CIRCADIAN RHYTHM OF PLASMA CORTISOL IN ANTARCTICA

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ABSTRACT. The levels of plasma cortisol were studied in four subjects while living at Halley Bay, Antarctica, during the year 1972. The British Antarctic Survey station, Halley Bay (lat. 75° 36′ S., long. 26° 38′ W.), is situated on the Caird Coast of the Weddell Sea. The station is buried, with the men living underground and subjected to an artificial light/dark environment. These subjects lived a normal daily routine during the summer months, asleep from 24.00 to 08.00 hr. and awake from 08.00 to 24.00 hr. This pattern was basically similar during the winter months, April–August, although there were some slight individual differences. Venous blood samples were taken every 4 hr. during a period of 24 hr. in February, April and August.

These results were compared with those obtained from ten subjects studied at South Georgia (lat. 54° 17′ S., long. 36° 30′ W.) in February 1972; although living in an isolated community with an Antarctic environment, these men were not subjected to periods of continuous darkness or continuous daylight.

In February at Halley Bay the individual peak levels of plasma cortisol occurred at the time that the subjects awoke and the nadir occurred at the time the subjects retired to bed. The circadian rhythms at Halley Bay were very similar to those found at South Georgia. However, at the end of winter (in August) following a 3 month period of continuous environmental darkness, the peak level of plasma cortisol occurred at 12.00 hr. and not at the time the subjects awoke.

EXCEPT in the far Northern and Southern Hemispheres, Man is exposed to periods of daylight and darkness with overall lengths of 24 hr. In polar regions the annual period of winter darkness and of continuous summer daylight are each of about 4 months' duration with a 2 month period of normal light day/dark night at each equinox. During these different periods of light/dark the normal sleep/wake and rest/activity cycles continue. The environment at Halley Bay provides an ideal background for further studies on the effects of altered light/dark patterns on Man. Recently, workers (Berson and Yalow, 1968; Hellman and others, 1970; Krieger and others, 1971; Weitzman and others, 1971; Gallagher and others, 1973) have shown that cortisol is secreted episodically with maximum plasma cortisol levels between 04.00 and 08.00 hr., with a low cortisol secretion rate from 4 hr. before to 2 hr. after the onset of sleep.

Studies by the author (to be published) have shown that alterations of the sleep/wake schedule for a period of 10 days resulted in a new circadian rhythm for plasma cortisol. It is difficult to determine whether this sleep/wake reversal is solely responsible for the shift of the rhythm or whether other factors such as the light/dark or rest/activity cycles play a significant part in the causation of this new thythm. Aschoff (1963) has shown that light is an important "Zeitgeber" in animals, and in the last decade there has been conflicting evidence to support the hypothesis that light is an important "Zeitgeber" in Man (Krieger and others, 1969; Orth and Island, 1969; Aschoff and others, 1971; Osterman, 1974).

MATERIALS AND METHODS

Four healthy male subjects, aged 24–37 years, were studied on three separate occasions throughout the year 1972 (February, April and August) at Halley Bay. Two of the subjects, PJ and AS, were occasionally subject to irregular sleep/wake patterns due to their occupations as meteorologists, but these patterns were of a very short duration, 2–3 days. The subjects were all studied while continuing their normal daily work routines. Subjects in the summer months arose at 08.00 hr. and retired to bed about 24.00 hr. The amount of time spent out of doors at Halley Bay, according to Norman (1965), is 13 per cent during the months of continuous light (October–April) and 5 per cent during the months of darkness (May–September). During the remainder of the time the subjects are exposed to an artificial light/

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dark environment. In the winter there was a slight variation in that subjects tended to awake slightly later, 09.00–10.00 hr., and retired about 24.00–01.00 hr. The subjects slept in complete darkness.

Ten healthy male subjects, aged 25–35 years, were studied during the same year at South Georgia. Their heights varied from 1·57 to 1·83 m. and their weights ranged from 59·0 to 80·5 kg. These subjects were studied under normal conditions of daylight and darkness (February 1972) and provided a control group for comparison with subjects studied at Halley Bay. The experimental format was similar at both stations.

Venous blood samples for the measurement of plasma cortisol were collected from the antecubital fossa in all experiments. Plasma was separated from the cells by centrifuging at 2,500 r.p.m. for 10 min. Duplicate aliquots, each of 1 ml. were placed in small stoppered tubes and 0·1 ml. of 0·25 N sodium hydroxide was added to each tube. Each aliquot was then made up to 2 ml. with 0·9 ml. distilled water. The duplicates were stored in separate freezers at −20 °C until analysis on return to the United Kingdom.

The circadian variation in plasma cortisol was examined by sampling every 4 hr. during a 24 hr. period, commencing at 08.00 hr. (09.00 hr. in February at Halley Bay) and finishing at 08.00 hr. the following day. During this period the subjects rested as much as possible immediately prior to the sampling time.

Plasma cortisol was analysed using a sulphuric acid induced fluorescent technique. Cortisol and corticosterone were first extracted into 5 ml. of dichloromethane. A 4 ml. aliquot of the dichloromethane extract was then evaporated to dryness and the residue so obtained dissolved in 0·5 ml. of alcohol and 1·5 ml. distilled water. Vermuelen and van der Straeten (1964) have found that the partition coefficient for corticosterone is 0·75 and that for cortisol is 120 in a water/carbon tetrachloride partition. They found that the addition of corticosterone to aliquots of plasma to produce concentrations of 3·3 and 6·6 μg./100 ml. resulted in a 90 ± 4 per cent ($n = 8$) removal of the corticosterone by carbon tetrachloride. It is thus possible to separate the two hormones by washing the aqueous extract, containing both cortisol and corticosterone, twice with 10 ml. of carbon tetrachloride. The cortisol, remaining in the aqueous layer, was extracted again into 5 ml. of dichloromethane. The fluorescence of cortisol was measured on an Aminco-Bowman spectrophotofluorimeter (American Instrument Co. Inc.) after treatment with 1·5 ml. of the appropriate ethanol/sulphuric acid reagent, using an excitation wave-length of 470 nm. and a fluorescent wave-length of 530 nm. The mean results of the simultaneous determination of plasma cortisol in ten aliquots taken from the same plasma pool on three different occasions gave a mean coefficient of variation of 5·9 per cent for cortisol. Statistical analysis was carried out using Student's *t*-test for paired observations.

RESULTS

The individual and mean circadian rhythms for plasma cortisol in ten subjects at South Georgia are shown in Fig. 1. The peak levels of plasma cortisol occurred at 08.00 hr. with a nadir at 24.00 hr.

The individual circadian rhythms for plasma cortisol for four subjects at Halley Bay in February are shown in Fig. 2. Each subject showed a well-marked circadian rhythm and in three subjects the peak level occurred at 09.00 hr. with a nadir at 01·00 hr. The results for plasma cortisol levels in April (Fig. 3) demonstrate an early morning peak level at 08.00 hr. for subjects KA, JF and AS, similar to that found during the summer months. Subject PJ has an inverted rhythm with a peak at 20.00 hr. However, in all four subjects there is a further peak at 20.00 hr. which is not significantly different from the 08.00 hr. value ($n = 5, p > 0{\cdot}1$).

The individual results for plasma cortisol in August (Fig. 4) demonstrate a peak level at 12.00 hr. There is a significant difference between the mean 12.00 hr. value $22{\cdot}7 \pm 3{\cdot}0$ μg./100 ml. and the 08.00 hr. value $13{\cdot}8 \pm 5{\cdot}8$ μg./100 ml. ($n = 6$, $t = 2{\cdot}65$, $0{\cdot}05 < p < 0{\cdot}02$).

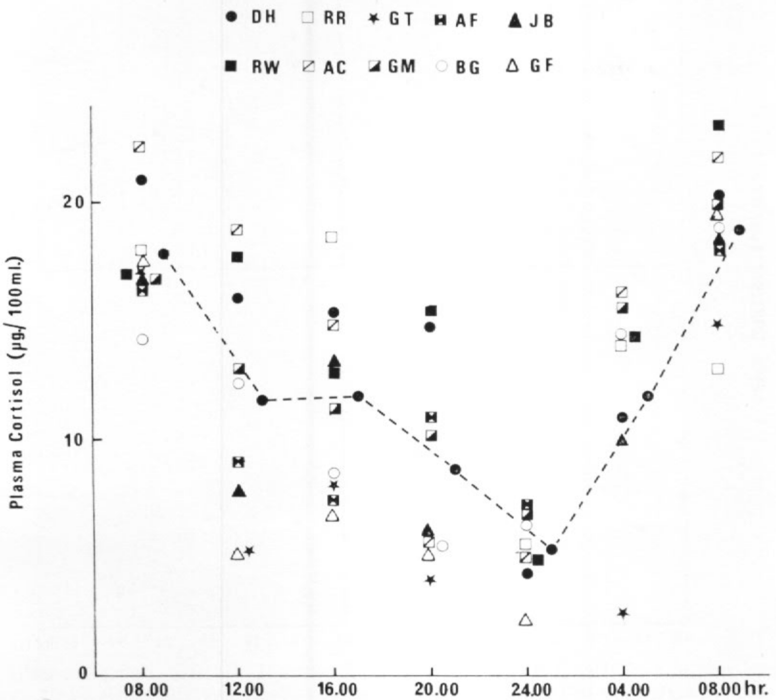

Fig. 1. Individual values of plasma cortisol (μg./100 ml.) for ten subjects at South Georgia, 1972. The mean circadian rhythm is indicated by the dashed line.

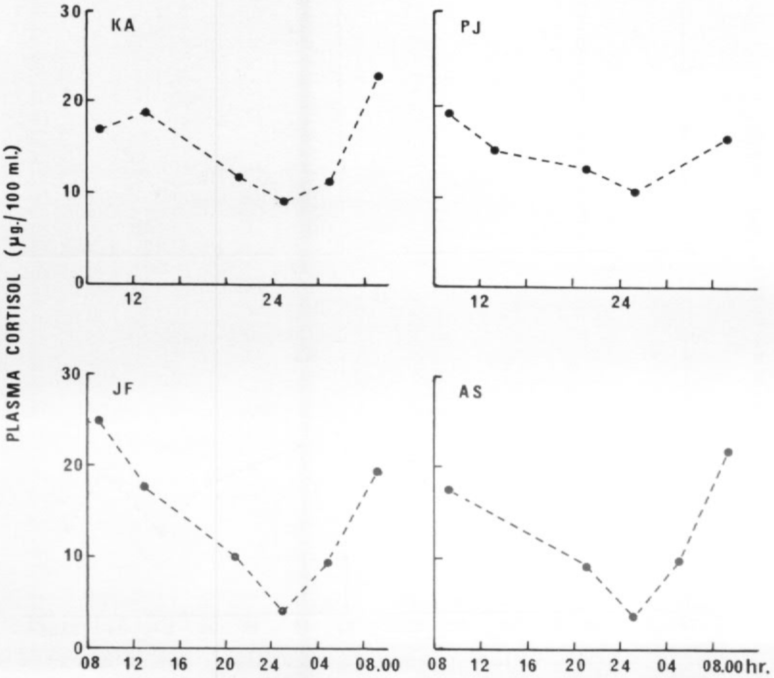

Fig. 2. Individual rhythms of plasma cortisol for four subjects at Halley Bay during the summer (February 1972). There is no 13.00 hr. result available for subject AS and no 05.00 hr. result for subject PJ.

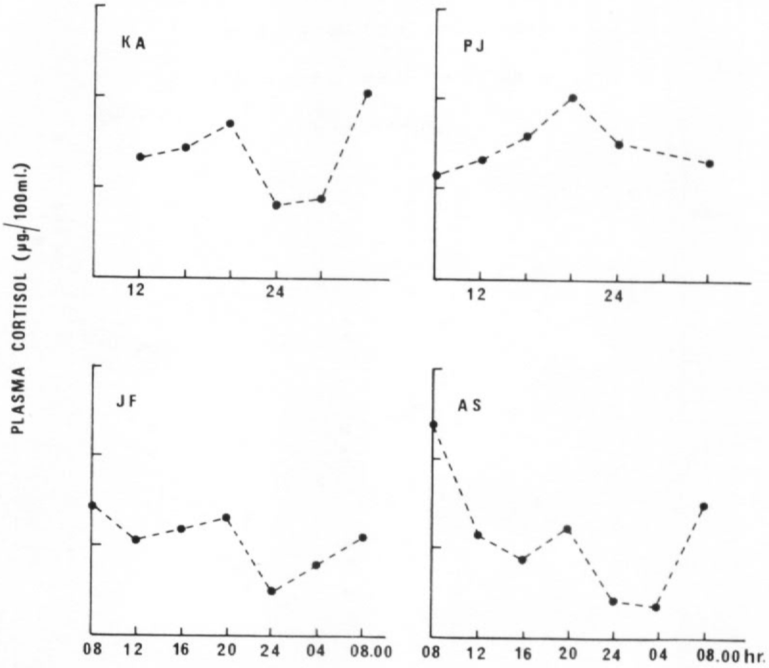

Fig. 3. Individual rhythms of plasma cortisol for four subjects at Halley Bay during the early part of winter (April 1972). There is no 08.00 hr. result available for subject KA and no 04.00 hr. result for subject PJ.

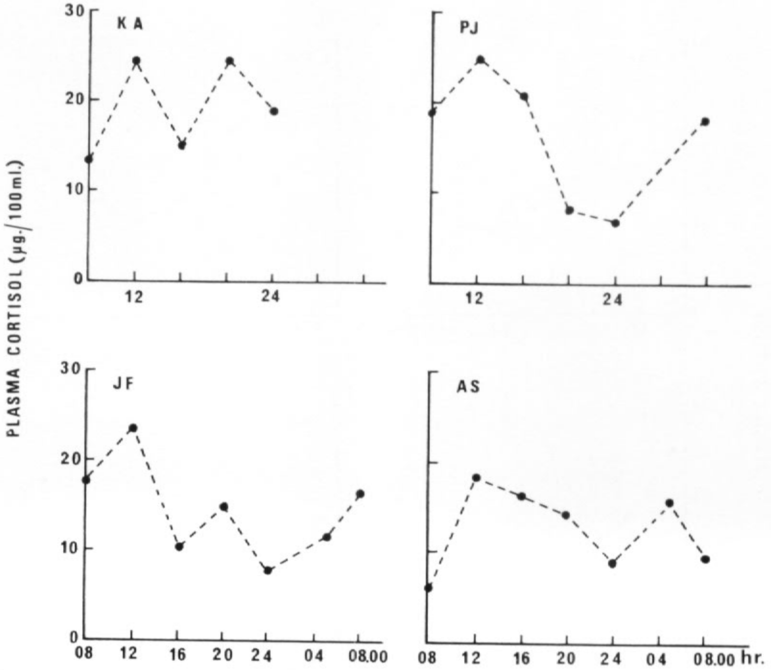

Fig. 4. Individual rhythms of plasma cortisol for four subjects at Halley Bay at the end of winter (August 1972) after 3 months of continuous environmental darkness. There are no 04.00 and 08.00 hr. results available for subject KA nor is there an 04.00 hr. result for subject PJ.

Subject KA had a further high level at 20.00 hr. The nadir occurred at 24.00 hr., the time at which the subjects retired to bed.

The amplitude of the mean plasma cortisol rhythm in August is lower than in February. Perkoff and others (1959), in their sleep-reversal studies, found that the amplitude of the diurnal variation of plasma 17 hydroxycorticosteroids was much less following the period of sleep reversal. Table I shows the mean values for plasma cortisol in the control group at South Georgia and also the mean values at Halley Bay in February, April and August 1972.

TABLE I. CIRCADIAN RHYTHMS OF PLASMA CORTISOL AT SOUTH GEORGIA AND HALLEY BAY, 1972

Time of day	*Plasma cortisol* Mean ± S.D. (μg./100 ml.)			
	South Georgia February	*Halley Bay* February	*Halley Bay* April	*Halley Bay* August
	Number of subjects			
	10	4	4	4
08.00	17·8±2·3	19·7±3·8 (09.00)*	16·5±6·6	13·8±5·8
12.00	11·7±5·2	17·2±2·0 (13.00)*	12·1±1·4	22·7±3·0
16.00	11·8±3·9	–	12·7±3·0	15·6±4·5
20.00	8·7±4·3	11·1±1·8 (21.00)*	15·7±3·7	15·5±6·7
24.00	5·3±1·4	7·0±3·5 (01.00)*	8·8±4·6	10·5±5·7
04.00	12·1±4·5	10·4±1·2 (05.00)*	6·8±3·0	13·8±3·4 (05.00)*
08.00	18·9±3·1	20·4±3·0 (09.00)*	14·9±4·1	14·7±4·6

* Modified times at Halley Bay given in brackets.

DISCUSSION

This study demonstrates that there is a circadian rhythm in plasma cortisol during the summer months in these high-latitude regions. These findings are in agreement with those of de Rodriguez and others (1972), who studied four subjects at the Argentine Antarctic station, Almirante Brown (lat. 64° 53′ S., long. 62° 53′ W.). However, the rhythm at Halley Bay is disturbed during the months of darkness. In the early part of winter there is irregularity of the rhythm with a bimodal pattern, peak levels occurring at 08.00 and 20.00 hr. By the end of winter a definite new rhythm has emerged. This follows a period of 3 months environmental darkness living underground in an artificial light/dark environment and with no communication with the outside world. The peak levels of plasma cortisol occur at 12.00 hr. instead of the usual early morning peak at the time of waking. There is a gradual decline in levels during the day to reach a nadir at 24.00 hr. The levels then rise during the period of sleep. It is difficult to state emphatically that the levels are still rising from 08.00 to 12.00 hr. and that this peak level is not due to a single episodic secretion of plasma cortisol. However, this is an unlikely explanation since all four subjects demonstrate a peak plasma cortisol level at this time. Further work with more frequent blood sampling and a larger group of subjects would be necessary to establish this validity.

Weitzman and others (1975) have carried out a study in which a more frequent blood-sampling technique was employed. They studied seven healthy male subjects, who were living in an environment similar to that of Antarctica at Tromsö in Norway (within the

Arctic Circle). The sleep patterns and 24 hr. plasma-cortisol and growth-hormone patterns were studied during four different seasons of the year. Blood samples were taken every 20 min, from an intravenous catheter during a 24 hr. period on one occasion during each season. All the subjects were found to have a normal pattern of episodic secretion of plasma cortisol with minimal variation in the mean circadian patterns for each season of the year. However, they found that there was a significant difference in the mean 24 hr. concentration of cortisol between the winter and summer, and autumn and winter values ($p < 0 \cdot 01$, $p < 0 \cdot 02$, respectively (t-test)). They also demonstrated that there was very little variation in the amount of time spent in bed during the summer and winter months in these regions, with no variation in the sleep patterns.

In the studies carried out by the author the lowest levels of plasma cortisol occurred at the transition from light to dark, active to rest, and wake to sleep. Orth and Island (1969) carried out experiments in which subjects followed a schedule in which the period of darkness began at 22.00 hr., the hour of retiring, but was prolonged for 2 hr. after awakening, ending at 10.00 hr. They demonstrated a 4 hr. delay in the rise of plasma cortisol, with the peak level occurring at the time of change from dark to light. Osterman (1974) carried out similar work and showed that during a period of 10 days, when darkness was artificiall extended from 06.30 to 10.30 hr. with the subjects being woken at 06.30 hr., a shift occurred in the rhythm of plasma hydroxycorticosteroids.

During the winter months at Halley Bay subjects may not adhere to the strict sleep/wake activity schedules of the summer months. The usual light stimulus of the environment (daylight) is absent. Each subject adjusts his own light/dark environment by switching on his light when he wakes in the morning, and with these ideal conditions there should be complete retinal darkness until the subject awakes. Thus during the winter months the subject may awake at a slightly later hour, between 09.00 and 10.00 hr. Vermikos-Danellis and others (1972) have demonstrated that bed rest reduces the amplitude of the plasma-cortisol rhythm but the circadian rhythm persists. Their experiment was conducted over a period of 56 days. Grim and others (1974) have shown that there is no change in the circadian rhythm of plasma cortisol with changes in posture. It would appear that a further period of bed rest in the morning would not account for a raised level of plasma cortisol at mid-day, i.e. on the basis of an extended period in the prone position. Coincidental secretory episodes are also an unlikely explanation owing to the fact that plasma cortisol levels for all four subjects are significantly elevated.

In this study there is dissociation of the light/dark cycle from the sleep/wake cycle in the true physical sense. However, as in the experiments of Osterman (1974), the subjects in these studies were in complete darkness until they awoke, but this is in fact true for 12 months at Halley Bay. A possible explanation for this delay in the rise of plasma cortisol might be the abrupt transition from dark to light that occurs in these subjects as suggested by Orth and Island (1969). If this was true, there should be a similar shift in the peak levels of plasma cortisol in the summer and early winter. It is possible that during the summer months the stimulus of the external environment (daylight) is sufficient to maintain the normal circadian pattern of these hormones.

However, Aschoff and others (1971) were of the opinion that the light/dark cycle is not essential to entrain human circadian rhythms. They put forward the hypothesis that social activities appeared to be sufficient "Zeitgebers" in the entrainment of human circadian rhythms. In the present studies, subjects lived underground for 3 months in an artificial light/dark environment. They were not subject to the stresses of a normal community life, although different forms of stress may have been exerted within their small isolated community.

The findings of the author are not in agreement with those of Weitzman and others (1975). It is also interesting that Natani and others (1970) and Paterson (1975) have both shown changes in the sleep patterns of subjects on Antarctic bases, whereas Weitzman and others

(1975) found no such disturbances in the Arctic community. The two communities, in the Arctic and Antarctica, are subject to similar light/dark patterns throughout the year with prolonged periods of light during the summer months and prolonged periods of darkness during the winter months. It may well be that the difference in the two communities is due to the psychological conditions that may be prevalent in Antarctic bases, i.e. the isolation during the winter months. Paterson, in his studies at Halley Bay, has suggested that the extremes of daylight in polar regions may be responsible for the change in sleep patterns observed on Antarctic bases and that this may be mediated by changes in arousal systems.

The social environment and psychological conditions at Halley Bay, Antarctica, may result in the disturbance of the circadian patterns of plasma cortisol. These findings would tend to support the views of Aschoff and others (1971) and Weitzman and others (1975) that social activities and sleep patterns are the most important "Zeitgebers" in the maintenance of circadian rhythms in Man.

It is conceivable that many factors including dark/light transition, social activities, sleep patterns and psychological conditions interact in the development and maintenance of human circadian rhythms in plasma cortisol.

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