Circadian Rhythms in Man

A self-sustained oscillator with an inherent frequency underlies human 24-hour periodicity.

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It is well known even to the layman that each day the body temperature reaches a highest value toward the evening and a low point early in the morning. Since this phenomenon was described by Gierse in 1842 (see *1*), numerous clinical and physiological studies have shown that there is apparently no organ and no function in the body which does not exhibit a similar daily rhythmicity. Whether we measure, hour by hour, the number of dividing cells in any tissue, the volume of urine excreted, the reaction to a drug, or the accuracy and the speed with which arithmetical problems are solved, we usually find that there is a maximum value at one time of day and a minimum value at another. All these rhythms are expressions of a so-called "physiological clock" which we have to consider as a basic feature in nearly all living systems, including unicellular organisms (*2*). As a result of extensive work by zoologists and botanists, especially during the last 15 years, we now understand some of the mechanisms involved and have good reason to assume that the rhythm originates primarily in the organism itself. The results of experiments with human subjects which I report on here give support to this hypothesis. In order to introduce the problem, I shall briefly refer to an experiment with birds which may stand as an example for many similar findings.

The Biological Background

An easy technique for following the rhythmic behavior of an animal without disturbing it is to measure its locomotor activity. Caged finches, kept in the laboratory in an artificial light-dark cycle with 12 hours of light and 12 hours of darkness, display a clear 24-hour rhythm with a typical pattern, all activity being restricted to the 12 light hours (Fig. 1, left). If this rhythmic behavior were a mere passive reaction to the periodicity of the environment, that is, to the light-dark cycle, it should cease in conditions of constant illumination. The two curves of Fig. 1, representing sections of much longer records, demonstrate that this is not the case. The activity of the birds continues to be cyclic in continuous dim light (1.8 lux), as well as in brighter light (120 lux). Furthermore, there are differences which claim our attention. Besides changes in the total amount of activity—the birds are less active in dim light, and for shorter spans of time, than in bright light—the length of the period (τ) is of special interest. In a physical oscillation, we measure the period as the time interval between any two identical phases. In biological oscillations, because of the variability in the shape of the curve and its unavoidable "noise," the situation is more complex. In many cases the sharp onsets of activity have proven to be useful phases from which to measure the period. In light of 1.8 lux, the bird starts to be active each day at about 5 a.m.; its period is very near to, but not exactly, 24 hours. Contrary to this, in light of 120 lux the bird becomes active each day 2 hours earlier than on the preceding day; its period is 22 hours.

These two facts, the undamped continuation of the rhythm and the deviation of the period from 24 hours, allow only one plausible explanation: that this rhythm is not imposed on the organism by the environment but is truly endogenous. We are dealing with a self-sustained oscillation which is free-running under constant conditions and has its own inherent frequency. An increasing number of experimental results demonstrated that we are right in using this technical terminology and that the "clock" on which the overt rhythms are based has all the characteristics of an active oscillator, as was first outlined by Pittendrigh (*3*). [The opposing hypothesis, that in constant conditions the rhythm is controlled by an unknown exogenous 24-hour input of, possibly, cosmic origin, has been criticized several times (*4*) with sufficient thoroughness; I therefore refrain from discussing it again.]

To signify that under constant conditions the frequency of the biological oscillator deviates more or less from 24 hours, Halberg (*5*) introduced the term "circadian," derived from the Latin *circa* and *dies*. Under natural conditions, the circadian period is synchronized with (or entrained to) the period of the earth's rotation by means of periodic factors in the environment, called Zeitgebers. The day-night changes in illumination and temperature are the most important of these. To illustrate this point, in Fig. 2 I have graphed the results of a second experiment in another way. The horizontal black bars represent the times when the bird is active (corresponding to α in Fig. 1). The experiment starts with 5 days of entrainment by an artificial light-dark cycle. Thereafter the bird is free-running in constant illumination of 0.4 lux. On the 33rd day, the Zeitgeber is reintroduced for 15 days, entraining the bird immediately. Finally, the bird is again placed under constant conditions, but in light of 120 lux, instead of the 0.4 lux used earlier. It is evident from the graph (Fig. 2) that the free-running period is longer than 24 hours in dim light and shorter in bright light. This type of behavior is not unique to the bird. Experiments with many species in several laboratories gave rise to the following "circadian" rule: With increasing intensities of illumination, the circadian period is shortened in diurnal (light-active) animals and lengthened in nocturnal (dark-active) animals. Recently, Hoffmann reviewed the facts on which the rule is based (*6*).

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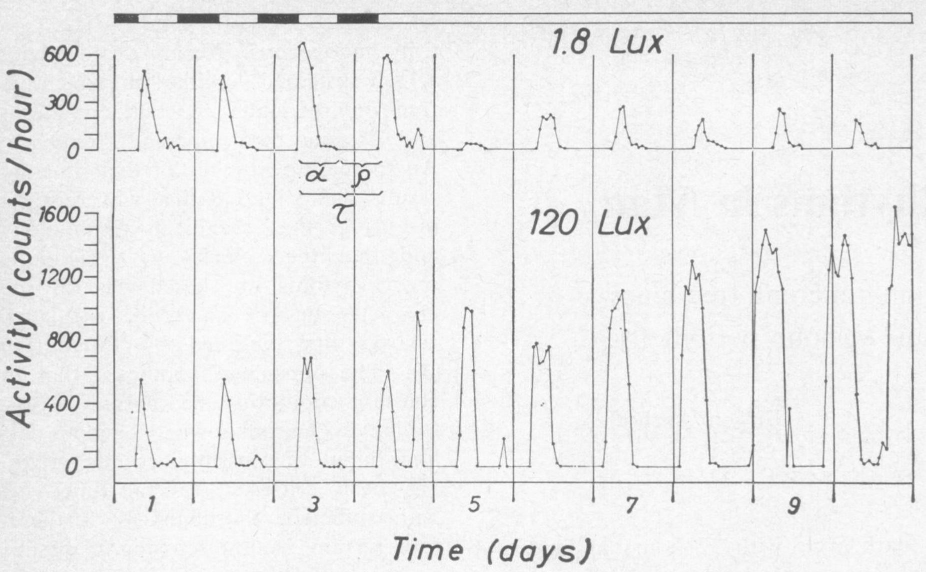

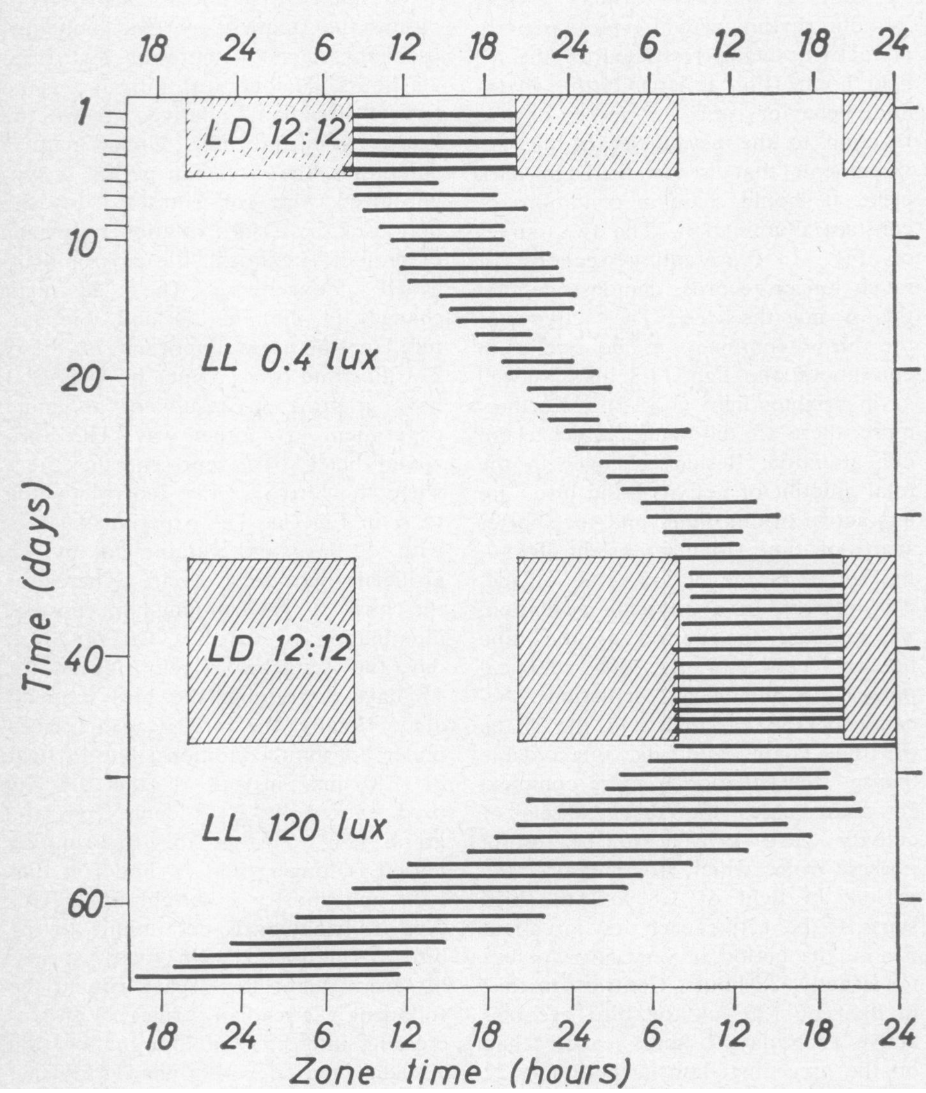

Fig. 1 (top). Activity rhythms of two chaffinches (*Fringilla coelebs*). The birds were kept 3 days in an artificial light-dark cycle, and thereafter in continuous illumination, one in light with an intensity of 1.8 lux (above), and the other in 120 lux (below). τ, Period; α activity time; ρ, rest time; ordinate, perch-hopping activity, recorded by means of print-out counters. Fig. 2 (bottom). Activity rhythm of a chaffinch (*Fringilla coelebs*) in a light-dark cycle with 12 hours of light and 12 hours of darkness (*LD 12:12*) and in continuous illumination (*LL*) with an intensity of 0.4 lux and of 120 lux. Black bars, activity time; shaded area, darkness.

Human Subjects without Time Cues

The older hypothesis of exogenous causation of diurnal rhythms was based, to some extent, on studies of human subjects. The rhythm of night workers was found not to be shifted as compared to the rhythm of those working in the daytime; scientists who had traveled with sailboats along latitudes reported that the rhythm of their body temperature was always "in phase" with local time. These and other observations suggested an unknown (cosmic) control of the rhythm in man. When the circadian nature of the oscillation and its endogenous origin were demonstrated in animals, my co-workers and I became eager to know whether man also possesses a circadian clock. In order to investigate this properly, it became necessary to separate single isolated subjects from all possible factors which could act as Zeitgebers. Therefore we had to exclude natural daylight, as well as any other periodic input from the environment which might contain information on time. For some pilot experiments, a soundproof underground operation room left over from the wartime was used at first. Recently we were able to build our own underground bunker with more sophisticated facilities. Here the subjects live in complete isolation for up to 3 or 4 weeks. Attached to a rather comfortable bed-sitting room are a shower and a small kitchen. The occupant of the bunker prepares his own meals. We ask him to lead a "regular" life, that is, to have three meals in a normal sequence, not to nap after lunch, and to perform a few psychological tests. Otherwise, he is allowed to do what he wishes. Many subjects are students who cram for an examination. Listening to recorded music is their favored entertainment.

The setup for registration is outside the bunker. We measure continuously the subject's body temperature (by means of a rectal probe), his activity pattern, his time estimation (of intervals of 20 seconds and of hours), and his movements in the bed. The subject also collects his urine at intervals of his own choice. There is a locked double door at the entrance. In the small room between the two doors, an icebox serves for a first storage of urine samples. Through the same channel we supply the subject with fresh food and other necessities, such as one bottle of Andechs beer daily. A magnetic catch permits only one of the two doors to be opened at a time. The only way in which the subject can communicate with the outside world is by sending and receiving letters. The times at which experimenters enter the double door from outside to deliver supplies and so forth are randomized. Usually we allow the subjects to turn the light off when they go to bed and to turn it on when they get up. The intensity of illumination, however, is controlled from outside. The temperature in the room can be set by the subject himself.

The curves in Fig. 3 give an impression of the behavior of a subject under those conditions (7). There is a clear cycle of sleep and wakefulness which is reflected also in the rhythms of body temperature and of urine excretion. The maxima of the four functions measured are not always exactly "in phase" with each other and with the activity cycle, but they all have, on an average, the same free-running period of about 25.0 hours. Since in this case I myself was the subject, I can add a few remarks on personal feelings. After a great curiosity about "true" time during the first 2 days of bunker life, I lost all interest in this matter and felt perfectly comfortable to live "timeless." From the knowledge of animal experiments I was convinced that I had a period shorter than 24 hours; when I was released on day 10, I was therefore highly surprised to be told that my last waking-up time was 3 p.m. In the "mornings," I had difficulty in deciding whether I had slept long enough. On day 8, I got up after only 3 hours of sleep (see Fig. 3). Shortly after breakfast I wrote in my diary: "Something must be wrong. I feel as if I am on dogwatch." I went to bed again and started the day anew after three more hours of sleep. Judging from the curve of body temperature, my first start happened to coincide with the worst phase of the circadian period, that is, with the low point of temperature. I was mistaken by an effort of will and put to order by my physiological clock.

The regularity of the circadian rhythm and its steady drift against local time become more evident from Fig. 4. Corresponding to the activity times of the bird in Fig. 2, the black bars here represent the times when the subject is awake, the span being measured from the moment when he turns the lights on to the moment when he turns them off. Initially, the subject lives in contact with the outside world and keeps his normal phase relationship. After the beginning of isolation, some irregularities appear; two extremely long activity times (more than 20 hours of wakefulness) are followed by a very short one. Thereafter, the rhythm becomes remarkably stable, with a mean period of 25.9 hours. The maxima of urine excretion (obtained by averaging the hours at which the maxima of water, potassium, calcium, and sodium occur) follow the same trend; the mean period differs only by 0.3 hour from that of the activity cycle. After 18 physiological days in confinement, the subject wakes up at 8 p.m.; he has "lost" 1.5 real days. Readjustment to the correct phase relationship with local time occurs with a "jump," produced by an interposed activity time of more than 30 hours duration.

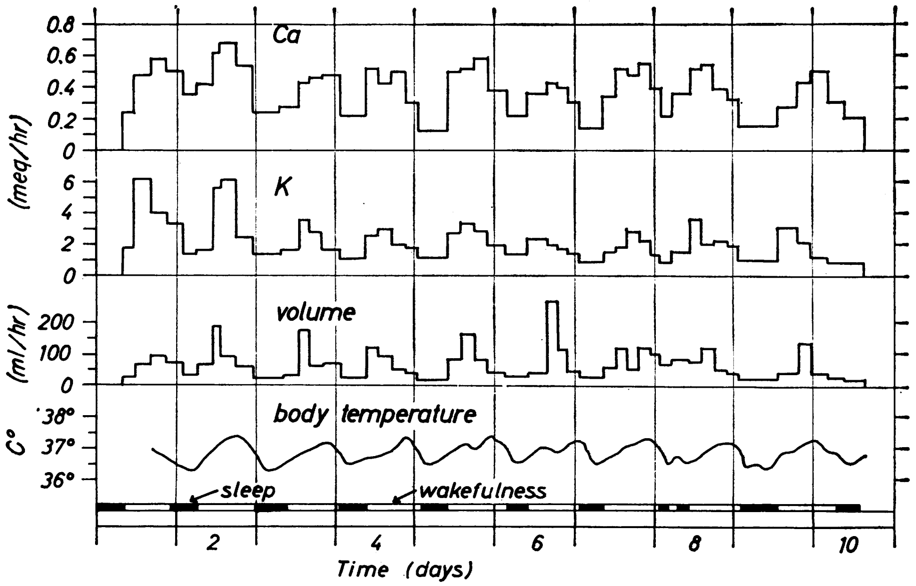

Fig. 3. Circadian rhythm of urine excretion (calcium, potassium, and water), body temperature, and sleep-wake cycle in a human subject kept without timepiece in complete isolation from the outside world.

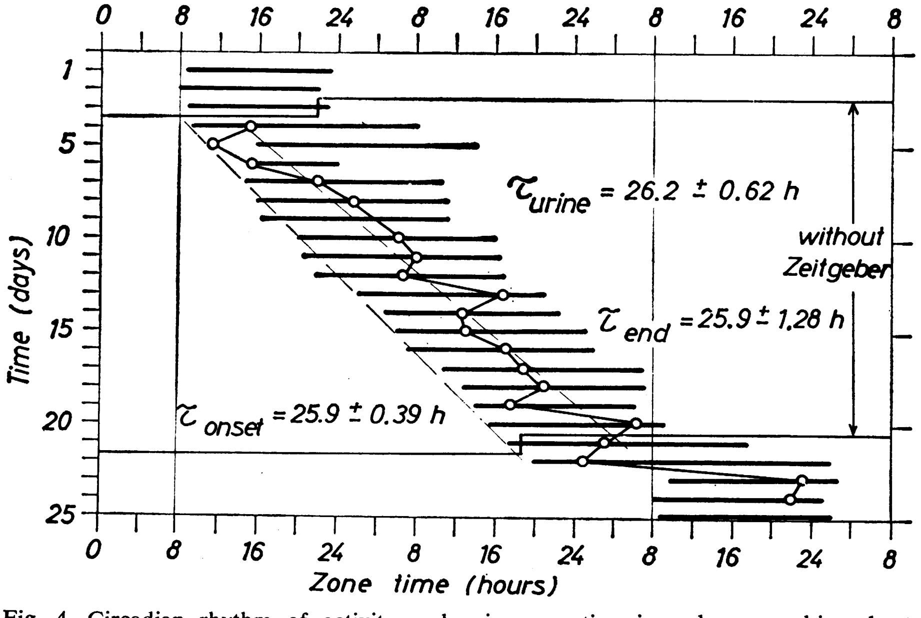

Fig. 4. Circadian rhythm of activity and urine excretion in a human subject kept for 3 days under normal conditions, then for 18 days in isolation, and finally again under normal conditions. Black bars, times of being awake; circles, maxima of urine excretion; τ, mean values of period for onset and end of activity and for urine maxima.

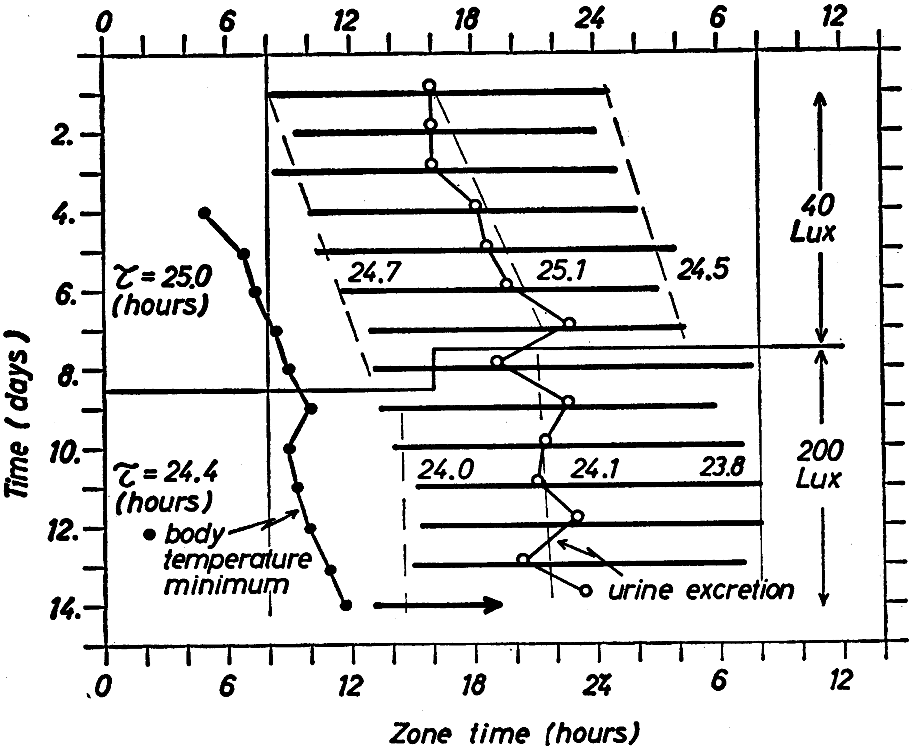

Fig. 5. Circadian rhythm of activity (black bars), urine excretion (open circles, maxima), and body temperature (closed circles, minima) of a human subject kept in isolation with illumination of 40 lux and later of 200 lux intensity. τ, Mean value of period.

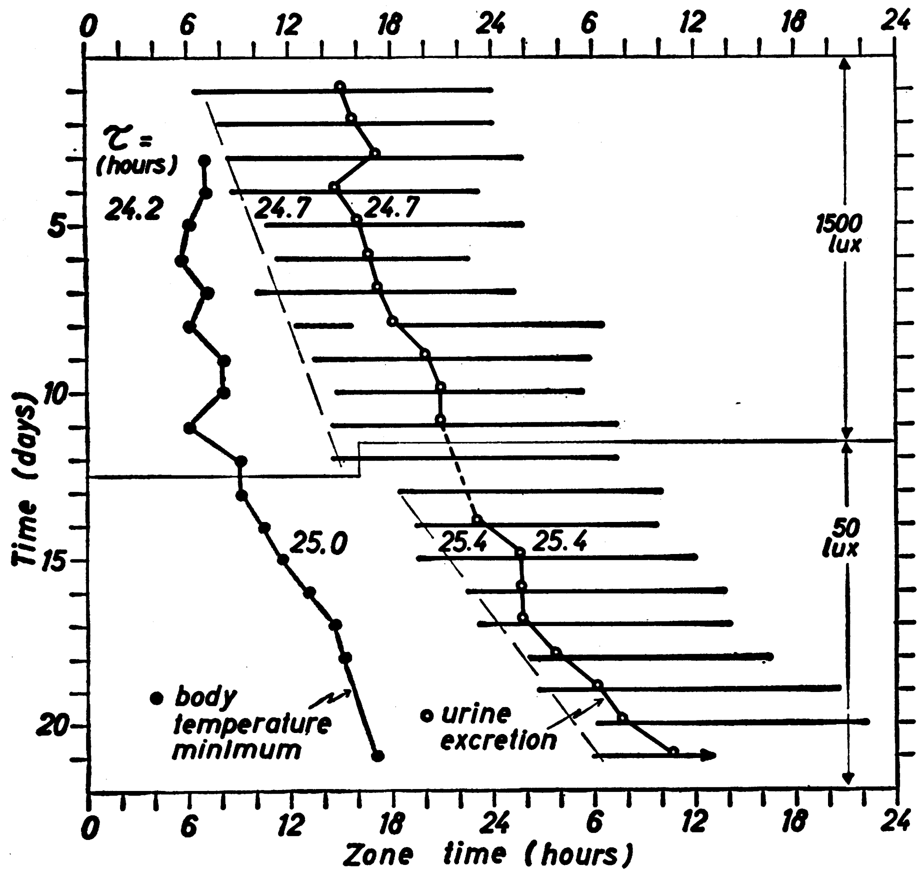

Fig. 6. Circadian rhythm of activity (black bars), urine excretion (open circles, maxima), and body temperature (closed circles, minima) in a human subject kept in isolation with illumination of 1500 lux and later of 50 lux intensity. τ, Mean value of period.

Influence of Light Intensity

The importance of light as the main Zeitgeber makes it of interest to know whether in man period length depends on light intensity as it does in animals (compare Fig. 2). By nature, man belongs to the group of light-active organisms. We therefore expected that his period would be shortened when the intensity of illumination was increased, and we were able to verify this expectation in a few preliminary experiments. Two examples are given in Figs. 5 and 6. In the first case, we started with a rather dim illumination of 40 lux. The period was close to 25.0 hours for the activity cycle, for body temperature, and for urine excretion. When we increased the light intensity to 200 lux, the periods were shortened by 0.7 to 1.0 hour. In the second case, we used the reverse procedure and offered the subject first 1500 lux for 11 days and then 50 lux for the rest of the time. There was again good agreement between the periods of activity and those of other functions. All lengthened by 0.7 to 0.8 hour with the decrease in light intensity. I must admit that we have so far the results of only a limited number of experiments of the type demonstrated in Figs. 5 and 6. In a few cases, the data are inconclusive or, in one case, even contradictory to the rule. The positive results prevail, however, and are suggestive. If we can confirm the dependence of period length on light intensity, we have a first demonstration that light influences the overall organization of human beings. Such findings might well be of more general interest than only for the field of circadian studies.

Only four out of a total of 26 subjects showed periods shorter than 24 hours. One was a student who lived for 10 days in continuous dim illumination of 0.3 lux (similar to moonlight); he had a period of 23.6 hours. Another student, whom we kept for the whole time in an illumination of 1500 lux and who turned the lights on and off, exhibited a period of only 19.0 hours during the first 10 days of confinement; he then lengthened the period consciously (as he told afterwards) and reached 25.8 hours. The possibility of manipulating the period to some extent by intentionally stretching the activity time contributes to the variability in our results and may sometimes mask the influence of light intensity. There is, on the other side, the surprising fact that 85 percent of all subjects studied so far had periods considerably longer than 24 hours. This is in agreement with Siffre's records on his own activity cycle in a cave, and with the data published recently by Mills (*8*). The following circumstances might be causes of the long periods: (i) The rather dim illumination, as compared with natural conditions; in agreement with the circadian rule, this could slow down the oscillator. (ii) The monotony of the situation, those stimuli which we experience under natural conditions, such as noise and social contacts, being absent; experiments with birds show that grouped animals with mutual stimulation have a higher circadian frequency than those kept in isolation. (iii) The feedback between the subject's endogenous activity cycle and the self-selected periodic stimuli—that is, turning the lights on and off. The last explanation is supported by results from experiments with birds which we kept in a situation similar to that of the bunker. By means of a special apparatus, bright light was turned on by the bird itself shortly after it had started to jump from perch to perch; when the bird went to rest, the bright light was turned off and only a dim background illumination was left. Under those conditions, the period of the bird's activity cycle was longer than under constant conditions with either dim or bright light. More data are necessary to allow a final decision.

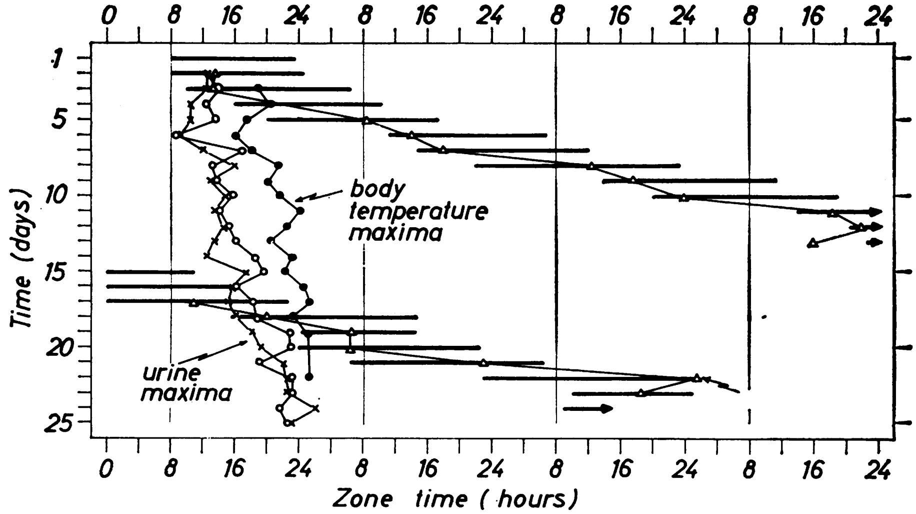

Fig. 7. Desynchronization of circadian rhythms in a subject living in isolation without time cues. Black bars, times of wakefulness; open triangles, maxima of calcium excreted in urine; open circles and crosses, maxima of water and potassium (respectively) excreted in urine; closed circles, maxima of body temperature.

Dissociation and Desynchronization

The adaptive significance of circadian rhythmicity is that it enables the organism to master the changing conditions in a temporally programmed world—that is, to do the right thing at the right time. This could be achieved, to some extent, by an exogenous rhythm. But by developing a self-sustained oscillation of approximately the same frequency as that of the environment, the organism, in its own organization, anticipates the respective states which will enable it to react properly to the environmental conditions which will ensue—it is prepared in advance. Another prerequisite, of course, is the entraining Zeitgeber which provides a distinct and species-specific phase relationship between the periodicity of the organism and that of the environment. Entrainment, furthermore, supports the temporal organization of a multiplicity of oscillating variables in the organism itself (*9*). All the rhythmic functions, a few of which I have mentioned, keep a more or less fixed phase relationship to each other. The graphic representation of this has been called the "phase-map" (*10*). Even under constant conditions, the temporal order of the phase-map may on the whole be maintained. But sometimes slight differences in the τ values of different functions occur, resulting in a small but steady change of internal phases (see Fig. 4). This need not be proof for the existence of several clocks running with different frequencies and independent of each other. It is rather likely that under constant conditions, that is, without the "correcting" stimuli of a Zeitgeber, the rhythmic functions tend toward a new phase relationship, different from that under conditions of entrainment. In this interpretation the differences in τ values are considered to be transitory dissociations which will finally result in a new steady state of full internal synchronization.

But there are also cases in which the organism becomes truly desynchronized. One subject (Fig. 7) showed an extremely slow rhythm of sleep and wakefulness, with a mean period of 32.6 hours. Contrary to this, the rhythms of body temperature and of excretion of water and potassium in the urine had a period of only 24.7 hours. Surprisingly, calcium excretion did not follow either of these patterns but was bound to the activity cycle instead. This subject exhibited two frequencies even in the same organ, the kidney. The rhythms of activity and calcium excretion regained their original (normal) phase with the rhythms of the three other functions every third to fourth day. The times when all functions were in phase coincided with occasional diary notes in which the subject indicated that he felt especially well and fit. The question whether both the frequencies shown in Fig. 7 represent true circadian clocks is difficult to decide. It might be that we have to consider the unusual long behavioral periods as a kind of "artifact," while the primary clock system is reflected in the more nearly daily rhythms of body temperature and of excretion of water and potassium.

We do not yet have enough data on hand to make strong statements about the probability with which we have to expect major dissociations and desynchronizations in free-running systems, or about the consequences for the organism of such phenomena if they last for a longer time. Such disturbances would be of minor practical interest if they were restricted to organisms in constant conditions. However, a dissociation of functions may also occur in the entrained organism. This has been demonstrated by Lobban (*11*). She observed two groups of students who lived at Spitzbergen, on either a 21- or 27-hour day. In a few of the subjects whose body temperature and water excretion adapted perfectly to the new routine, the potassium excretion remained on a 24-hour rhythm. Similar investigations may be of major interest for future studies of circadian rhythm, especially with regard to problems in applied physiology.

Applications

Industry makes allowance for the fact that workers have a circadian rhythm of efficiency. Accidents and errors in tending machines or in reading indicator boards are most likely to happen at 3 a.m. (*12*). There are only slight phase differences in these rhythms between night workers and day workers. But night workers are in general less efficient and suffer psychologically and physiologically during night shifts. They live in a situation of conflict between two tendencies in the circadian system: to remain entrained by the social Zeitgebers of the normal environment, and to become adjusted to the shifted work-rest cycle. These unnatural conditions are reflected in a narrowed range (amplitude) of the body temperature curve of nightworkers (*13*). Of course, in conditions without conflicting Zeitgebers, that is, in more rigorously controlled environments of isolation, or after a trip along latitudes in a jet airplane (see below), a 180-degree shift of the circadian rhythm will be accomplished in about 6 days (*14*). It is still an open question whether night shifts should last long to make use of some adaptation which may occur, or whether frequent changes between night and day shifts, requiring repeated readjustments, are preferable.

The situation becomes even more complicated with a work-rest schedule of 4 hours on duty and 4 hours off, as may be used for a crew in a spacecraft. Under those conditions, the circadian system could become synchronized in a 1-to-3 ratio with the 8-hour period of the artificial Zeitgeber. The few groups of subjects studied on those and similar schedules showed a clear circadian rhythm. There was, however, a continuous drift in phase of the human rhythm against clock time. The experiments conducted so far did not last long enough to determine whether the circadian system was free running, or entrained with a tendency toward a new phase relationship to the Zeitgeber (*15*). The answer would be of importance for further decisions on work-rest schedules and other living conditions for crews.

A common experience in the jet century may serve as a third example. If we travel by air from Paris to New York, our circadian clock keeps on going with European time. Arriving at Kennedy Airport, we are out of phase by about 6 hours. Entrainment to local time takes 2 to 3 days, during which we feel tired and less efficient (*16*). As we could demonstrate in birds, the time necessary for readjustment is not the same when we shorten the light-dark cycle once by 6 hours as when we lengthen it by the same amount. Applying these results to man, we have to expect different durations of indisposition after long-distance flights eastward and westward. Obviously, it is of interest for civil purposes as well as for military use to know how we can shorten the time required for resynchronization. One way seems to be to influence the natural frequency of the circadian oscillator; this frequency determines to some extent the speed with which the system regains its natural phase relationship to the environment, at least in birds. Manipulation of artificial Zeitgebers—for example, the insertion of relatively short times of light and dark after extreme long flights—might be another possibility. The discussion of these problems has only reached a state of theoretical analysis and of preliminary experimentation with animals.

All three examples mentioned above show that we still lack much necessary information, but they indicate that the self-sustained circadian oscillator has to be taken into account. Its main properties seem to be the same in human beings as in all other organisms. We have to study them before we can discuss practical problems successfully. As always in science, a better understanding of the basic phenomena will be the first step toward a proper application in practice.

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