

# Human Puberty

## SIMULTANEOUS AUGMENTED SECRETION OF LUTEINIZING HORMONE AND TESTOSTERONE DURING SLEEP

R. M. BOYAR, R. S. ROSENFELD, S. KAPEN, J. W. FINKELSTEIN,  
H. P. ROFFWARG, E. D. WEITZMAN, and LEON HELLMAN

*From the Institute for Steroid Research, Departments of Oncology, Pediatrics,  
Psychiatry, and Neurology, Montefiore Hospital and Medical Center,  
Bronx, New York 10467*

**ABSTRACT** Plasma luteinizing hormone (LH) and testosterone (T) were measured by radioimmunoassay in nine pubertal boys and three sexually mature young men at 20-min intervals for 24 h. Plasma LH and T were also measured in one boy during a delayed sleep onset study. Polygraphic monitoring was carried out to identify precisely sleep onset, wakefulness, and specific sleep stages. In all nine pubertal boys the plasma T concentration fluctuated and was significantly higher during normal nocturnal sleep as compared to daytime waking. This increased T secretion during sleep was temporally linked to the characteristic pubertal sleep augmentation of LH secretion. To define further the relationship of this increased T secretion to sleep, plasma LH and T were also measured in three of the pubertal boys after acute (1-day) reversal of the sleep-wake cycle. One of these boys was also studied after 3 days of sleep-wake cycle reversal. The results of these studies showed that plasma T was now augmented during the reversed daytime sleep period; the mean T concentrations during this period were significantly higher ( $P < 0.001$ ) than during nocturnal waking in all four studies. Measurement of plasma LH and T in the three sexually mature young men showed episodic secretion of LH and T during both waking and sleep periods; there was no consistent significant augmentation of LH or T secretion during sleep. This study demonstrates that (a) in normal pubertal boys and sexually mature young men plasma T fluctuates epi-

sodically; (b) there is marked augmentation of T secretion during sleep in pubertal boys, which is dependent on increased LH secretion; (c) this pubertal LH-T secretory "program" is dependent on sleep, since it shifts with delayed sleep onset and reversal of the sleep-wake cycle; and (d) this demonstrable tropic effect of LH on T is evident only during puberty, since sexually mature young men fail to show any consistent relationship between LH and T secretion either awake or asleep.

### INTRODUCTION

Recent use of the combination of plasma sampling at frequent intervals, sensitive and specific radioimmunoassays, and polygraphic recording of sleep has led to a revised perspective of hormone-secretory dynamics. These procedures, employed throughout the complete 24-h sleep-wake cycle, allowed the recognition of the episodic secretion of cortisol (1-3), ACTH (4, 5), luteinizing hormone (LH)<sup>1</sup> (6-13) and follicle-stimulating hormone (FSH) (8, 11, 12) in adult men and women. The polygraphic monitoring of sleep showed the important role of sleep in the secretion of human growth hormone (14-17), human prolactin (18-20), LH and FSH in normal pubertal girls (21-24), and LH in pubertal boys (22, 23).

<sup>1</sup> *Abbreviations used in this paper:* CNS, central nervous system; FSH, follicle-stimulating hormone; LH, luteinizing hormone; 2nd IRP-HMG, second international reference preparation of human menopausal gonadotropin; NREM, non-rapid eye movement; REM, rapid eye movement; RIA, radioimmunoassay; T, testosterone.

A preliminary portion of this work has been reported as an abstract (1973. *J. Clin. Invest.* 52: 11a).

Received for publication 19 October 1973 and in revised form 26 February 1974.

Since the synchronous augmentation of LH secretion with sleep is found consistently in pubertal and sometimes in "late" prepubertal boys, an attempt was made to establish the importance of this phenomenon in initiating testosterone (T) secretion that results in the development of male secondary sexual characteristics during puberty. To establish firmly the importance of sleep in the control of nocturnal LH-T secretory program, plasma LH and T were measured by radioimmunoassay (RIA) in six normal pubertal boys at 20-min intervals throughout a control 24-h sleep-wake cycle, in one boy during a delayed sleep-onset study, and in three additional boys during control and acute sleep-wake cycle reversal study periods. To determine whether young adult men also show a consistent rise in plasma LH and T during sleep, three sexually mature young men had control studies following a protocol identical to that of the pubertal boys.

## METHODS

*Subjects and design.* Nine healthy pubertal boys (12–15 yr), with no history of medical illness and who were taking no medications, volunteered and were admitted to the sleep laboratory for a control study. The parents of the subjects gave informed, written consent. Three normal young men (19–24 yr) were also studied (one subject was studied on two occasions). During the control study, each subject was adapted to the sleep laboratory by sleeping with surface electrodes during the nocturnal period (2300–0700 h) before the blood sampling procedure. The following morning between 0800 and 1000 h a catheter was inserted into an antecubital vein, and 3-ml blood samples for measurement of plasma LH and T were obtained every 20 min for the next 24 h by a technique developed in our laboratory and published in detail (1–3). Before normal nocturnal sleep, the subjects were again fitted with surface electrodes for polygraphic monitoring of sleep electroencephalogram, chin electromyogram, and electrooculogram. In three of the subjects, plasma LH and T were measured during acute sleep-wake cycle reversal carried out approximately 1 mo after the control study. During this study, the subjects were kept awake the entire night before the insertion of the catheter. The following morning between 0900 and 1000 h, a catheter was inserted into an antecubital vein, and the subjects were allowed to sleep undisturbed for a period of 8 h while sleep was monitored polygraphically. When the subjects awoke from their daytime sleep, they were kept awake until blood samples had been taken for a complete 24-h period. One of these subjects was also studied after 3 days of acute sleep-wake reversal. The samples were immediately centrifuged, and plasma was frozen until processing. Sleep stages were scored in 30-s periods according to standardized criteria (25). Student's *t* test was used to assess statistical significance between the mean LH and T concentrations during sleep and waking periods. Pubertal stage was assigned according to the criteria of Tanner (26) from preadolescent (P1) to almost complete sexual maturity (P5). The intermediate stages (P2, P3, and P4) are based on progressive enlargement of the scrotum, testes, and penis.

*Plasma LH measurement.* Plasma LH was measured by the double-antibody radioimmunoassay described by Midg-

ley (27) as modified in our laboratory (9, 23). The highly purified LH (LER 960) used for radioiodination was provided by the National Institute of Arthritis, Metabolic, and Digestive Diseases. The specificity of the anti-human chorionic gonadotropin antibody (rabbit) has been reported previously (9). Parallel dose response curves were obtained with the second international reference preparation of human menopausal gonadotropin (2nd IRP-HMG), LER 907, and serum samples in the range reported in this study using our anti-human chorionic gonadotropin antibody. Results are expressed in terms of the 2nd IRP-HMG. 5  $\mu$ g of LH (LER 960) was iodinated with 1 mCi of  $^{125}$ I (Union Carbide Corp., New York) according to the method of Hunter and Greenwood (28). The reaction mixture was purified by cellulose adsorption chromatography (29). The intra-assay coefficient of variation for duplicate samples for the range of values reported in this study was 6–8%. All samples from each 24-h study were assayed in duplicate and in the same run to negate interassay variability. A 20% incremental change in successive LH values between 4 and 20 mIU/ml was considered outside the range that could be ascribed to assay variability and was considered a major LH secretory episode. Incremental changes of greater magnitude are required for establishing the validity of LH secretory episodes when the values fall between 1.0 and 3.5 mIU/ml. It should be recognized that if no LH secretion occurred, the succeeding plasma LH concentration should fall in accordance with the known half-life of this hormone. Therefore, even though an incremental rise in LH is not sufficient for statistical validity, it does not rule out the possibility of a secretory episode.

*Plasma T measurement.* [1,2,6,7- $^3$ H]T (sp act 100 Ci/mM) was obtained from New England Nuclear, Boston, Mass. It was purified on a Sephadex LH 20 column (35  $\times$  1 cm) with toluene:methanol 85:15 as the solvent phase. Radiochemical homogeneity (> 95%) was verified by reverse isotope dilution techniques. The anti-T (rabbit) antibody was prepared by immunizing with 3-carboxymethoximino-T hemocyanin (30). Scatchard (31) analysis gave a straight line over a considerable binding range,  $K_{diss}$ ,  $0.6 \times 10^{-10}$  M at 30°C. Portions of undiluted antiserum were kept in the deep freeze (–20°C). They were thawed and diluted 1:100 with 0.01 M Na phosphate buffer, and the primary dilution was frozen. Before use in the radioimmunoassay, a portion of this initial dilution of antibody was further diluted 1:120 (1:12,000) in phosphate buffer. The specificity of the antibody was evaluated by determining the amount of possible cross-reacting steroids that displaced 50% of the labeled T from the antibody. Of a wide range of steroids investigated, only dihydrotestosterone (100%), 19-nortestosterone (39%), androstenediol (20%), and androsterone (18%) gave a cross-reaction greater than 1%. To achieve maximal specificity of the T immunoassay, plasma extracts were chromatographed on Celite micro-columns that excluded all of the above cross-reacting steroids. Celite (Johns Mansville Products Corp., Celite Div., Denver, Colo.) was dried at 1,000°F overnight. The material (20 g) was then mixed with 10 ml of 1:1 ethylene glycol:propylene glycol by grinding with a glass rod for into a 5-ml Kimble disposable pipette (Kimble Products 10 min. Approximately 0.5 g of Celite was packed tightly Div., Owens Illinois, Inc., Toledo, Ohio) to 5 cm above a glass bead placed in the tip. 3.5 ml of iso-octane was then added to the column; the pipette tip was capped, and the unit was stored until used.

After the addition of 1,500–2,000 cpm [1,2,6,7- $^3$ H]T to 400  $\mu$ l-1 ml plasma in scintillation vials, 10 ml of anhydrous

ether was added. The vials were vortexed for 60 s and immersed in dry ice and acetone until the aqueous phase had frozen, and then the ether solutions were decanted into 15-ml conical centrifuge tubes, which were placed in a water bath at 45°C under nitrogen until the solvent was removed. The dried residues were resuspended in 1 ml iso-octane (freshly distilled) in the same tubes. After the Celite column was washed twice with 3.5 ml iso-octane and draining the solvent to the top of the packing, the 1 ml iso-octane extracts were applied, followed by a 0.5 ml iso-octane rinse of the tubes. When the level of the iso-octane extract had descended to the top of the Celite, stepwise elution was carried out with 3.5 ml of ethyl acetate:iso-octane (1:9) to remove the dihydrotestosterone fraction. This was followed by 3.5 ml of ethyl acetate:iso-octane (2:8) for elution of the T fraction. The T extract was then concentrated to dryness in a 45°C water bath under nitrogen and redissolved in 1 ml of a 0.01 M phosphate buffer containing 9.0 g NaCl, 1.0 g gelatin (pharmacy grade), 0.1 g sodium azide, 50 ml basic phosphate buffer (0.2 M, pH 7.8), and 950 ml deionized H<sub>2</sub>O. Duplicate 100- $\mu$ l samples were transferred to 10  $\times$  75-mm disposable glass tubes for immunoassay, and 500  $\mu$ l was removed for counting to estimate recovery. If increased sensitivity is required (i.e. <9 ng/100 ml), the dried extract can be redissolved in 300  $\mu$ l of buffer and 100- $\mu$ l samples in duplicate transferred for RIA and 100  $\mu$ l utilized for estimation of recovery. This would increase the sensitivity to approximately 3 ng/100 ml.

**Radioimmunoassay.** The two samples were gently mixed with 100  $\mu$ l of the diluted antibody and 4,000 cpm of [<sup>3</sup>H]T in 100  $\mu$ l of buffer. T standards (Steraloids, Inc., Pawling, N. Y.) with antibody and tracer testosterone were used to construct a six-point standard curve from 6.25 pg to 200 pg in quadruplicate. Control tubes (no antibody), maximal binding tubes (no standard T), T standards, and unknowns were then incubated at 60°C for 10 min (facilitating rapid approach to equilibrium) before immersion in a 30°C water bath for 30 min. All tubes were then chilled in an ice bath, and 0.5 ml of 1% charcoal (1 g Norit A, American Norit Co., Jacksonville, Fla., particle size 4–5  $\mu$ m, which had been washed with deionized water, dried, and suspended in 100 ml phosphate buffer) was added rapidly with an Oxford pipettor (Oxford Labs, Foster City, Calif.). The tubes were allowed to stand for 10 min in an ice bath with frequent gentle shaking and centrifuged at 2,000 rpm for 5 min at 4°C. The supernates were decanted into scintillation vials which contained 10 ml of scintillation liquid (25 g 2,5-diphenyl oxazole, 2.5 g dimethyl-1,4-bis-[2-(5-phenyloxazolyl)]benzene dissolved in 10 liters redistilled toluene) and were counted for 20 min in a Packard Tricarb Scintillation Counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Interpolation of the radioactivity in the sample from the standard curve utilizing the logit-log transformation permitted quantification of T in unknowns. Recoveries ranged from 58 to 96% with a mean  $\pm$ SD of 81.1 $\pm$ 7.9. Water blanks and plasma from an adrenalectomized-oophorectomized woman (blank plasma) showed consistently less than 6.25 pg of T. The least detectable dose in 400  $\mu$ l-1 ml of plasma, calculated from the least detectable dose in the standard curve, multiplied by sampling factors and procedural losses, varied from 9–20 ng/100 ml, but could be increased to 3–5 ng/100 ml by assaying 100  $\mu$ l of the 300  $\mu$ l redissolved plasma extract. The addition of unlabeled T to blank plasma at 100, 200, 400, and 800-pg dose levels yielded recoveries of (mean

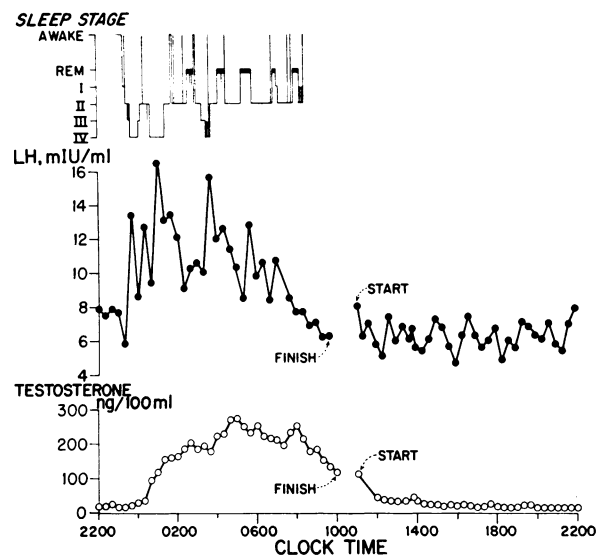


FIGURE 1 Plasma LH and testosterone every 20 min in subject 3. The histogram displaying sleep stage sequence is depicted above the period of nocturnal sleep. Sleep stages are REM (—) with stages I–IV shown by depth of line graph. Plasma LH (●—●—●) is expressed as mIU/ml 2nd IRP-HMG. Plasma T (○—○—○) is expressed as ng/100 ml.

$\pm$ SD) 106.2 $\pm$ 21.4, 189.4 $\pm$ 4.6, 386.9 $\pm$ 9.6, and 762.6 $\pm$ 14.7. The intra-assay and interassay coefficient of variation for T was 7.2% ( $n=20$ ) and 13.1% ( $n=14$ ), respectively. The 24-h mean plasma T concentration  $\pm$ SD (obtained by averaging the 72 results obtained during the course of a 20-min interval 24-h study) in adult men ( $n=8$ ) was 466  $\pm$ 149 ng/100 ml, and 20.6 $\pm$ 3.4 ng/100 ml in premenopausal women ( $n=5$ ).

## RESULTS

**Simultaneous augmented LH and T secretion during sleep.** Fig. 1 shows the 24-h LH and T secretory patterns derived from 20-min interval plasma sampling in one of the early pubertal subjects (subject 3). The sleep stage sequence is depicted above the period of nocturnal sleep (2320–0830). The augmented LH secretion associated with sleep resulted in a mean LH concentration of 11.0 mIU/ml compared with 6.3 mIU/ml during waking ( $P < 0.001$ ). The onset of augmented LH secretory activity at 2320 was synchronous with the onset of sleep, with the LH concentration rising from 5.9 to 13.4 mIU/ml. Plasma T began to rise at 0020 from 30 to 95 ng/100 ml at 0100. This rise in plasma T continued until 0240, reaching a T concentration of 240 ng/100 ml. The plasma T concentration was maintained while LH secretory episodes occurred at 2400 (8.6  $\rightarrow$  12.7 mIU/ml) and 0040 (9.4  $\rightarrow$  16.5 mIU/ml). The major LH secretory episode at 0320 (10.1  $\rightarrow$  15.6 mIU/ml) was followed 20 min later by an increase in the plasma T concentration from 180

TABLE I  
Mean Plasma LH and T in Pubertal Boys Asleep and Awake\*

Subject control	Age	Pubertal stage	LH†		T	
			Asleep	Awake	Asleep	Awake
	yr		mIU/ml		ng/100 ml	
1	15	P1	§3.7±0.9	2.3±0.42	26.5±8.8	<19.0
2	13	P2	§14.4±6.0	6.1±1.8	§70.8±20.0	33.0±14.7
3	14	P2	§11.0±2.3	6.3±1.2	§187±71.0	43.1±46.9
4	12	P3	§6.2±2.2	2.5±0.7	§278±92.7	180±72.9
5	15	P4	§6.0±1.7	2.8±1.1	§427±137	272±128
6	15	P4	§12.3±3.9	7.7±1.3	§371±122	288±122
Sleep delay study						
7	14	P3	¶4.9±2.1	4.0±0.7	§297±40.1	193±40.1

\* Mean values±SD.

† 2nd IRP-HMG.

§ Mean LH or T asleep significantly greater than mean LH or T awake ( $P < 0.001$ ).

|| All T values less than 19 ng/100 ml during waking. Insufficient plasma to repeat with sensitized method.

¶ Mean LH asleep significantly greater than mean LH awake ( $P < 0.05$ ).

to 285 ng/100 ml. The next major LH secretory episode occurred at 0520, with plasma LH rising from 8.5 to 12.8 mIU/ml. This was followed by only a small change in plasma T from 235 to 255 ng/100 ml at 0520. The last sleep-associated LH secretory episode was initiated at 0640, with plasma LH increasing from 8.4 to 10.7 mIU/ml. Plasma T began to rise 20 min after the LH peak, from 195 to 225 ng/100 ml. After the subject's awakening at 0835, plasma LH gradually fell from 10.7 to 6.3 mIU/ml at the termination of the

study. Plasma T also gradually fell from 255 to 120 ng/100 ml. During the awake period, there were lesser increases in LH at 1220 (5.1→7.4 mIU/ml), 1420 (5.4→7.4 mIU/ml), 1600 (4.7→7.4 mIU/ml), and at 2120 (5.4→7.9 mIU/ml), which failed to cause significant incremental rises in plasma T. The time of onset of T secretion after peak LH concentrations was usually 20 min. It should be noted that after the study was started (2305), plasma T gradually fell from 115 to 45 ng/100 ml. This fall after insertion of the catheter is frequently observed. It is important to note that the three major LH secretory episodes initiated during sleep occurred when the plasma T concentration was rising and close to the maximum concentration reached during the entire 24-h period. During sleep, the mean plasma T concentration was 187 ng/100 ml, compared with 43 ng/100 ml during waking ( $P < 0.001$ ). The other six pubertal boys also showed simultaneous augmented secretion of LH and T associated with sleep that resulted in significantly increased mean LH and T concentrations during sleep compared with waking (Table I).

*LH and T secretion in mid-puberty.* The 24-h LH and T secretory patterns of subject 5 in midpuberty are shown in Fig. 2. With the onset of sleep at 0025, plasma LH rose from 4.4 at 0100 to 8.9 mIU/ml. This slow, steady rise of the plasma LH concentration was followed by a gradual rise of the plasma T concentration from 175 ng/100 ml at 0120 to 500 ng/100 ml at 0300. This peak plasma T concentration occurred 20 min after the peak LH concentration at 0240. The similarity of the slopes of the LH and T curves is striking. Although the plasma T concentration was maintained

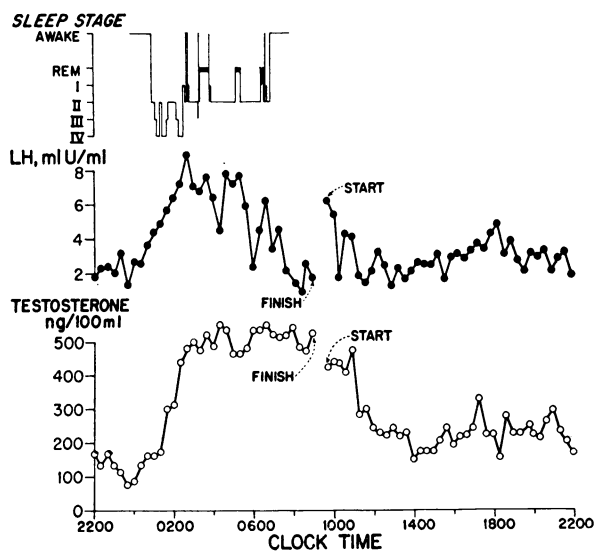


FIGURE 2 Plasma LH (●-●-●) and T (○-○-○) concentrations sampled every 20 min in subject 5, in mid-puberty.

between 455 and 550 ng/100 ml during the remainder of the sleep period, LH-secretory episodes were still initiated at 0200, 0320, and 0600. The first two LH-secretory episodes resulted in significant rises of plasma T from 490 to 550 ng/100 ml and 465 to 530 ng/100 ml, while the third resulted in a small rise from 530 to 550 ng/100 ml. Although the magnitude of these LH-secretory episodes was not dissimilar to the initial major LH secretory episode, the magnitude of the corresponding rise in T was much less. During waking, the plasma T concentration gradually fell to 150 ng/100 ml at 1400. Despite this low plasma T concentration, no major LH secretory episodes were initiated. Small incremental rises in plasma LH occurred during the day that were temporally related to small increases in T which occurred at 1040, 1500, 1600, 1820, 2020, and 2340. The augmentation of T secretion during sleep in this subject resulted in a mean  $\pm$ SD T concentration of  $427 \pm 137$  ng/100 ml compared with  $272 \pm 128$  ng/100 ml during waking ( $P < 0.001$ ). An additional mid-pubertal subject's LH and T secretory patterns are shown in Fig. 3. The mean T concentration  $\pm$ SD asleep was  $278 \pm 92.7$  compared with  $180 \pm 72.9$  awake ( $P < 0.001$ ).

*The effect of delayed sleep onset on T secretion.* To show that the increased T secretion during sleep is dependent on the sleep-associated augmented LH secretion, a delayed sleep onset study is shown in Fig. 4. During this study, sustained sleep did not occur until 0200 h, 3 h after this subject's usual sleep onset time. Note that with the first sustained period of stage IV sleep, a major LH secretory episode occurred, with plasma

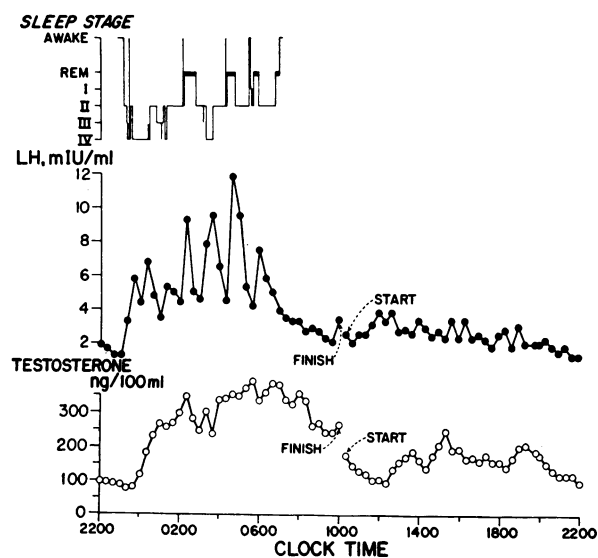


FIGURE 3 Plasma LH (●-●-●) and T (○-○-○) concentrations sampled every 20 min in subject 4 in mid-puberty.

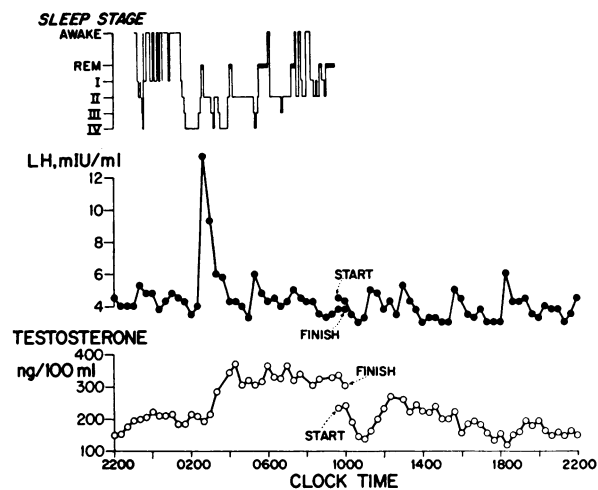


FIGURE 4 Plasma LH (●-●-●) and T (○-○-○) sampled every 20 min in subject 7. Because of frequent awakenings, sustained sleep onset did not occur until 0200 h. Premature frequent arousals also occurred from 0630 h until awakening.

LH rising from 4.0 to 13.3 mIU/ml. 20 min after the peak LH concentration of this secretory episode, plasma T rose from 215 to 370 ng/100 ml. The plasma T concentration was maintained at this higher nocturnal concentration by small rises in plasma LH occurring at 0500 (3.3  $\rightarrow$  6.0 mIU/ml) and 0640 (4.0  $\rightarrow$  5.0 mIU/ml). These were followed by small increments in the plasma T concentration. Although there were LH-secretory episodes during the day (1100, 1240, 1520, and 1800) equivalent in magnitude to those occurring during the later part of the night, the plasma T concentration continued to fall to its lowest concentration during the entire 24-h period at 1820 (120 ng/100 ml). The mean plasma T concentration during sleep was 297 ng/100 ml compared with 193 ng/100 ml during the awake period ( $P < 0.001$ ).

*The effect of acute sleep-wake reversal on T secretion.* To elucidate further the interrelationship of the increase in T secretion during the nocturnal hours to sleep and LH secretion, three of the pubertal subjects (subjects 7-9) had plasma LH and T measured after acute (1-day) reversal of the sleep-wake cycle. Subject 9 was also studied after 3 days of sleep-wake cycle reversal. The mean plasma T concentrations during sleep and waking in the control, 1-day, and 3-day reversal studies are shown in Table II. In all three subjects, LH (24) and T were significantly increased during both nocturnal (control) and diurnal (reversal) sleep periods compared with plasma LH and T during diurnal (control) and nocturnal (reversal) waking periods. The augmentation of LH secretion during diurnal sleep is similar to what we have previously re-

TABLE II  
Mean Plasma T Asleep and Awake during Control and Sleep-Wake Reversal\*

Subject 1-day reversal	Age	Pubertal stage	Control T		Reversal T	
			Asleep	Awake	Asleep	Awake
	yr				ng/100 ml	
7	14	P4	‡336±27.6	290±78	§322±55.8	264±44.4
8	14	P4	575±65.7	536±91	§590±36.1	355±109
9	14	P3	§333±99.8	186±106	§416±55.3	270±76.5
3-day reversal						
9	14	P3	§333±99.8	186±106	§314±121	178±98.1

\* Mean values±SD.

‡ Mean T asleep significantly greater than mean T awake, ( $P < 0.01$ ).

§ Mean T asleep significantly greater than mean T awake; ( $P < 0.001$ ).

|| Mean T asleep significantly greater than mean T awake; ( $P < 0.05$ ).

ported after acute shifting of the time of sleep (21); however, during nocturnal waking a persistence of augmented LH secretion was found. This failure to completely obliterate augmented LH secretion during nocturnal waking after 1-day sleep-wake reversal has been a consistent finding in pubertal boys and is the subject of a separate report from this laboratory (24).

In an attempt to obtain more complete reversal of the LH and T secretory patterns, Subject 9 was restudied after 3 days of sleep-wake cycle reversal. The

results of this study are shown in Fig. 5. After insertion of the catheter, the plasma T concentration fell from 310 to 180 ng/100 ml. Between 0940 and 1300, the plasma T concentration varied from 140 to 220 ng/100 ml. The first major LH-secretory episode was initiated at 1200 with plasma LH rising from 6.5 to 20.5 mIU/ml. 20 min after this peak LH concentration, plasma T rose from 190 to 290 ng/100 ml. A further rise in plasma T occurred at 1400 (260–360 ng/100 ml), associated with a change in the rate of decline of the LH concentration. It has been clearly shown in 5-min interval plasma-sampling studies that a slope of plasma cortisol (5) or LH<sup>2</sup> decline that exceeds the half-lives of these hormones is uniformly associated with small secretory episodes missed by the 20-min interval sampling mode. 20 min after the peak LH concentration of the second major LH-secretory episode at 1440 (8.2→13.6 mIU/ml), plasma T rose from 250 to 480 ng/100 ml. The third and most dramatic LH-secretory episode resulted in a rise of plasma LH from 11.7→45.6 mIU/ml. The plasma T-secretory episodes that resulted from this LH secretory episode were evident at 1640 (300→410 ng/100 ml) and 1740 (210→720 ng/100 ml). After the subject's awakening, the plasma T concentration gradually fell from 720 ng/100 ml at 1840 to 70 ng/100 ml at 0040. During nocturnal waking, LH secretory episodes were evident at 2340, 0320, 0640 and were followed by T-secretory episodes at 2440 (70→190 ng/100 ml), 0400 (70→210 ng/100 ml), 0500 (160→210 ng/100 ml), 0640 (90→150 ng/100 ml), and 0720 (100→240 ng/100 ml). The mean±SD LH and T concentrations during daytime sleep were 15.8±8.3 mIU/ml and 314±121 ng/100 ml, compared with 9.4±1.9 mIU/ml and 178±98.1 ng/100 ml during nocturnal waking ( $P < 0.001$ ). Comparison of these

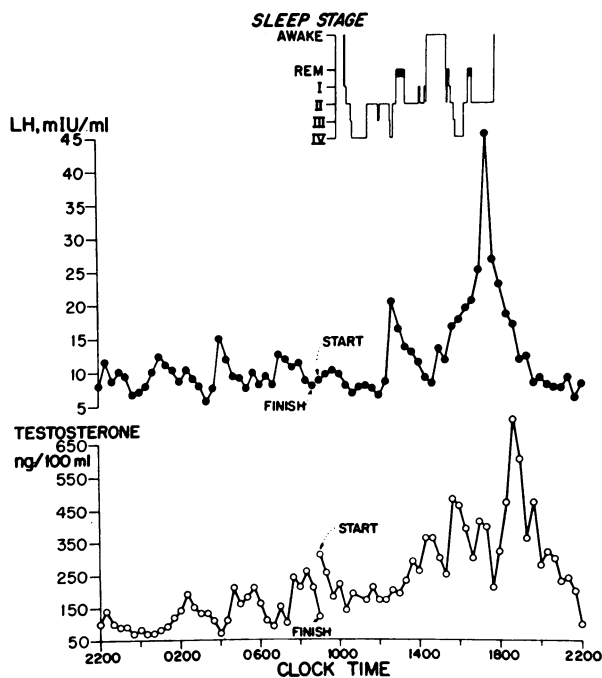


FIGURE 5 Plasma LH (●-●-●) and T (○-○-○) sampled every 20 min in subject 9 after 3-day sleep-wake cycle reversal. The sleep histogram shows that diurnal sleep onset occurred at 1025 h and awakening at 1750 h.

<sup>2</sup> M. Perlow, and R. M. Boyar. Unpublished observations.

TABLE III  
Mean Plasma LH and T in Sexually Mature Young Men Asleep and Awake\*

Subject	Age	LH†		T	
		Asleep	Awake	Asleep	Awake
	yr	mIU/ml		ng/100 ml	
10	19	6.1 ± 1.2	7.1 ± 1.7	399 ± 80	413 ± 153
	19	§6.3 ± 1.2	5.3 ± 1.5	291 ± 58.3	277 ± 126
11	24	8.6 ± 2.0	8.4 ± 1.7	376 ± 59.6	346 ± 57.3
12	23			§502 ± 50.5	452 ± 72.0

\* Mean values ± SD.

† 2nd IRP-HMG.

§ Mean LH or T asleep significantly greater than mean LH or T awake ( $P < 0.02$ ).

mean T concentrations with this subject's control study showed a complete reversal of the mean T concentrations (Table II).

*LH and T secretion in sexually mature young men.* Plasma LH and T were also measured at 20-min intervals in three young sexually mature men (19–24 yr) by a protocol identical to the control studies carried out in the pubertal subjects (Table III). In agreement with our earlier work (9), these young adult men showed no consistent augmentation of LH secretion associated with sleep. Further, the major T-secretory activity was no longer associated with the sleep period but rather was spread equally throughout the entire 24-h period. Only in one of the four studies was there a statistically significant increase in T secretion during sleep (subject 12,  $P < 0.02$ ). Subject 10 was studied over two 24-h periods and there was no consistency between the 24-h mean plasma T concentrations or secretory patterns from the first 24-h study to the second 24-h study. During all four studies, there was episodic secretion of T but no obvious consistent relationship between LH and T secretory activity. There were frequent periods when plasma T was low and LH secretory activity was also quiescent, and as we observed in the pubertal subjects, LH-secretory episodes were initiated when the plasma T concentration was rising.

## DISCUSSION

Puberty in normal boys is characterized by an increase in the rate of growth and the development of secondary sexual characteristics. The mechanism(s) responsible for the initiation of puberty are incompletely understood. Although LH and FSH have been detected by both bioassay (32) and RIA (33) in the urine of prepubertal children, it is not known whether a sharp rise in either or both hormones occurs coincident with the development of secondary sexual characteristics. With the development of sensitive and specific RIA methods for the measurement of LH (27) and FSH

(34) in plasma, it became possible to measure these hormones at frequent intervals during a 24-h period. A previous study from this laboratory (21) reported the synchronization of augmented LH secretion with sleep in pubertal children. This increased pulsatile LH-secretory activity during sleep showed a similar periodicity to the rapid-eye movement (REM)-non-REM sleep cycle. Since this LH-secretory program was not consistently found in prepubertal children or sexually mature adults, it provided a new biologic index for the identification of puberty (21).

To show the importance of this pubertal LH-secretory program in stimulating the development of secondary sexual characteristics, it was critical to show that LH-related increases in T occurred during sleep in pubertal boys. The results of this study clearly show that sleep-related increases in T occur during puberty and are stimulated by the pubertal sleep-related secretory activity of LH. This dramatic temporal relationship between LH and T during sleep in pubertal boys was not consistently found in prepubertal children (35), or sexually mature men.<sup>3</sup> The interrelation of sleep, LH, and T was further documented by finding that a delay in the onset of sustained sleep resulted in a corresponding delay in the onset of LH and T secretion and that reversal of the normal sleep-wake cycle was accompanied by a reversal of the higher mean LH and T concentrations to the daytime sleep period.

These results show the important role of the central nervous system (CNS) in initiating the development of secondary sexual characteristics characteristic of the pubertal state. The mechanism determining what sets this sleep-related LH-secretory program off at the age of puberty is completely unknown. Earlier studies from this laboratory have shown that two patients with untreated congenital adrenal hyperplasia (8.5 and 5.8 yr) with ad-

<sup>3</sup>H. P. Roffwarg, E. J. Sachar, F. Halpern, and L. Hellman. Plasma testosterone and sleep: relationship to sleep stage variables. Submitted for publication.

vanced bone age showed augmented LH secretion synchronous with sleep, similar to normal older pubertal boys. These observations suggested that the prolonged exposure to increased concentrations of adrenal androgens may result in premature CNS maturation and earlier activation of the pubertal LH program. An alternative explanation is that in this disorder the rapid increase in body weight due to excessive adrenal androgen secretion causes earlier attainment of the "critical body weight" for human development (36), which in some way activates the pubertal LH program. The suggestion has been made that the attainment of the critical body weight is associated with a change in the metabolic rate that reduces the sensitivity of the hypothalamic-pituitary gonadotropin axis to sex steroids (37). This sequence of events has been suggested to be responsible for the initiation of gonadotropin secretion at the onset of puberty.

The pubertal LH-T secretory relationship described in this study is difficult to explain solely on the basis of negative feedback control involving the gonadal-pituitary axis. A similar problem concerning the adrenocortical-pituitary axis was encountered in attempting to understand the mechanism controlling ACTH and cortisol secretion when it was found that both hormones were secreted episodically and synchronously in close temporal proximity (2-5). In the present study concerning the secretion of LH and T, there were many instances when high concentrations of T failed to suppress or delay the initiation of LH secretion. Indeed, during nocturnal sleep periods LH-secretory episodes were often maximal when plasma T concentrations were highest. On the other hand, there were instances when plasma T was low or declining and LH secretion was not initiated. These observations are more compatible with a CNS-regulated secretory program than the "classical" feedback endocrine mechanism controlling the secretion of LH and T in the pubertal boy. Although this sleep-synchronized LH-secretory program would appear to be of hypothalamic origin via the episodic secretion of gonadotropin-releasing hormone, recent data in monkeys (38) show that LH secretion may be episodic with constant gonadotropin-releasing hormone stimulation, suggesting a possible role of the pituitary in the pulsatile secretion of LH.

The absence of a consistent temporal relationship between LH and T secretion in sexually mature young men in this study as well as in the adult men studied by others (39, 40) is puzzling. It is possible that with advancing sexual maturation, the sleep-related pubertal LH-T secretory program matures or is modified to become in part governed by feedback influences. This hypothesis is supported by the decrease in the 24-h mean LH concentration in adult men compared with late-pu-

bertal boys (21). Therefore, the interplay between two hormone secretory control mechanisms (CNS program and classical feedback) in the adult male makes for a more complex system, so that a tropic effect of LH on T is not always discernible. Perhaps more frequent plasma sampling (e.g. 5-min intervals) than the 20-min intervals utilized in this study will better characterize the LH-T secretory relationship in sexually mature men.

The absence of a consistent increase in the mean sleep T concentration compared with waking in the adult men in this study has been extended to additional subjects in a recent report from this laboratory.<sup>3</sup> Although a nocturnal rise in T was found in four subjects reported by Judd, Parker, Rakoff, Hopper, and Yen (41), there were few waking samples obtained to compare adequately to the mean T concentrations during sleep. Although a nocturnal rise in T occurs in some adult men, it is not a consistent phenomenon, nor does it uniformly result in higher mean sleep T concentrations compared with waking. The paucity of 24-h frequent sampling studies with polygraphic monitoring of sleep makes most previous studies inadequate in terms of providing additional useful data.

The results of this study clearly establish that the sleep-associated augmentation of LH secretion stimulates T secretion in pubertal boys. Since this sleep-related increase in T is sometimes found in prepubertal boys (e.g. subject 1) before "clinical" puberty, it appears to be an additional biologic index for the identification of puberty initiation. Longitudinal studies during sexual maturation and into adulthood should lead to a more complete characterization of the sequential changes in this unique sleep-related LH-T relationship.

#### ADDENDUM

After these studies were submitted, Judd, Parker, Siler, and Yen (*J. Clin. Endocrinol. Metab.* **38**: 710, 1974.) submitted a Rapid Communication concerning similar findings in a pair of early pubertal twin boys.

#### ACKNOWLEDGMENTS

The authors appreciate the technical assistance of Anne Thalassinos, Mira Fein, Sue Durling, and Marilyn Hurler. Dr. E. Nieschlag, Dusseldorf Germany, provided the testosterone antibody and the National Institute of Arthritis, Metabolic, and Digestive Diseases provided the highly purified LH (LER 960).

This work was supported by the following U. S. Public Health Service research grants: CA 07304, ROH 00331, HD 06209, HL-14734, and RR-53.

#### REFERENCES

1. Weitzman, E. D., H. Schaumberg, and W. Fishbein. 1966. Plasma 17-hydroxycorticosteroid levels during sleep in man. *J. Clin. Endocrinol. Metab.* **26**: 121-127.
2. Hellman, L., F. Nakada, J. Curti, E. D. Weitzman, J. Kream, H. Roffwarg, S. Ellman, D. Fukushima, and



- T. Gallagher. 1970. Cortisol is secreted episodically by normal man. *J. Clin. Endocrinol. Metab.* **30**: 411-422.
3. Weitzman, E. D., D. Fukushima, C. Nogeire, H. Roffwarg, T. F. Gallagher, and L. Hellman. 1971. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J. Clin. Endocrinol. Metab.* **33**: 14-22.
  4. Berson, S. A., and R. S. Yalow. 1968. Radioimmunoassay of ACTH in plasma. *J. Clin. Invest.* **47**: 2725-2751.
  5. Gallagher, T. F., K. Yoshida, H. D. Roffwarg, D. K. Fukushima, E. D. Weitzman, and L. Hellman. 1973. ACTH and cortisol secretory patterns in man. *J. Clin. Endocrinol. Metab.* **36**: 1058-1068.
  6. Midgley, A. R., Jr., R. B. Jaffe. 1971. Regulation of human gonadotropins: X. Episodic fluctuation of LH during the menstrual cycle. *J. Clin. Endocrinol. Metab.* **33**: 962-969.
  7. Nankin, H. R., and P. Troen. 1971. Repetitive luteinizing hormone elevations in serum of normal men. *J. Clin. Endocrinol. Metab.* **33**: 558-560.
  8. Rubin, R. T., A. Kales, R. Adler, T. Fagan, and W. Odell. 1972. Gonadotropin secretion during sleep in normal men. *Science (Wash. D. C.)*. **175**: 196-198.
  9. Boyar, R. M., M. Perlow, L. Hellman, S. Kapen, and E. Weitzman. 1972. Twenty-four hour pattern of luteinizing hormone secretion in normal men with sleep stage recording. *J. Clin. Endocrinol. Metab.* **35**: 73-81.
  10. Kapen, S., R. Boyar, L. Hellman, and E. D. Weitzman. 1973. Episodic release of luteinizing hormone at mid-menstrual cycle. *J. Clin. Endocrinol. Metab.* **36**: 724-729.
  11. Yen, S. S. C., C. C. Tsai, F. Naftolin, G. Vandenberg, and L. Ajabor. 1972. Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. *J. Clin. Endocrinol. Metab.* **34**: 671-675.
  12. Krieger, D. T., R. Ossowski, M. Fogel, and W. Allen. 1972. Lack of circadian periodicity of human serum FSH and LH levels. *J. Clin. Endocrinol. Metab.* **35**: 619-623.
  13. Kapen, S., R. Boyar, M. Perlow, L. Hellman, and E. D. Weitzman. 1973. Luteinizing hormone: changes in secretory pattern during sleep in adult woman. *Life Sci.* **13**: 693-701.
  14. Takahashi, Y., D. M. Kipnis, and W. H. Daughaday. 1968. Growth hormone secretion during sleep. *J. Clin. Invest.* **47**: 2079-2090.
  15. Honda, Y., K. Takahashi, S. Takahashi, K. Azumi, M. Irie, M. Sakuma, T. Tsushima, and K. Shizume. 1969. Growth hormone secretion during nocturnal sleep in normal subjects. *J. Clin. Endocrinol. Metab.* **29**: 20-29.
  16. Sassin, J. F., D. C. Parker, J. W. Mace, R. W. Gotlin, L. C. Johnson, and L. G. Rossman. 1969. Human growth hormone release: relation to slow-wave sleep and sleep-waking cycles. *Science (Wash. D. C.)*. **165**: 513-515.
  17. Parker, D. C., J. F. Sassin, J. W. Mace, R. W. Gotlin, and L. G. Rossman. 1969. Human growth hormone release during sleep: electroencephalographic correlation. *J. Clin. Endocrinol. Metab.* **29**: 871-874.
  18. Sassin, J. F., A. G. Frantz, E. D. Weitzman, and S. Kapen. 1972. Human prolactin: 24-hour pattern with increased release during sleep. *Science (Wash. D. C.)*. **177**: 1205-1207.
  19. Parker, D. C., L. G. Rossman, and E. F. VanderLaan. 1973. Sleep-related, nyctohemeral and briefly episodic variation in human plasma prolactin concentrations. *J. Clin. Endocrinol. Metab.* **36**: 1119-1124.
  20. Sassin, J. F., A. G. Frantz, S. Kapen, and E. D. Weitzman. 1973. The nocturnal rise in prolactin is dependent on sleep. *J. Clin. Endocrinol. Metab.* **37**: 436-440.
  21. Boyar, R., J. Finkelstein, H. Roffwarg, S. Kapen, E. Weitzman, and L. Hellman. 1972. Synchronization of augmented luteinizing hormone secretion with sleep during puberty. *N. Engl. J. Med.* **287**: 582-586.
  22. Boyar, R., J. W. Finkelstein, R. David, H. Roffwarg, S. Kapen, E. Weitzman, and L. Hellman. 1973. Twenty-four patterns of plasma luteinizing hormone and follicle-stimulating hormone in sexual precocity. *N. Engl. J. Med.* **289**: 282-286.
  23. Boyar, R., J. Finkelstein, H. Roffwarg, S. Kapen, E. D. Weitzman, and L. Hellman. 1973. Twenty-four-hour luteinizing hormone and follicle-stimulating hormone secretory patterns in gonadal dysgenesis. *J. Clin. Endocrinol. Metab.* **37**: 521-525.
  24. Kapen, S., R. M. Boyar, J. W. Finkelstein, L. Hellman, and E. D. Weitzman. 1974. Effect of sleep-wake cycle reversal on LH secretory pattern in puberty. *J. Clin. Endocrinol. Metab.* **39**: 283-289.
  25. Kales, A., and A. Rechtschaffen, editors. 1968. U. S. Public Health Service Manual of Standardized Terminology, Techniques and Scoring Systems for Sleep Stages of Human Subjects. U. S. Government Printing Office, Washington, D. C.
  26. Tanner, J. M. 1962. Growth at adolescence: with a general consideration of the effects of heredity and environmental factors upon growth and maturation from birth to maturity. 2nd edition. Blackwell Scientific Publications, Ltd., Oxford. 32.
  27. Midgley, A. R., Jr. 1966. Radioimmunoassay: a method of human chorionic gonadotropin and human luteinizing hormone. *Endocrinology*. **79**: 10-18.
  28. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.)*. **194**: 495-496.
  29. Jeffcoate, S. L. 1971. Purification of <sup>125</sup>I-LH and <sup>125</sup>I-FSH by cellulose adsorption chromatography. In Radioimmunoassay methods. K. E. Kirkham and W. M. Hunter, editors. The Williams & Wilkins Company, Baltimore. 30-37.
  30. Nieschlag, E., and D. L. Loriaux. 1972. Radioimmunoassay for plasma testosterone. *Z. Klin. Chem. Klin. Biochem.* **10**: 164-168.
  31. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**: 660-672.
  32. Rifkind, A. B., H. Kulin, and G. T. Ross. 1967. Follicle-stimulating hormones (FSH) and luteinizing hormone (LH) in the urine of prepubertal children. *J. Clin. Invest.* **46**: 1925-1931.
  33. Rifkind, A. B., H. E. Kulin, P. L. Rayford, C. M. Cargille, and G. T. Ross. 1970. 24-hour urinary luteinizing hormone (LH) and follicle stimulating hormone (FSH) excretion in normal children. *J. Clin. Endocrinol. Metab.* **31**: 517-525.
  34. Midgley, A. R., Jr. 1967. Radioimmunoassay for human follicle-stimulating hormone. *J. Clin. Endocrinol. Metab.* **27**: 295-299.
  35. Boyar, R. M., R. Rosenfeld, J. Finkelstein, S. Kapen, H. Roffwarg, E. D. Weitzman, and L. Hellman. 1974. The ontogeny of luteinizing hormone (LH) and testosterone

- (T) secretion during puberty. *Clin. Res.* **22**: 556. (Abstr.)
36. Frisch, R. E., and R. Revelle. 1970. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science (Wash. D. C.)*. **169**: 397-399.
37. Frisch, R. E., and R. Revelle. 1969. The height and weight of adolescent boys and girls at the time of peak velocity of growth in height and weight: longitudinal data. *Hum. Biol.* **41**: 536-559.
38. Ferin, M., S. Khalaf, M. Warren, I. Dyrenfurth, R. Jewelewicz, W. White, R. L. VandeWiele. Effects of continuous infusions of synthetic LH releasing hormone (LRH) in Rhesus monkeys. In program of the 55th Annual Meeting of the Endocrine Society. Chicago, Ill. June 1973. 198. (Abstr.)
39. Murray, M. A. F., and C. S. Corker. 1973. Levels of testosterone and luteinizing hormone in plasma samples taken at 10-minute intervals in normal men. *J. Endocrinol.* **56**: 157-158.
40. Naftolin, F., H. L. Judd, and S. S. C. Yen. 1973. Pulsatile patterns of gonadotropins and testosterone in man: the effects of clomiphene with and without testosterone. *J. Clin. Endocrinol. Metab.* **36**: 285-288.
41. Judd, H. L., D. C. Parker, J. S. Rakoff, B. R. Hopper, and S. S. C. Yen. 1974. Elucidation of mechanism(s) of the nocturnal rise of testosterone in men. *J. Clin. Endocrinol. Metab.* **38**: 134-141.