

sexine 2–3 times thicker than nexine; lumina 2–13  $\mu\text{m}$  across,  $\pm$  polygonal (irregular if compressed) with reticulate/granulate pattern formed of ectonexine; muri straight or curved, simplipilate, may be thickened at joints, with or without pila heads at each joint.

The slides and negatives have been deposited in the repository of the Birbal Sahni Institute of Palaeobotany, Lucknow, India.

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## The menstrual cycle in a human female under social and temporal isolation is not coupled to the circadian rhythm in sleep–wakefulness

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We investigated the circadian rhythm in sleep–wakefulness (SW rhythm) and in the rectal-temperature profile (Temp rhythm) of a 24-year-old female subject under conditions of social and temporal isolation. The subject stayed in the isolation facility, fitted with basic living amenities but devoid of time cues, for 35 days. In isolation the Temp rhythm of the subject freeran with a period of  $25.1 \pm 0.8$  h, but her SW rhythm freeran with a period of  $45.9 \pm 2.1$  h (circadian), resulting in desynchronization of the two rhythms. In 35 calendar days the subject experienced only 22 subjective, sleep–wakefulness days. Interestingly the menstrual cycle of this subject was normal, i.e. two episodes of onset of menses occurred 28 calendar days apart. We conclude in this first report on the subject that the menstrual cycle in the human female may not be coupled to the circadian rhythm underlying sleep–wakefulness while under social and temporal isolation.

NEARLY a hundred bodily functions in humans show 24-h daily rhythms that are all in synchrony with the sleep–wakefulness (SW) cycle<sup>1</sup>. All these rhythms are entrained by light–dark (LD) cycles and the social cues that accompany day and night<sup>2,3</sup>. In human subjects living in isolation, screened from the LD cycles of nature and social and other *zeitgebers* (time cues), the bodily rhythms persist and 'freerun' with periods close

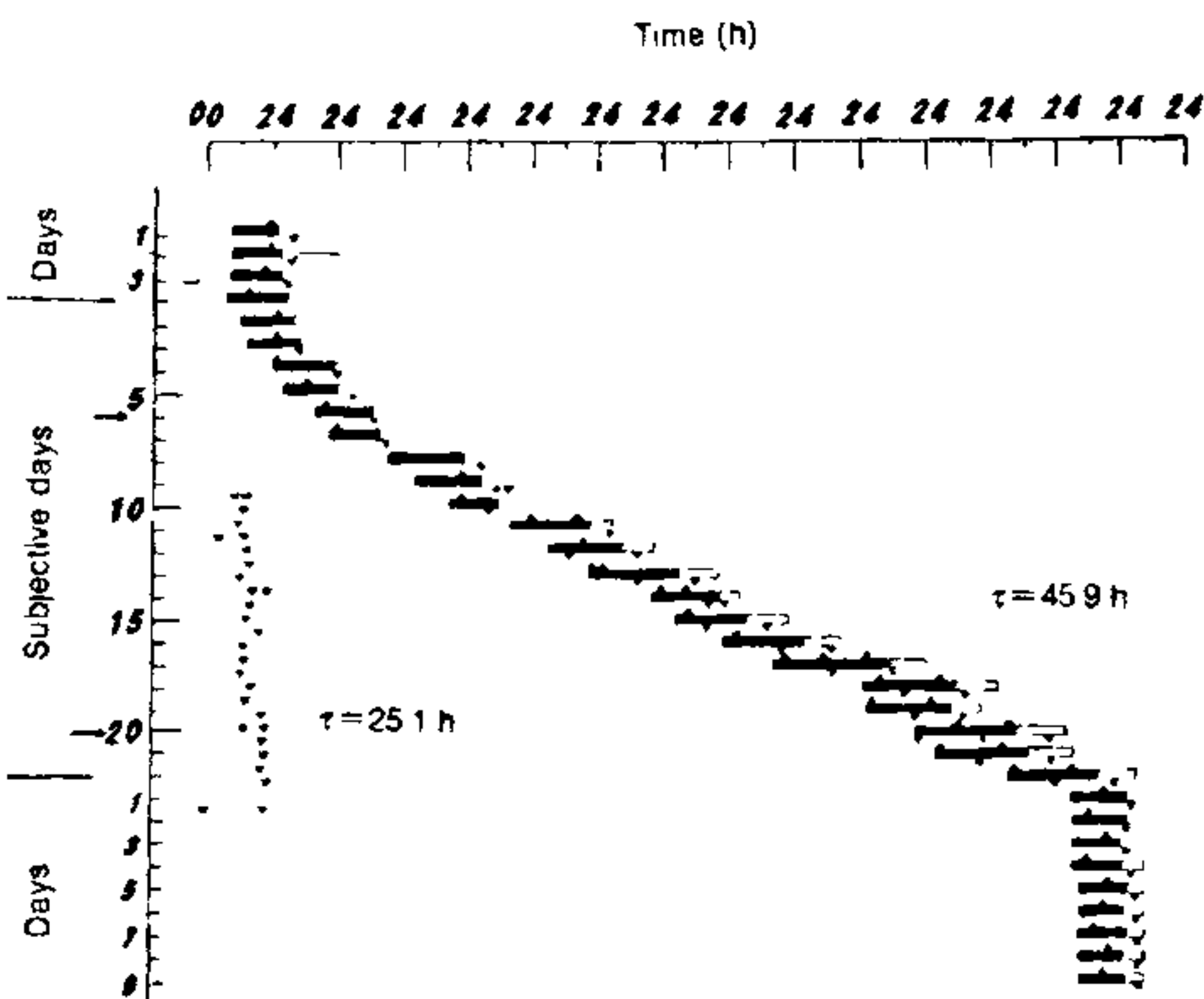
to 24 h (circadian), advertising their endogenous (genetic) origin. The best markers of the circadian organization in humans are the SW rhythm and the rectal Temp rhythm, which are also easily and reliably measured. The two parameters maintain mutual synchrony in entrainment and freerun. But in roughly 30% of humans studied in isolation facilities and bunkers the SW and Temp rhythms dissociated and freeran with markedly different periods, causing 'internal desynchronization'<sup>4</sup>. In addition to the circadian rhythms in the human female there is a circalunar monthly menstrual rhythm. The relationship between the circadian rhythm in physiological processes and the menstrual rhythm has not been investigated. In fact, a monograph written on the subject in 1981, *Circadian Rhythms and the Human*<sup>5</sup>, does not mention the menstrual cycle in the text even once. It has, however, been reported that onset of oestrus in the golden hamster does have a circadian component and that the period of the oestrous cycle also lengthened under freerunning conditions and oestrus occurred once in four circadian cycles of the activity rhythm<sup>6</sup>. The present report appears to be the first scientific one on this subject for humans even though it is based on only 47 'woman-day' data (including four days prior to and eight days after the 35 days of isolation) and the experiment was carried out on a single female human subject.

The isolation facility consisted of a double-walled bunker impervious to natural light and external noise. It had a large living area, a kitchenette and a bath. Fluorescent tubelights constituted the light source. The temperature was held constant around 25°C and stored water of uniform temperature was available for use. The isolation facility was devoid of potential *zeitgebers*, viz. clocks, radios, TV, current periodicals, etc. The occasional food and other requirements of the subject were placed in an antechamber, and there was no social contact for the duration of the experiment. Communication with the outside was mostly through scribbled notes.

The subject was a 24-year-old, presumably healthy female with a history of regular, predictable menstrual cycles of about 27–29 days. The core body temperature of the subject was measured using a rectal temperature probe. Ambulatory movements were measured by a wrist monitor. The temperature and activity levels were sampled at six-minute intervals and data stored in a battery-powered solicorder device. Data retrieval was done at random intervals to avoid giving inadvertent time cues to the subject. The subject activated individual buttons on a wall-mounted panel for different functions like 'to bed', 'sleep', 'wake-up', 'food', 'exercise', etc., which were conveyed to an Esterline Angus event recorder, thereby providing a function–time profile for each subjective day.

Pre-isolation or societal-day data on the SW rhythm and the Temp rhythm were acquired for four days. The subject entered the isolation facility in mid-luteal phase (4–12 days before menses) of her menstrual cycle. The experiment was designed to last till the second episode of menses occurred. On the whole, the subject underwent 35 days of isolation in the absence of time cues. The measurements were continued for eight days after the subject emerged from isolation. The ambient lighting experienced by the subject ranged from 1300 to 1500 lux depending on the distance from the light source during the light phase of the self-selected LD cycle, while absolute darkness prevailed during the dark phase.

Prior to isolation, under societal conditions the subject had a SW cycle with a period length of about 24 h ( $24.1 \pm 0.4$  h). During the first 10 subjective days of isolation the subject's SW cycle freeran, with a period length of  $30.5 \pm 1.9$  h (Figure 1). The temperature minimum on each subjective day occurred during the sleep phase and the Temp rhythm had a period of  $26.0 \pm 2.1$  h. In spite of this slight disparity in the period lengths of SW and Temp rhythms they were still



**Figure 1.** Circadian and circabidian rhythms of sleep and wakefulness (white and black bars) and of rectal temperature (maxima ▲ and minima ▼) of a 24-year-old female human subject living in social and temporal isolation in self-selected light–dark regime for a period of 35 calendar days. Data for days 1–3 before isolation and for days 1–9 after isolation are depicted as calendar (24 h) days. The experiment lasted a total of  $(3 + 35 + 9 =)$  47 days. During her social and temporal isolation of 35 calendar days the subject experienced only 22 subjective SW days. The Temp rhythm desynchronized from the SW rhythm on subjective day 11. The Temp rhythm had periods of  $26.0 \pm 2.1$  h before desynchronization and  $25.1 \pm 0.8$  h after desynchronization; the SW rhythm had periods of  $30.5 \pm 1.9$  h before desynchronization and  $45.9 \pm 2.1$  h after desynchronization. The upper and lower arrows indicate onset of the first and second episodes of menses on subjective days 6 and 20 respectively. Data on the SW rhythm during isolation are plotted in 'subjective days' whereas the Temp rhythm is plotted on both calendar- and subjective-day scales.

internally synchronized. From subjective day 11 onwards the SW rhythm showed gradual lengthening, reaching a maximum period length of 55.9 h, but the period length of the Temp rhythm remained virtually the same. Thus, in the second phase of the experiment, the SW rhythm of the subject freeran with an average period length of  $45.9 \pm 2.1$  h (circabidian<sup>7</sup>), while the Temp rhythm had a period length of  $25.1 \pm 0.8$  h. Thus the SW rhythm and the Temp rhythm were internally desynchronized. Under this condition the temperature minima consistently occurred more than once during a subjective day—once during the sleep phase and again during the wakefulness phase. The duration of wakefulness ( $\alpha$ ) prior to desynchronization was  $21.9 \pm 4.1$  h, while after desynchronization it showed a dramatic increase to  $32.1 \pm 5.0$  h. There was a similar change in the duration of the rest phase ( $\rho$ ), which increased from  $8.6 \pm 4.2$  h to  $13.8 \pm 3.0$  h under conditions of desynchronization. As a consequence of the increased period length of the SW rhythm the subject experienced only 22 subjective days as against the actually elapsed 35 calendar days. The first episode of menses in isolation occurred on subjective day 6 and the second episode 28 calendar days later. Owing to the circabidian period of the SW rhythm (period  $45.9 \pm 2.1$  h) the female subject was experiencing subjective day 20 when the second spell of menses set in. Interestingly the highest  $\rho$  values, 15.9 h and 18.3 h, occurred just prior to the onset of menses. It is clear from these results that the circadian/circabidian rhythm is not involved in the timing of the menstrual cycle of the human female, in contrast to the involvement of the circadian rhythm in the oestrus cycle of hamsters<sup>6</sup>. It must also be pointed out that it is not known if the SW rhythm and the menstrual cycle are coupled in human females living under normal societal conditions. Our methodology appears to be the only way to test if there is any coupling. Normal human subjects show, on average, 28 episodes of sleep and wakefulness in the course of a menstrual cycle. Our human subject experienced only 14 episodes of (circabidian) sleep and wakefulness. That is the novelty. The period of the Temp rhythm after isolation is very close to the geophysical lunar day of 24.8 h. But the menstrual cycle in the human females is not entrained or synchronized by lunar events. We are now in the process of writing a report that, in contrast to the unrelatedness between the SW rhythm and the menstrual cycle, there is a direct correlation between the circadian SW rhythm and two-hour-time-interval estimation in subjects studied in our isolation facility (Chandrashekar *et al.*, in preparation).

The dissociation of the SW rhythm and the Temp rhythm in our female subject coincided with onset of menstruation. This phenomenon of dissociation of the SW rhythm and the Temp rhythm has been reported by Wever for a 22-year-old female subject studied in the

Erling Andechs (Germany) bunker but the experiment in that case was not continued long enough to test the relationship of these rhythms to the menstrual cycle<sup>1</sup>. The impressive dissociation of the SW rhythm and the Temp rhythm in humans has not been reported in the case of other mammals investigated under comparable circumstances. In humans the nocturnal peak of the hormone melatonin has been shown to be highest during the pre-menstrual period, including the first day of bleeding<sup>8</sup>. In the present study, the subject had the longest duration of sleep during the pre-menstrual period, which may be correlated to higher levels of melatonin.

The multiple-oscillator model of Wever suggests the presence of two oscillators, one controlling SW rhythm and the other the Temp rhythm, which are coupled and freerun together during states of internal synchronization. Eventually these oscillators uncouple and freerun more or less independently, revealing their own natural periods, leading to spontaneous internal desynchronization. While desynchronized, the two oscillators assume a 'compromise period' closer to the natural period length of the stronger oscillator, which happens to be the temperature oscillator. The temperature oscillator in nearly all subjects has a period close to 25 h, ranging from 24 to 27 h. Those subjects with SW oscillator in the circadian range, between 20 h and 30 h, usually do not show desynchronization. Only subjects who possess a SW oscillator on the fringes of this range (below 20 h or beyond 30 h) eventually show desynchronization<sup>9</sup>. There are other models that attempt to explain the phase relationship between various circadian rhythms in humans. The data from the present study reinforce the multiple-oscillator theory. In addition to the temperature minimum that occurs during sleep phase there is yet another temperature minimum that indicates the point at which sleep onset would have occurred if the SW rhythm had remained synchronized with the Temp rhythm. The freerunning Temp rhythm, over a few days, shows modulation of frequency in the manner of 'relative coordination', suggesting that there may still exist some coupling forces between the two oscillators.

Our study on a young human female subject has provided interesting information on the state of the different circadian rhythms and the menstrual cycle under conditions of desynchronization. Further studies of the interaction between menstrual cycle and other circadian physiological parameters, especially neuro-endocrine rhythms, promise interesting and significant results.

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## Influence of nutrient stress on pyrethrin production by cultured cells of pyrethrum (*Chrysanthemum cinerariaefolium*)

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**Pyrethrum (*Chrysanthemum cinerariaefolium*) produces the insecticidal pyrethrins. Callus cultures of pyrethrum were subjected to nutrient stress to study its effect on growth and pyrethrin production. Nitrogen stress induced two-fold increase in pyrethrin level in two weeks, sugar stress resulted in reduction of pyrethrin level, and phosphate stress did not alter pyrethrin production. We discuss here the implications of these results.**

PYRETHRIN production by tissue cultures has been reported in pyrethrum and *Tagetes*<sup>1,2</sup>. We have recently reported the production of high-pyrethrin-yielding callus cultures derived from high-yielding plants<sup>3</sup>. With a view to enhancing the yield potential of pyrethrum callus cultures, we studied the influence of nutrient stress on pyrethrin production.

Callus culture was initiated from leaf explants of elite plants of *C. cinerariaefolium* by the method reported earlier<sup>3</sup>. The callus was maintained on Murashige and

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