CIRCADIAN PERIODICITY OF PLASMA CORTISOL LEVELS: EFFECT OF RANDOM LIVING SCHEDULE IN MAN

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Abstract. Six normal subjects lived for two weeks on disorganized schedules in which they were in bed for four 2-h periods per day at random intervals. Meals were also served on a random schedule. Subjects varied in their ability to adapt their sleep to such schedules. One subject achieved an excellent adaptation, one very good, and two fairly good. Circadian periodicity of plasma corticosteroid levels was not greatly affected by the experiment, but became 'noisier' in most subjects, possibly because of irregularity of individual cortisol secretory episodes within the circadian cycle. The results do not encourage the belief that circadian adrenal periodicity depends upon the cumulative effects of regular living schedules.

The circadian periodicity of hypothalamic-pituitary-adrenal function in man has several characteristics of endogenous rhythms. It persists and retains its original phase through several days of bed rest (Katz, 1964), constant light (Krieger *et al.*, 1969), constant darkness (Aschoff *et al.*, 1971), or constant waking (Rubin *et al.*, 1969). It undergoes a gradual phase shift following shifts in environmental and sleep schedules (Flink and Doe, 1957; Perkoff *et al.*, 1959; Sharp *et al.*, 1961; Haus *et al.*, 1968), and free runs during prolonged isolation in constant environments (Halberg and Reinberg, 1967; Ghata *et al.*, 1968).

These observations establish that the adrenal cycle is not an acute response to any of these schedules, but they do not exclude the possibility that it may be due to a cumulative effect of regular schedules of habit, sleep, and environmental changes. During prolonged isolation in constant environments, free running adrenal cycles have remained internally synchronized with the sleep activity cycle (Halberg and Reinberg, 1967; Ghata *et al.*, 1968). If the sleep schedule is capable of acting as a synchronizer, then the free running adrenal cycle could be attributed to the cumulative effect of the sleep-wake cycle. This possibility is supported by the findings of Orth *et al.*, (1967) that several cycles of sleep-wake schedules of 12, 19, or 33 h resulted in adrenal cycles with corresponding periods. Contrary evidence was reported by Simpson and Lobban (1967), who found that a 24-h adrenal cycle persisted for several weeks in subjects on 21-h sleep-wake schedules.

If the adrenal cycle depends upon cumulative effects of regular synchronizer schedules, it should gradually disappear on random or grossly irregular schedules. The present report describes an investigation of this hypothesis. On random environmental schedules rats (Holmquest *et al.*, 1966) and micro-organisms (Edmunds and Funch, 1969) appear to develop free-running circadian rhythms, and a lizard became immobile and died (Taylor and Tschirgi, 1960). To our knowledge man has not pre-

viously been studied on random schedules, and there have been no attempts to randomize the activity schedule of any species.

Of possible importance is the fact that the daily rise and fall of plasma cortisol is not smooth but occurs as a series of spikes (Weitzman et al., 1966). Hellman et al. (1969) showed that these are due to episodic bursts of cortisol secretion, and that secretion ceases completely in the intervals between bursts. The effect of random living schedules on plasma cortisol spikes has also been investigated in a limited way.

1. Subjects and Methods

Six normal subjects (4 men, 2 women), age 19–32 yr, were admitted to a small clinical research ward for total periods of 21 days each. The first 7 days comprised a control period, during which the subjects followed the normal ward routine, being in bed from 2200 to 0600 hours each day and up from 0600 to 2200. Meals were served at 0630, 1200, and 1730. The first week was followed by 14 days of random schedules which had been determined in advance by card shuffling. The schedules were such that each 24-h day contained four 2-h periods in bed with the opportunity to sleep, and eight 2-h periods out of bed with no sleep allowed, the periods occurring in random sequence. Three meals were served each day at random intervals during the periods out of bed.

Time in bed was spent in private, quiet, dark rooms, the windows having been sealed so as to admit no light from the outside. During times out of bed, all areas, including sleeping rooms, were well illuminated by fluorescent lights, or, where available, by natural daylight. While out of bed the subjects engaged in self-selected activities such as reading, receiving visitors, or watching television. During these times they were also exposed to the other members of the ward population, who followed the normal diurnal living schedule of the ward. The total ward population at any one time varied from 6 to 10 persons, including the subjects of this study.

Subjects 1 and 2 (males) were studied as individuals, each at a time when no one else on the ward was undergoing this experiment. They each followed the schedule depicted in Figure 1. Subjects 3, 4, 5, and 6 were studied simultaneously as a group, following the schedule depicted in Figure 2. Sleep deprivation was not a desired result of the experiment but served as a means of forcing sleep into unnatural schedules. The more perfectly a subject adapted to the schedule the less sleep he lost.

Toward the end of the Control period and again toward the end of the Experimental period, intravenous catheters were placed in a forearm vein and fitted with three-way stopcocks according to the technique of Orth et al. (1967) so as to allow blood sampling with minimal disturbance to the subject. These remained in place for approximately $2\frac{1}{2}$ days at a time, during which blood was sampled hourly.

Because of technical difficulties blood samples were not obtained from Subject 1 during the initial control period. Series of samples were collected from him at the end of the first and second weeks of the random schedule, and he returned to the ward several months later to contribute control samples while living on the Control schedule. Subject 6 first entered the experiment at the beginning of the random schedule, and he
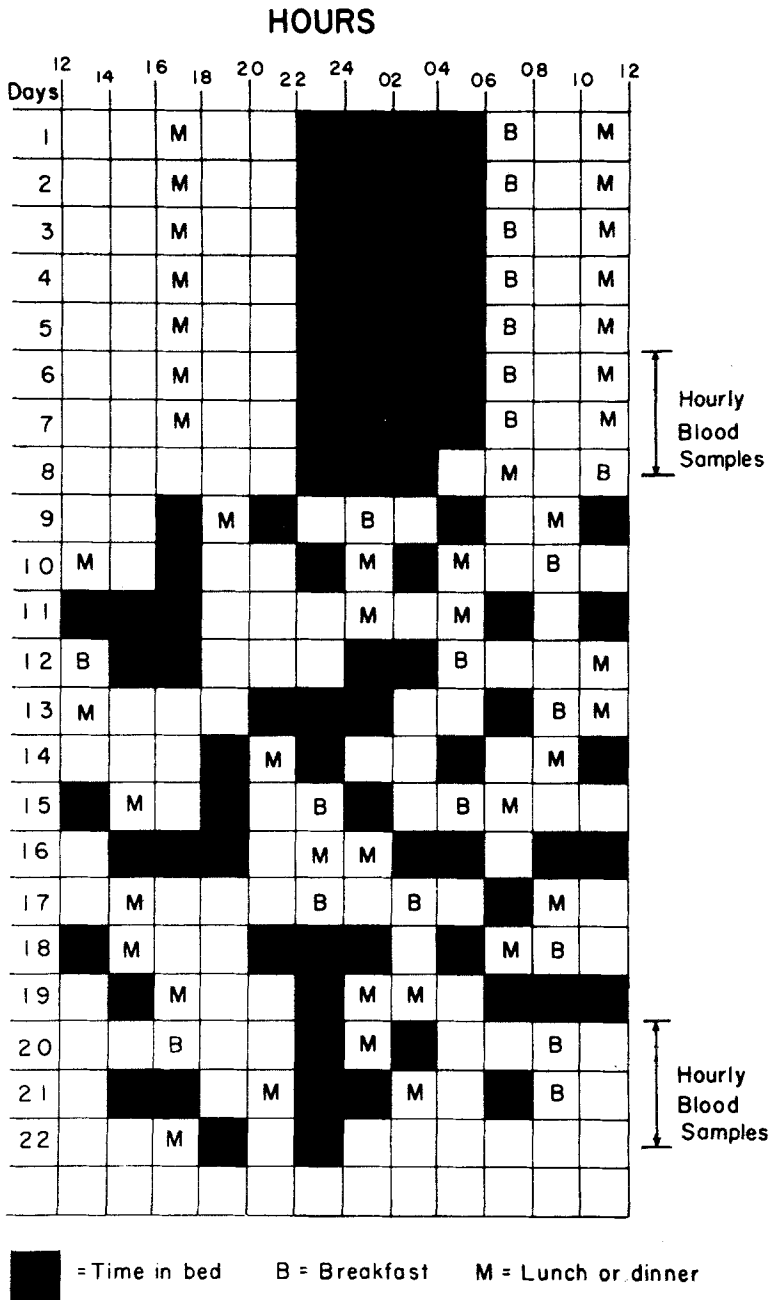
HOURS

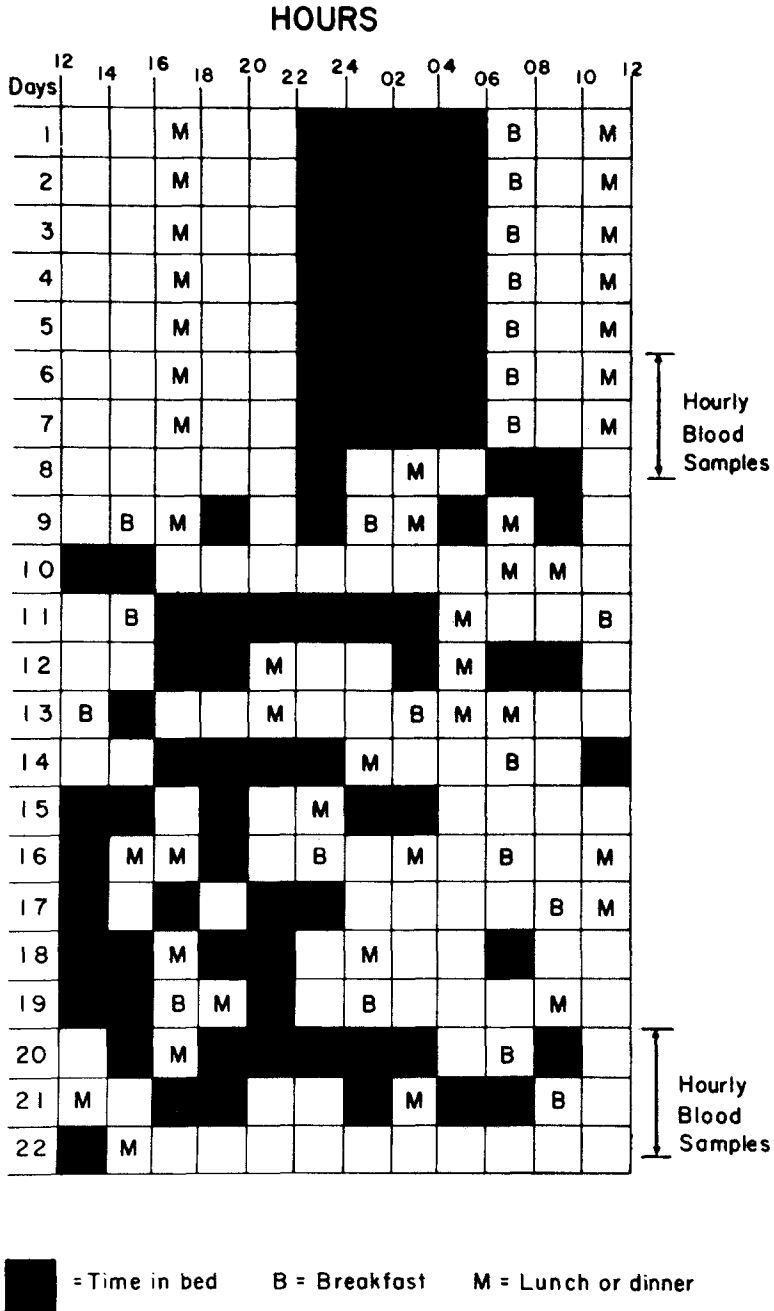Fig. 1.   Living schedule of Subjects 1 and 2.

Fig. 2.   Living schedule of Subjects 3, 4, 5, and 6.

also returned several months later for control measurements. Several samples from subjects 3, 4, and 5 were missed during the Control period because of technical difficulties.

Plasma cortisol was measured in duplicate by competitive protein binding with equilibrium dialysis (Murphy *et al.*, 1963 ; Jones and Mason, 1966). Standard deviations obtained by repeated assay of various plasma pools were approximately 0.7 μg %.

2. Results

Subjects varied in their reported ability to sleep at irregular times. Though reports may be inaccurate regarding the presence or absence of sleep at a particular time, reports of overall good or poor sleep are borne out by electroencephalographic studies (Mendels and Hawkins, 1967; Monroe, 1967). Hence, the subjects' reports of overall good or poor sleep may have value in the present context. Subject 1 reported consistently good sleep, being able to fall asleep promptly at any time and to remain asleep until called to get up. Subjects 3 and 4 reported fairly good sleep throughout. Subjects 2 and 6 reported moderate difficulties and Subject 5 reported considerable difficulty. Most of the reported difficulties concerned time spent in bed during daytime hours.

Graphs of the plasma cortisol data from each subject are presented in Figure 3. Hourly measurements of plasma cortisol do not precisely identify individual secretory episodes as described by Hellman *et al.* (1969), who considered measurements every 20 min to be necessary. Nevertheless, the spiking pattern and the overall configuration of the curve during the control period appeared to be characteristic for each individual, and to be fairly reproducible on consecutive days in those subjects from whom complete data were obtained. Maximum values always occurred around 0700, and there was usually a deep trough during the evening, with values near zero. Plasma cortisol levels did not return to near zero levels between the larger spikes in the morning and early afternoon.

At the end of the Experimental period, a basic circadian pattern was still clearly present, though the pattern of individual spikes was less regular. The largest spikes still occurred around 0700 and lower values still occurred in the evening. However, evening troughs were more frequently broken by small spikes, and plasma cortisol fell to lower levels between the larger morning spikes. Spikes tended to occur shortly after, rather than during, periods in bed, suggesting that times in bed may have tended to abort or postpone individual secretory episodes. Two unusual occurrences were the failure of Subject 1 to produce a major spike during the last night of his study and the occurrence of a major spike at 0100 on the last day of Subject 4's study. Inspection of the graphs leads to the conclusion that circadian periodicity survived the Experimental schedule in all subjects, but became somewhat 'noisier' in most subjects.

Mathematical analyses support this conclusion. Table I presents the results of cosinor analysis (Halberg *et al.*, 1967) of the group data. This is a computer method for estimating the parameters of the cosine curve which best fits the data. No curve derived by this method, either for an individual or for the group, had a period which was
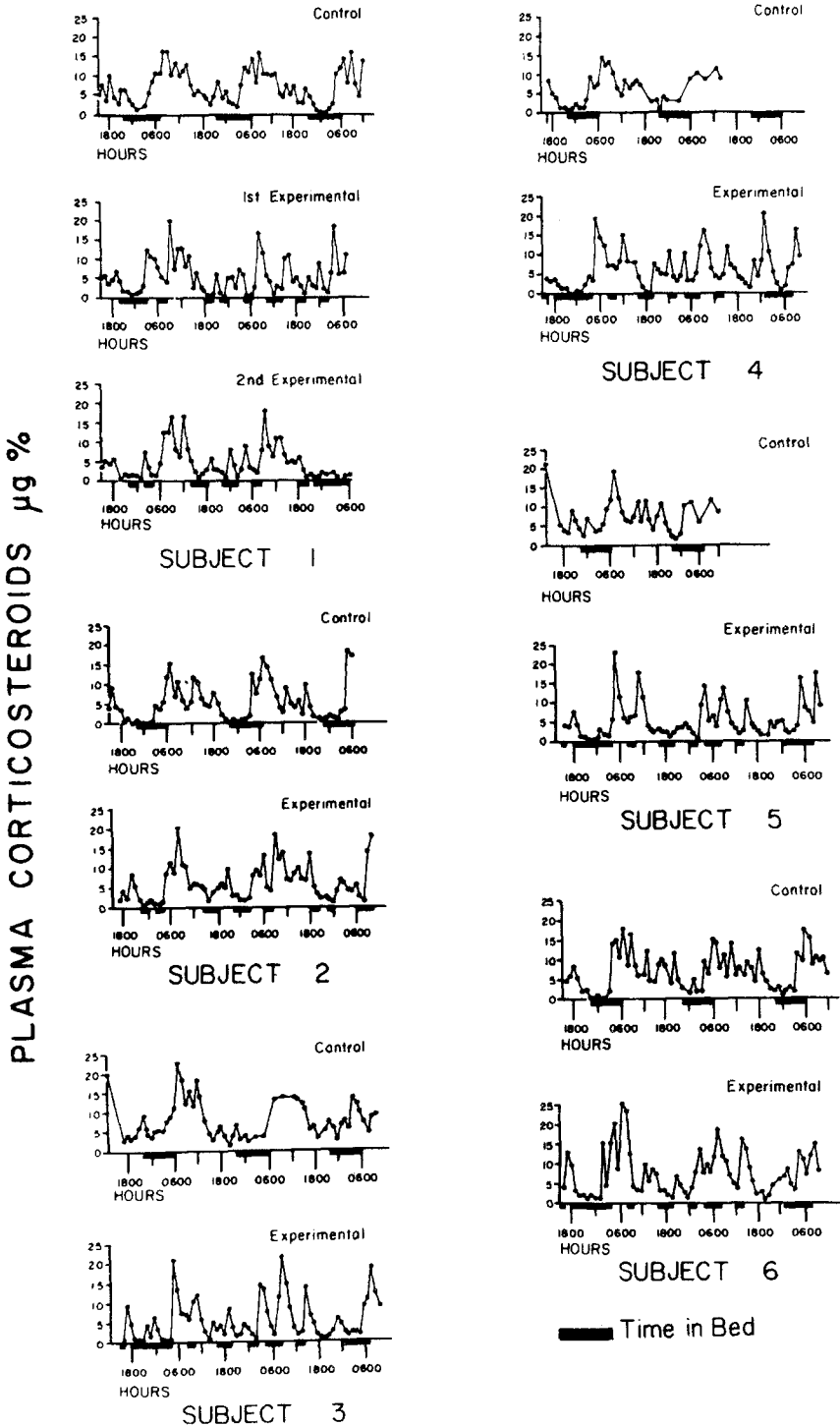
Fig. 3.   Plasma corticosteroid levels of subjects on Control and Experimental schedules.

statistically different from 24-h. Therefore, the results in Table I are based on the fitting of curves with periods of exactly 24-h. Highly significant 24-h periodicities with approximately the same phase were present under Control and Experimental conditions.

TABLE I

Cosinor analysis, giving parameters of best fit cosine curves ($\tau = 24$ h) for group plasma cortisol data under Control and Experimental conditions

	Amplitude (C)[a] $\mu g\%$	Phase (ϕ)[b] Angular Degrees	Significance of Rhythm Detection p
Control (6 subjects)	4.10 (2.97, 5.22)	-148 (-136, -159)	< 0.001
Experimental (6 subjects)	3.63 (2.74, 4.52)	-138 (-113, -166)	< 0.001

[a] With 95 % confidence interval.
[b] Expressed as angular delay of wave crest relative to local midnight.

TABLE II

Autocorrelation coefficients of plasma cortisol data for each subject under Experimental and Control conditions

	Number of Data Points	r for lag 24 h	$\bar{z}$ for lags 20–28 h
Subject 1			
Control	70	0.77[b]	0.50[b]
Exptl. 1st wk.	65	0.13	0.14
Exptl. 2nd wk.	62	0.55[b]	0.39[c]
Subject 2			
Control	63	0.70[b]	0.48[b]
Exptl.	66	0.40[b]	0.26[a]
Subject 3			
Control	58	0.50[b]	0.46[b]
Exptl.	67	0.24	0.31[c]
Subject 4			
Control	38	0.80[b]	0.68[c]
Exptl.	65	0.14	0.19
Subject 5			
Control	37	0.22	0.24
Exptl.	67	0.15	0.23
Subject 6			
Control	69	0.67[b]	0.38[b]
Exptl.	66	0.54[b]	0.35[c]

[a] Significantly different from zero at $p < 0.06$.
[b] Significantly different from zero at $p < 0.01$.
[c] Significantly different from zero at $p < 0.025$.

In the Experimental situation the amplitude of the average derived curve was slightly smaller and the amplitude and phase more variable. Table II presents the results of auto-correlation analyses of the data from each individual. With the exception of Subject 5 from whom very few Control data points were obtained, all subjects had highly significant circadian periodicities during the Control period, as indicated by the autocorrelation coefficient for 24-h lags (r_{24}) or by the average of z scores ($\bar{z}_{20-28}$) derived from r's for lags from 20 to 28 h, spanning the circadian spectrum. In all subjects r_{24} and z_{20-28} were smaller in the Experimental than in the Control data. Non-significant r_{24}'s were obtained from the Experimental data of 4 subjects, and non-significant $\bar{z}_{20-28}$'s were obtained from the experimental data of 4 subjects.

Subject 1 is of special interest, both because of being the best sleeper by self-report, and also because of an extra series of data after only one week of random schedule. These show a maximum reduction of autocorrelation values after one week with partial recovery after two weeks.

Cosinor analyses were also performed on the data from each subject individually. These results have not been presented in tabular form, but were consistent with the results of the autocorrelation analyses; i.e. small changes in autocorrelation coefficients corresponded with small changes in the same direction in amplitude of the best fitting cosine curve.

3. Discussion

Circadian adrenal periodicity was not greatly altered by the random schedule, though it did become somewhat more irregular or 'noisy' in most subjects. This may have been due to some effect on individual episodes of cortisol secretion within the daily cycle.

The increased noisiness could be interpreted as early weakening of the circadian periodicity due to weakening of the cumulative effect of regular synchronizer schedules. The subjects who reported better, and therefore more nearly random, sleep (Subjects 1, 3, 4) were approximately the same as those (Subjects 1, 2, 3, 4) whose adrenal cycles became noisier on the random schedule. This view would imply that continuation of the random schedule for a longer period would have resulted in progressive deterioration of the adrenal cycle. However, the data from Subject 1 showed the opposite; disorganization was maximal after one week on the random schedule, and some reorganization had occurred by the end of the second week. Furthermore, the two-week interval was more than that required for phase shifting (Perkoff *et al.*, 1959; Sharp *et al.*, 1961) or for Orth *et al.* (1967) to establish non-circadian periods. If extrapolation from these situations is permissible, then two weeks should have been adequate to establish whatever effect the schedule was to have.

It may be objected that sleep was not strictly random, since some subjects apparently tended to lie awake more when in bed at times outside their accustomed sleep schedules than otherwise. Subject 1's report of near-perfect adherence to the sleep schedule argues against non-random sleep in his case, whereas this objection bears more weight

with respect to Subject 5. However, sleep was broken and irregular even in those subjects who probably had unequal distributions of sleep between day and night.

It is conceivable that the social and organizational routines of the remaining persons on the ward may have served as secondary synchronizers for the adrenal cycles of these subjects. Weitzman *et al.* (1968) failed to obtain phase reversal by reversing sleep without isolating subjects from the social milieu, and night shift workers may also fail to shift their adrenal cycle (Migeon *et al.*, 1956; Conroy, 1967). Thus the possibility that secondary synchronizers may have maintained the adrenal cycle in this study cannot be ruled out. On the other hand, Orth *et al.* (1967) who reported establishing non-circadian adrenal periods, did not describe any measures to isolate their subjects from surrounding 24-h social routines.

The findings favor the view that adrenal periodicity is a true circadian rhythm, and that noisiness during the Experimental condition was due to acute effects of the irregular schedule. Such acute effects may have acted on the individual secretory episodes within the circadian pattern. The possibility that the surrounding social routines served as a secondary synchronizer would also support this view, since the ability to entrain to secondary synchronizers is a characteristic of circadian rhythms.

One piece of evidence which fits awkwardly with this interpretation is the apparent ease with which Orth *et al.* (1967) established non-circadian adrenal periods by imposing non-24-h sleep-wake cycles. It is not clear how this finding can be reconciled with the other evidence.

Acknowledgements

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