Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 793–801. Pergamon Press. Printed in Great Britain

# RHYTHMS IN THE SECRETION OF GONADOTROPINS AND GONADAL STEROIDS

H. W. G. BAKER, R. J. SANTEN, H. G. BURGER, D. M. DE KRETSER, B. HUDSON, R. J. PEPPERELL and C. W. BARDIN

Medical Research Centre, Prince Henry's Hospital and Howard Florey Institute of Experimental Physiology and Medicine, Melbourne, Australia and Department of Medicine, Division of Endocrinology, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, Pennsylvania 17033, U.S.A.

## SUMMARY

The occurrence of short term variations in the blood levels of pituitary gonadotropins and sex steroids is reviewed.

1. The oscillations of LH levels are regular in timing and form and are almost certainly the result of episodic secretion by the pituitary in response to pulses of LRH from the hypothalamus.
2. Sleep modifies the pattern of LH secretion in pubertal subjects and in women during the follicular phase of the menstrual cycle.
3. The frequency and amplitude of the LH pulses are altered during the menstrual cycle and following testosterone and estradiol administration suggesting that the gonadal steroids modulate the episodic secretion of LH.
4. Some patients with hypogonadotropism have LH pulses of normal proportional amplitude even though the mean levels are low, others have diminished or absent pulses with normal or low mean levels.
5. Although the levels of FSH are less varied than those of LH, the oscillations tend to coincide.
6. Testosterone levels exhibit an irregular circhoral rhythm in men and the oscillations are not consistently related to the LH pulses. It is possible that there is a variable time lag in the Leydig cell response to LH and it seems clear that factors other than LH contribute to the variability of the testosterone levels.
7. There is a small amplitude circadian rhythm of testosterone secretion, at least in young men but its mechanism is uncertain.
8. There is some evidence for episodic fluctuations of estradiol levels in women and there may also be a circadian rhythm.
9. The biological significance of the high frequency rhythms in the levels of these hormones must remain speculative at present. It may be that the oscillations allow the transmission of a signal more efficiently than would a constant hormone level.

## INTRODUCTION

The recent availability of sensitive and precise radioligand assays has facilitated study of hormonal rhythms. Recent investigations have demonstrated that many hormones are secreted episodically rather than continuously. The present review will concentrate on the short term fluctuations in the circulating levels of pituitary gonadotropins and sex steroids. After a brief comment on analytical methods, some aspects of the rhythms of LH, FSH, testosterone and estradiol levels in healthy and diseased humans will be examined. Then, the possible significance of such rhythms will be considered. Work performed in our laboratories in Melbourne and Hershey will be emphasized but it is acknowledged that important contributions in this field have come from a number of other laboratories, particularly those of Yen and Weitzman.

*General definitions.* A rhythm may be defined broadly as a repetitive sequence. A systematic terminology for bio-rhythms based upon their periodicity has been recommended by Halberg[1]; however, at present, a number of terms are used synonymously (Table 1). High frequency rhythms with periods of approximately one hour are called circhoral rhythms. This term was used by Dierschke *et al.*[2] to describe the hourly oscillations of LH levels in castrate monkeys and has also been used for the similar fluctuations in man even though the periodicity frequently exceeds 1 h. Circadian rhythms have a period of approximately 24 h and may be divided into those rhythms either tightly or loosely entrained by the sleep–wake cycle. The menstrual cycle is a well known example of a rhythm with a longer period. There is also evidence for low frequency rhythms of testicular function with periods ranging from several days to one year [1, 3–5]. The latter may represent a seasonal rhythm in man. Further information on human reproductive rhythms may be found in a recently published book [5].

### *Methods of examination of hormonal rhythms*

*General principles.* The principles of data collection and several approaches to analysis have been reviewed by Halberg[1]. When dealing with hormone levels in blood, the necessity of a sufficiently short sampling interval in relation to the circulating half-time of the hormone and a sufficiently long duration

Table 1. Terminology of rhythms

| Approximate period | Names |
|---|---|
| 1 hour | Circhoral (Ultradian) |
| 24 hours | Circadian (Diurnal, Nycthemeral) |
| 1 month | Menstrual (Circatrigintan) |
| 1 year | Annual (Circannual, Seasonal) |

of study with respect to the period of the rhythm under consideration are self-evident. Definition of hormonal rhythms is dependent upon assay precision. Thus, it is essential to establish that the biological changes under investigation are outside the range of method error. In this regard, if the amplitude of the hormonal rhythm is relatively small, it is often useful to confirm the pattern by reassaying the samples, preferably in a different order (Fig. 1).

*Sample collection.* Two techniques have been applied to detect fluctuations in hormone levels—(a) frequent single blood samples obtained at 10 to 20 min intervals or (b) continuous blood collections over 20 and 40 min intervals [6, 7]. The continuous sampling procedure may be performed with a double lumen catheter in a forearm vein. A dilute solution of heparin is infused through the smaller lumen to prevent clotting while blood is withdrawn through a larger lumen at a constant rate with a peristaltic pump into a fraction collector [7]. Heparin impregnated catheters have also been used by Kowarski *et al.*[8] and are now available commercially. The continuous sampling procedure allows measurement of mean or integrated hormone levels over the period of collection.

*Analytical methods.* Methods of analysis of hormone rhythm data may be simple or complex. Inspection alone is convincing for the relationship between ACTH and cortisol levels in man [9] and for the relationship between LH and testosterone levels in bulls and rams [10–11]. Standard statistical methods are frequently used to indicate the significance of a time effect. Other techniques have been used for specific purposes. A useful approach has been developed for examining the significance of changes in the pattern of LH levels. Mean pulse amplitude, frequency, decay time and overall mean levels are analyzed by computer. A pulse is defined as a rise in LH levels from nadir to peak of greater than 20%. Decay time refers to the "apparent" half-time of disappearance following a pulse, provided the disappearance rate is exponential over 40 min [6]. Co-variance, auto- and cross-correlation analyses may also be useful for indicating significant rhythms and relationships between different hormones [7, 12].

More complex methods of time series analysis involving curve fitting and vector representation and their statistical evaluation have been used and developed by Halberg[1] and others [5]. These methods have been applied although not extensively, to recent data on rhythms of hormone levels in plasma [5, 12–15]. Models of episodic secretion of LH and other hormones have been developed which may be applied to the measurement of secretion rates provided certain characteristics of distribution and clearance of the hormone are assumed [16–18].

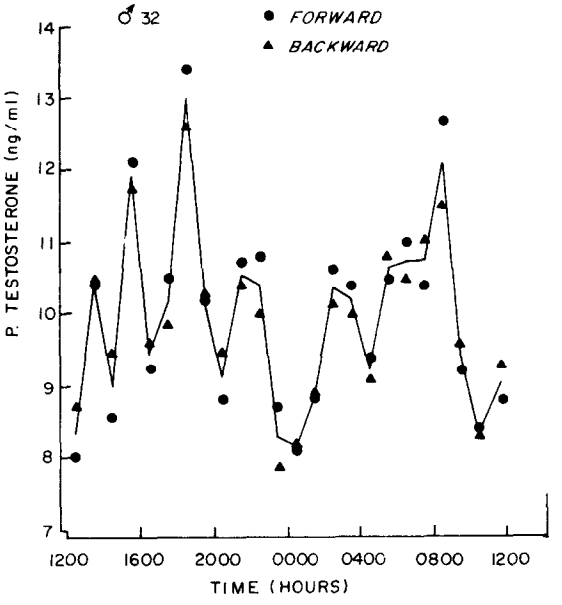


Fig. 1. Plasma testosterone levels in a healthy man aged 32 years. Venous blood was withdrawn at a constant rate for 24 h and collected at hourly intervals. Plasma testosterone was measured in two assays with samples in reverse order.

## I. *Rhythms in healthy subjects*

A. *Gonadotropin rhythms.* 1. Circhoral rhythm of LH and FSH levels. The occurrence of high frequency oscillations in circulating LH levels was first clearly demonstrated in castrate monkeys by Dierschke *et al.*[2] and a number of elegant studies has been performed with this model [19]. Nankin and Troen[20] and Midgley and Jaffe[21] found similar pulses in men and women and subsequently the pattern of changes during the day and night has been defined by a number of groups [6, 7, 13, 22–26]. In men there is general agreement about the pattern and the form of the LH pulses which may be summarized as follows (Fig. 2). Typical LH pulses are initiated with a brisk upstroke over 5–40 min to reach a peak 30 to 300% higher than the preceding levels before declining gradually over 1–4 h. The decay time is frequently longer than the estimated half-time of plasma disappearance of LH. These pulses occur every 1–6 h with a mean period of 100 min [6].

In women there are changes in the circhoral rhythm during the menstrual cycle [6, 21, 25, 27, 28]. The LH oscillations maintain a period of 1–2 h during the follicular phase and at midcycle but in the luteal phase the period lengthens to 2–6 h. The relative amplitude of the pulses decreases progressively

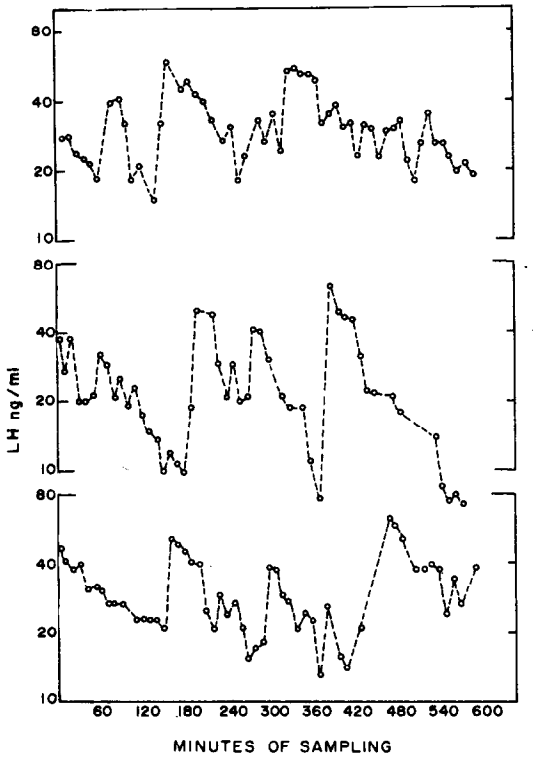


Fig. 2. Serum LH levels measured at 10 min intervals over 10 h during the day in three healthy men (Logarithmic LH scale).

in the follicular phase, increases during the midcycle surge and is greatest in the luteal phase.

An interesting discovery was made in pubertal subjects by Boyar *et al.*[29]. They found that the LH oscillations first appear during the night. Similar rises in LH levels were found in subjects who slept during the day suggesting that the nocturnal secretion of LH during puberty is triggered by sleep [30].

FSH levels exhibit less marked oscillations which may be difficult to detect. There is usually a significant correlation between LH and FSH levels in a time series and when pulses of both hormones are obvious, they frequently but not always coincide [6, 7, 25]. Santen and Bardin [6] have shown that the greater the amplitude of the LH pulse the more likely it is to be accompanied by a significant FSH rise. Concordant rises of both LH and FSH levels during sleep have been found in children of pubertal age with gonadal dysgenesis by Boyar *et al.*[31].

2. Circadian rhythm of LH and FSH levels. Although some previous studies involving 4–6 h sampling intervals had suggested that there were circadian rhythms of LH and FSH levels [13, 32, 33], recently, analysis of the patterns of these hormones obtained by sampling at 20 min intervals in adults has not revealed consistent cross-subject circadian or other clock-time related rhythms [7, 13, 23, 24]. However, as already mentioned, there are sleep related rises of LH and FSH levels in pubertal subjects. Inspection of the LH patterns during the night in young men reveals upward trends towards morning in some subjects [23, 34] and in women in the late follicular phase of the cycle, there may be a tendency for increased amplitude of LH pulses about the time of awakening [7, 27, 28]. Kapen and Weitzman [35] have found significant reductions in LH levels following sleep onset during the follicular phase and have confirmed this with sleep reversal and 3-h sleep–wake cycle experiments. Thus, it is possible that there are some sleep and time-related influences on gonadotropin secretion in adults.

B. *Sex steroid rhythms.* 1. Circhoral rhythm of testosterone levels. The use of short interval sampling has also revealed frequent oscillations in testosterone levels in men [7, 26, 35–43]. The pattern is more irregular than the circhoral rhythm of LH levels and it is difficult to define a testosterone "pulse". These changes in blood levels almost certainly represent rapid changes in the secretion rate of testosterone by the Leydig cells because the metabolic clearance rate of testosterone remains relatively constant [44].

2. Circadian rhythm of testosterone levels. Despite controversy in the past [for references, see 7, 45] and the inability of our group in Melbourne to demonstrate a consistent circadian rhythm with the continuous blood sampling technique (Fig. 3), evidence from other recent studies such as the measurement of hourly integrated levels by DeLacerda *et al.*[46] or measurement of testosterone levels at 20 min intervals overnight [40, 43] suggests that there is a circadian rhythm of testosterone secretion in young men. The acrophase of this rhythm is about the time of awaking and the nadir is in the evening. However, the amplitude is small (10–25%) and DeLacerda *et al.*[46] found that the time effect only accounted for 20% of the total variance of the hourly integrated testosterone levels.

It is probable that the main reason for the lack of consistency in the findings of the studies using infrequent sampling and small numbers of subjects is the relatively low amplitude of the rhythm compared to the amplitude of the circhoral oscillations. However, it is also possible that the rhythm may disappear with advancing age since most studies showing a circadian rhythm involve young adults under 25 or 30 years of age; whereas some of those which failed to show the rhythm have included older subjects (*e.g.* 45, Fig. 3). It is now established that there are sleep-related rises in LH and testosterone levels during puberty in boys [29, 30, 47]. Thus, there may be a persistence of a sleep-related rise in plasma testosterone levels in young adult males as a feature of continuing maturation.

3. Circhoral rhythm of estradiol levels. There are relatively few investigations directed at the elucidation of circadian or higher frequency rhythms in women or men. A study of the hormonal changes in 20 min continuous blood samples during the night in two women (follicular phase of the cycle) revealed several short term fluctuations in estradiol levels, suggesting that estrogens may be secreted episodically by the ovary [7]. Korenman and Sherman[48] have

found marked variation in estradiol and estrone levels measured at 4-h intervals in women in the immediate preovulatory phase.

4. Circadian rhythm of estradiol levels. Several studies have suggested that there is a circadian rhythm of estrone and estradiol levels in late pregnancy which has been interpreted as reflecting the circadian rhythm of secretion of adrenal precursors [49]. In view of the important contribution of peripheral conversion of testosterone to estradiol in men [50], and the apparent simultaneous secretion of testosterone and estradiol by the testis when stimulated by HCG [51], a similarity in the patterns of testosterone and estradiol levels during the day might be expected. Baird and Guevara[52] found no evidence of a circadian rhythm of estradiol levels in healthy men and women but Bodenheimer *et al.*[53] reported that the estradiol levels were significantly higher at 8 a.m. in seven blind men. However, these studies involved infrequent single blood samples. The patterns of hourly integrated levels of testosterone and estradiol in three men are shown in Fig. 3. There was no significant correlation between the levels of the two hormones. In two subjects the estradiol levels appeared to be higher during the night. Similar results have been found in four men by Leymarie *et al.*[41]. Using 30 min blood samples over 24 h, they found relatively slight changes in estradiol levels with a possible rise at night. While these studies require confirmation it seems that there are not synchronous changes in testosterone and estradiol levels in men and the occurrence of circhoral and circadian rhythms of estradiol production remains uncertain.

II. *Circhoral rhythms in disease states*

In normal subjects there is a relatively constant "saw-tooth" pattern of LH levels. In women the amplitude and frequency of the LH pulses change during the menstrual cycle. There is an irregular circhoral rhythm of testosterone levels in men and in women, estradiol levels may also exhibit circhoral oscillations. Fluctuations of FSH levels are less marked than those of LH. Alterations in the normal circhoral rhythms which might be found in disease states include: reduction or accentuation of the amplitude of the oscillations, variations in frequency and changes in mean levels. While few studies have been performed on the patterns of sex steroid levels, some interesting abnormalities of the pulsatile secretion of LH have been found.

A. *Secondary hypogonadism.* In 3 patients with partial hypopituitarism with hypogonadism, the mean levels of LH were low but the circhoral rhythm was still in evidence [6]. A blunting or absence of the episodic fluctuation in LH levels was found in women with anorexia nervosa [6] and this has been confirmed by Boyar *et al.*[54], who also showed that some patients had sleep-related pulsatile secretion of LH similar to that seen in pubertal children. LH patterns with clearly detectable but constant LH levels may be seen in boys with hypogonadotropic eunuchoidism, in some men with hepatic cirrhosis,

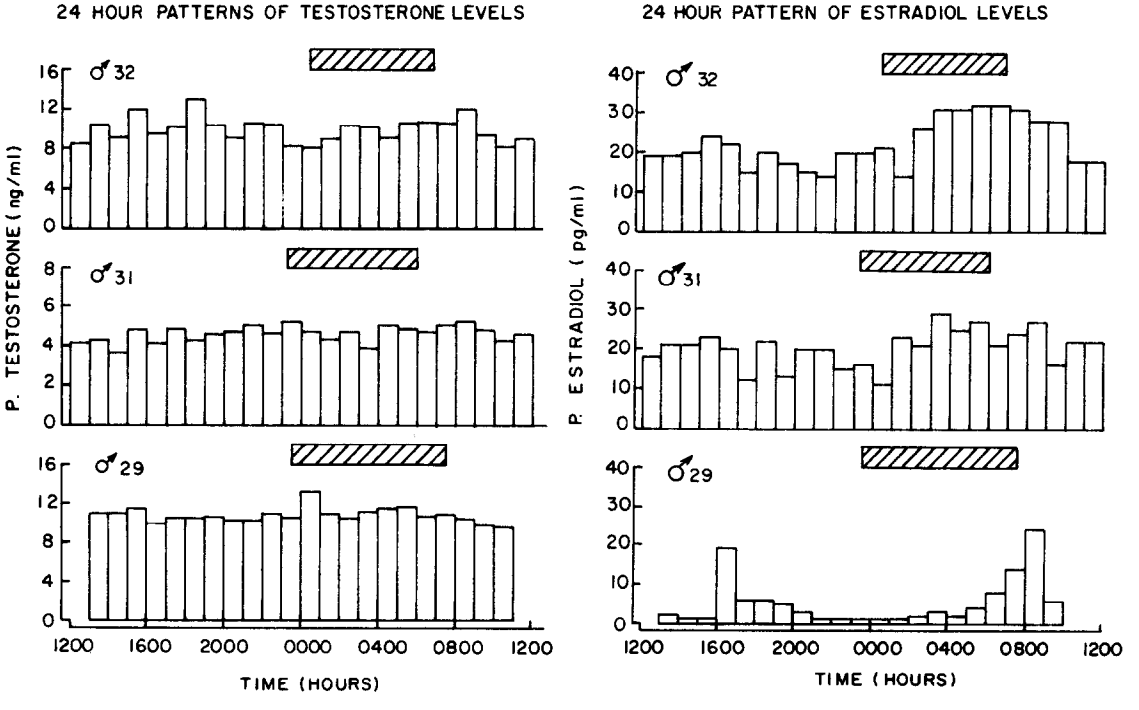


Fig. 3. Twenty-four hour pattern of hourly integrated testosterone and estradiol levels in three healthy men. The time of sleep is indicated by the hatched rectangles.

and in women with various forms of hypogonadotropic amenorrhea [55–57]. Examples of the patterns in these disorders are shown in Fig. 4.

B. *Primary hypogonadism.* In hypergonadotropic states such as in gonadal dysgenesis and ovariectomized or postmenopausal women the episodic fluctuations of LH and FSH occur with a period of about 1–2 h [6, 25, 31, 58–60]. In patients with Klinefelter's syndrome the fluctuation appeared to be irregular or of small magnitude in 4 of 5 subjects. Root *et al.*[60] also showed a somewhat irregular pattern of LH levels in one man with Klinefelter's syndrome.

C. *Other disorders.* The circhoral patterns of the gonadotropins and sex steroids have been studied in patients with azoospermia or oligospermia not due to gonadotropin deficiency by de Kretser *et al.*[61]. Despite the occurrence of elevated mean FSH and LH levels and low mean testosterone levels in some subjects, the oscillations in the hormone levels were similar to those of normal men. Santen and Bardin[6] have found that the LH pulses display a pattern intermediate between those of the follicular and luteal phases in women with hirsutism and with psychogenic secondary amenorrhea and others have also noted the persistence of episodic secretion of LH in various forms of secondary amenorrhea [54, 56].

While these observations must be confirmed and extended before the abnormalities of the circhoral rhythms of the gonadotropins and sex steroids can be usefully classified, it does appear that there is a blunting or absence of the LH pulses in anorexia nervosa and in some patients with hepatic cirrhosis or primary amenorrhea whereas the pulsatile secretion of LH may continue in gonadotropin deficiency due to pituitary lesions.

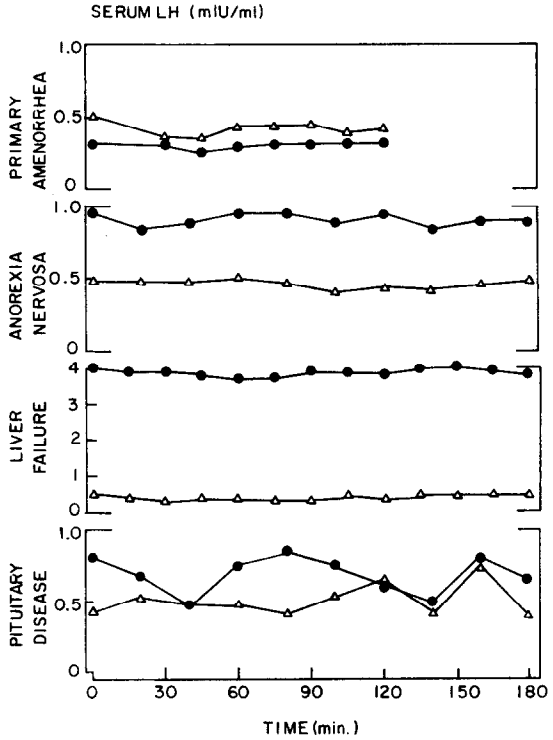


Fig. 4. LH patterns in hypogonadotropic disorders. The LH pulses are blunted or absent in hypogonadotropic primary amenorrhea, anorexia nervosa and in some men with hepatic failure but are preserved in pituitary disorders (Sheehan's syndrome and pituitary tumor).

III. *Mechanisms of the rhythms of gonadotropins and sex steroids*

A. *Circhoral rhythm of gonadotropin levels.* Although it is clear that the LH peaks result from episodes of increased secretion from the pituitary gland and not from phasic changes in the rate of clearance [6, 16, 25], the precise mechanisms for control of secretory episodes are not known. Dopaminergic and α-adrenergic neurons in the hypothalamus are believed to be involved in the control of LRH secretion which in turn stimulates the release of LH and FSH from the pituitary. Gonadal steroids may exert inhibitory or facilitory effects on the hypothalamus or pituitary. Thus, there are several possible sites of origin and modulation of the circhoral rhythm of gonadotropin secretion. The neural mechanism, LRH and the sex steroids will be discussed.

1. Neural mechanisms. The finding of prompt inhibition of the circhoral rhythms of LH levels during the administration of dopaminergic and α-adrenergic blocking drugs to castrate monkeys provides strong evidence for neural control of the pulsatile secretion of LH [19]. However, several investigators [6, 62] were unable to demonstrate a similar suppressive effect of phentolamine or chlorpromazine on the LH pulses in man. It is probable that the monkeys were given relatively larger doses of these agents and therefore it is not known whether the lack of effect in humans was due to the dosage or to a species difference.

2. LRH. There is considerable indirect evidence that the episodic secretion of LH is neurally controlled *via* LRH. For example, the suppression of the LH circhoral rhythm in monkeys with α-adrenergic and dopaminergic blocking drugs has been interpreted to result from inhibition of the neurons concerned with the elaboration of LRH [19]. The correlation between LH and FSH levels and the concordance of pulses may be construed as evidence for LRH activity as this hormone releases both gonadotropins [6, 7]. Measurements of LRH in peripheral blood of man and animals has revealed only isolated detectable levels which would be in keeping with an intermittent discharge of LRH [63–65].

3. Sex steroids. The individual gonadotropin pulses do not appear to result from steroid feedback because of the lack of a close relationship between their respective oscillations. In addition, the LH pulses continue during the administration of gonadal steroids and after gonadal ablation. However, estradiol appears to modulate the pulsatile secretion of LH. In the castrate monkey model, Knobil and his colleagues[19] have demonstrated almost immediate cessation of the circhoral discharges of LH with injections

or short infusions of estradiol and the suppression persisted for some hours after estradiol had disappeared from the circulation.

In humans, it seems likely that the negative feedback effect of estrogens is slower and less dramatic than in the monkey. Administration of pharmacological doses of estrogens to subjects with ovarian failure reduces the basal levels but may not abolish the LH pulses in some [58, 59, 66]. As in the monkey the suppression persists for some hours after cessation of estrogen infusion. Santen[67] has studied the effects of more physiological manipulations in men—infusing testosterone or estradiol over 6-h in doses equivalent to twice the mean daily production rates. Although both steroids produced a similar degree of suppression of mean LH levels, the frequency of the pulses decreased during testosterone infusion. By contrast, during estradiol infusion, the amplitude of pulses decreased but the frequency did not change. That the effects of estradiol have some physiological significance are suggested by studies using clomiphene citrate. This anti-estrogen increases the LH pulse amplitude in men [16, 36]. These results have added further evidence that the sex steroids modulate the pulsatile secretion of LH and also suggest that testosterone and estradiol exert different effects on the male LH secretory mechanism.

B. *Circhoral rhythm of testosterone levels.* Because of the known feedback relationship between LH and testosterone secretion some effort has been expended to determine whether the testosterone oscillations result from the LH circhoral rhythm.

1. Relationship between the circhoral rhythms of LH and testosterone levels. Boyar *et al.*[30] and Judd *et al.*[48] have found a close temporal relationship (20–80 min) between the nocturnal rises of LH and testosterone in pubertal subjects suggesting that the Leydig cells respond rapidly to LH. However, there are differences in interpretation of the patterns of LH and testosterone levels measured at 10–20 min intervals in adult males. Naftolin *et al.*[36] reported that each rise in testosterone levels of 2 ng/ml or more was preceded by a LH peak by 45–80 min but that only one-third of the LH peaks were followed by a rise in testosterone levels and in another study, Judd *et al.*[40] found that 12 of 14 testosterone pulses were preceded by LH spikes by 20–140 min. This type of analysis by inspection alone may be criticized because the fluctuations in LH and testosterone levels either occur so frequently that such a wide time relationship may not have great meaning, or, as shown in Fig. 5, the pattern of testosterone levels may vary from day to day making it difficult to detect significant oscillations. There is general agreement that testosterone rises do not follow each LH pulse and a statistically significant relationship between LH and testosterone levels has not been established in data obtained at short intervals [7, 26, 43]. However, in a study using 3-h continuous blood samples, there was a positive correlation between testosterone levels and LH levels in the preceding sample, suggesting that the testosterone response might be delayed by 2–4 h [68]. Cross-correlation analysis of the hourly integrated LH and testosterone levels in three men is shown in Fig. 6. There was a significant correlation between LH and testosterone levels 4 h later in two of the subjects, but in the third, there was no significant correlation at any time. Despite the absence of complete consistency, these results do suggest that there may be a considerable time lag in the Leydig cell response to LH in adult males.

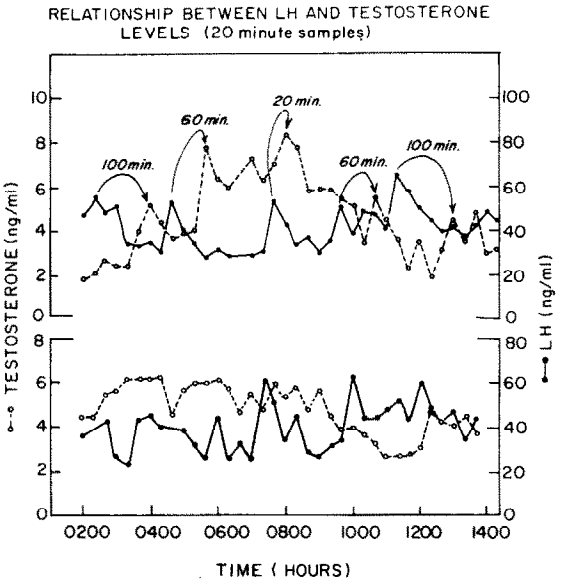


Fig. 5. Patterns of LH and testosterone levels in a healthy man studied on two days. Possible time relationships between the LH and testosterone pulses are indicated in the upper panel. On the second day the fluctuations in the levels of testosterone were less marked and there is not such an obvious relationship.

2. Kinetics of the Leydig cell response. It might be expected that measurement of testosterone levels after the induction of rises in circulating LH activity would indicate the limits of timing and magnitude of the testosterone secretory response. As yet, such studies have not produced clear-cut or consistent results.

a. HCG and LH administration. Following intravenous infusions of HCG, significant rises with plasma testosterone levels have been found after 4–6 h [69]. Santen [43] found that injections of pharmacological doses of LH in healthy men produce a gradual rise in mean testosterone levels which at 2 h is only 20% above the mean pretreatment level. Only two of the five subjects studied had a rise in testosterone levels above the upper limit of the normal male range and there were oscillations in the testosterone levels. Similar testosterone responses to injected LH have been found by Marshall *et al.*[70].

b. LRH administration. Examination of the changes in plasma testosterone levels following LH peaks induced by administration of LRH have produced variable results. Some authors have not been able to find rises in plasma testosterone levels

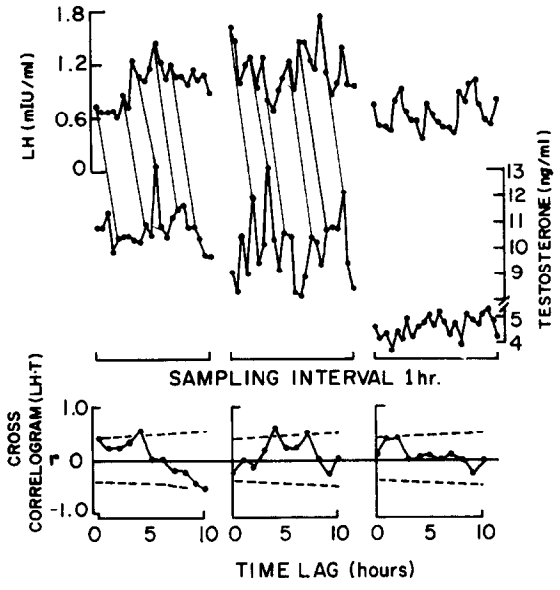


Fig. 6. Relationship between hourly integrated levels of LH and testosterone in three men. In the lower panel portion of the cross-correlograms, including the 5% confidence intervals, are shown. There was a significant positive correlation between the two hormones when testosterone levels were offset by 4 h in two of the subjects. This relationship is indicated in the upper panel by lines joining the LH peaks to the testosterone levels 4 hours later. In the third subject, there was no significant correlation at any time.

[71, 72]. Others have found significant rises but there is a wide range in timing. For example, Roth *et al.*[73] found peak testosterone levels to occur in most subjects 4 h after the injection but in some subjects the peaks occurred at 8 h, whereas Judd *et al.*[74] have found testosterone rises 30–100 min after the LH peak in seven of nine men. To some extent, the difference in these results may have been caused by the methods of analysis used. Similar studies have been performed in our laboratories. In five men aged between 19 and 30 years given 100 μg of Hoechst LRH, there was a gradual upward trend in testosterone levels which was significantly elevated above the pretreatment levels at 60 min after the injection or 45 min after the LH peak (Fig. 7). Yet, over the 4-h period of the study, there was no instance of a rise of testosterone levels outside the normal male range despite a 5-fold increase in LH levels. There were also marked variations in the testosterone levels.

These data highlight the problems of interpretation of such studies, particularly whether a clear-cut testosterone peak or merely a significant increase in mean levels should be used to assess the timing of the Leydig cell response. It may be that there is both time lag and variability in the Leydig cell responsiveness to LH and while further studies will be necessary to define the kinetics, it is obvious that Leydig cell response to LH in men is unlike the adrenal response to ACTH where there is a very close relationship between rises of ACTH and cortisol [9, 75]. It would also appear that the response is different from that found in bulls and rams where there usually are close relationships between spontaneous rises of LH and testosterone [10, 11, 76]. Because of the lack of clear-cut relationships between the oscillations of LH and testosterone levels and the persistence of the testosterone fluctuations in the face of a pharmacological elevation of LH levels, other factors must be involved in the causation of the circhoral rhythm of testosterone levels in men. Alterations in testicular blood flow may be one source of such variability [77].

C. *Circadian rhythm of testosterone levels.* Studies have been performed on the origin of the circadian rhythm of testosterone. It is not abolished by dexamethasone treatment and is, therefore, not tied to the circadian rhythm of adrenal precursors of testosterone [13, 47]. The rhythm is present in blind men [53] and unlike certain seasonal breeding animals, the light and dark cycle would not appear to be an important determinant of this rhythm in man. Sleep reversal studies have revealed that nocturnal rise of LH and testosterone secretion is sleep-related in pubertal subjects [30] and although the evidence is incomplete, it seems likely that the nocturnal rise is also sleep-related in men [46, 78].

If there is no rise in LH levels during the night in adults, the cause of the rise in testosterone levels might be related to increased Leydig cell sensitivity to LH or to other hormonal changes occurring at this time of day. For example, prolactin levels rise in parallel to the nocturnal rise in testosterone and at least in rodents this hormone potentiates LH activity on the Leydig cell [47, 79]. Although Southren *et al.*[44] found no change in the metabolic clearance rate of testosterone at night, a decrease in testosterone clearance during sleep has not been excluded.

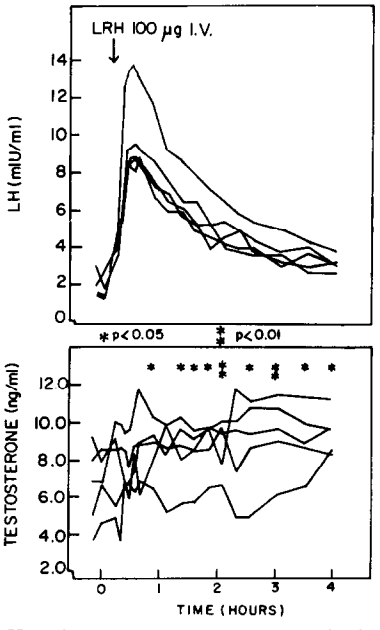


Fig. 7. LH and testosterone responses to the intravenous injection of LRH in five healthy men. The testosterone levels were variable but they were significantly elevated 45 min after the LH peak (paired *t* test).

*Biological significance of circhoral rhythms of gonadotropins and sex steroids*

Do these intriguing high frequency oscillations in hormone levels have any functional significance or are they unimportant, being merely the result of general instability in secretory mechanisms? While this question cannot be answered at present, it is of interest that Bogumil *et al.*[80], in developing a mathematical model of the menstrual cycle found that it was necessary to insert short term random oscillations in gonadotropin levels to simulate the normal slight irregularities in cycle length and form of the midcycle gonadotropin surge. Thus, in a way, these authors predicted the circhoral rhythms of gonadotropins. In discussing physical models of biological systems, Iberall and Cardon[81] indicated that oscillating signals allow a margin of safety in a control system, whereby short term perturbations are usually not followed by violent, self-destructive swings. Bogumil[18] has also suggested that an oscillating system provides precision in control.

How an oscillating level of a tropic hormone in the circulation would influence the target gland is difficult to conceptualize, particularly in view of the apparent long half-lives of receptor–hormone complexes and the occurrence of "spare" receptors [82]. The role of the short term fluctuations in the levels of the sex steroids is even more uncertain. It would seem unlikely that there are circhoral oscillations in target tissue function. Further studies of the patterns in disease states and of the effects of hormones administered in a pulsatile fashion to mimic the circhoral rhythms may shed some light in this area.

*Acknowledgements*—The authors wish to thank Mrs. Marlene Brinser for secretarial assistance. Supported in part by PHS Androgeny Grant No. H.D. 05276.

## REFERENCES

1. Halberg F.: *Ann. Rev. Physiol.* **31** (1969) 675–725.
2. Dierschke D. J., Bhattacharya A. N., Atkinson L. E. and Knobil E.: *Endocrinology* **87** (1970) 850–853.
3. Ismail A. A. A. and Harkness R. A.: *Acta endocr., (Copenh.)* **56** (1967) 469–480.
4. Lagoguey M., Dray F., Chauffournier J. M. and Reinberg A.: *Int. J. Chronobiol.* **1** (1973) 91–93.
5. Ferin M., Halberg F., Richart R. M. and Vande Wiele R. L.: *Biorhythms and Human Reproduction.* Wiley, New York (1974).
6. Santen R. J. and Bardin C. W.: *J. clin. Invest.* **52** (1973) 2617–2628.
7. Alford F. P., Baker H. W. G., Burger H. G., de Kretser D. M., Hudson B., Johns M. W., Masterton J. P., Patel Y. C. and Rennie G. C.: *J. clin. Endocr. Metab.* **37** (1973) 848–854.
8. Kowarski A., Thompson R. G., Migeon C. J. and Blizzard R. M.: *J. clin. Endocr. Metab.* **32** (1971) 356–360.
9. Gallagher T. F., Yoshida K., Roffwarg H. D., Fukushima D. K., Weitzman E. D. and Hellman L.: *J. clin. Endocr. Metab.* **36** (1973) 1058–1068.
10. Katongole C. B., Naftolin F. and Short R. V.: *J. Endocr.* **50** (1971) 457–466.
11. Sanford L. M., Winter J. S. D., Palmer W. M. and Howland B. E.: *Endocrinology* **95** (1974) 627–631.
12. Vagnucci A. H., Wong A. C. and Liu T. S.: *Proc. 56th Meeting of The Endocrine Society* (1974) Abstr. 123.
13. Faiman C. and Winter J. S. D.: *J. clin. Endocr. Metab.* **33** (1971) 186–192.
14. Rubin R. T., Kales A., Adler R., Fagan T. and Odell W.: *Science* **175** (1972) 196–198.
15. Winget C. M., Hetherington N. W., Rosenblatt L. S. and Rambaut P. C.: *J. appl. Physiol.* **33** (1972) 635–639.
16. Boyar R. M., Perlow M., Kapen S., Lefkowitz G., Weitzman E. and Hellman L.: *J. clin. Endocr. Metab.* **36** (1973) 561–567.
17. Rebar R., Perlman D., Naftolin F. and Yen S. S. C.: *J. clin. Endocr. Metab.* **37** (1973) 917–927.
18. Bogumil R. J.: In *Biorhythms and Human Reproduction* (Edited by M. Ferin, F. Halberg, R. M. Richart and R. L. Vande Wiele). Wiley, New York (1974) pp. 107–131.
19. Knobil E., Dierschke D. J., Yamaji T., Karsch F. J., Hotchkiss J. and Weick R. F.: In *Gonadotropins* (Edited by B. B. Saxena, C. G. Beling and H. H. Gandy). Wiley, New York (1972) pp. 72–86.
20. Nankin H. R. and Troen P.: *J. clin. Endocr. Metab.* **33** (1971) 558–560.
21. Midgley A. R. Jr. and Jaffe R. B.: *J. clin. Endocr. Metab.* **33** (1971) 962–969.
22. Naftolin F., Yen S. S. C. and Tsai C. C.: *Nature New Biol.* **236** (1972) 92–93.
23. Boyar R., Perlow M., Hellman L., Kapen S. and Weitzman E.: *J. clin. Endocr. Metab.* **35** (1972) 73–81.
24. Krieger D. T., Ossowski R., Fogel M. and Allen W.: *J. clin. Endocr. Metab.* **35** (1972) 619–623.
25. Yen S. S. C., Tsai C. C., Naftolin F., Vandenberg G. and Ajabor L.: *J. clin. Endocr. Metab.* **34** (1972) 671–675.
26. Murray M. A. F. and Corker C. S.: *J. Endocr.* **56** (1973) 157–158.
27. Kapen S., Boyar R., Hellman L. and Weitzman E. D.: *J. clin. Endocr. Metab.* **36** (1973) 724–729.
28. Naftolin F., Yen S. S. C., Perlman D., Tsai C. C., Parker D. C. and Vargo T.: *J. clin. Endocr. Metab.* **37** (1973) 6–10.
29. Boyar R., Finkelstein J., Roffwarg H., Kapen S., Weitzman E. and Hellman L.: *New Engl. J. Med.* **287** (1972) 582–586.
30. Boyar R. M., Finkelstein J., Kapen S., Roffwarg H., Weitzman E. and Hellman L.: *J. clin. Invest.* **52** (1973) 11a–12a Abstr. 40.
31. Boyar R. M., Finkelstein J. W., Roffwarg H., Kapen S., Weitzman E. D. and Hellman L.: *J. clin. Endocr.* **37** (1973) 521–525.
32. Midgley A. R. Jr. and Jaffe R. B.: *J. clin. Endocr.* **28** (1968) 1699–1703.
33. Saxena B. B., Leyendecker G., Chen W., Gandy H. M. and Peterson R. E.: In *Karolinska Symposia on Research Methods in Reproductive Endocrology*, First Symposium Immunoassay of Gonadotropins (Edited by E. Diczfalusy) Bogtrykkeriet forum, Copenhagen (1969) pp. 185–206.
34. Nankin H. R. and Troen P.: *J. clin. Endocr. Metab.* **35** (1972) 705–710.
35. Kapen S. and Weitzman E. D.: *Proc. 56th Meeting of the Endocrine Society* (1974) Abstr. 277.
36. Naftolin F., Judd H. L. and Yen S. S. C.: *J. clin. Endocr. Metab.* **36** (1973) 285–288.
37. West C. D., Mahajan D. K., Chavré V. J., Nabors C. J. and Tyler F. H.: *J. clin. Endocr. Metab.* **36** (1973) 1230–1236.
38. Tunbridge D., Rippon A. E. and James V. H. T.: *J. Endocr.* **59** (1973) xxi Abstr.
39. Wieland R. G., Hallberg M. C., Koepke K. R. and Zorn E. M.: *Fertil. Steril.* **24** (1973) 644–647.
40. Judd H. L., Parker D. C., Rafkoff J. S., Hopper B. R. and Yen S. S. C.: *J. clin. Endocr. Metab.* **38** (1974) 134–141.

41. Leymarie P., Roger M., Castanier M. and Scholler R.: *J. steroid. Biochem.* **5** (1974) 167–171.
42. Rosenfeld R. S. and Boyar R. M.: *Proc. 56th Meeting of The Endocrine Society* (1974) Abstr. 5.
43. Santen R. J.: unpublished observations.
44. Southren A. L., Gordon G. G., Tochimoto S., Pinzon G., Lane D. R. and Stypulkowski W.: *J. clin. Endocr. Metab.* **27** (1967) 686–694.
45. Boon D. A., Keenan R. E. and Slaunwhite W. R. Jr.: *Steroids* **20** (1972) 269–278.
46. DeLacerda L., Kowarski A., Johanson A. J., Athanasiou R. and Migeon C. J.: *J. clin. Endocr. Metab.* **37** (1973) 366–371.
47. Judd H. L., Parker D. C., Siler T. M. and Yen S. S. C.: *J. clin. Endocr.* **38** (1974) 710–713.
48. Korenman S. G. and Sherman B. M.: *J. clin. Endocr. Metab.* **36** (1973) 1205–1209.
49. Townsley J. D., Dubin N. H., Grannis G. F., Gartman L. J. and Crystle C. D.: *J. clin. Endocr. Metab.* **36** (1973) 289–295.
50. Longcope C., Kato T. and Horton R.: *J. clin. Invest.* **48** (1969) 2191–2201.
51. Weinstein R. L., Kelch R. P., Jenner M. R., Kaplan S. L. and Grumbach M. M.: *J. clin. Invest.* **53** (1974) 1–6.
52. Baird D. T. and Guevara A.: *J. clin. Endocr. Metab.* **29** (1969) 149–156.
53. Bodenheimer S., Winter J. S. D. and Faiman C.: *J. clin. Endocr. Metab.* **37** (1973) 472–475.
54. Boyar R. M., Kapen S., Finkelstein J. W., Fukushima D. K., Weitzman E. D. and Hellman L.: *J. clin. Invest.* **53** (1974) 9a Abstr. 33.
55. Yen S. S. C., Rebar R., Vandenberg G. and Judd H.: *J. clin. Endocr. Metab.* **36** (1973) 811–816.
56. Boyar R. M., Kapen S., Finkelstein J. W., Perlow M., Sassin J. F., Fukushima D. K., Weitzman E. D. and Hellman L.: *J. clin. Invest.* **53** (1974) 1588–1598.
57. Johanson A.: *J. clin. Endocr. Metab.* **39** (1974) 154–159.
58. Yen S. S. C., Tsai C. C., Vandenberg G. and Rebar R.: *J. clin. Endocr.* **35** (1972) 897–904.
59. Wallach E. E., DeCherney A. H., Russ D., Duckett G., Garcia C. R. and Root A. W.: *Obstet. Gynecol.* **41** (1973) 227–233.
60. Root A., DeCherney A., Russ D., Duckett G., Garcia C.-R. and Wallach E.: *J. clin. Endocr. Metab.* **35**, (1972) 700–704.
61. de Kretser D. M., Baker H. W. G., Burger H. G., Hudson B. and Keogh E.: *Proc. Annual Meeting of the Australian Endocrine Society* (1973) Abstr. 21.
62. Yen S. S. C., Vandenberg G., Tsai C. C. and Parker D. C.: In *Biorhythms and Human Reproduction* (Edited by M. Ferin, F. Halberg, R. M. Richart and R. L. Vande Wiele). Wiley, New York (1974) pp. 203–218.
63. Seyler L. E. Jr. and Reichlin S.: *J. clin. Endocr. Metab.* **37** (1973) 197–203.
64. Arimura A., Kastin A. J. and Schally A. V.: *J. clin. Endocr. Metab.* **38** (1974) 510–513.
65. Foster J. P., Holland D. T., Jeffcoat S. L. and Crighton D. B.: *J. Endocr.* **61** (1974) LXII Abstr.
66. Rosenfield R. L., Fang V. S., Dupon C., Kim M. H. and Refetoff S. *J. clin. Endocr. Metab.* **37** (1973) 574–580.
67. Santen R. J.: *Proc. 56th Meeting of The Endocrine Society* (1974) Abstr. 4.
68. Alford F. P., Baker H. W. G., Patel Y. C., Rennie G. C., Youatt G., Burger H. G. and Hudson B.: *J. clin. Endocr. Metab.* **36** (1973) 108–116.
69. Maurer W., Volkwein U. and Tamm J.: *Acta endocr., Copenh.* **72** (1973) 615–624.
70. Marshall J. C., Anderson D. C., Fraser T. R. and Harsoulis P.: *J. Endocr.* **56** (1973) 431–439.
71. Kley H. K., Wiegelmann W., Nieschlag E., Solbach H. G., Zimmerman H. and Krüskemper H. L.: *Acta endocr., Copenh.* **75** (1974) 417–427.
72. Wollesen F., Swerdloff R. S. and Odell W. D.: *Proc. 56th Meeting of The Endocrine Society* (1974) Abstr. 53.
73. Roth J. C., Grumbach M. M. and Kaplan S. L.: *J. clin. Endocr. Metab.* **37** (1973) 680–686.
74. Judd H. L., Rebar R., Vandenberg G. and Yen S. S. C.: *J. clin. Endocr. Metab.* **38** (1974) 8–13.
75. Krieger D. T., Allen W., Rizzo F. and Krieger H. P.: *J. clin. Endocr. Metab.* **32** (1971) 266–284.
76. Katongole C. B., Naftolin F. and Short R. V.: *J. Endocr.* **60** (1974) 101–106.
77. Eik-Nes K. B.: *Recent Prog. Horm. Res.* **27** (1971) 517–535.
78. Evans J. I., MacLean A. W., Ismail A. A. A. and Love D.: *Nature* **229** (1971) 261–262.
79. Hafiez A. A., Bartke A. and Lloyd C. W.: *J. Endocr.* **53** (1972) 223–230.
80. Bogumil R. J., Ferin M., Rootenberg J., Speroff L. and Vande Wiele R. L.: *J. clin. Endocr. Metab.* **35** (1972) 144–156.
81. Iberall A. S. and Cardon S. Z.: *Ann. N.Y. Acad. Sci.* **117** (1964) 445–518.
82. Catt K. J. and Dufau M. L.: *Nature New Biol.* **244** (1973) 219–221.