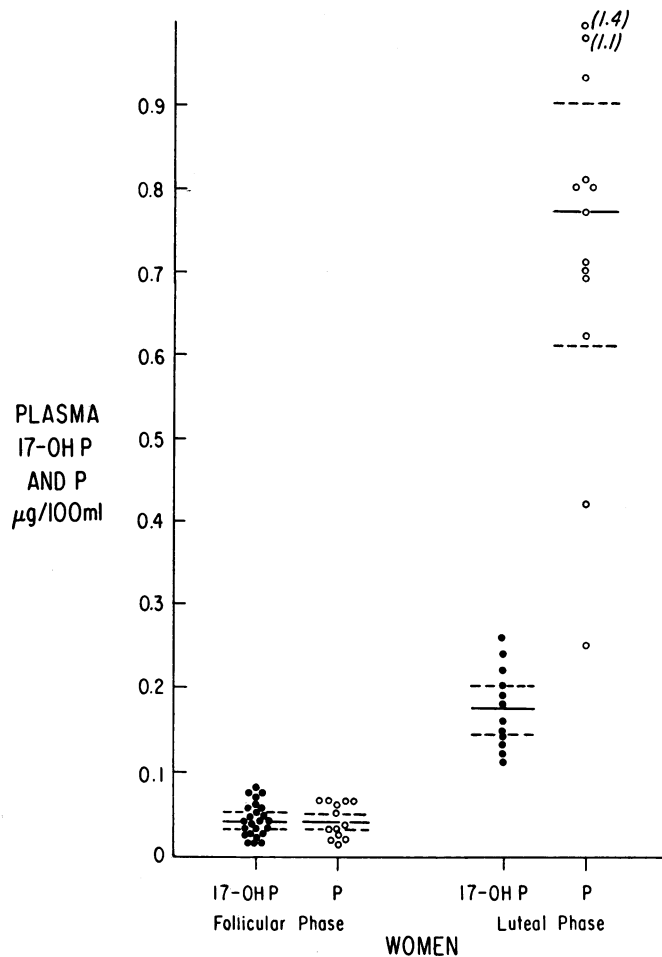


FIGURE 2 Plasma concentration of 17-hydroxyprogesterone (17-OHP) and progesterone (P) in normal women during the follicular and luteal phases of the menstrual cycle. The solid and broken lines for each set of data represent the mean and 95% confidence limits.

of the hypothalamic-pituitary Leydig cell system, we measured plasma 17-OHP and LH concentrations every 6 hr in the nine normal men for a period of 2 days. The results of these studies are presented in Fig. 5 and Table I. There was a marked variation in plasma 17-OHP levels, the peak occurring about 8 a.m. and the nadir at 8 p.m. On 2 successive days, the 8 p.m. plasma 17-OHP level was 47 and 40% of the respective 8 a.m. values. By contrast, plasma LH did not vary throughout the day. Since changes in plasma levels could result from alterations in metabolic clearance rates, 17-OHP-³H was perfused for 10 hr. There was no consistent change in the metabolic clearance rates calculated from the morning or afternoon specimens.

To ensure that there was no adrenal component responsible for these diurnal shifts, plasma 17-OHP con-

centrations were measured every 8 hr for 2 days in three normal men who received 1 mg of dexamethasone every 6 hr. As shown in Fig. 6, the diurnal variation of plasma 17-OHP was not altered by a dose of dexamethasone sufficient to suppress completely adrenal cortical function and presumably ACTH secretion.

Progesterone in plasma was measured concurrently in all studies. Its concentration was not affected by administration of HCG or fluoxymesterone. No circadian variation was detected.

Although 17-OHP is derived almost exclusively from the testis in normal men, the adrenal cortex too has the capacity to synthesize and secrete 17-OHP and progesterone. This can be demonstrated by stimulating the adrenal cortex with ACTH and measuring the increase in plasma steroids. Eight normal women in the follicular

phase of the menstrual cycle were given 40 U of ACTH intravenously for 8 hr and plasma concentrations of cortisol, progesterone, and 17-OHP were measured before and after the infusion. Plasma cortisol and progesterone increased almost four times and 17-OHP (omitting the 0.86 value) was increased five fold (Fig. 7). There were similar proportionate increases of lesser magnitude when 0.25 mg of Cortrosyn (B₁₋₂₄ corticotrophin) was given intramuscularly to five normal men.

Studies in CAH. Plasma 17-OHP in five untreated patients with CAH ranged from 4 to 22 $\mu\text{g}/100\text{ ml}$ (Fig. 8). Plasma progesterone was also high and ranged from 0.2 to 1.1 $\mu\text{g}/100\text{ ml}$. When 40 U of ACTH were infused for 8 hr, the plasma 17-OHP and progesterone concentrations doubled in two patients and were essentially unchanged in three. Two patients had normal concentrations of both steroids while receiving glucocorticoids.

The metabolic clearance rates and production rates of progesterone and 17-OHP were measured in three normal men and two patients with CAH, a 5 yr old boy and a 16 yr old girl (Table II). The data on the first sub-

ject have been reported previously (5). The progesterone metabolic clearance rates in normal subjects averaged 2100 liters/24 hr. The absolute progesterone metabolic clearance rates in the patients with CAH were low but were within the normal range when expressed as a fraction of body surface area. The progesterone plasma production rate (product of the plasma concentration and the metabolic clearance rate) was 0.3 mg/24 hr in the normal men and 3.3 and 6.5 mg/24 hr in the subjects with CAH.

The mean 17-OHP metabolic clearance rate in the three normal men was 2000 liters/24 hr. When the clearance rates in the patients with CAH were corrected for body surface area, one was clearly normal and the other lower than the three normals. The 17-OHP plasma production rate was calculated using a "mean" plasma 17-OHP concentration, 67% of the 8 a.m. value. This correction factor was derived by calculating a mean 17-OHP plasma concentration from the nine subjects whose levels were measured every 6 hr for 2 days taking each 8 a.m. value as 100%. Thus, the corrected plasma 17-OHP

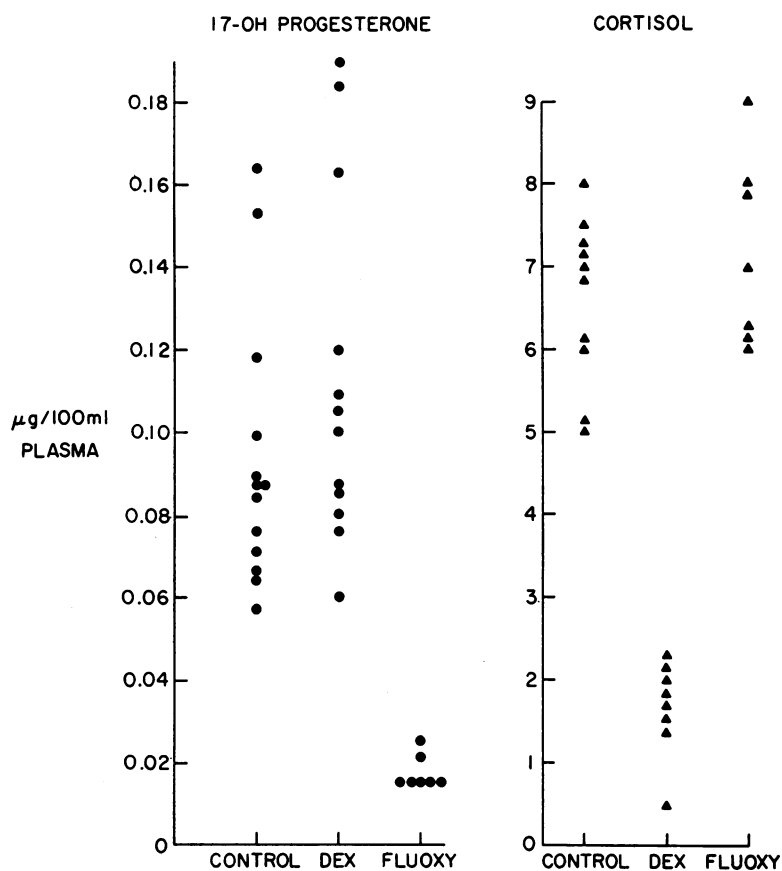


FIGURE 3 Plasma concentration of 17-hydroxyprogesterone and cortisol in normal men during a control period and after the administration of dexamethasone (DEX) and fluoxymesterone (FLUOX).

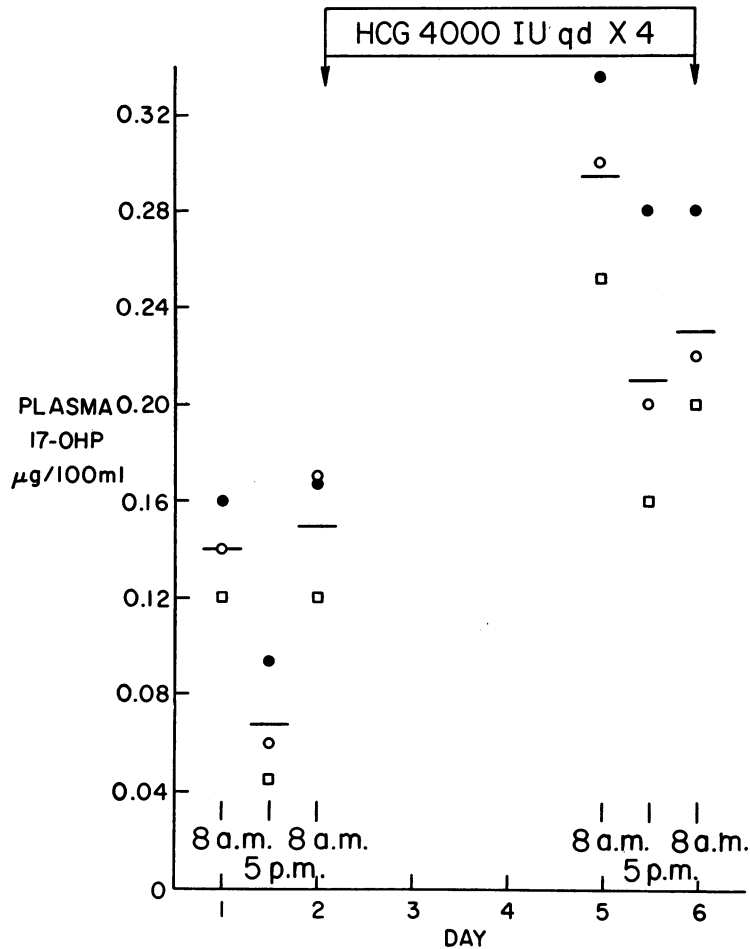


FIGURE 4 Plasma concentration of 17-hydroxyprogesterone (17-OHP) in three normal men before and during the administration of 4000 IU of HCG daily for 4 days. Each subject is represented by the same symbol. The solid lines for each set of data represents the mean.

production rate averaged 1.8 mg/24 hr. In CAH, production rates of 55 and 113 mg/24 hr were noted. No correction factor for plasma 17-OHP was used in these two studies.

DISCUSSION

There are many data suggesting that a small fraction of each intermediate in steroid biosynthesis "leaks" into the effluent vein and thereby becomes a secretory product. Thus, each intermediate in cortisol biosynthesis has been detected in the adrenal cortical venous effluent and many of the intermediates in testosterone and estrogen biosynthesis have been identified in the venous effluent from the testis and ovary respectively. Nevertheless, the production rate of 17-OHP is an unusually large fraction of the testosterone production rate. The mean testosterone production rate of normal men in this laboratory is 7

mg/24 hr and the 17-OHP production rate was 1.8 mg/24 hr or 25% of the testosterone production rate.

The reasons for this large production rate of 17-OHP by the testis are obscure. In the testis, 17-OHP is metabolized further only by the lyase catalyzing the conversion of 17-OHP to androstenedione. By contrast, in the adrenal cortex 17-OHP is hydroxylated and utilized for cortisol biosynthesis as well, and the lyase may, therefore, be of lesser importance in the further metabolism of 17-OHP. As we have shown, little 17-OHP is normally secreted by the adrenal cortex.

The results of our studies in normal men are clearly consistent with the origin of 17-OHP from the testis. This does not prove testicular secretion of 17-OHP since these data would also be consistent with secretion of a precursor and subsequent peripheral conversion to 17-OHP. This explanation would require a high secretion

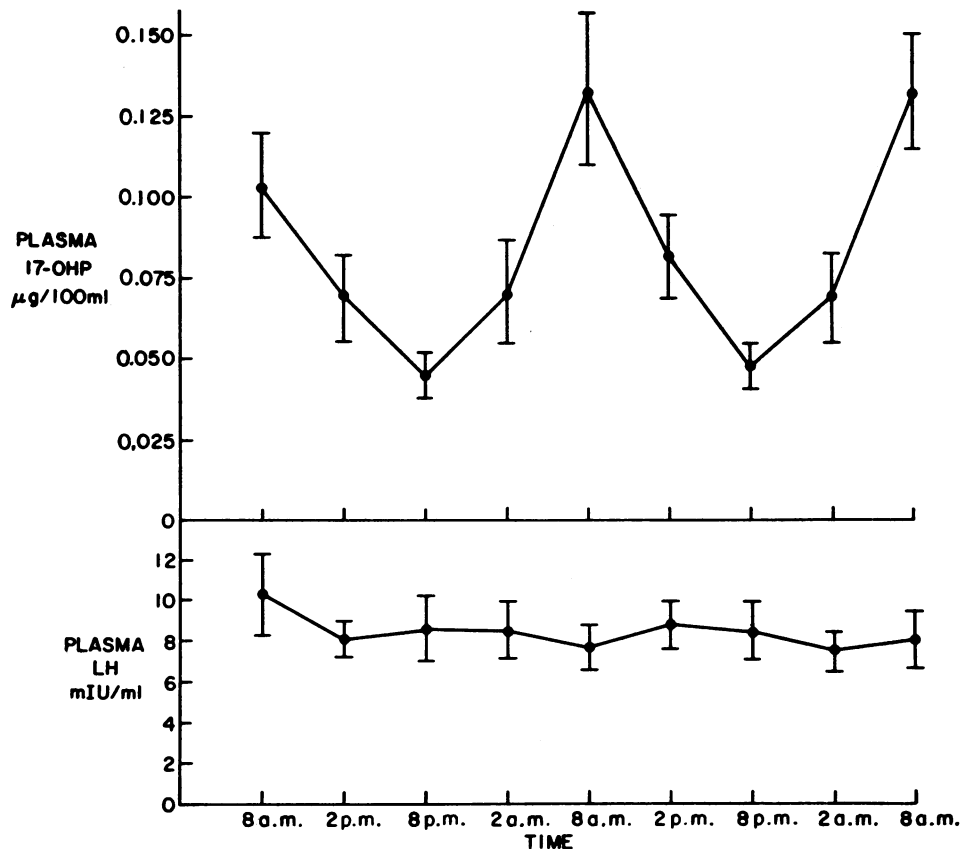


FIGURE 5 The mean plasma concentration ± 1 SE of 17-hydroxyprogesterone (17-OHP) and luteinizing hormone (LH) measured every 6 hr in nine normal men.

TABLE I
Circadian Variation of Plasma 17-Hydroxyprogesterone Levels

Subject	8 a.m.	2 p.m.	8 p.m.	% of 8 a.m. value	2 a.m.	8 a.m.	2 p.m.	8 p.m.	% of 8 a.m. value	2 a.m.	8 a.m.
	$\mu\text{g}/100 \text{ ml}$			%	$\mu\text{g}/100 \text{ ml}$			%	$\mu\text{g}/100 \text{ ml}$		
1	0.118	0.079	0.061	52	0.015	0.270	0.071	0.079	29	0.103	0.178
2	0.080	0.110	0.056	70	0.101	0.163	0.091	0.063	39	0.126	0.163
3	0.055	0.036	0.036	66	0.037	0.083	0.070	0.040	48	0.030	0.207
4	0.087	0.039	0.040	46	0.107	0.129	0.119	0.034	26	0.047	0.120
5	0.115	0.086	0.036	32	0.040	0.131	0.148	0.036	27	0.047	0.171
6	0.180	0.082	0.050	28	0.114	0.163	0.096	0.043	26	0.061	0.121
7	0.101	0.038	0.026	26	0.025	0.054	0.035	0.054	100	0.019	0.047
8	0.165	0.130	0.089	54	0.150	0.174	0.080	0.072	41	0.141	0.143
9	0.030	0.018	0.014	47	0.051	0.040	0.027	0.010	25	0.049	0.050
Mean	0.103	0.069	0.045	44	0.071	0.134	0.082	0.048	36	0.069	0.133
SD	0.047	0.038	0.022		0.047	0.070	0.035	0.021		0.043	0.055
SE	0.016	0.013	0.007		0.016	0.023	0.013	0.007		0.014	0.018

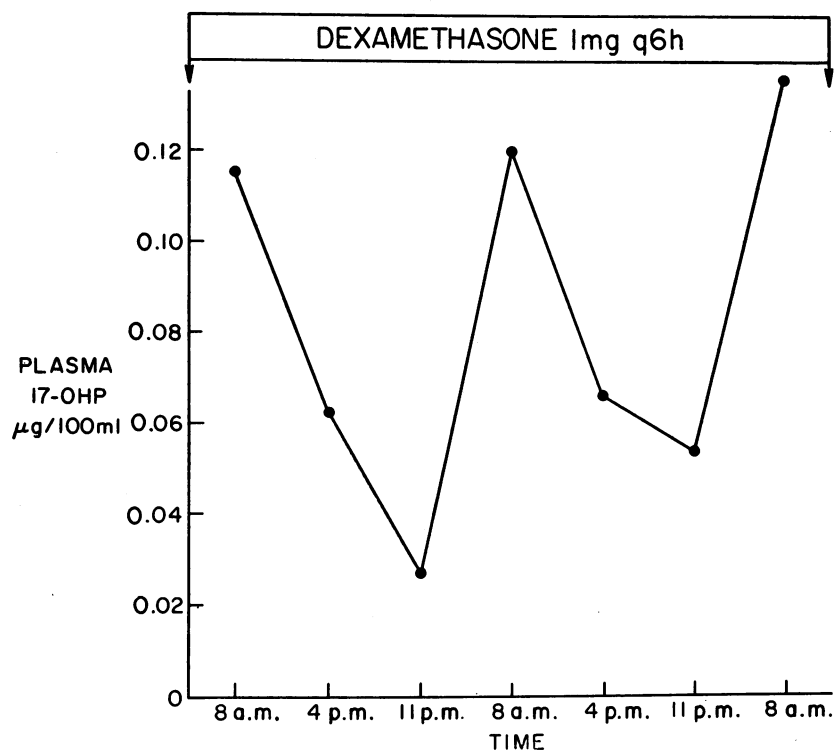


FIGURE 6 The mean concentration of plasma 17-hydroxyprogesterone (17-OHP) in three normal men receiving dexamethasone.

rate of the precursor and a large fractional conversion rate. The only ancillary evidence available is the analogous metabolism of the 3β -hydroxy Δ^5 function of dehydroepiandrosterone to the 3-keto Δ^4 group of androstenedione (6) where the fractional conversion rate was 6%. If the fractional conversion rate for 17-hydroxypregnenolone to 17-OHP were also 6%, then the production rate of 17-hydroxypregnenolone would have to be 3 mg to account for as much as 10% of the 17-OHP production rate. The possible role of 17-hydroxypregnenolone sulfate in 17-OHP production may be minimized since dehydroepiandrosterone sulfate is a poor precursor of androstenedione peripherally (7). It is therefore probable that 17-OHP is secreted rather than produced peripherally from a testicular precursor.

Previous studies of urinary pregnanetriol (8) have demonstrated mean differences in its excretion between men and women as we would predict but the ranges overlapped. This may be due in part to random sampling throughout the menstrual cycle and to methodologic problems in the analysis of urinary pregnanetriol. The finding that 17-OHP is derived from the Leydig cell was anticipated by the report that HCG increased urinary pregnanetriol excretion in men (9).

The circadian rhythm of plasma 17-OHP is of greater magnitude than that reported for plasma testosterone.

In those studies, the 7 to 10 p.m. testosterone concentrations were 63% (10), 61% (11), 73% (12), and 69% (13) of the 7 to 9 a.m. values. The reason for this difference is unknown. Since the plasma samples in this study were obtained after at least an hour's rest, the metabolic clearance rates could not have varied as a result of altered hepatic blood flow. Since there was no consistent change of the metabolic clearance rate from 9:00 a.m. to 7:00 p.m., it is unlikely that the profound swings in plasma 17-OHP are due to comparable changes in clearance rate; rather they must reflect the secretory activity of the Leydig cell.

The question of the existence of a circadian rhythm of plasma LH has been examined by many investigators and there is substantial agreement that there is no (4, 13-15) circadian rhythm. In two studies (13, 15) plasma testosterone and LH levels were measured simultaneously and, although there was a fall in plasma testosterone concentrations, plasma LH values did not parallel them. In our study, the LH measurements were made in a single radioimmunoassay; the coefficient of variation within an assay is 7-8%. Thus, only a circadian variation of this magnitude would fall within the error of the method and could thus remain undetected.

There are several possible explanations of the phenomenon of circadian variations of steroid secretory

products without concurrent changes in the plasma levels of the trophic hormone. First, there may be another trophic hormone that influences Leydig cell function. We have shown that it is not ACTH since the circadian rhythm was not interrupted by dexamethasone, and a large number of experiments in animals and humans make it unlikely that FSH is important for Leydig cell function. Furthermore, those maneuvers designed to increase or decrease plasma LH caused 17-OHP to change in the same direction as testosterone. Thus, there is no evidence that another trophic hormone is responsible for the circadian rhythm. Second, the postulated small and not detectable changes in plasma LH might be amplified in the reacting system, the Leydig cell, to give the observed changes in secretion rate. In the only analogous mammalian system, ACTH-adrenal cortex-cortisol, the changes in cortisol secretory rates or plasma levels are roughly proportional to plasma ACTH levels (16, 17). Finally, there remains the possibility that there is an intrinsic rhythm within the Leydig cell responsible for

the cyclic variations in testosterone and 17-OHP secretion.

The result of our studies in patients with CAH largely confirm the conclusions derived from measurements of urinary pregnanetriol and pregnanediol. Thus, the hallmark of the simple virilizing type of CAH due to 21-hydroxylase deficiency is the excretion of large amounts of pregnanetriol, the chief urinary metabolite of 17-OHP. The five subjects with CAH had high plasma 17-OHP concentrations, 50–200 times those of normal adult men. The comparison of the 17-OHP concentrations in CAH to those of adult men may be misleading since four of the five subjects with CAH were below 6 yr of age. It is probable that plasma 17-OHP concentration in normal children is below that of women in the follicular phase. Thus, plasma 17-OHP in CAH is probably more than 100–200 times that of normal subjects of comparable age. Since the corrected 17-OHP metabolic clearance rates were approximately the same in normal subjects and in patients with CAH, the plasma level of

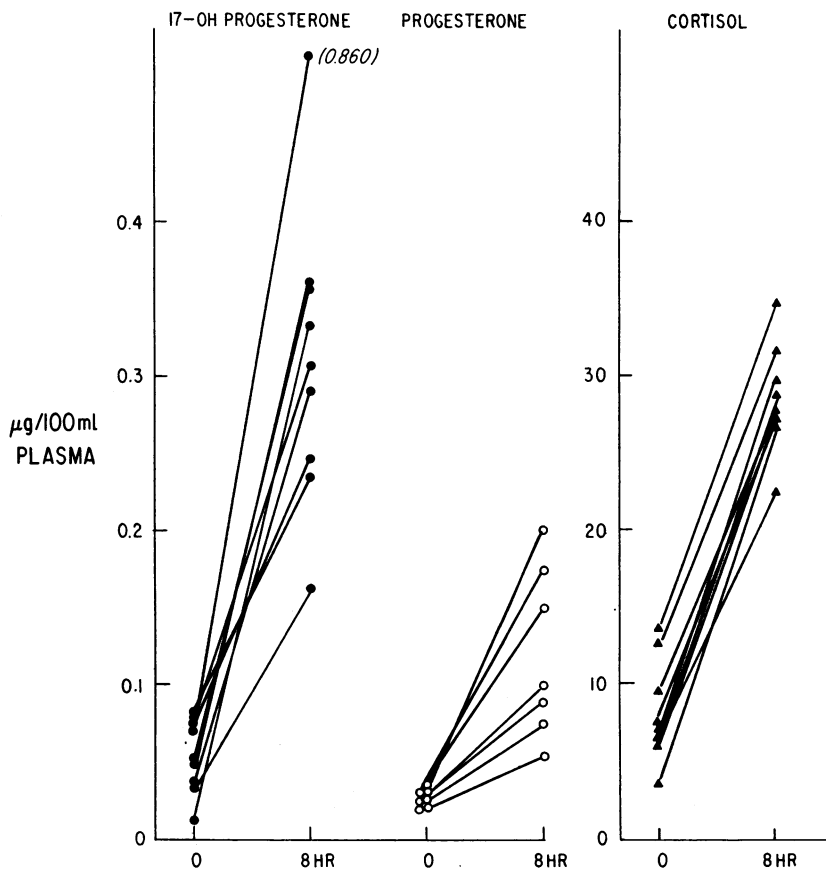


FIGURE 7 Plasma concentration of 17-hydroxyprogesterone, progesterone, and cortisol in normal women in the follicular phase of the menstrual cycle before and at the end of an 8 hr infusion of 40 U of ACTH.

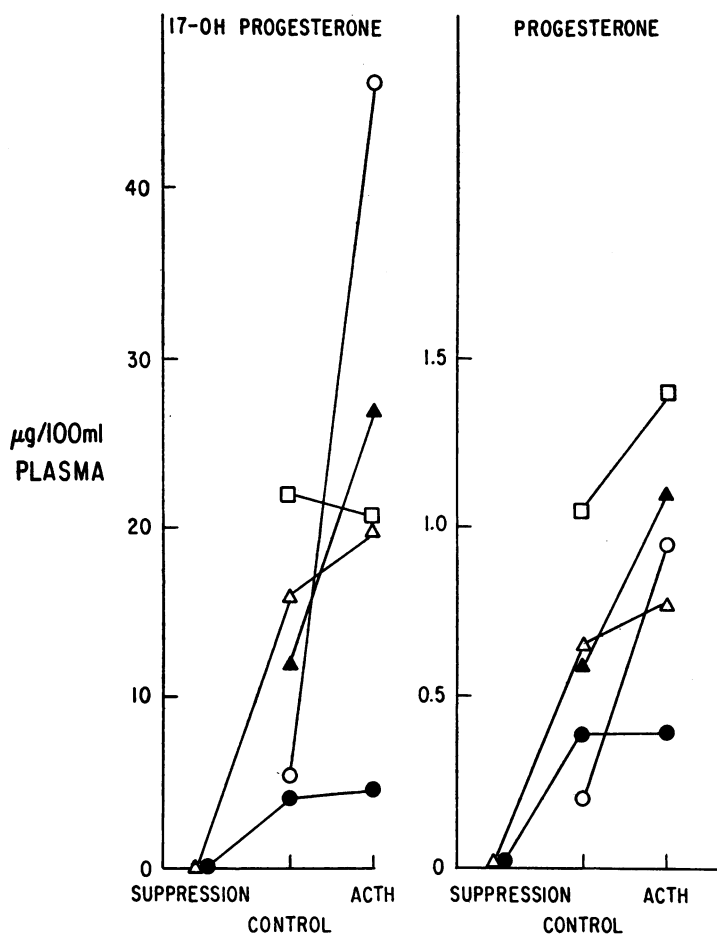


FIGURE 8 Plasma concentration of 17-hydroxyprogesterone and progesterone in five patients with CAH. Like symbols indicate the same patient. During the control period the patients received no therapy. 40 U of ACTH were infused over an 8 hr period and blood was drawn at the end of the infusion.

TABLE II
Plasma Clearance and Production Rates

	17-Hydroxyprogesterone					Progesterone				
	Normal men			CAH		Normal men			CAH	
	A	B	C	D	E	A	B	C	D	E
Plasma level, ($\mu\text{g}/100\text{ ml}$)	0.108	0.145	0.143	12.0	5.5	0.018	0.012	0.014	0.600	0.200
Calculated mean plasma level*	0.072	0.097	0.095							
Metabolic clearance rate (liters/24 hr)	1700	2100	2300	940	1100	2080	2060	2170	1090	1590
(liters/24 hr/m ²)	1100	1070	1190	1090	700	1370	1040	1120	1270	990
Blood production rate‡ (mg/24 hr)	1.1	1.8	1.9	113.0	55.0	0.360	0.250	0.300	6.5	3.3

* 67% of 8:00 a.m. value.

‡ Product of MCR and calculated mean plasma level.

17-OHP must be directly proportional to its production rate.

We have not examined the question of a circadian rhythm of plasma 17-OHP in CAH. We have assumed there was none, since stimulation of the adrenal cortex by ACTH as in Cushing's syndrome due to adrenal hyperplasia, abolishes the diurnal rhythm. We, therefore, used the 8 a.m. values for calculating production rates.

A production rate of 240 mg was reported in one subject with CAH using the urinary isotope dilution method (18). This should have been accurate since we have shown that urinary pregnanetriol in this disease is derived exclusively from plasma 17-OHP (5). The 17-OHP production rates obtained in normal subjects by the same method (18, 19) have been in the same range as ours suggesting that in normal subjects too urinary pregnanetriol is derived to a large extent from plasma 17-OHP.

There are surprisingly few data about pregnanediol excretion in CAH. Bergstrand and Gemzell (20) reported that it was increased in five children with CAH and it has been isolated in large amounts by several investigators (21). Our estimates of the plasma progesterone concentration and production rate show that the increased excretion of urinary pregnanediol in CAH results from the high production rate. It is probable that this increase is due to the inability of the 17 α -hydroxylase to convert all the precursor presented to it.

The variable response to exogenous ACTH in CAH is a consequence of the degree of endogenous stimulation of the adrenal cortex since the maximally stimulated gland will not respond to more ACTH. Plasma progesterone and 17-OHP in CAH decreased to normal with the administration of glucocorticoids as would be predicted from the physiology of the disease.

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