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# RHYTHMS OF ACTH AND CORTICOSTEROID SECRETION IN HEALTH AND DISEASE, AND THEIR EXPERIMENTAL MODIFICATION

DOROTHY T. KRIEGER

Division of Endocrinology and Metabolism of the Department of Medicine,
Mount Sinai School of Medicine, N.Y.C., N.Y., U.S.A.

**SUMMARY**

Detailed (q 5–30 min) sampling studies of both plasma ACTH and cortisol concentrations in normal human subjects demonstrates an early morning major circadian rise and a decline over the subsequent 24 h period, on which are superimposed lesser, episodic fluctuations (both synchronous and asynchronous) of plasma ACTH and cortisol concentrations. Marked concordance of bioassayable and immunoassayable plasma ACTH concentrations is present. The results of studies of ACTH–cortisol periodicity in patients with localized CNS disease or ocular blindness support the thesis of a neural origin of such periodicity. It is suggested that such a periodicity is endogenous, with the observed time of peaking related to some aspect(s) of the sleep–wake cycle, but synchronized by the dark–light cycle. Animal studies indicate that altered feeding schedules (without alteration of light–dark cycles) can alter the phase of corticosteroid periodicity, perhaps by alteration of the sleep–wake cycle. The presence of abnormal pituitary–adrenal periodicity in patients with Cushing's disease in remission lends support to the thesis of a neural etiology of this disease. Alteration of neonatal corticosteroid levels in the rat is associated with only temporary disruption of the normal periodicity of plasma cortocosteroid levels, and alterations of neonatal CNS amine content is not associated with any disruption of such periodicity.

The existence of a circadian periodicity of plasma corticosteroid and ACTH levels has been well documented in normal human subjects [1–4] and in infrahuman mammalian [5] and sub-mammalian species [6, 7]. For the most part, these studies (which have been based on a sampling frequency of every 4–6 h) have characterized this circadian pattern as being one in which peak hormone levels occur prior to or at the time of awakening, with a progressive decline over the remainder of the 24 h period. This peak, therefore, is seen in the early morning hours in human subjects, and in the early evening in nocturnal animals. Reversal of the light–dark, and consequently the wake–sleep schedule, results in a phase reversal of the circadian pattern within a period of approximately 8 days [1, 8].

There is conflicting data with regard to altered circadian adrenal responsiveness to ACTH [9, 10], and although there is also some evidence of a circadian periodicity of adrenal corticoid release in cultured hamster adrenal glands, it is accepted that the predominant factor regulating the circadian periodicity of adrenal corticoid secretion is the circadian periodicity of ACTH release. Periodicity of plasma ACTH levels is present in the absence of the adrenal glands [11–13]. With the growing awareness of the role of the central nervous system in the regulation of pituitary function, it has become increasingly evident that the primary modulator of such periodic ACTH release resides within the central nervous system. Further studies have revealed that the neuroendocrine pathways involved in the regulation of such hormonal periodicity are different than those involved in basal hormone secretion, stress mediated hormone secretion and perhaps in feedback modulation of hormone secretion.

There are still many unanswered questions with regard to the synchronizer(s) of such periodicity and the specific anatomical and chemical nature of the pathways involved. The purpose of the present article is to characterize more fully the normal circadian patterns of human plasma corticosteroid and ACTH levels and their alteration in various disease states. In addition, data will be presented on the results of some recent animal studies on the experimental alteration of plasma corticosteroid levels, performed in an attempt to elucidate the physiological bases of such periodicity.

*Periodicity of plasma ACTH and corticosteroid concentrations in normal human subjects*

A circadian periodicity of plasma corticosteroid concentrations is not present until 3 to 8 years of age [14] similar to the findings in animal studies, demonstrating that the development of such periodicity does not occur until the equivalent of the mid-prepubertal period is attained [15–17]. Once established, this periodicity is remarkably resistant to change, persisting under conditions of prolonged bed rest [18], fasting [19], continuous feeding [3] and 2- to 3-day periods of sleep deprivation [20].

To further study the periodicity of plasma corticosteroid concentrations in the normal subject, it became necessary to establish the reproducibility of such periodicity, and criteria for normalcy. In view of the 60 to 90 min half life of cortisol, it was realized that

more frequent sampling (q. 20 to 30 min) was necessary. Such sampling was therefore performed over a 48 h period. It became apparent in these studies that the circadian rise and fall of plasma ACTH and corticosteroid concentrations did not occur in a linearly smooth manner [21, 22]. Episodic, relatively synchronous peaks of plasma ACTH and corticosteroid concentrations were evident throughout the day. The majority of such peaks, however, occurred in the period from 0300 to 0900, describing a gradual upward course during that time period. Upon awakening, there was a subsequent downward trend, characterized by episodic peaking (but not to the height of the early morning level) interspersed with quiescent periods. Such episodic peaking was most evident between 1100 and 1300 and 1700 and 1900. These half-hourly sampling patterns were utilized in constructing criteria for establishing the presence of normal periodicity under clinical conditions where less frequent sampling is feasible. Normal periodicity of plasma corticosteroid concentrations was defined as one in which all corticosteroid values after 0800 were less than 75% of the 0800 concentration, excluding consideration of noontime values, in view of the great variability in the amount and height of peaking at this time. Noon values 15% or more *greater* than the 0800 concentration, however, were considered abnormal. Utilizing such criteria, a survey of 106 normal subjects gave a false positive rate (*i.e.* characterization of a given pattern as abnormal) of 2%. Age (between 15 and 95 years), sex, and hospitalization had no effect on the circadian pattern. In a given subject, corticosteroid concentration and pattern were reproducible over 1- to 120-day intervals.

Although in these half-hourly sampling studies the major corticosteroid peaks were always accompanied by ACTH peaks, these data also revealed the presence of additional ACTH peaks during "quiescent" periods of low plasma corticosteroid levels. In addition, in any given individual, there was a lack of proportionality between increments in plasma ACTH and corticosteroid concentrations. It became necessary, in view of the short half life of ACTH in the human (8 to 18 min as determined by bioassay, and 11 to 25 min as determined by immunoassay) [23], to resort to sampling at 5 min intervals. These studies [24], performed in two subjects, and including the time periods 1000–1300, 1600–2300 and 0400–0800, again confirmed the general nature of the circadian pattern of plasma corticosteroid and ACTH concentrations as previously described (Fig. 1). No new regular, ultradian frequency of ACTH periodicity was observed, although more frequent episodes of secretion were seen. In these studies, plasma ACTH concentrations were determined both immunoassay (I) and bioassay (B). A highly significant correlation was present between I- and B-ACTH concentrations at all times. An unexpected, and still unexplained, finding was the

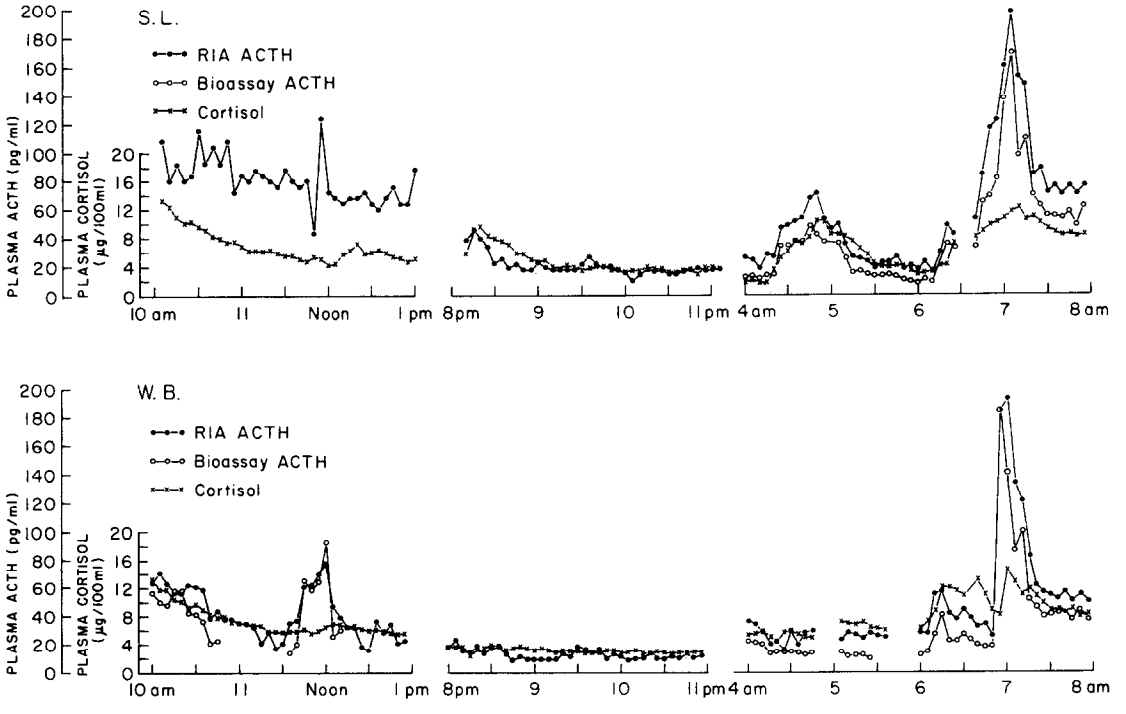


Fig. 1. Plasma ACTH (RIA = radioimmunoassay, bioassay) and cortisol concentrations in two normal male subjects sampled at 5 min intervals via an indwelling intravenous catheter over 3–4 h spans, sampling periods separated by intervals of at least one month. In this figure the depicted sampling periods are 10 a.m.–1:00 p.m. (subjects fasting), 8:00 p.m.–11:00 p.m. (meal at 6:00 p.m.), and 4:00 a.m.–8:00 a.m. (subjects asleep, blood samples obtained from an adjoining room via the indwelling intravenous catheter, sleep onset at 12:30 a.m.). Note parallelism between immunoassayable and bioassayable ACTH concentrations, as well as episodes in the first and third sampling spans where major rises in ACTH concentrations are accompanied by no, or slight changes in cortisol concentrations.

observation in both subjects of 30 to 50 min episodes during which marked rises in both I- and B-ACTH concentrations occurred without concomitant or with markedly diminished increments in plasma cortisol concentrations.

*Periodicity of plasma ACTH and cortisol concentrations in disease and following drug administration*

1. *In disease not involving the central nervous system.* Abolition of the circadian pattern of both plasma free and conjugated corticosteroid concentrations and of urinary free and total corticosteroids has been reported in patients with liver disease [25]. This was assumed to be secondary to the decreased rate of cortisol removal from the plasma in such patients; the effect of possible alterations in protein binding was not investigated. The reported absence of corticosteroid circadian periodicity in patients with chronic congestive failure (all of whom had right-sided failure) may probably be ascribed to the hepatic involvement in such patients [26]. Patients with acute illness without mental confusion and chronically ill patients without liver or kidney disease exhibited normal circadian variation [27]. In this last report it was noted that alert, ambulatory lung cancer patients exhibited a slightly decreased circadian variation, whereas circadian variation was absent (there was no drop from normal peak concentrations) in patients with advanced bronchogenic carcinoma. It has been recently demonstrated [28] that afternoon plasma ACTH concentrations are elevated in 50% of patients with bronchogenic carcinoma without clinical Cushing's syndrome—lack of clinical symptomatology perhaps being due to the fact that the ACTH measured was predominantly "Big ACTH" which has decreased biological activity as compared to that of "normal, little" ACTH [29]. Continued secretion of this less active form, especially should it be shown to have a longer half life than "little" ACTH, could explain the lack of circadian variation noted in the patients with bronchogenic carcinoma.

2. *In organic central nervous system (CNS) disease.* Patients demonstrating disturbance of consciousness with either acute [27] or chronic diffuse CNS disease [2] had been reported to show absence or alteration of corticosteroid periodicity. In view of the animal studies demonstrating the importance of hypothalamic–limbic system areas in the regulation of such periodicity, it was felt that abnormal periodicity might be encountered in conscious patients with disease delimited to these areas.

To date [30–32] we have studied 43 conscious patients with radiographically and clinically localized hypothalamic or limbic system disease, of whom 53% had abnormal (phase reversal, or peaking at normally quiescent times of day) corticosteroid patterns, as determined either by sampling at half-hourly or four-hourly intervals. Plasma ACTH concentrations, determined in one half-hourly study on a patient with a hypothalamic tumor who exhibited an abnormal corticosteroid pattern, varied concordantly with corticosteroid concentrations. (There was minimal evidence of any other disturbance in basal or stimulated endocrine function in the vast majority of these patients.) In contrast, only one of 21 conscious patients with localized CNS disease outside the hypothalamic–limbic system area had an abnormal pattern. Abnormal patterns were encountered in 31% of 21 patients with nonfunctioning pituitary tumors, and in only 1 of 8 acromegalic subjects.

These studies would indicate that pathways involved in the regulation of circadian corticosteroid periodicity in the human occupy a delimited CNS area, roughly similar to that demonstrated in animal lesion studies.

3. *In psychiatric illness.* Studies of circadian periodicity of corticosteroids in depressive states have yielded somewhat conflicting results. There now, however, appears to be general agreement that such periodicity is normal, though maintained at higher plasma cortisol concentrations [33–35]. A recent study utilizing 20 min sampling intervals [36] demonstrated an increase in the number, magnitude and duration of cortisol secretory episodes in such patients but with the major rise still occurring in the early morning hours. A study of manic patients also reports normal periodicity, but there were only two sampling points over the 24 h period [37]. Recently concluded studies (unpublished observations) with half-hourly sampling over 48 h each during a manic and depressed stage in a patient with monthly/manic-depressive cycles revealed normal periodicity during the depressed phase and an arrhythmic series of peaks with no circadian rise during the manic stage. Whether the alteration during the manic stage is related to the lack of sleep is problematical. A normal periodicity (again based on two sampling points) has been reported in schizophrenic subjects [38].

4. *In Cushing's disease.* The absence of a circadian periodicity of plasma corticosteroid concentrations (as determined by relatively infrequent sampling) in Cushing's disease and syndrome is well known [39]. Corticosteroid concentrations are depicted as being essentially unchanged over the 24 h period. In half-hourly sampling studies on patients with Cushing's disease, we have demonstrated [3] that these concentrations are not constant, but instead show continuous irregular oscillatory patterns. These patients, in contrast to normal subjects, also do not display a greater proportion of peaks during the early morning hours. Such abnormal periodicity is present in patients with Cushing's disease when both clinically active and when in remission [40]; a normal pattern was seen in a patient with Cushing's syndrome following removal of an adrenal adenoma, but only after a year had elapsed post-surgery [41].

Studies of plasma ACTH periodicity in patients with Cushing's disease (sampling 8·5 min over a 3 h period) revealed no greater frequency of peaking than seen in the normal subjects. Highly significant correlations between I- and B-ACTH concentrations were obtained over the entire sampling span [24]. Again,

in these subjects, similar to the normal, there were rather prolonged spans during which marked rises in both I- and B-ACTH concentrations occurred without concomitant increases in plasma cortisol concentrations. This phenomenon was also seen in half-hourly sampling studies performed over a 10 h period (Fig. 2).

The absence of a clearly defined circadian surge, and the presence of abnormal corticosteroid and ACTH periodicity even when these patients are in clinical remission (together with evidence of altered sleep EEG stages and periodicity of growth hormone release in patients with both active disease and in remission [40]), would suggest a malfunction of a central nervous system locus as being etiological in this disease.

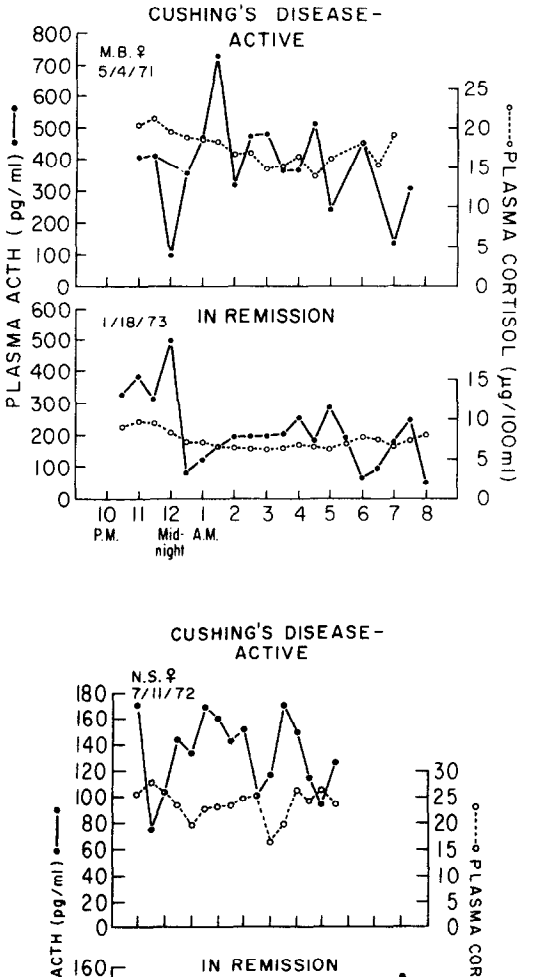


Fig. 2. Plasma immunoreactive ACTH and cortisol levels in 2 patients with Cushing's disease before and after remission induced by pituitary irradiation. Note decrease in plasma cortisol levels and persistence of elevated plasma ACTH levels in remission (more marked in patient M.B.), and lack of parallelism between increments in plasma ACTH and plasma cortisol levels.

5. *In blind subjects (also normal subjects exposed to constant light or constant dark).* Determination of corticosteroid periodicity in 19 blind subjects revealed abnormal patterns in 13 [42]. Subjects were selected with blindness secondary to intraocular diseases of varying etiology, with exclusion of those with blindness secondary to central nervous system disease in view of the previously cited alterations in hormone periodicity in such patients. There was no correlation of abnormality of periodicity with etiology, duration or age of onset of blindness: female patients, however, had a higher frequency of abnormality than males (86% vs 55%). In the majority of patients with abnormal patterns the early morning rise of plasma corticosteroid concentrations occurred. However, there were abnormal secondary peaks during the course of the day and a lack of the normal day-to-day reproducibility of circadian patterns. Two of three blind patients reported by Orth and Island[43] and 5 of 7 blind patients reported by Bodenheimer *et al*[44] showed abnormalities similar to those that we have reported.

These studies should be considered in context with those performed on normal subjects, who underwent changes in clock time of exposure concomitantly with changes in clock time of wake–sleep, as well as studies in which attempts were made to alter these variables independently. The normal early morning circadian rise still occurred in normal subjects exposed to either 21 days of constant light [45] or 4 [46] or 10 days [43] of constant darkness. (In the latter instance there was a one-hour period of light from 1800–1900; in the constant light studies even though the same intensity of illumination was present during the times when the subject was awake or asleep, eyelid closure may have diminished the amount of light (impinging upon the retina).) These studies would indicate a minor role of light–dark influences on the circadian corticosteroid rise unlike the animal studies to be reported subsequently. Before ascribing such changes completely to sleep–wake changes, it should be pointed out that subjects after 7 "days" on a 19 h day schedule (one-sixth of each day being spent in sleep) besides showing a rise in corticosteroid concentrations on awakening (which was also associated with dark–light transition at either midnight, 1700, 1900 or noon on the days of study), also showed persistence of the early morning circadian rise at a time when there was no dark–light of sleep–wake transition [47]. These observations would suggest a rhythmicity in the nervous system independent of either sleep–wake or dark–light.

There are other studies, however, which indicate some role for light–dark. In the constant dark (23 h) studies cited above [43], an additional corticosteroid

rise could be produced by the lights on period at 1800–1900. Furthermore, normal, awake subjects after 13 days of light–dark reversal (but not sleep–wake reversal) also showed a corticosteroid rise following lights on at 1800 in addition to the early morning circadian rise [43]. In further studies by these investigators, it can be noted that when the period of dark was prolonged for 4 h after morning awakening, that although the early morning circadian rise began during sleep, its time of peaking was delayed until light onset. This might indicate, as suggested above, a role for light merely in entrainment of the rhythm.

Taken together, all the above studies would appear to indicate that there is an endogenous 24 h periodicity of corticosteroid concentrations, basically related to sleep–wake, in which light can act either as an entraining agent, or independently (outside of the period of the major circadian rise) cause elevation of corticosteroid concentrations.

6. *Effect of drugs.* To date it has not been possible to block the circadian rise in plasma corticosteroid concentrations by the administration of either atropine (3–6 mg subcu. administered between midnight and 0200) or sodium phenobarbital (400 mg po. similarly administered). (These drugs are effective in blocking such a rise in the experimental animals [48].)

Administration, in a therapeutic regimen, over a 2-week period of reserpine, chlordiazepoxide, meprobamate, or chlorpromazine, or of diphenylhydantoin over a 2- or 8-week period, was also ineffective in blocking the circadian rise [49].

*Recent animal studies on the experimental alteration of plasma corticosteroid concentrations*

1. *Effect of constant light and constant dark.* Rats have been reared (a) neonatally under different lighting regimens (normal light–dark, constant light, constant dark, or post-orbital enucleation) until adulthood or (b) transferred at different postnatal ages from either normal light–dark to constant light or dark, or from constant conditions to normal light–dark [50]. These studies have indicated that there is no critical period in development during which normal light–dark alternation has to be present for normal corticosteroid periodicity to occur: *i.e.* animals exposed to constant light or dark until adulthood (and manifesting abnormal periodicity at that time) can regain normal periodicity when exposed to normal light–dark conditions. Conversely, animals reared in normal light–dark until adulthood, and then exposed to constant light or dark, lose their previously normal corticosteroid periodicity. Such alteration in periodicity does not appear to be secondary to the development of a free-running state, and is not characterized by the flattening of the curve reported in lesioned animals. Animals sacrificed under constant light conditions had higher daily mean levels of plasma cortisol, while those reared in constant dark had lower levels. Enucleated animals also showed altered periodicity. It has been reported that constant light exposure permanently damages the outer layers of retinal cells, while sparing the inner and pigment cell layers, and also blocks photically evoked electrical responses from the optic tract [51]. Therefore, the occurrence of normal corticosteroid periodicity in animals reared under prolonged constant light and then transferred to normal light–dark, is supportive of the concept that a non-visual, photically stimulated retinal mechanism is involved in such periodicity.

2. *Effect of alteration of neonatal hormone levels.* Our laboratory has reported that circadian periodicity of plasma corticosteroid concentrations of 30-day-old animals was suppressed if corticosteroids were administered systemically between days 2 to 4 of neonatal life but not if they were given between days 12 to 14 of neonatal life [52]. Neonatal administration of testosterone or reserpine had no effect on the subsequent development of corticosteroid periodicity. Early neonatal administration of corticosteroid had no effect on other parameters of the CNS–pituitary adrenal axis (*i.e.* stress responsiveness, responsiveness to exogenous ACTH). This would indicate that the function of a specific neural pathway involved in the regulation of periodicity had been affected.

Such neonatal steroid suppression of corticosteroid periodicity is not a permanent one, however (unpublished observation). Eighty-day-old animals to whom corticosteroids were administered on days 2 to 4 of neonatal life had normal corticosteroid periodicity, although the peak concentrations attained in the male animals were lower than those of normal 80-day-old males. This might indicate a different mechanism of alteration of periodicity than seen with neonatal androgen administration [53] where the loss of cyclicity of luteinizing hormone release in the female rat persists throughout adult life.

3. *Effect of alteration of neonatal CNS–neurotransmitter content.* The extent of circadian variation of plasma corticosteroid concentrations, when studies at 30 days of age, was normal in rats who received either intraventricular 6-OH dopamine or 5,6-dihydroxytryptamine on day 3 of life. When studied at 30 days of age, there was significant depletion of 0800 and 2000 levels of norepinephrine in the cortex, hypothalamus, hippocampus and amygdalae of the 6-OH dopamine treated animals and similar depletion of serotonin in these areas in the 5,6-dihydroxytryptamine treated animals. In the injected animals, there was no difference in the amine levels seen at these two times in any of these areas, in contrast to the variation seen in uninjected animals, in whom 0800 levels of both amines in all areas were higher than 2000 levels. These data demonstrate that the circadian variation of plasma corticosteroid concentrations can develop in the presence of either marked norepinephrine or serotonin depletion in CNS areas that have been implicated in the regulation of such periodicity.

4. *Effect of altered feeding schedule.* A recent report [54] noted that restriction of the water intake of rats to a brief period within daytime hours is

associated with elevated morning corticosteroid levels and perhaps an altered rhythmicity of these levels. (These animals were sampled at different clock times over a 1-month period to construct the 24 h pattern.) Our laboratory confirmed this observation with either food restriction alone or food and water restriction to a 2 h period between 0930 and 1130 [55]. (In these studies a given animal was serially studied over a 24 h period.) Reversal of the normal circadian pattern of plasma corticosteroid concentrations was noted in these studies with a similar reversal in the time of the peak of body temperature. Alteration of patterns of running activity, with a marked increase in daytime running activity, was also present in such food and water restricted animals. There was also a reversal of a.m./p.m. ratios of hippocampal norepinephrine and serotonin concentrations in these animals. These findings demonstrate that presence of normal light–dark alternation is not sufficient for the maintenance of normal circadian periodicity of plasma corticosteroid concentrations. It is possible that disruption of the sleep–wake pattern induced by food restriction may be a factor responsible for the observed changes.

## CONCLUSION

It is apparent that there are still many unanswered questions with regard to the genesis and the functional role of the circadian aspects of pituitary adrenal function. There is general agreement that such periodicity is a reflection of CNS "processes" involved in the regulation of periodic corticotrophin-releasing factor (CRF), and consequently of periodic ACTH release. Among other possibilities, these CNS "processes" may involve periodic variation in neurotransmitter synthesis and release, or periodic variation in the "threshold of activation" of the CRF secretory cell. In some species (including the human) the circadian periodicity of pituitary-adrenal secretion appears to be an endogenoous one, with the observed time of peaking related to some aspects of the sleep–wake (rest–activity) cycle, but also modulated by the dark–light cycle. It is not known whether either of these cycles "drives" the hormonal one, or whether all of them are "driven" by a common controller, acting independently on each of these variables. In either case the nature of the "controller" is unknown. It should also be stressed that it is entirely possible that in different species different neurotransmitter substances and different synchronizers (*i.e.* light or dark) may be involved in such "control". Answers to the above and related questions should contribute to fundamental understanding of neural and neuroendocrine processes.

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