Twenty-four Hour Pattern of the Episodic Secretion of Cortisol in Normal Subjects

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ABSTRACT. Plasma cortisol was measured for seven 24-hr periods using the frequent sampling technique (every 20 min) in 6 normal acclimated subjects. A defined sleep-wake schedule was established over 3–5 consecutive nights with polygraphic definition of their sleep patterns. A mean of 9 secretory episodes (range 7–13) occurred over the 24-hr sampling time, the subjects spending an average of 24% of the time in active secretion. It was estimated that, on the average, 16 mg of cortisol was secreted over the 24 hr, with a mean of 66 min half-life of cortisol decay. Although great variability was found in both the amount of cortisol secreted and the time spent in secretory activity/hr, the secretory rate was quite constant at approximately .05 mg/min. A temporal pattern of episodic secretion was recognized, and the 24-hr sleep-wake cycle could be divided into 4 unequal temporal phases: *Phase 1.* A 6-hr period of "minimal secretory activity" (4 hr before and 2 hr after lights out); *Phase 2.* A 3-hr period called "preliminary nocturnal secretory episode" (3rd to 5th hr of sleep); *Phase 3.* A 4-hr period, the "main secretory phase" (6, 7, 8 hr of sleep and 1st hr after awakening); and *Phase 4.* The 11 hr of "intermittent waking secretory activity." No evidence for a "basal level" or "steady state" of cortisol concentration was found. Changes in cortisol output during the 24-hr day appear to be due to differences in frequency and duration of secretory episodes and not to major changes in secretory rate. (*J Clin Endocr* **33:** 14, 1971)

IN A RECENT STUDY carried out in our laboratories, using 20-minute sampling intervals, and measuring the changes in the specific activity of ^{14}C-labeled cortisol, it was demonstrated that cortisol was secreted in a series of brief episodes (1). It was also found that, if the total amount of cortisol secreted over 24 hours was estimated solely from the successive secretory episodes using the 20-minute sampling interval, this calculated value agreed closely with the daily cortisol secretion rate determined by the isotope dilution method in the same subject.

It is now well established that there are variations in the plasma concentration of cortisol during the 24 hours (2–8). In earlier studies, in which sampling was carried out at infrequent intervals, every four hours or less (2–5) a rapid rise in plasma corticosteroid concentration was described as occurring during the latter half of sleep, with a maximum reached just prior to the time of arousal, and followed by a more gradual decline in concentration during the waking period, reaching a minimum at the start of the sleep period. Later studies, in which sampling was done at 30-minute and one-hour intervals, demonstrated that the daily change in cortisol concentration was not a smooth curve but was in fact a jagged line with sharp rises and falls (6–9). In addition, in a study using a 30-minute sampling interval it was found that corticosteroid concentration varied so drastically during sleep that the curves suggested episodic secretion of the steroid rather than a steady nocturnal rise in concentration (8). It was subsequently reported that when cortisol and ACTH were both measured in plasma samples obtained at 30-minute intervals over a 24-hour period the abrupt elevations in ACTH concentration were closely followed by rises in cortisol concentration (10). These

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findings confirmed previously reported evidence that rapid changes occurred more frequently late in the sleep period, and at the time of arousal, but also indicated that such fluctuations were occurring during the rest of the day.

This demonstration of secretory episodes spread throughout the 24-hour period raised the important question whether or not there was a consistent pattern marking the time of occurrence and intensity of the episodes.

The data obtained from 20-minute samples of seven 24-hour studies are analyzed in this report.

The results confirm the episodic nature of cortisol secretion in normal man, the ability to locate and estimate the intensity of these episodes, and suggest that there is a consistent temporal pattern of secretory episodes in normal man during the 24-hour sleep-waking cycle.

Materials and Methods

Subjects. Six healthy young adult subjects (5 male, 1 female, ages 23–40) were employed in this study. All reported a normal sleep-waking cycle with 7–8 hr of regular nocturnal sleep. All subjects were adapted to the laboratory with 3–5 consecutive night sleep recordings prior to the 24-hr sampling period. They were admitted to a special sleep-research unit on a clinical center and were allowed 8 hr of sleep at approximately the same time each night (onset 11–12 PM). They ate all meals on the unit and were ambulatory during the day, with no scheduled program of activities except for meal times. Their activities consisted of reading, watching television and engaging in conversation with visitors and staff. Just prior to each 8-hr nocturnal sleep period, scalp EEG electrodes were applied, (C_3, C_4, O_1, A_1, A_2), as well as electro-oculogram and chin electromyographic electrodes. The subjects were recorded and slept in the dark, in a sound-attenuated, air-conditioned room. After 8 hr of darkness the lights were turned on, and the electrodes were removed. After this adaptation period, a small plastic catheter was inserted into an antecubital vein during the middle of the waking period, usually about 1 PM. This was attached to a longer polyethylene catheter (0.4 mm i.d.) which extended into an adjoining room where blood samples were removed. The catheter was filled with heparinized normal saline (5000 U heparin in 1 liter N saline) between samples. The catheter was insulated from contacting the skin so as to prevent awareness by the subject of temperature differences between the blood and skin. Each sample of blood was obtained by first withdrawing all the saline in the catheter, as well as 0.5–1 cc of blood to eliminate dilution. A new plastic syringe was then used to withdraw 5 cc of blood. The tubing was then flushed with the heparinized saline and sealed until the next sample. Each sample was transferred immediately to heparinized tubes and then centrifuged for 10 min. The plasma was then separated and frozen until ready for chemical assay. Samples were obtained every 20 min for a 24-hr period. During the 16-hr waking portion, the subjects sat in a bed or in a chair. Meals were served at regular times (breakfast 8–9 AM, lunch 12–1 PM, dinner 5–6 PM). Between meals they engaged in activities similar to those on the adaption days, except that they did not leave their rooms. One such 24-hr plasma sampling study was carried out for 5 subjects; the sixth subject (L.C.) was studied twice, the 2nd study performed 2 months after the first. Little difficulty was encountered in obtaining the plasma samples as outlined, except for 2 occasions (subj. R.S.) when the catheter stopped functioning early in the sleep period. Lights were turned on for 15 min in order to correct this problem and the subject was awake for approximately 50 min. The rest of the 24-hr period was uneventful.

Plasma cortisol measurement. The plasma cortisol was measured by the competitive protein binding method of Murphy *et al.* (11), with modifications as described in Hellman *et al.* (1).

Estimation of cortisol secretory episode. A secretory episode was defined as occurring when the plasma cortisol concentration rose in a successive sample by at least 2 μg/100 ml and when the next concentration was also higher than the initial one. The episode was considered to have terminated at the first time point when the concentration fell by at least 1 μg/100 ml, provided that the fall was consistent for the next sampling points. As previously reported (1), the amount of cortisol secreted in each episode was approximated from the product of the volume of distribution and the corrected increment in plasma cortisol concentration.

Equation I: Amt. of cortisol secreted

$$= V\left[(c_t - c_0) + \frac{(c_0 + c_t)}{2}\,\frac{\Delta t}{\bar{T}}\right].$$

V = volume of distribution (assumed to be 18L, thiocyanate space);
c_0 = initial plasma cortisol (μg/l);

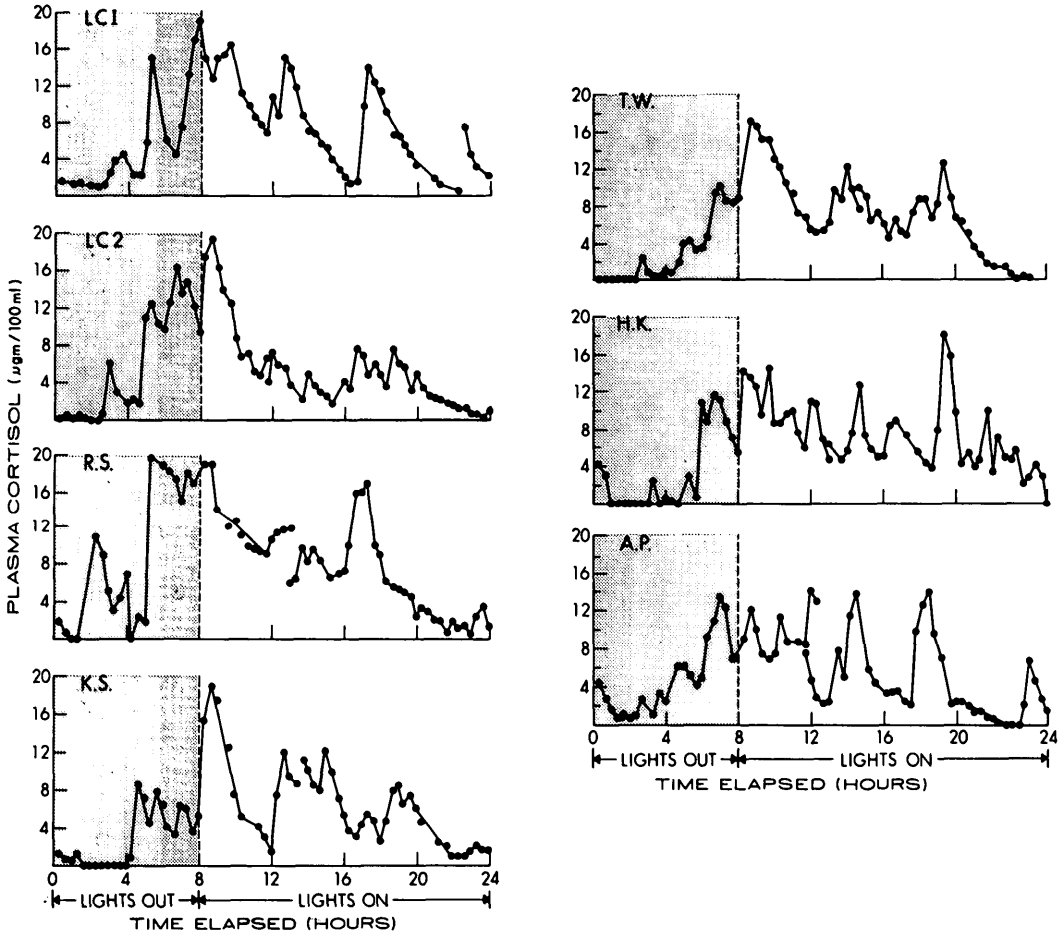

FIG. 1. Plasma cortisol values of normal subjects for 24-hr periods of study. Samples obtained every 20 min. Period of time of "lights out" is sleep period available.

c_t = peak plasma cortisol ($\mu g/l$);
Δt = duration of the episode (min);
$\bar{T}$ = mean life of cortisol (min) ($1.44 \cdot t_{\frac{1}{2}}$);
$t_{\frac{1}{2}}$ = half-life of cortisol. A value of 70 min was used, based on disappearance curve of cortisol-4-^{14}C reported in our previous report.

Results and Discussion

All subjects studied demonstrated a series of major fluctuations in plasma cortisol concentration throughout the 24-hour sleep-wake cycle. These fluctuations were characterized by sharp rises followed by a slower, generally smooth, decline, in most cases closely following an exponential curve. Indeed, the concentration of cortisol was never constant except when the value had reached zero or near zero in the period just before and/or after the onset of sleep. The intervals between these elevations were not regular but ranged from 40 minutes, the minimum separation detectable in our sampling paradigm, to 4 hours and 40 minutes within the period of active daytime secretion, and over 8 hours between the start of the last waking episode to the onset of the first nocturnal one (Fig. 1). In addition, the concentrations at which these rises were initiated varied greatly, beginning at values of zero up to 17 $\mu g/100$ ml. The peak values obtained also varied over a wide range, from less than 5 to almost 20 $\mu g/100$ ml.

These findings appear to be at variance with many published studies describing the 24-hour plasma cortisol concentration as an essentially smooth (2–5) or progres-

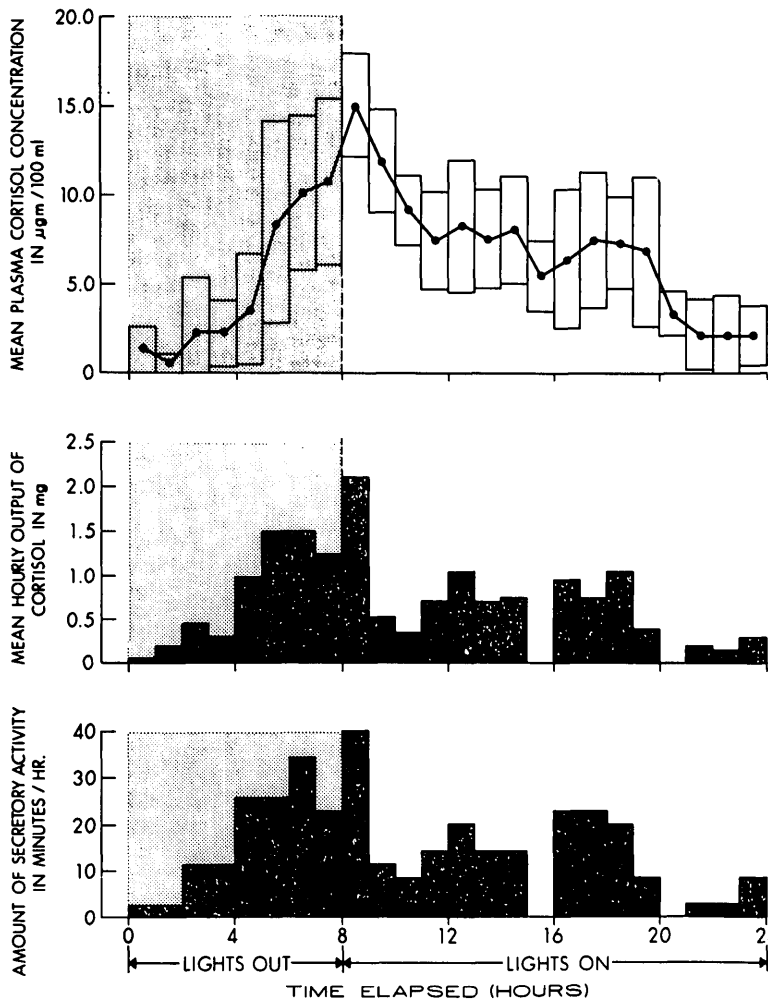

FIG. 2. *Top.* Mean and standard deviation of plasma cortisol concentration for 24-hr period. The hourly mean value includes all plasma values obtained for each hour of all 7 studies. *Center.* Mean hourly output of cortisol for the 7 studies based on the amount secreted in the episodes or parts of episodes falling within each of the 24 hr. *Bottom.* Mean min/hr of secretory activity determined from the number and duration of episodes or parts of episodes falling within each of the 24 hr.

sive "circadian" (6, 7) curve. However, when these same data are averaged for one-hour intervals and for all seven studies, the resultant curve conforms quite closely to the often described deceptively smooth circadian curve (Fig. 2, top). The data obtained in this study are therefore fully consistent with those reported earlier. The introduction of the frequent sampling technique demonstrates that the averaged "circadian" function results from a combination of the frequency and amplitude of these intermittent rises. The plasma cortisol concentration was consistently elevated in all seven studies in the hour following arousal, as confirmed by the peak and small variation of the mean concentration at this time. However, similar sharp increases often equal to or greater than these individual peaks were found during the subsequent waking period but differing by their wide spacing. During the nine-hour period of the day (11–20 hr), the mean concentration curve tended to plateau, instead of demonstrating a continuous decline. Since the individual secretory episodes occurred irregularly, when averaged they therefore tended to cancel one another. Indeed, in four of the seven studies (Fig. 1), the maximum or near-maximum plasma cortisol concentration of the 24 hours was present during this portion of the waking period. Cortisol concentration does not truly begin to fall toward its minimum until the fourth hour before the start of the sleep period.

TABLE 1. Estimates of total daily secretory output of cortisol, time spent in secretory activity, and mean half-life of the disappearance of plasma cortisol

Subject	No. of secretory episodes	Total cortisol secreted over 24 hr (mg)	Mean half-life of cortisol decay (min)	Total time spent in secretory activity 24 hr (min)	% of 24 hr
K.S.	8	14	58	320	22
L.C.(1)	7	16	67	340	24
L.C.(2)	9	13	59	240	17
R.S.	9	18	73	420	29
T.W.	7	12	86	340	24
H.K.	13	19	61	420	29
A.P.	9	21	69	380	26
Mean of 7 Studies:	9	16	66	350	24

A series of 7 to 13 episodes of cortisol secretion may be defined for each of these 24-hour sampling studies. This is based on our previous finding that cortisol is secreted during each instance of a sharp rise in plasma concentration, and not during the fall in concentration. This was demonstrated utilizing changes in the specific activity of plasma cortisol following the intravenous injection of ^{14}C-labeled cortisol during the latter half of the sleep period (1).

The definition of a secretory episode is described under Materials and Methods. The amount of cortisol produced for each episode was determined from the product of the corrected increment of the plasma concentration and the volume of distribution. On the basis of these calculations, the following results were obtained, and are shown in Tables 1–3. The total cortisol secreted for each of the 24-hour studies is in close agreement with the previously reported values using isotope dilution techniques based on the specific activities of the urinary metabolites of cortisol. Since the termination point of a secretory episode was defined, both time spent in secretory activity and the metabolic half-life were determined. The half-life was calculated for the 36 of 53 episodes where there were at least three successive falling points. The average half-life value of 66 minutes for all studies is in close agreement with the 68 minutes determined by the isotope dilution method. Although these values are less than the value of 83 minutes originally reported by Peterson and Wyngaarden (12), the differences are not incompatible, if one takes into consideration the range of variation present in both studies and the time of day when the half-life was determined. Peterson and Wyngaarden's values ranged from 50 to 148 minutes, and our range was from 45 to 142. A mean of 93 minutes for the seven half-life determinations was obtained in our studies during the comparable three-hour morning interval in which Peterson and Wyngaarden per-

TABLE 2. Mean cortisol secretory output and secretory rate for seven 24-hour studies, for each hourly period subsequent to "lights out"

Hr	Mean cortisol secreted in mg/hr	Mean min of secretory activity/hr	Secretory rate in mg/min
1	—	—	—
2	—	—	—
3	.44	11.4	.04
4	.30	11.4	.03
5	.97	25.7	.04
6	1.48	25.7	.06
7	1.48	34.3	.04
8	1.23	22.9	.05
9	2.12	40.0	.05
10	.53	11.4	.05
11	.32	8.6	.04
12	.70	14.3	.05
13	1.04	20.0	.05
14	.70	17.1	.04
15	.74	14.3	.05
16	0	0	—
17	.94	22.9	.04
18	.81	22.9	.04
19	1.05	20.0	.05
20	.39	8.6	.05
21	—	—	—
22	—	—	—
23	—	—	—
24	.28	11.4	.02

TABLE 3. Cortisol secretory activity analyzed by descriptive phases

Phase	Title	Start to end of phase (hr) (0 hr is time of sleep onset)	Duration (hr)	Min of secretory activity/hr	Average total cortisol secreted phase (mg)
I	Minimal secretory activity	−4 to 2	6	2	.28
II	Preliminary nocturnal episode	3 to 5	3	16	1.7
III	Main secretory	6 to 9	4	31	6.3
IV	Intermittent waking activity	10 to −5	11	15	7.2

formed their studies. Estimate of the total time spent in secretory activity also indicated close agreement among subjects with a mean value of 25% of the 24 hours. On the basis of these figures, the adrenal cortex when actively secreting cortisol is doing it at a rate of approximately .05 mg/min.

The approximate rate of secretion of cortisol was calculated from determinations of the average amount of cortisol secreted and the duration of secretory activity for each hour of the 24-hour period. The finding of a remarkably constant secretory rate during periods of both high and low cortisol output indicates that it is the number and duration of the secretory episodes and not a change in secretory rate that determines the amount of cortisol secreted for a given time period. This suggests that the adrenal cortex functions as an all-or-none mechanism when stimulated and releases cortisol at a rate close to .05 mg/min. The two exceptions to this, namely, the fourth and twenty-fourth hour of the study, were both periods of low total cortisol secretion and therefore may be explicable by the limitation of 20-minute sampling technique. Since a secretory episode which lasts less than or is equal to 20 minutes may be missed or underestimated during such hourly periods of low total secretory activity, the rate will erroneously be determined to be low.

There are some instances (Fig. 1, R.S., T.W., K.S.) where the slope of the declining portion of a secretory episode yields a half-life (approximately 140 min) which is longer than the 60- to 80-minute value demonstrated for plasma cortisol. This finding can be explained by assuming either that cortisol continued to be secreted during the declining portion of the episode, although at a much lower rate than during the rise, or alternatively that secretory episodes, small in both duration and magnitude, occur in the period between the 20-minute samples. Preliminary data from our laboratories suggest that the latter can occur, since small secretory episodes have been found to occur during the 20-minute period when samples are obtained at five-minute intervals (unpublished observations). Further studies are necessary with concomitant measurement of the daily production rate using isotope dilution techniques and five-minute sampling before the daily production rate and the estimate obtained from Equation I can be accurately compared. A more frequent sampling rate might well yield a somewhat higher rate of secretion. Indeed, the importance of using frequent sampling techniques is illustrated in Fig. 2, comparing the clear phasic pattern of hourly output and secretion time to the smooth curve of averaged concentrations.

When the time of occurrence and the duration of the secretory episodes are displayed (Fig. 3), and compared with Fig. 1, a temporal pattern of activity emerges. These data suggest that adrenal cortical activity over the 24-hour sleep-wake cycle of normal man may be divided into four unequal temporal phases (Table 3).

Phase 1 (period of minimal secretory activity). During the six-hour period including four hours before and two hours after the onset of sleep, cortisol secretion is negligible and the pituitary-adrenocortical mechanism for the secretion of this hormone is in a period of inactivity. Cortisol elaborated earlier is steadily removed from the plasma and during the last one or two hours of

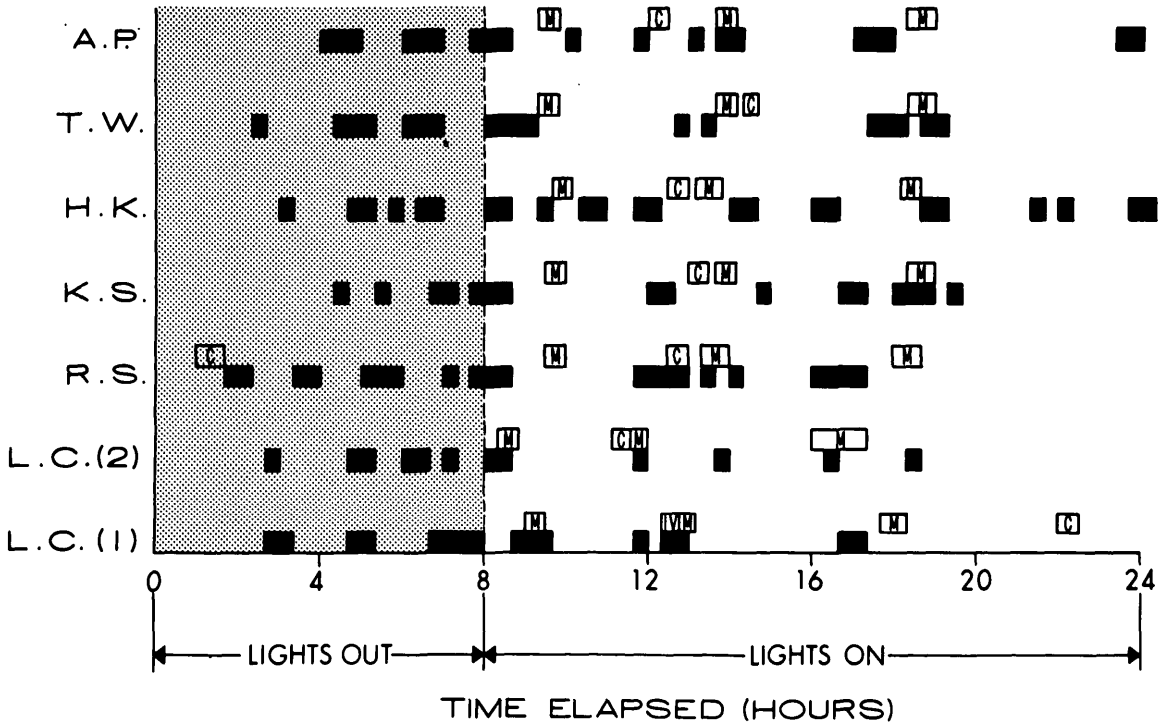

FIG. 3. Duration and timing of individual secretory cortisol episodes for each 24-hr study period. M is time of meals; C is time of insertion or reinsertion of the venous catheter; IV is time of intravenous injection of tracer dose of ^{14}C-cortisol [L.C. (1)].

this phase there is almost no circulating cortisol detectable.

Phase 2 (preliminary nocturnal secretory episode). A single isolated secretory episode was found to occur in five of the seven studies during the third through fifth hours of sleep. Of the remaining two studies, in one a brief arousal due to catheter trouble was followed by two secretory episodes within this time period. In the sixth study an episode occurred at the end of the phase and was followed rapidly by a second secretory burst. In addition to the above, this isolated preliminary nocturnal episode could be recognized in data published previously using a 30-minute sampling schedule. It can be seen in five of six eight-hour sleep studies from graphs published by Weitzman *et al.* (8), and appears to be present for both cortisol and ACTH in four of the eight graphs shown by Berson and Yalow (10).

Phase 3 (main secretory phase). During the 6th, 7th and 8th hours of sleep and continuing through the first hour of wakefulness, a series of three to five secretory episodes occurred. This fourth period represents the most intense time of adrenal cortical activity, and accounts for nearly half the total hormone secreted over 24 hours. It should be emphasized that this major cortisol secretion phase is largely due to the close clustering of secretory episodes.

The sleep patterns were normal, with the six subjects sleeping an average of 438 minutes each night or 90% of the eight-hour period. They spent an average of 10% of their sleep time in stage I sleep, 52% in stage II, 11% in stage III, 8% in stage IV and 19% in REM sleep. None of the subjects showed any unusual sleep patterns. The general correlation between increased rapid eye movement (REM) sleep, decreased slow wave sleep and increased

adrenocortical activity in the latter half of the sleep period presented in a previous study (8) was reaffirmed. As mentioned in that report, the parallel elevation of mean hormone level and mean REM time during the latter half of the night was "not sufficient evidence to clearly indicate a causal relationship between them." Indeed, evidence from a number of studies including our own does not support a one-to-one REM-hormone causal relationship (9, 13, 14). When subjects were totally sleep deprived for one or two nights, the nocturnal episodic secretion of cortisol was not prevented. In addition, when the sleep-waking cycle was acutely inverted, a significant delay in the re-establishment of the circadian 17-OHCS occurred and a dissociation between sleep stage patterns and plasma cortisol levels was present. These findings indicated that the temporal correlation of REM sleep and episodic cortisol release during the latter third of the night can be dissociated and therefore an obligatory relationship is not supported by the evidence.

Phase 4 (intermittent waking secretory activity). Between four and nine secretory episodes were found in this 11-hour compartment, spanning the second through twelfth hours of the waking period. There was considerable variability in the secretory output, duration and spacing of these episodes, with a suggestion of a bimodal distribution (Fig. 3), around the fifth and tenth hours of this phase. Although these were roughly coincident with the subjects' meal times, no specific correlation between meals and secretion could be recognized when the studies were examined individually. However, a more precise timed-analysis searching for a possible relation between plasma cortisol and meal-related blood glucose changes should be carried out in light of a recent report of a decrease in plasma ACTH after oral and intravenous glucose administration in normal subjects (15). Other possible environmental factors such as telephone calls, conversations, reading, visitors and changes in emotional state were not rigorously defined and therefore their contribution, if any, to the timing of the waking secretory episodes must await future studies specifically designed to answer this question.

Five of the seven studies showed a prolonged cessation of secretory activity in the second, third and fourth hours of the waking period [Fig. 1 and 3, L.C. (1), L.C. (2), R.S., K.S., T.W.]. This post-secretory pause marked an abrupt discontinuation of activity after the maximum secretory phase, with the maximum or near-maximal concentrations attained in the first hour of waking falling off smoothly and rapidly. Waking secretory activity then resumed for these subjects at the end of the fourth or in the fifth hour of waking. Four or five episodes then occurred, with no definite regularity, with the secretory activity ceasing abruptly by the twelfth hour of waking. Subjects A.P. and H.K. showed no such post-secretory pause and continued their secretory activity throughout the entire waking day, with episodes occurring in the hour immediately before the lights-out time. Both subjects had the maximum plasma cortisol concentrations of the 24 hours recorded during this period.

The two complete 24-hour studies done on the same subject at an interval of two months [Fig. 1, 3, L.C. (1) and (2)] demonstrate major differences in secretory activity during the waking period. In the first study, three major secretory episodes occurred at the 2nd, 5th and 10th hours of wakefulness, producing peak concentrations of 16, 15 and 14 μg/100 ml, respectively. One additional episode was a small "shoulder" to the subsequent larger episode. During the long periods between episodes, cortisol concentration fell smoothly. By contrast, during the second 24-hour study [L.C. (2)], a major elevation of concentration occurred in the first hour of waking, with a peak value of 20 μg/100 ml. This was followed by a rapid series of four discernible small episodes during which the concentration varied between 2 and 8 μg/100 ml.

The common features in these two daytime patterns are the presence of a postsecretory pause and the apparent absence of secretory activity during the last four hours of wakefulness, a feature shared with three of the five other studies.

It is important to emphasize that the data presented above, as well as previous reports by ourselves and others, seriously challenge the concept that a "steady state" or "basal level" of cortisol is present during any extended time compartment of the 24-hour cycle. Indeed, only when the cortisol concentration fell to near zero was there any prolonged time of constancy; this occurred during the two to four hours in proximity to the onset of sleep. This can hardly be called a basal level, however, since there is essentially no cortisol being secreted by the adrenal gland. Moreover, these studies demonstrate great variability in both the lag time between secretory episodes and the plasma concentration at which the episodes are initiated. Therefore, any theory which proposes a closed feedback loop and/or a variable regulator ("set-point") mechanism must account for the apparent absence of a "steady state" concentration and the great variability over short time periods of cortisol and ACTH concentration throughout the entire 24-hour period (16). There is certainly strong evidence that the concentration of cortisol in blood and certain brain areas is effective in dampening the ACTH-cortisol secretory system. However, as was emphasized in our previous paper, the temporal sequence of episode initiation appears to be under central nervous system control, with a fairly consistent pattern under stable cyclic circadian conditions.

Finally, these results emphasize the importance of considering biological time in contrast to physical or clock time in defining physiologic and pathologic processes. The timed clustering of secretory episodes of the ACTH-cortisol system is only one component of the temporal relationship of a wide variety of neural, hormonal and metabolic processes. The new discipline of chronobiology advocated by Franz Halberg has fully documented the rhythmic complexity of physiologic patterns which take place in man (17, 18). The implications of these and other experimental findings in the issue of the timing of metabolic-tissue phase synchronization, of symptoms and pathological changes and of treatment with ACTH and adrenal cortical steroids, must be seriously considered.

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