# Persistent 24-h variations of urinary 6-hydroxy melatonin sulphate and cortisol in Antarctica

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*Summary.* Bright light (2000–3000 lux) of sufficient intensity to suppress human melatonin secretion, acts as a strong zeitgeber in the entrainment of circadian rhythms in man. In polar conditions, light of this intensity is not experienced for several weeks during the winter. The entrainment of human circadian rhythms, in particular that of melatonin, is clearly of interest in these circumstances. Urinary 6-hydroxy melatonin sulphate (aMT6s) is a good index of melatonin secretion in man. In a limited study of seven male volunteers living on an Antarctic base the overall 24-h rhythm of aMT6s excretion was maintained at four different times of year (spring, summer, autumn and winter) and no significant seasonal effects were noted. Cortisol excretion, appeared to be markedly affected by the season although other factors such as social and environmental stress cannot be discounted. These observations suggest that in the absence of a strong light-dark cycle melatonin production may be entrained by other factors.
*Key words.* Human circadian rhythms; melatonin; cortisol; polar environment.

Circadian rhythms in man as in other species are synchronized, or entrained, to the 24-h day by a number of time cues or 'zeitgebers'. The most important of these, generally speaking, is the light-dark cycle, although in man in particular social cues and clock time are of importance.

A qualitative difference is evident in the response of the human circadian system to light: very intense artifical light (2000–3000 lux) will entrain human 24-h rhythms to a 29-h 'day'[1], whereas with artificial light equivalent to normal domestic illumination (i.e. 500 lux) the integrity of the circadian system is maintained only up to a daylength of 27 h. Low intensity light is thus considered to be a weak 'zeitgeber'.

One potentially important biochemical difference in the effects of light of different intensities is that > 2500 lux will suppress human melatonin secretion at night whereas 500 lux will not[2]. Melatonin may be an effective synchronizer of at least some 24-h rhythms in the rat[3] and preliminary studies suggest that it may entrain the 24-h fatigue rhythm in man[4].

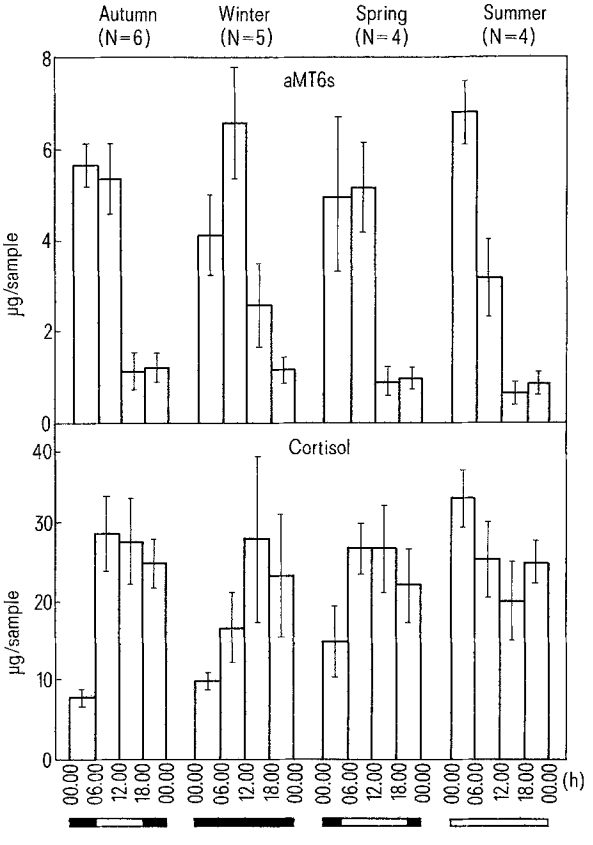
Base personnel in Antarctica live with exclusively artifical light (maximum 500 lux) for lengthy periods during the winter, and in these circumstances frequently experience mild depression, apathy and a reduction of slow-wave sleep[5,6]. A recent report describing the successful treatment of 'winter depression' with bright light (2500 lux)[7], together with the evident interest of assessing 24-h rhythms in such an unusual environment, has led us to investigate the melatonin metabolite, 6-hydroxy melatonin sulphate (aMT6s), and cortisol excretion in healthy volunteers living on an Antarctic base. aMT6s is reported to be the major metabolite of melatonin in man[8,9]. Its urinary excretion is a good index of melatonin production[10].

*Methods.* Volunteers were members of a British Antarctic Survey team living at Rothera Point, Adelaide Island (lat. 67°34', long. 68°07' W).

A total of 19 24-h collections of urine were obtained from seven male caucasians aged 23–33 years, selected from a base complement of 13. Four collections were made throughout the year, at approximately 3-monthly intervals. Urine was passed into individually labeled, plastic, 1-l wide-necked bottles which were collected at 6-h intervals (06.00, 12.00, 18.00 and 24.00 h). After thorough mixing, the volume of each collection was noted and 30 ml decanted into a glass universal bottle for immediate storage at −18 °C. No preservative was added. Urine was transported, frozen, back to the United Kingdom for analysis. On collection days, volunteers arose at 08.00–09.00 h and performed normal work, retiring to their curtained, darkened bunk rooms at 23.00–24.00 h. Indoor lighting was provided by standard fluorescent strip lighting (Thorn; Warm white, Daylight) giving a maximum of 500 lux at approximately eye level. Outdoor exposure at midwinter consisted of brief sorties for specific tasks, whilst exposure was considerably greater in December and January due to the increased outdoor workload and, in leisure time, sunbathing.

Samples were assayed for aMT6s by a novel, direct radioimmunoassay[10]. The method was validated for human urine by classical and chromatographic techniques. aMT6s is extremely stable in frozen urine (unpublished results). Urinary cortisol was assayed by radioimmunoassay[11].

*Results.* Table 1 shows the natural daylength characteristics at the times of urine collection. The sun remains below the horizon from 18 May to 25 July in midwinter and does not set from 17 November to 24 January in midsummer. Thus in winter base personnel experience 68 days without a strong light-dark cycle (less than 500 lux light intensity at any time during this period), and at the equinoxes, a very rapid rate of change of daylength. The mean level of aMT6s and cortisol excretion at each time of




Mean levels of aMT6s (µg/sample) and cortisol (µg/sample) excretion ± SEM, at each time of day for each season. 6-hourly sequential urine collections were obtained at each time of year, sampling dates as shown in tables 2 and 3.

day for each season is shown in the figure. The number of subjects from whom data was available at each season is shown in brackets. Inspection of this figure suggests that while both measures exhibited a circadian rhythm; that in aMT6s was relatively constant over the seasons, while that in cortisol varied considerably across the four seasons.

In order to estimate the parameters of each individual's rhythms in each season, cosine curves were fitted by a least squares method[12] to each set of four 6-hourly samples. The resultant phase (in hours and tenths of an hour) and amplitude estimates are shown in tables 2 and 3. These tables also show the phase and amplitude estimates derived from cosine curves fitted to the groups' mean scores. In the case of aMT6s excretion (table 2), both the mean phase and the mean amplitude estimates were relatively constant across the four seasons. In contrast, the cortisol rhythm showed a low amplitude and considerable phase displacement during the summer as compared with the other seasons.

The reliability of these effects was assessed by means of analyses of variance based on the raw data from the three subjects from whom complete records were available. These confirmed that there was a significant 'time of day' effect in aMT6s excretion ($F = 14.644$; $df = 3.6$; $p < 0.01$), but no main effect of season ($F = 1.067$; $df = 3.6$; $p > 0.25$), or interaction between time of day and season ($F = 1.206$; $df = 9.18$; $p > 0.25$). However, cortisol excretion showed no significant main effect of time of day ($F = 1.025$; $df = 3.6$; $p > 0.25$) or season ($F < 1$) but a significant interaction between time of day and season ($F = 2.703$; $df = 9.18$; $p < 0.01$).

*Discussion.* Any seasonal effects related to daylength should be enhanced when under the influence of extreme daylength as in the Antarctic, although clearly interpretation of observations is complicated by other factors such as the socially and physically stressful nature of life in confined and unusual circumstances. Surprisingly the aMT6s rhythm persisted throughout the year with little significant seasonal change, albeit with a slightly broader peak in winter. Previous reports have suggested a lower amplitude melatonin rhythm in both spring and autumn[13], or an overall decline with decreasing daylength[14]. Whilst there is as yet no information on possible variations in melatonin metabolism at different times of year, such variations are likely to be small in comparison to the amplitude of the circadian rhythm. The persistent 24-h rhythm in aMT6s suggests that maintenance of the melatonin rhythm does not necessarily require a bright light-dark cycle. Evidence from animal work indicates that the melatonin response to light is relative and may depend on previous exposure in terms of intensity[15]. Such phenomena may be of importance in the human response to light.

The 24-h cortisol rhythm was also maintained during the Antarctic year, with little change in amplitude, but the timing of peak levels varied greatly with season.

The fact that rhythms in melatonin as assessed by excretion of its major metabolite and cortisol were differently affected by season may suggest that they are functionally independent and not causally related. This would be consistent with previous observations, for example, of their rate of adaptation to time-zone change[14]. They may be controlled by different underlying rhythm-generating processes[16,17]. No firm conclusions can be drawn however in view of the possible influence of stress on cortisol levels and the small population number.

This study is clearly limited in both numbers of volunteers and sampling times. Nevertheless it suggests that no gross changes in melatonin 24-h rhythms are present in the exceptional light-dark conditions of Antarctica. Whether the observed change in the timing of peak cortisol levels in the course of the year is related to the physical environment or to events on the Base remains an open question.

Table 1. Outdoor light/dark cycle at Rothera Point

| Collection date | Twilight** | Sunrise | Sunset | Twilight | Photoperiod (twilight to twilight, h, min) |
|---|---|---|---|---|---|
| 23 March 1981 (autumn equinox)* | 04.31 | 05.29 | 18.14 | 19.12 | 14.41 |
| 29 June 1981 (mid-winter) | 09.19 | S.B.H. | S.B.H. | 14.44 | 5.25 |
| 12 October 1981 (2 weeks post-vernal equinox) | 03.05 | 04.14 | 19.50 | 20.58 | 17.53 |
| 20 January 1982 (4 weeks post mid-summer) | S.A.H. | S.A.H. | S.A.H. | S.A.H. | 24.00 |

* Note reversal of seasons in southern Hemisphere; ** civil twilight; sun 6° below horizon; S.A.H.: sun above horizon; S.B.H.: sun below horizon. (Figures obtained from Reeds Nautical Almanac.)

Table 2. aMT6S. The 'peak' acrophase (in hours and tenths of hours) and amplitude (in µg per sample) estimates (shown in brackets) of the aMT6s rhythm for each individual in each season. $\bar{x}(1)$ is the mean of the individual fits while $\bar{x}(2)$ are the estimates derived from cosine curves fitted to the averaged raw data

| | Autumn (23 March) | Winter (29 June) | Spring (12 October) | Summer (20 January) |
|---|---|---|---|---|
| A.H. | 6.0 (3.3) | 8.9 (3.6) | 8.7 (2.1) | 5.6 (3.3) |
| P.G. | 7.1 (3.9) | 7.6 (3.4) | 6.6 (4.0) | 6.9 (1.8) |
| N.H. | 5.4 (2.5) | 4.6 (1.2) | 4.6 (3.8) | 3.7 (3.3) |
| A.T. | 3.8 (2,7) | 8.1 (2.1) | 5.2 (2,7) | – |
| T.G. | 5.6 (3.5) | 7.9 (4.5) | – | – |
| B.A. | 6.2 (3.2) | – | – | – |
| J.H. | – | – | – | 3.4 (3.2) |
| $\bar{x}(1)$ = | 5.7 (3.2) | 7.4 (2.9) | 6.3 (3.2) | 4.9 (2.9) |
| $\bar{x}(2)$ = | 5.8 (3.1) | 7.9 (2.8) | 6.0 (3.0) | 4.7 (2.7) |

Table 3. Cortisol. The 'peak'/acrophase (in hours and tenths of hours) and amplitude (in µg per sample) estimates (shown in brackets) of the cortisol rhythm for each individual in each season. $\bar{x}(1)$ is the mean of the individual fits while $\bar{x}(2)$ are the estimates derived from cosine curves fitted to the averaged raw data

| | Autumn (23 March) | Winter (29 June) | Spring (12 October) | Summer (20 January) |
|---|---|---|---|---|
| A.H. | 10.0 (10.6) | 13.8 (30.7) | 12.0 (18.8) | 07.6 (9.3) |
| P.G. | 16.4 (17.8) | 16.3 (13.4) | 01.6 (7.8) | 22.0 (9.9) |
| N.H. | 10.2 (7.3) | 15.8 (2.9) | 13.3 (5.1) | 03.8 (11.3) |
| A.T. | 17.8 (14.9) | 21.0 (18.0) | 16.0 (14.4) | – |
| T.G. | 12.2 (15.9) | 19.5 (3.2) | – | – |
| B.A. | 14.9 (17.0) | – | – | – |
| J.H. | – | – | – | 02.2 (4.9) |
| $\bar{x}(1)$ = | 13.6 (13.9) | 17.3 (13.6) | 16.7 (11.5) | 02.9 (8.9) |
| $\bar{x}(2)$ = | 14.3 (10.4) | 16.3 (9.8) | 13.6 (6.5) | 03.1 (6.8) |

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## The lymphatic route. 1) Albumin and hyaluronidase modify the normal distribution of interferon in lymph and plasma[1]

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*Summary.* When human recombinant interferon-$\alpha_2$ diluted in saline was injected s.c. into rabbits, the total amount recovered in thoracic lymph was less than 0.4%. Recoveries increased from 2- to 8-fold if interferon was injected in 4% albumin or with hyaluronidase, respectively. Albumin added to interferon acts as an interstitial fluid expander, thus favoring interferon absorption through lymphatics rather than blood capillaries. This strategy may increase the therapeutic index of interferon.
*Key words.* Interferon; immunomodulator; catabolism; pharmacokinetics; administration routes.

The problem of distribution of interferon (IFN) in lymph and plasma has attracted little attention so far[4], but there is no doubt about the occurrence of transcapillary passage of IFN. After i.v. administration of IFN into rats, its concentration in lymph was similar to that in the plasma[5]. However, because of renal filtration[6,7] and hepatic catabolism[8] IFN has a very short half-life in plasma so that, when IFN is administered via i.m. and s.c. routes that favor blood capillary rather than lymphatic absorption, it is likely that lymphatic organs may exchange little IFN with the plasma pool[9]. This uneven distribution may represent an important drawback as it remains uncertain whether in cancer therapy IFN acts more as a cytostatic drug or as an immunomodulatory agent. Thus, in order to reproduce the physiological distribution of IFN[10], one of us has proposed[11] that lymphatic absorption should be facilitated and expanded as far as possible, in order to minimize IFN plasma levels and to improve the interaction of IFN with effector cells. In this report we investigated the distribution of human recombinant interferon-$\alpha_2$ (rec. IFN-$\alpha_2$) in plasma and thoracic lymph in the rabbit after s.c. injection of IFN, diluted either in saline, or in 4% human albumin (ALB) solution, or in saline with the addition of 75 U hyaluronidase (HYAL).

*Materials and methods.* Human rec. IFN-$\alpha_2$ was obtained through the courtesy of Dr I. I. A. Tabachnich (Schering Corp. Bloomfied, N. J.). It had a potency of $4.2 \times 10^9$ IU/ml and it was at least 98% pure. 20 adult male rabbits (3.488±0.285 kg) were randomly assigned to one of the 4 groups necessary for the investigation.

The animals were anesthetized with Nembutal throughout the experiment: after cannulation of the thoracic duct and a femoral artery, lymph and blood samples were collected at predetermined times. A constant flow of lymph was ensured by a slow infusion of saline into a marginal vein of the ear and by passively moving the extremities of the animals every hour. Rabbits of group A received a single s.c. injection (0.5 ml) in the hind leg of 11 mega units (MU) human rec. IFN-$\alpha_2$ (from E. coli) in saline. Groups B, C, D were injected at 5 sites (0.1 ml/site) of the hind legs with the same amount of IFN diluted in saline (B), in saline-4% ALB. solution (C) and in saline containing 75 U (0.5 ml) of HYAL (D).

Lymph and blood were collected in heparinized vials and, after centrifugation, the volumes of the cell-free lymph and plasma were measured and stored at −20 °C until IFN determination. IFN titration was carried out by virus plaque-assay using HEp 2 cells and VSV as challenge virus[12]. All samples were assayed at least twice in duplicate, and the titres were referred to an international standard of IFN-$\alpha$. The results presented here are means ± standard error of the means. Statistical evaluation presented in the table was made by using the t-test.

*Results and discussion.* The figure shows amounts of IFN recovered hourly in thoracic lymph and profiles of the IFN plasma levels during 8 h after s.c. injection of human rec. IFN-$\alpha_2$. The peak of IFN concentration in the lymph was reached within 4 h irrespective whether IFN was injected at 1 or 5 sites. However, in the latter case, IFN concentration was almost 1 log unit higher than after a single administration. The amount of IFN recovered hourly in lymph increased when IFN was injected with either 4% ALB or HYAL. This enzyme was used as a well-known means of favoring lymphatic absorption of proteins, so that its effects could be compared with those of albumin. Clearly, HYAL cannot be injected in cancer patients because it can favor cancer cell metastatization. In all cases, lymph-to-plasma IFN concentration ratios were above 1 and were increased when IFN was injected with ALB and particularly with HYAL.

The table summarizes cumulative recoveries of IFN in lymph expressed as percentages of the administered dose. Simultaneous administration of either IFN and ALB, or IFN and HYAL, increased IFN yields in lymph between 2- and 8-fold, respectively. This result indicates that an increase of the oncotic pressure of the interstitial fluid, like the one obtained here with 4% albumin, is hardly comparable to the HYAL effect and there-

Recoveries of human rec. IFN-$\alpha_2$ in thoracic lymph of rabbits after s.c. injection

| Group | Total units recovered in 8 h (means ± SE) | % of dose (means ± SE) |
|---|---|---|
| A (saline, 1 site) | 11,266 ± 6,519 | 0.10 ± 0.06 |
| B (saline, 5 sites) | 41,406 ± 34,405 | 0.38 ± 0.32 |
| C (4% ALB, 5 sites) | 91,397 ± 19,829 | 0.83 ± 0.18 |
| D (75 U HYAL, 5 sites) | 360,386 ± 58,747 | 3.28 ± 0.53 |

B vs C: NS, B vs D: $p < 0.05$.