

## Twenty-Four-Hour Rhythms in Relation to the Natural Photoperiod: A Field Study in Humans

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**Abstract** The daily rhythms of salivary melatonin, salivary cortisol, and axillary body temperature were measured in nine healthy volunteers in midsummer, around the autumn equinox, and in midwinter, at a latitude of 60°N. The aim was to find out whether these rhythms were dependent on variations of the natural daylength. The samples were collected every 2 hr during 24-hr periods in everyday conditions. The individual rhythms were characterized with the acrophase estimates of the best-fitting cosine curve models and with the half-rise and half-decline times calculated from the raw data. The melatonin and cortisol rhythms were delayed significantly (about 1 hr) in midwinter as compared with summer and autumn. The most advanced rhythms were found in autumn. The shifts of the melatonin and cortisol rhythms could be explained as a result of the changes of natural illumination. The overt temperature rhythms did not differ significantly among the sampling months. The lack of seasonal patterns in temperature rhythms probably primarily reflected the socially determined rest-activity cycles of the subjects.

**Key words** circannual, seasonal, melatonin, cortisol, body temperature, lighting

More than 10 years ago, Aschoff (1981) analyzed the annual rhythms of human mortality, suicides, and conception rates. He concluded that, in addition to sociocultural factors, there must be environmental (natural) factors that control these rhythms. Since then, the significance of light in human biology has been increasingly emphasized. It has been shown in several laboratory studies that circadian rhythms in humans can be affected by bright light (e.g., Wever et al., 1983; Czeisler et al., 1986; Lewy et al., 1987; Illnerová et al., 1993). The biological responsiveness of humans to light seems qualitatively similar to that of other mammals. In addition to circadian rhythms, human physiology and behavior may be affected by seasonal variations in lighting conditions.

In mammals, both circadian and annual rhythms are regulated by photic information mediated by the retinohypothalamic pathways to the suprachiasmatic nuclei. A homologous system may function in humans, although seasonality is not a prominent feature in human physiology. Recently, as evidence for the biological nature of human seasonality, clear annual variations were described in the vasopressin neuron population of the human suprachiasmatic nuclei (Hofman et al., 1993; Hofman and Swaab, 1993).

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The secretion of melatonin from the pineal gland serves as a signal transducing environmental lighting information to the body. In animals, it participates significantly in the regulation of the annual rhythms of breeding, renewal of fur, body weight, and so forth. In controlled laboratory conditions, the duration of nocturnal melatonin secretion in humans was longer in a short than in a long photoperiod (Wehr, 1991; Buresová et al., 1992; Wehr et al., 1993). Under natural conditions in a region with a strong seasonal contrast in luminosity (65°N), the duration of the melatonin peak was lengthened during the dark season (Kauppila et al., 1987).

In subjects living under their usual lighting conditions, seasonal variation has been found in the melatonin peak amplitude, in the daytime or nocturnal plasma concentrations, or in the daily excretion of melatonin in urine (Arendt et al., 1977, 1979; Touitou et al., 1984; Martikainen et al., 1985; Kauppila et al., 1987; Kivelä et al., 1988; Stokkan and Reiter, 1994). In some studies, differences in concentrations were not found; instead, the main seasonal change observed was the advance of the melatonin rhythm in summer as compared with winter (Illnerová et al., 1985; Kennaway and Royles, 1986; Broadway et al., 1987; Bojkowski and Arendt, 1988; Matthews et al., 1991; Honma et al., 1992). Recently, an opposite phase difference between January and June has been described in Arctic urban residents: Melatonin peaked about 2 hr later in summer during the continuous sunshine than in winter, when daylight was only 2 hr of twilight (Stokkan and Reiter, 1994). The authors suggest that this exceptional finding was caused by the subjects' social rhythm, which was delayed in summer.

Seasonal variations in urinary corticosteroid excretion and plasma cortisol levels measured at a certain time of the day have repeatedly been reported (for a review, see Aschoff, 1981). In the early studies, the seasonal variation of ambient temperature was considered the major factor responsible for the annual changes. However, the effect of lighting conditions was not excluded. A phase shift of the daily cortisol rhythm resembling that of melatonin has been described between winter and summer (Kennaway and Royles, 1986).

Body temperature is the variable that is most often measured in the studies on human daily rhythms, but we are aware of only a few studies on seasonal variations of this rhythm. In natural or seminatural conditions, in which lighting and ambient temperature vary, the body temperature rhythm phase was delayed in summer as compared to winter (Touitou et al., 1986; Maruta et al., 1987; Jeong and Tokura, 1989). However, quite the opposite result was obtained in an experiment in which the ambient temperature was controlled and only the photoperiod was allowed to change (Honma et al., 1992). In one study, there was no consistent seasonal variation in the body temperature rhythm in a group of young healthy controls (Touitou et al., 1986).

In the present study, we measured the daily rhythms of salivary melatonin, salivary cortisol, and axillary temperature in healthy volunteers during their normal everyday lives. The samples were collected in midsummer, around the autumn equinox, and in midwinter. The rhythms were related to the natural light and dark periods, in order to find out whether the seasonal variations in these rhythms could be explained by the changes of the natural lighting conditions. The volunteers were urban residents with fairly regular, normal lifestyles. Considerations influencing the choice of variables for the measurements were that the variables might be affected by lighting conditions and that the samples could be collected almost anywhere.

## METHODS

### *SUBJECTS*

Nine healthy volunteers (two males and seven females; age range = 23–52, with a mean age of 38 years) participated in the study. They were students, researchers, and other personnel of the Institute of Biomedicine at our university. Informed consent was obtained from all subjects after the nature of the study had been explained.

### *COLLECTION OF THE SAMPLES*

The subjects were instructed to collect the samples during 24-hr periods of usual working days (not immediately after exceptional journeys, holidays, parties, etc.). Each subject collected 5–7 ml of saliva without stimulation into a plastic tube every 2 hr, starting at 1000 hr. At the same time points, they measured their axillary temperature with a standard clinical thermometer. At night the subjects slept between the samplings. During the 60 min preceding each sampling, the subjects were not allowed to eat, drink, brush their teeth, or smoke. Each subject collected samples in midsummer (June–July; referred to hereafter as June or Jun), around the autumn equinox (September or Sep), and in midwinter (December–January; December or Dec). The salivary samples were immediately frozen and stored at  $-20^{\circ}\text{C}$  until the hormone measurements were made.

### *NATURAL PHOTOPERIODS AND LOCAL TIME*

In midsummer at latitude of  $60^{\circ}\text{N}$ , the sun rises at about 0400 hr, and sets at about 2300 hr, resulting in a photoperiod of almost 19 hr. During the darkest season (the end of December and the beginning of January), sunrise and sunset occur at 0925 hr and 1520 hr, respectively; the resulting photoperiod is about 6 hr. Between these extremes, in September the sun rises at about 0700 hr and sets at about 1930 hr; thus, the natural light and dark periods are equal. All time points in this report are given as local times. In midsummer and in September, daylight savings time was in use (the time was advanced by 1 hr compared with standard time, which was used in midwinter). For example, if something happens at 0700 hr in summer, the corresponding time is 0600 hr in winter; if something happens at 0700 hr in winter, the corresponding time is 0800 hr in summer. Using the local time as a reference kept the social influences on the body rhythms as constant as possible.

### *MEASUREMENT OF MELATONIN*

Melatonin was extracted from 1.0 ml of saliva with chloroform and measured in duplicate with radioimmunoassay (RIA) (Vakkuri et al., 1984). This method has been validated for saliva samples (Vakkuri, 1985; Laakso et al., 1990). The nonspecific binding of the tracer was 4–7%. The lowest detectable concentration in the assays, defined as the apparent concentration at two standard deviations from the counts at maximum binding, was  $<3$  ng/liter ( $n = 6$  in each assay). Intra-assay variability calculated from the duplicate measurements was 7–17%, depending on the concentration range. The interassay variability during 21 months in 31 assays (including the assays of this study) was 18%, 16%, and 13% in standard

samples of 16, 40, and 400 ng/liter, respectively. All the samples collected during a 24-hr period by a subject were measured in the same assay.

### MEASUREMENT OF CORTISOL

The reagents used were from Amerlex Cortisol RIA Kits (Amersham, U.K.). The assay protocol given by the manufacturer for serum and urine samples was applied to the salivary samples as described earlier in detail (Laakso et al., 1993). The nonspecific binding of the tracer was 2–3% and the lower detection limit was 0.3–0.4  $\mu\text{g/liter}$ . The intra-assay variability was 7–12%, and the interassay variability during 13 months in 15 assays (including the assays of this study) was 13% and 7% for standard samples of 0.64 and 8.75  $\mu\text{g/liter}$ , respectively.

### CALCULATIONS AND STATISTICS

Single-cosinor analysis was applied to the computation of the circadian rhythm parameters for each 24-hr pattern of the measurements. The acrophase estimates were used in the evaluation of the rhythms. Because of the interassay variability of the hormone measurements, the mesors and amplitudes were not inspected. Instead, the melatonin levels of each 24-hr set of data were expressed as percentages of the maximum, and the cortisol levels were expressed as percentages of the daily mean. The axillary temperatures were expressed as deviations from the daily mean.

In many cases, the fitting of the data to the cosine curve model was poor; therefore, the raw data were used in addition in the evaluation of the rhythms. The melatonin half-rise and half-decline times were determined graphically from the individual patterns without smoothing the curves. They were defined as the time points when the half-maximal levels were reached at the ascending and descending phases of the pattern, respectively. The half-maximal level was defined as the halfway point between the peak level (mean of the three highest concentrations) and the baseline level (mean of the three lowest concentrations). The half-rise and half-decline times for cortisol were determined in the same way, with the exception that the peak and baseline levels were the highest and the lowest values, respectively. The half-rise and half-decline times for the temperature rhythms were the time points of the daily means in the ascending and descending phases, determined graphically from the individual curves.

The daily mean patterns of each variable were statistically evaluated by means of a two-way repeated-measures analysis of variance (ANOVA), and the rhythm characteristics by means of a one-way repeated-measures ANOVA. The pairwise comparisons were performed by means of the Tukey–Kramer multiple-comparisons test.

Among the subjects, there were two low secretors of melatonin. Because of very low or missing peaks, the melatonin patterns from these persons were excluded from the study. One pattern of body temperature was missed because of a broken thermometer during a sampling night; the temperature results of this person were excluded.

## RESULTS

### AVERAGE DAILY PROFILES

The average data show that the daily rhythms of salivary melatonin and cortisol, and even the rhythm of axillary temperature, were delayed in winter as compared with summer and

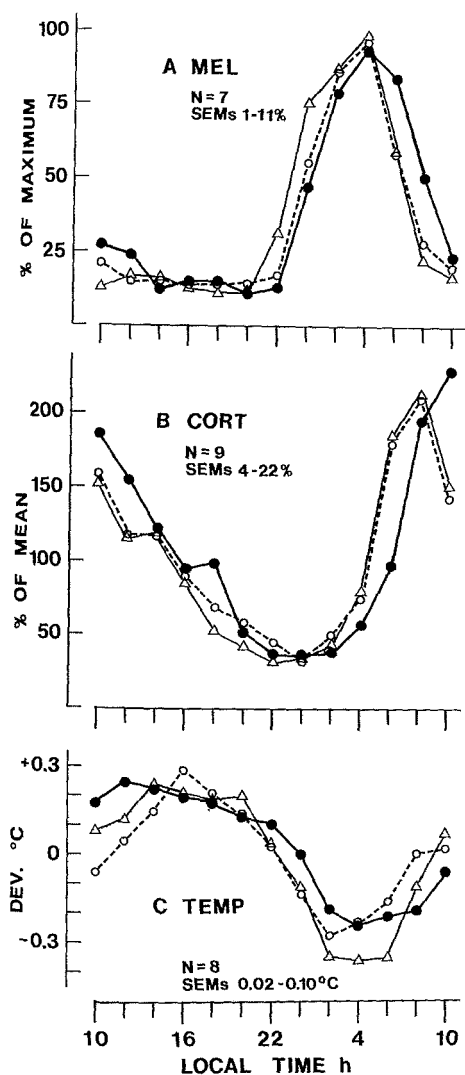


FIGURE 1. The 24-hr profiles of salivary melatonin (A), salivary cortisol (B), and axillary temperature (C) of healthy volunteers during three seasons: in midsummer (open circles), in mid-winter (filled circles), and around the autumn equinox (triangles). The number of subjects and the range of SEMs are given in the figure. The concentrations of melatonin are expressed as means of the percentages of the individual peak values during the sampling day, and the concentrations of cortisol are expressed as means of the percentages of the individual mean values during the sampling day. Axillary temperatures are expressed as means of the individual deviations from the daily mean values. Two-way repeated-measures ANOVAs: melatonin, season n.s., time of sampling  $p < 0.001$ , season  $\times$  time  $p < 0.001$ ; cortisol, season n.s., time  $p < 0.001$ , season  $\times$  time  $p < 0.001$ ; temperature, season n.s., time  $p < 0.001$ , season  $\times$  time n.s. Results of the pairwise comparisons of the profiles are given in the text.

autumn (Fig. 1). According to two-way ANOVAs with repeated measures, the differences among the three profiles of melatonin and among those of cortisol were significant, but the temperature patterns did not differ significantly. Pairwise two-way ANOVAs indicated that all three melatonin profiles were different (season  $\times$  time interactions: Jun vs. Sep,  $p < 0.001$ ; Jun vs. Dec,  $p < 0.01$ ; Sep vs. Dec,  $p < 0.001$ ). The cortisol patterns in summer and autumn did not differ from each other, but the profile in winter was different from both (Jun vs. Dec,  $p < 0.001$ ; Sep vs. Dec,  $p < 0.002$ ).

### RHYTHM CHARACTERISTICS

According to the cosine curve model, the highest levels of melatonin occurred about 1 hr later in winter than in summer or autumn (Table 1). The acrophase estimates in autumn were the most advanced, but they did not differ significantly from the values in midsummer.

TABLE 1. Rhythm Characteristics of Salivary Melatonin, Salivary Cortisol, and Axillary Temperature in Healthy Volunteers during Three Seasons

Month	Acrophase	Half-rise time	Half-decline time	Period from half-rise to half-decline
<u>Melatonin (n = 7)</u>				
Jun	3.3 ± 0.3	23.7 ± 0.2	6.6 ± 0.4	6.9 ± 0.4
Sep	2.6 ± 0.3	22.4 ± 0.2	5.9 ± 0.3	7.5 ± 0.3
Dec	4.2 ± 0.5	24.3 ± 0.5	7.9 ± 0.4	7.6 ± 0.3
ANOVA	p = 0.0010	p = 0.0022	p = 0.0001	n.s.
Tukey-Kramer				
Jun vs. Sep	n.s.	p < 0.05	n.s.	
Jun vs. Dec	p < 0.05	n.s.	p < 0.01	
Sep vs. Dec	p < 0.001	p < 0.01	p < 0.001	
<u>Cortisol (n = 9)</u>				
Jun	9.2 ± 0.2	5.0 ± 0.4	11.6 ± 0.7	6.6 ± 0.7
Sep	8.9 ± 0.4	4.8 ± 0.3	10.6 ± 0.7	5.8 ± 0.7
Dec	10.6 ± 0.4	6.5 ± 0.4	11.9 ± 0.5	5.5 ± 0.5
ANOVA	p = 0.0005	p = 0.0005	n.s.	n.s.
Tukey-Kramer				
Jun vs. Sep	n.s.	n.s.		
Jun vs. Dec	p < 0.01	p < 0.01		
Sep vs. Dec	p < 0.001	p < 0.001		
<u>Axillary temperature (n = 8)</u>				
Jun	15.9 ± 0.5	9.7 ± 0.8	22.5 ± 0.4	12.8 ± 0.7
Sep	15.4 ± 0.4	8.9 ± 0.3	21.9 ± 0.7	13.0 ± 0.5
Dec	16.1 ± 0.4	9.7 ± 0.3	23.2 ± 0.5	13.5 ± 0.5
ANOVA	n.s.	n.s.	n.s.	n.s.

*Note.* The times are given as means ± SEMs (hours with decimals after midnight, local time). *n* = number of subjects. ANOVA, one-way repeated-measures analysis of variance; Tukey-Kramer, Tukey-Kramer multiple-comparisons test. The half-rise and half-decline times for melatonin and cortisol are defined as the time points of the half-maximal levels; those for axillary temperature are defined as the time points of daily means (see "Methods").

However, the rising phase of the melatonin synthesis was significantly advanced (1–2 hr) in autumn as compared with midsummer or winter (Fig. 1, Table 1). The decrease of the melatonin levels was significantly delayed (1–2 hr) in midwinter as compared with midsummer or autumn. The duration of the melatonin peak from the half-rise time to the half-decline time did not vary significantly among the seasons.

The acrophase estimates for the cortisol rhythm and the time points of the half-maximal levels of the cortisol profiles varied in much the same way as the melatonin patterns did: All characteristics were most advanced in autumn and most delayed in midwinter (Table 1). However, the differences between midsummer and autumn were small and not statistically significant. The rising phase and peak values were significantly delayed in winter as compared with summer and autumn. In the descending arms of the cortisol profiles, the interindividual variation was great during the active period of day, and there was no significant seasonal variation in the calculated half-decline times. Nor did the durations of the cortisol peaks differ significantly.

Although the average daily profiles of the axillary temperature suggested a somewhat delayed temperature rhythm in winter as compared with summer and autumn (Fig 1C), the means of the acrophase estimates and the means of the half-rise and half-decline times did not differ significantly (Table 1).

## PHASE RELATIONSHIPS

The average interval between the acrophase estimates of the melatonin and cortisol rhythms did not alter significantly during the sampling months (Table 2). The phase angle differences between the half-rise and half-decline times were also equal (data not shown). The difference between the acrophases of cortisol and body temperature was somewhat smaller in midwinter than in the other months, but the difference was not statistically significant. The difference between the acrophases of the melatonin and temperature rhythms was consistently smallest in midwinter, and this change reached statistical significance (Table 2).

## RELATIONSHIPS BETWEEN NATURAL LIGHTING AND RHYTHMS

In midsummer, the melatonin levels above the half-maximal levels were reached at about the time of sunset and maintained until a couple of hours after sunrise (Fig. 2). In autumn and winter, high levels were found only during the scotoperiod. In September the average half-rise time was 3 hr after sunset, when less than 30% of the scotoperiod had elapsed. In December the half-maximal levels were found 9 hr after sunset, when about 50% of the scotoperiod had elapsed. The average half-decline time occurred 1 hr before sunrise in September and 1.5 hr before sunrise in December (10% and 8% of scotoperiod left, respectively). Thus, the melatonin rhythm shifted toward sunrise in midwinter as compared with the rhythm in autumn.

The half-maximal levels of cortisol during the ascending phase were reached about 1 hr after sunrise in June, and 2–3 hr before sunrise in September and December (Fig. 2). In autumn, the average half-rise time occurred when 19% of the scotoperiod was left; in midwinter, it occurred when 15% was left. The average interval from the half-rise time of cortisol to the half-decline time of melatonin was 1–2 hr in all sampling months.

The half-decline time of the body temperature rhythm moved from the end of the light period to the middle of the dark period concomitantly with the decreasing photoperiod (Fig. 2). The half-rise time of temperature was not clearly connected with the changes of natural lighting.

TABLE 2. Phase Angle Differences between Acrophases of Salivary Melatonin, Salivary Cortisol, and Axillary Temperature Rhythms in Healthy Volunteers during Three Seasons

	Mel. – Cort. ( <i>n</i> = 7)	Cort. – Temp. ( <i>n</i> = 8)	Mel. – Temp. ( <i>n</i> = 7)
Jun	6.1 ± 0.2	6.5 ± 0.5	12.9 ± 0.6
Sep	6.4 ± 0.6	6.4 ± 0.6	12.5 ± 0.5
Dec	6.6 ± 0.6	5.5 ± 0.8	11.4 ± 0.5
ANOVA	n.s.	n.s.	<i>p</i> = 0.0396
Tukey–Kramer			
Jun vs. Sep			n.s.
Jun vs. Dec			<i>p</i> < 0.05
Sep vs. Dec			n.s.

Note. The values are hours with decimals ± SEMs. *n* = number of subjects. ANOVA, one-way repeated-measures analysis of variance; Tukey–Kramer, Tukey–Kramer multiple-comparisons test.

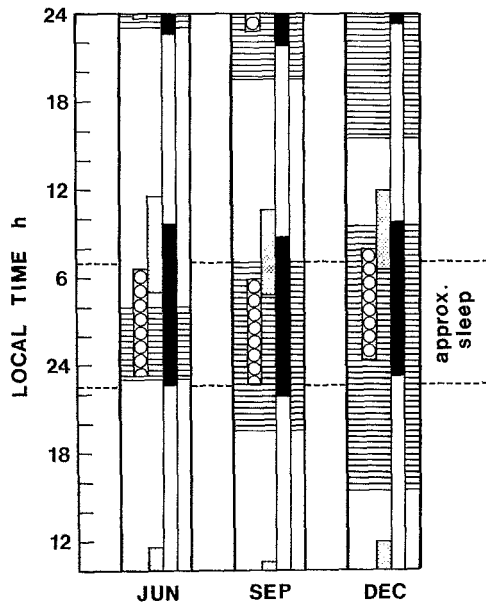


FIGURE 2. Relationships between natural lighting and the rhythms of melatonin, cortisol, and axillary temperature in midsummer (Jun), around the autumn equinox (Sep), and in midwinter (Dec). The average period when the salivary melatonin concentrations were above the half-maximal levels are indicated with circle-filled bars ( $n = 7$ ); the corresponding periods for cortisol are indicated with shaded bars ( $n = 9$ ). The average periods for the axillary temperature exceeding the daily mean are indicated with open bars; those for the temperature being below the daily mean are indicated with filled bars ( $n = 8$ ). The natural photo- and scotoperiods are indicated by open and hatched areas, respectively.

The approximate sleep time shown in Figure 2 is based on the logs kept by seven of the subjects of this study during another study (Laakso, Porkka-Heiskanen, Alila, and Johansson, unpublished). The mean times of awakening and going to bed on weekdays during 2 weeks were 0715 hr and 2345 hr in December, and 0700 hr and 2305 hr in August. Thus, the average sleep-wake cycle tended to be delayed in winter, despite the regular working hours. However, the differences between summer and winter were not statistically significant. The relationship of the body rhythms to this approximate social rhythm is taken into account below.

## DISCUSSION

The daily rhythms of melatonin and cortisol, using the local time as a reference, were delayed in winter by about 1 hr as compared with the patterns in summer. If the rhythm markers are expressed using standard time, the difference increases to 2 hr. The results agree with those of previous studies, in which a phase shift of the same magnitude and direction was found between midwinter and midsummer (Illnerová et al., 1985; Kennaway and Royles, 1986; Broadway et al., 1987; Bojkowski and Arendt, 1988; Matthews et al., 1991; Honma et al., 1992). The phase angle difference between the melatonin and cortisol rhythms remained constant during the phase shifts in our subjects, as in an earlier investigation (Kennaway and Royles, 1986).

In the present study, the rhythm of melatonin was, and the rhythms of cortisol and body temperature tended to be, most advanced around the autumn equinox. Previously

published results disagree on this point. In two studies performed in geographical areas where the seasonal variation of daylength is not as large as in the present study, the melatonin rhythm was not exceptionally advanced in autumn: In Japan at 35°N, the melatonin pattern in autumn resembled that in summer (Honma et al., 1992); in South Australia at 35°S, it resembled that in winter (Matthews et al., 1991). In Norway at 70°N, where a continuous night in winter and a continuous day in summer last several weeks, the melatonin rhythms in March and September were more advanced than in June but more delayed than in January (Stokkan and Reiter, 1994). Although there may have been some methodological differences that influenced these results, the different lighting conditions in the various geographical areas may have played a role too. Even the seasonal variations in the social habits of subjects affect their exposure to natural light, as suggested by Stokkan and Reiter (1994).

Our subjects were urban residents who artificially prolonged the light period when necessary to maintain the regular social daily rhythm, or continued the dark period in summer mornings by sleeping indoors sheltered from sunshine. Because the samples were collected during regular working periods, there was no great variation in the daily routines of the subjects from season to season. Nevertheless, their daily melatonin and cortisol rhythm, and possibly even the temperature rhythm, were delayed in winter and advanced in summer and autumn.

Figure 2, in which the average rhythm characteristics are related to natural dawn and dusk as well as to the approximate sleeping period of the subjects, helps to explain how natural lighting might have affected the daily rhythms. In midsummer at 60°N, the natural dusk and the usual bedtime coincide. The dawn occurs several hours before the wakeup time, but people are not exposed to strong illuminance because of their closed eyelids and the curtains that are often used on bedroom windows. Actually, the dark period extends from sunset to the wakeup time of the subjects. This is the probable reason for the maintenance of high melatonin levels after sunrise in midsummer. Around the time of autumn equinox, the usual wakeup time coincides with the natural dawn. Thus, the "lights-on" time remains equal to that in midsummer, but the dusk occurs some hours before the usual bedtime. Although electric lights are used in the evening, the intensity is below that of natural light. It is possible that the melatonin rhythm in autumn is more advanced than the rhythm in midsummer because the low-intensity evening light in autumn does not inhibit the rise of melatonin. The inhibition in midsummer may be due to the direct suppression of melatonin synthesis by light or to the compression of the rhythm toward morning. In midwinter the short photoperiod is prolonged by electric lights in both the evening and the morning. If the low-intensity artificial light does not effectively regulate the rhythms, a delay is to be expected. At least in rats, the melatonin peak tends to move toward dawn when the dark is prolonged at both ends of the scotoperiod (Ho et al., 1984; Illnerová and Vaněček, 1985). The dominance of dawn in adjusting the melatonin rhythm in long scotoperiods has been suggested.

Our finding that the most advanced rhythm occurs in autumn is in agreement with the finding of Binkley et al. (1990) that the most advanced wakeup times and bedtimes of four volunteers were concentrated around the autumn equinox, according to logs kept throughout a year. This time of the year has been found to be a turning point in some other annual rhythms in humans as well: The plasma testosterone levels in men are highest (Smals et al., 1976); in free-running conditions, the amount of sleep is greatest, the period of the temperature rhythm is longest, and the tendency to internal desynchronization may be highest (Wirz-

Justice et al., 1984); and the number of vasopressin neurons in the suprachiasmatic nuclei is greatest (Hofman et al., 1993; Hofman and Swaab, 1993). The possible interdependence of these phenomena will remain obscure until we have learned more about the system that regulates human annual rhythms.

Differing from the rhythms of melatonin and cortisol, the seasonal variations in the overt temperature rhythm were not statistically significant. Although the subjects were aware of the usual sources of error in measuring axillary temperature and were able to avoid them, the method is not considered as reliable as continuous recording of rectal or tympanic temperature. It is possible that more frequent measurements might have resulted in statistically significant differences of temperature patterns among the seasons.

Several factors may mask body temperature rhythm (e.g., physical activity, emotional excitement, or meals). It is probable that such factors caused a great intra- and interindividual variation of the temperature patterns in our investigation, and the possible effects of lighting conditions on the rhythm could not be determined. The temperature rhythm seemed to be more dependent on the social habits of the subjects than on the natural lighting conditions.

In conclusion, the seasonal variation of the daily melatonin rhythm in humans in everyday conditions could be explained by the changes in the natural lighting conditions. The daily rhythms of melatonin and cortisol were closely connected, but the overt temperature rhythm was probably affected by factors that might mask the possible lighting-dependent seasonal changes.

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