Temporal Coordination of Physiologic Function

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I. TIMING

A. CIRCADIAN SYNCHRONIZATION—WITH PHASE DIFFERENCES

The organism, as well as its parts, usually does not function at a constant rate along a 24-hour scale; many of its diverse, discrete changes do not recur at purely chance intervals. More often than might be expected from classical physiologic knowledge, we encounter circadian (circa, dies; [1–3]) repetitive sequences of events; this organization in time has apparently evolved through genetic adaptation of body metabolism to a terrestrial environment (for references see [1]).

An organism's circadian behavior seems to be plastic, rather than rigid; it involves the recurrence of like events in sequences that are not random and at intervals that are similar—rather than the repetition of identical changes after precisely fixed periods.

The circadian time structure, an adaptive feature of most species, is an aspect of functional integration in a given individual; its general characteristic resembles synchronization in the physical sense, i.e., frequency synchronization, rather than the coincidence in time of certain peaks or troughs.

Circadian synchronization with differences in phase (plus, zero or minus; Figs. 1–3) cannot be equated to synchrony; all physiologic events are not identically timed. This point deserves emphasis. It can be illustrated by many examples, so that the one following case may be left intentionally unidentified by this writer. Possible circadian changes in activity of two functions were explored. Two sets of samples were removed from groups of rats killed at the times of high and low motor activity, respectively. Significant within-day differences were found for one functional activity but not for another.

It was suggested that a rhythm characterized the former variable but not the latter—on the tacit assumption that circadian peaks and troughs of different functions are all necessarily synchronous (cf. Figs. 2 and 3). In the case cited, subsequent more-frequent sampling revealed that the activities of the two functions were periodic but they were synchronized with a phase difference.

B. INTERNAL AND EXTERNAL TIMING

Temporal coordination among physiologic functions, per se, can be described by the time relations of circadian rhythms to each other. This aspect in time of functional integration is denoted as t_i, or internal timing, in Fig. 1; t_i can be given as the difference in hours among the peaks or troughs of physiologic variables. Such internal timing of two or more functions may be described without causal implications as to their possible interrelations (see interval between peak incorporation of P_{32} into DNA of mouse liver and peak mitoses in hepatic parenchyma; $t_i \backsim 8$ hrs. in Fig. 2, bottom). Analytical statistical estimates of the variability of t_i, however, are desirable.

The time relations of circadian rhythms to environmental factors depict, in their turn, temporal aspects of adaptation. This external timing of rhythms is denoted as t_e in Fig. 1. External timing can be conveniently referred to a time base such as the conventional 24-hour clock. Such information on the temporal placement of a rhythm within the 24-hour time period (e.g., the clock time of a peak) becomes physiologically meaningful only when the environmental schedule dominating the t_e of rhythm is defined along the same (24-hour) time base (see peak hepatic mitoses at $\backsim$ 12:00, with light daily from 06:00 to 18:00 in Fig. 2, bottom).

C. THE "RIGHT TIME"

Physiologic regulation has been viewed as the whole of the body's provisions for supplying and transporting the "right amount" of the "right material" to the "right place." Evaluation of t_i and t_e adds a new temporal dimension to such regulation, i.e., the "right time" (Fig. 1).

To cite but a few aspects, the biochemist's "what" (as to the right compound), the pharmacologist's "how much" (as to the right dose), or the morphologist's "where" (as to the right organ or cell) are all pertinent to the physiologist's endeavors. Yet his research as to how the various tasks of organic regulation are accomplished and integrated with one another as well as with the environment also depends upon information on timing.

The physiologist builds not only upon the details of other sciences, including morphology, biophysics, biochemistry, and pharmacology, to name but a few (Fig. 1); equally pertinent to his task is the recognition of rules governing provisions for the "right time" as a function of physiologic state and ecologic condition. Information on a circadian organization in time (Figs. 1–3) need not take second place to the concern for compounds, doses, sites of action, age effects, etc. (cf. Fig. 3).

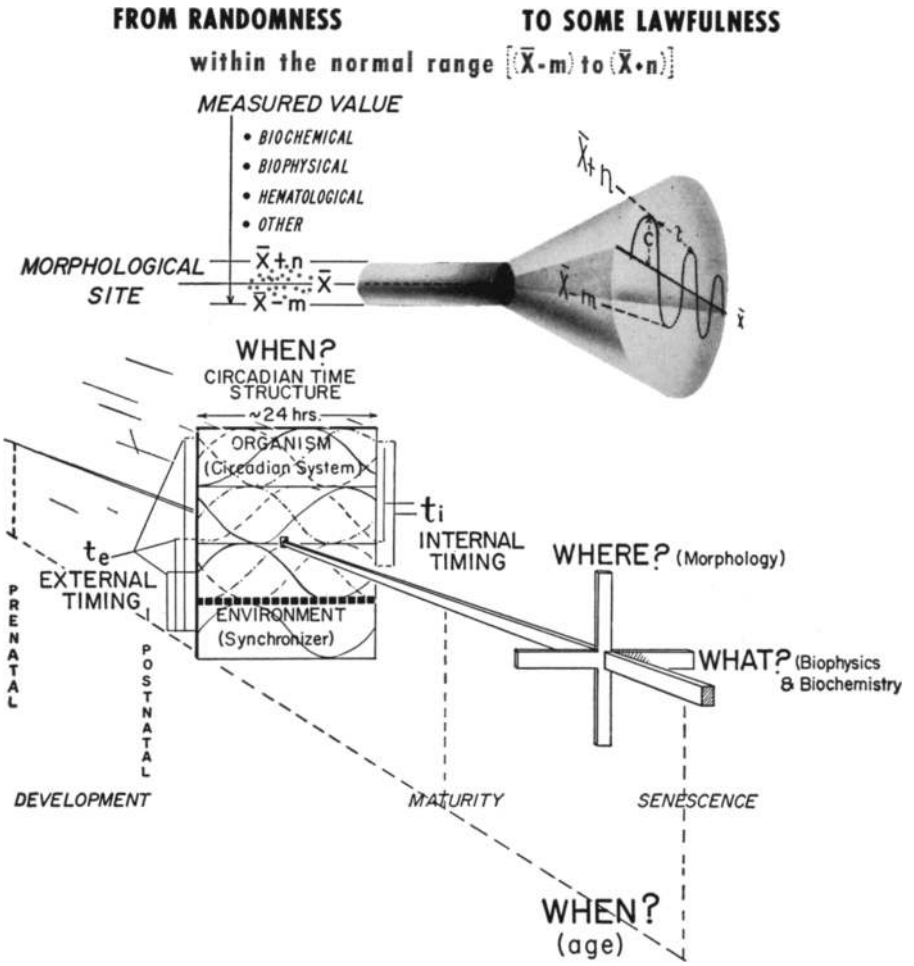


FIGURE 1. Circadian periodicity analysis—a tool for resolving temporal coordination of physiologic function. Cf. text, Sect. I and [1]. For t_e and t_i confront Figures 2 and 3.

II. CIRCADIAN RHYTHMS

A. GENERALITY, VARIABILITY, SIGNIFICANCE

Circadian periodic phenomena are not uncommon; along an age scale of human populations they are bracketed by births (Fig. 4) [4–7] and deaths (Fig. 5) [4, 5, 8, 9]. Pertinent, among others, is a paper published in 1848; breakdowns of births and deaths according to the hours of the day are included [4]. These authors refrained from interpreting the "periodicity" of their data [4], obviously a complex phenomenon (cf. Fig. 3).

Interest in medical aspects of circadian variations [10] was renewed following the discovery of the liver glycogen rhythm several decades ago; it led to the pioneering work of, *inter alios*, Forsgren, Jores, Kalmus, Menzel, and Möllerström (for references see [11]).

Figure 6 shows (on top) Ågren's early data on liver glycogen [12] plotted against clock hour. The middle and bottom sections of the same figure depict blood eosinophil variation in two stocks of mice studied by the writer [13]. Changes with time are unmistakable, yet the scatter must not be ignored. Such rhythms are statistical rather than deterministic entities [14]. This does not imply, however, that they are not reproducible, once conditions of observation and sampling are standardized [1].

Figures 7 and 8 (bottom), for instance, depict functions (cf. Fig. 6) that once were notorious as "unreliable" when they were studied in the intact animal. One reason for their "variability," however, is a circadian periodic component that can be readily standardized [13, 15–20]. Other rather basic functions, such as hepatic phospholipid, RNA or DNA metabolism, also reveal previously unsuspected circadian behavior which, again, is quite reproducible (Fig. 8) [11, 21].

Equally reliable and unexpected is the extent of change along a 24-hour scale in the susceptibility of the total organism to certain environmental agents of biophysical [22, 23], pharmacologic [24], endocrinologic [25], toxicologic [26], or bacteriologic [27] interest. In these particular instances, conditions can be standardized so that the circadian change in physiologic state of the organism significantly and reproducibly tips the balance toward death or survival (e.g., Fig. 9).

A few multivariate profiles are now available for

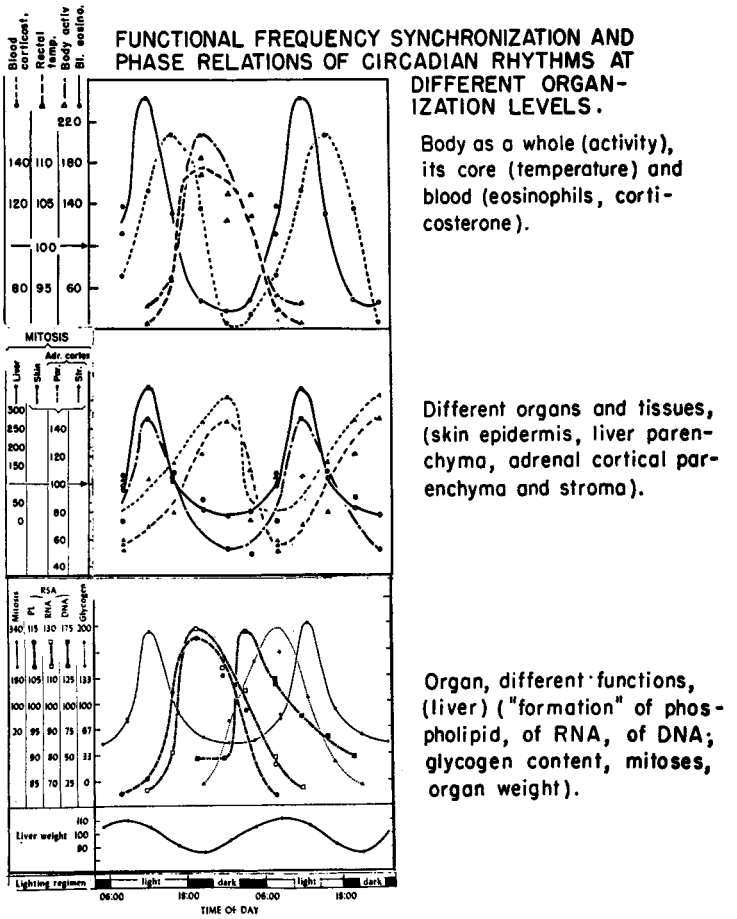


FIGURE 2. Some aspects of internal and external timing (t_i and t_e in Fig. 1)—at different levels of physiologic organization. Immature mouse. Ordinates are data expressed as per cent of 24-hour mean.

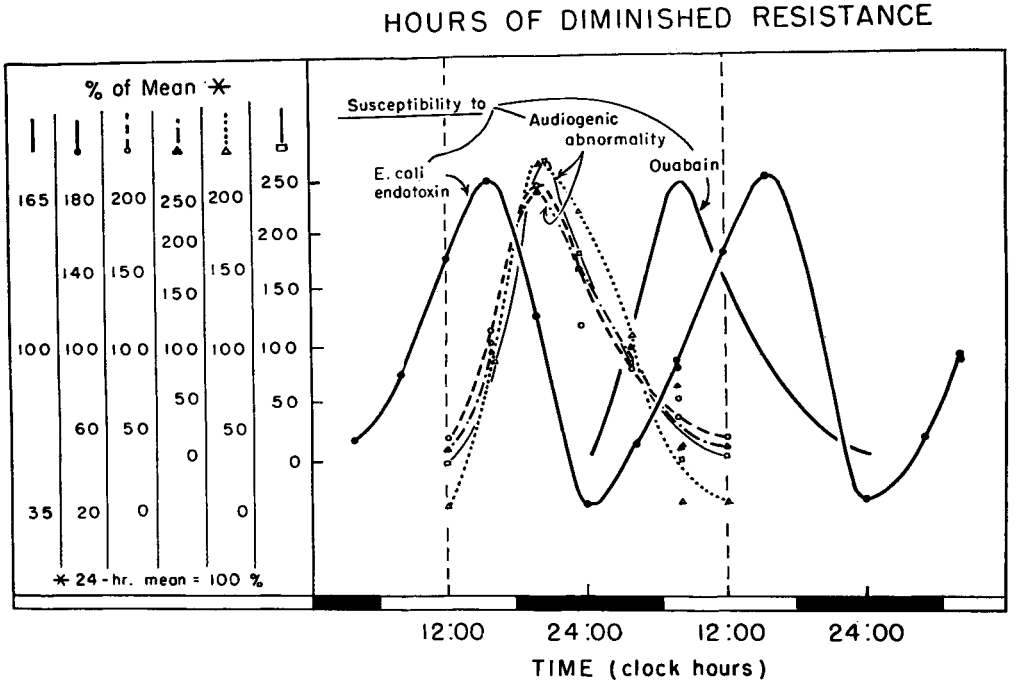


FIGURE 3. External and internal timing of circadian susceptibility rhythms. Cf. text, Sect I C and Fig. 1.

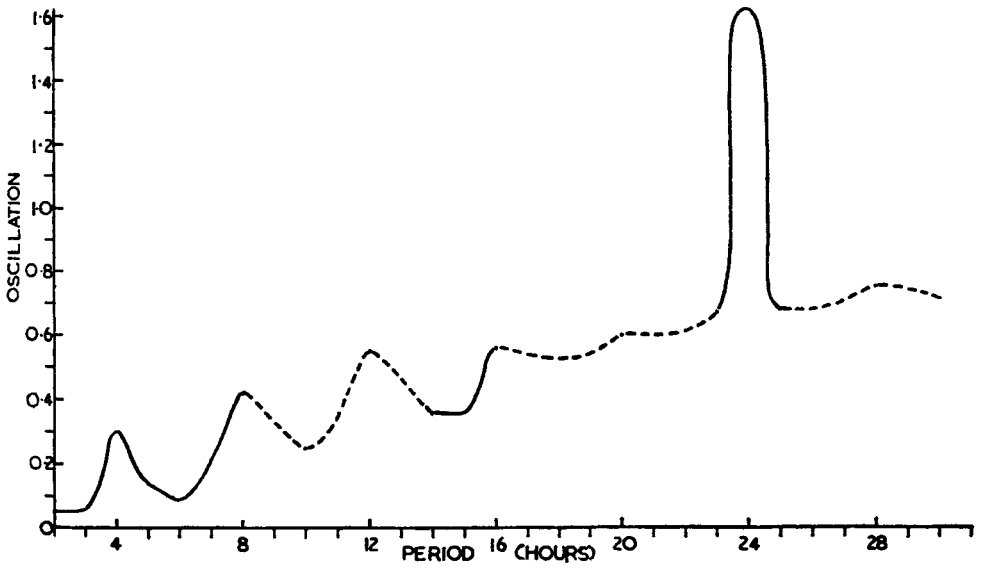


FIGURE 4. Incidence of human births, analyzed by periodogram [6].

mammals (e.g., Figs. 2 and 3) and even unicellulars (Fig. 1 in ref. [28]). Such time charts depict rhythms at different levels of organization, ranging from the resistance of the total organism to toxins to the metabolism in some of its cytoplasmic fractions.

Circadian charts that are reproducible for certain variables, species, sex, age, and some other conditions obviously are needed for studies of internal timing (t_i in Fig. 1); external timing (t_e), in its turn, is a function of both the environmental synchronizer [29–31] and t_i. Full understanding of either t_e or t_i therefore depends upon the multivariate approach. Up to now multivariate studies on rhythms were scarce since they involved much labor.

With reliable circadian maps available, however, various functions need not be fully recharted in each experiment; spotchecks, e.g., done at the known times of peak and trough, may suffice, just as a few landmarks en route are checked against geographic maps.

But the scope of circadian charts extends far beyond studies on rhythms. Spotchecks against such charts for definition of t_e and/or t_i may soon prove to be a *sine qua non* for any rigorous biologic work on normally periodic functions. It seems axiomatic that for work on rhythmic variables the "search for controls" implies a search for periodicity.

B. Noise

Standardization of circumstances of observation, sampling, etc., (cf. [1]), is not always feasible. In many time series, as usually obtained in clinic, field, or laboratory, rhythms are blurred by other variations, summarized for our purpose as noise. As a first approximation (cf. [32]), one may classify certain physiologic time series according to the prominence of their circadian rhythms:

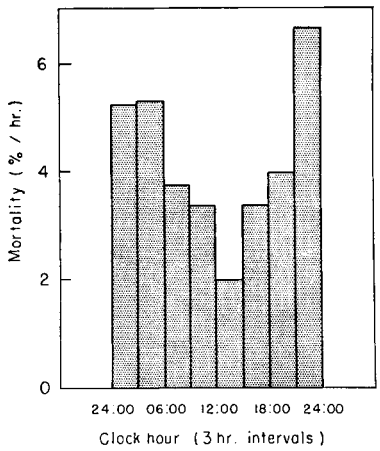


FIGURE 5. Post-surgical mortality at different clock hours (based on data from [9].)

Kind A data: periodicity stands out clearly; it is readily demonstrated by a plot against time; it involves statistically significant changes which are reasonably reproducible from one study to the next.

Kind B data: periodicity can be recognized, although it is distorted by noise. The changes found in any one study may be statistically significant but their timing (t_e and t_i) may vary greatly among studies done under presumably identical conditions.

Kind C data: periodicity, if any, is completely masked by other variations.

When faced with time series of Kind B or Kind C, one can choose or compromise between (1) using computational procedures for unmasking a possible circadian periodic component from interference, e.g., by autocorrelograms (cf. [33]) (Fig. 10), power spectra [32], among others (extensive data usually are needed

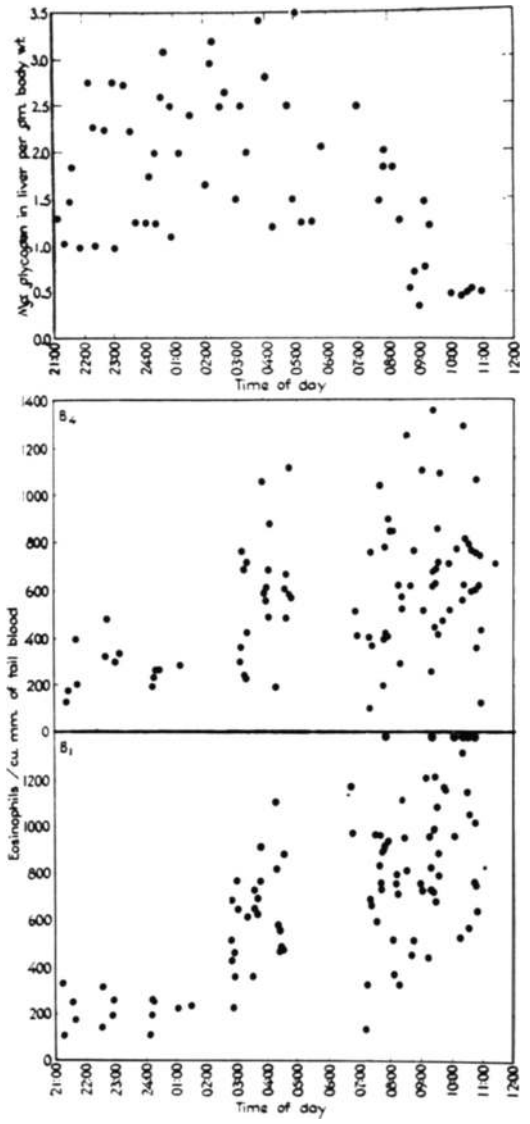


FIGURE 6. Nocturnal and diurnal variations in liver glycogen (top) [12] and blood eosinophils (middle and bottom) [13]. Cf. also text and Fig. 7.

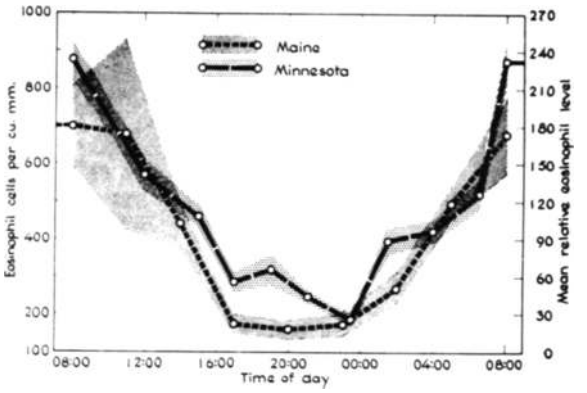


FIGURE 7. Data on eosinophil rhythm in the mouse—obtained in different laboratories under slightly different conditions [1, 18]. Note reproducibility.

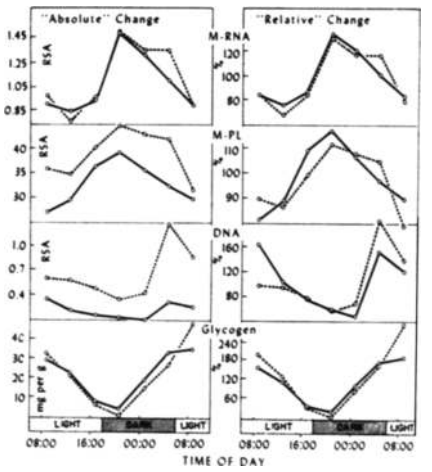


FIGURE 8. Metabolic circadian changes in separate experiments: left, "absolute" values; right, recomputed data (as percentage of series-mean). (*RSA* = relative specific activity; *M-RNA* = microsomal *RNA*; *M-PL* = microsomal phospholipid) [21].

for such computations); and (2) removing interfering variations to some extent at least, by standardized procedures.

C. Quantification

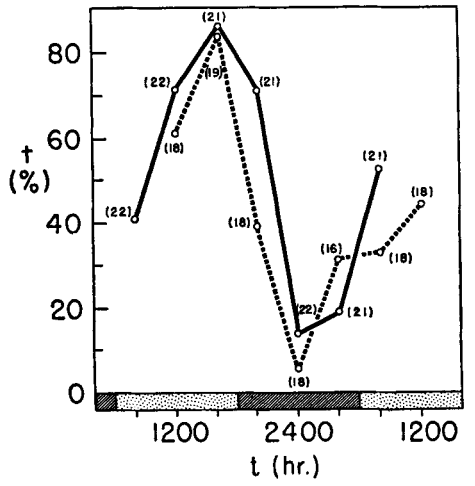
In studying a given circadian rhythm, numerical estimates are sought, i.a., of the period (τ), the amplitude (C), and the level ($\bar{X}$ in Fig. 1). The estimate of period is all too often based upon locating, by inspection, successive peaks or troughs (or motor activity onsets), a procedure which may be subjective. When a single frequency dominates the data, the cautious use [34] of periodograms represents a first step in our search for more objective numerical estimates of period, and it provides an amplitude estimate as well (Fig. 11). The necessity for inspecting the plots of data, however, can not be overemphasized. Beats of the envelope (Sect. II F below), among other features in the shape of a periodic function [35], are all pertinent aspects of analysis.

D. Procedure

Periodicity analysis may be done after an appropriate period provided for synchronization of circadian rhythms with certain environmental routines. The schedules used for such synchronization may be the routines of activity and rest in studies on human beings or primarily the alternation of light and darkness in the experimental animal's cage. Apart from reference to such schedules as cues [36], they have also been

defined as the Zeitgeber [37], entraining agent [38], clue [39], or synchronizer [31].

An appropriate synchronizer may be conveniently chosen to have a cycle length of 24 hours. Work may then be done in the absence of additional intentional stimulation, among several other simple precautions—the direct method—or by applying an unusual agent in different stages of rhythm—the indirect method.



Ordinate † (%) { % death of group of standardized mature C mice from E. coli lipopolysaccharide (Difco, 100 µg./20 gm., i.p.).
Abscissa t (hr.) { times of injection, in 2 experiments (injections begun at 2 different time points, during daily light period).
N of mice / group in parentheses.

FIGURE 9. Susceptibility rhythm of C mice to intraperitoneal injection of *Escherichia coli* endotoxin. A dose compatible with survival for most animals at one time is highly lethal when injected into comparable mice in a different stage of rhythm. Results of two experiments; see text. Note reproducibility [27].

Results from direct and indirect light-synchronized periodicity analysis [1] on mice were shown in Fig. 2 and 3, respectively. Figure 12 depicts, at the bottom, the rhythm in rectal temperature of a mature human subject synchronized by an institutional 24-hour routine providing for diurnal activities and meals and for nocturnal rest.

Equally synchronized by the social routine is the temperature rhythm of a younger patient in the same institution, shown in Fig. 13. During several weeks prior to severe chicken pox and after the febrile period, the 5 A.M. temperature of this subject constitutes the lower envelope of his rhythm; the synchronization of rhythm and routine was not obliterated during the period of incubation and during a later period complicated by pustules.

The profiles in Fig. 2 and 3 may be called transverse—many individuals were studied during one 24-hour period; while those in Fig. 12 and 13 are longitudinal—they involve sampling over many cycles in the same individual. Broader availability of longitudinal profiles for those varied functions of interest in clinic or laboratory awaits automation [40].

Non-synchronized (i.e., not intentionally synchronized) periodicity analysis, in turn, involves as far as

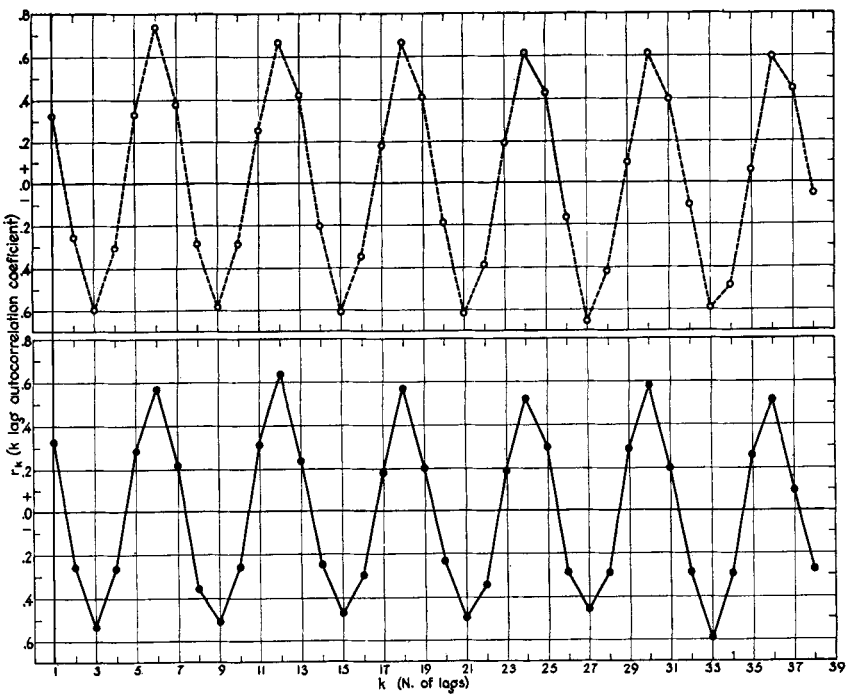


FIGURE 10. Autocorrelogram of rectal temperature data obtained at 4-hour intervals. Mouse. Work in cooperation with Professor L. Hurwicz and Mr. J. Smith.

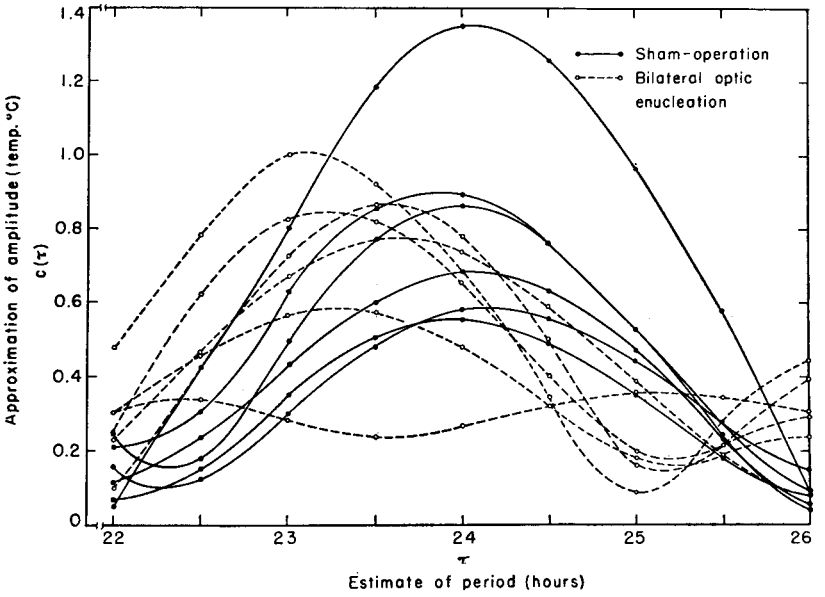


FIGURE 11. Periodogram of rectal temperature data from individual mice after blinding or sham operation (first month post-operative). See text Sect. II E.

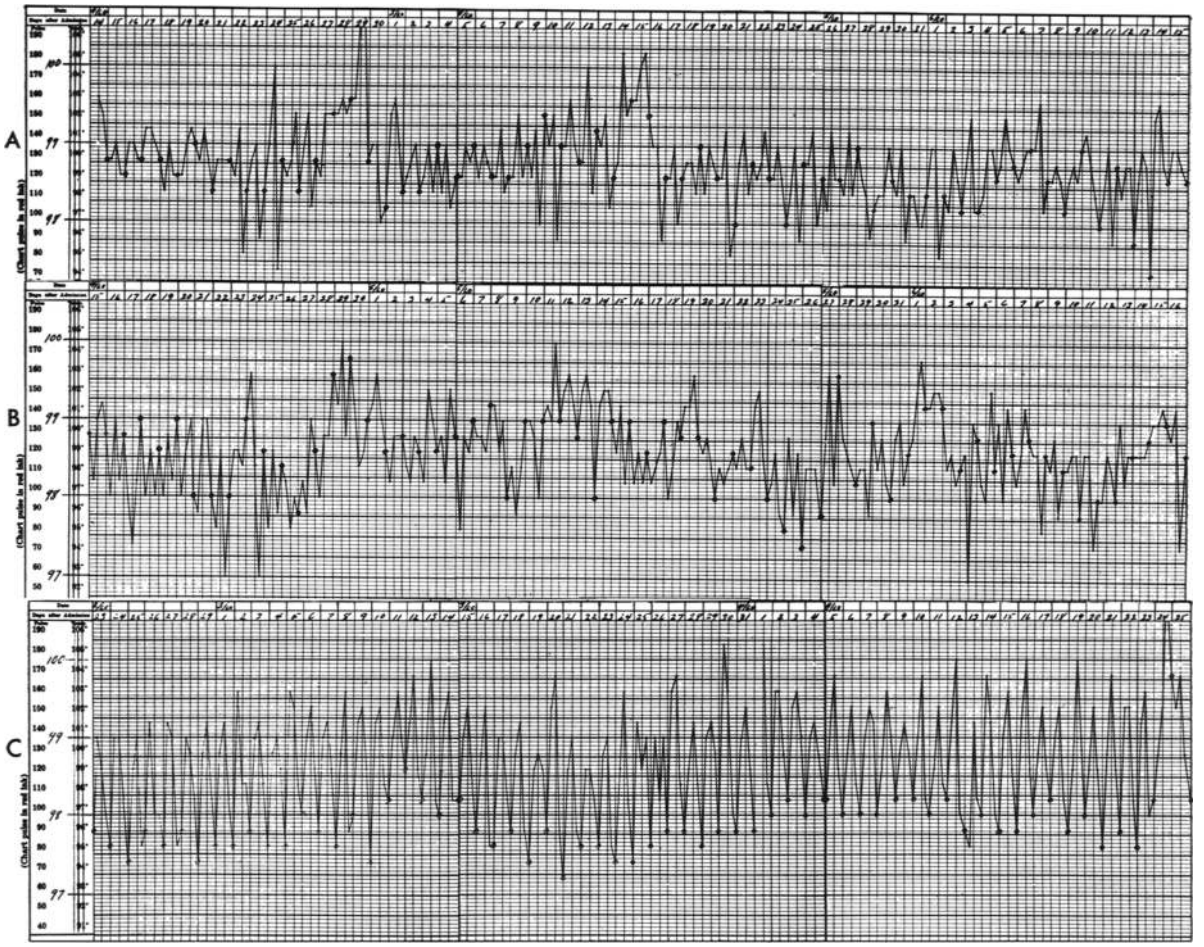


FIGURE 12. Rectal temperatures at 6-hour intervals over a two-month period in two cancer patients (top and middle) and a patient institutionalized for convulsive disorder but free of seizures during observation period. 5 A.M. temperatures are circled. Note synchronization with 24-hour institutional routine in the curve at the bottom of the figure; compare irregularities in the curves from cancer patients living on the same routine (top and middle of figure).

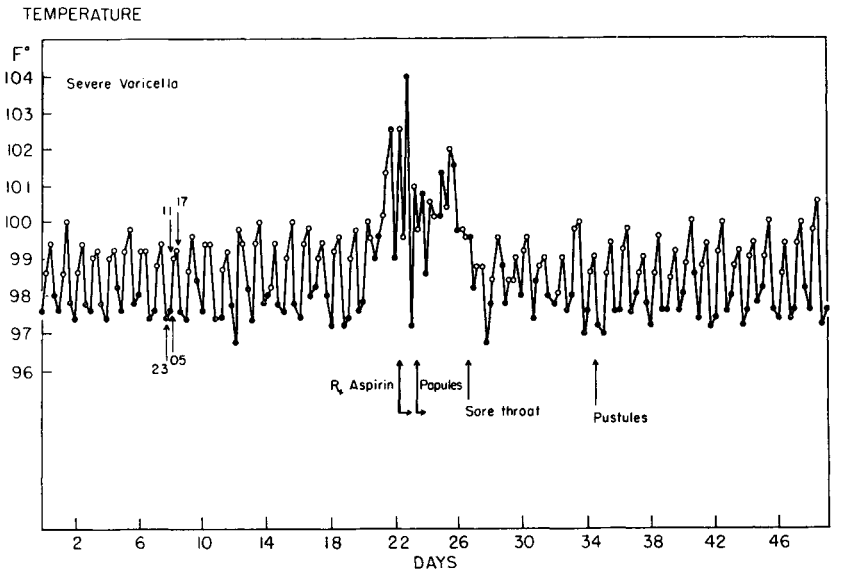


FIGURE 13. Synchronization of temperature rhythm with an institutional routine providing for diurnal activities and meals and for nocturnal rest—before and after a febrile period associated with severe chicken pox. 5 A.M. temperatures are black dots.

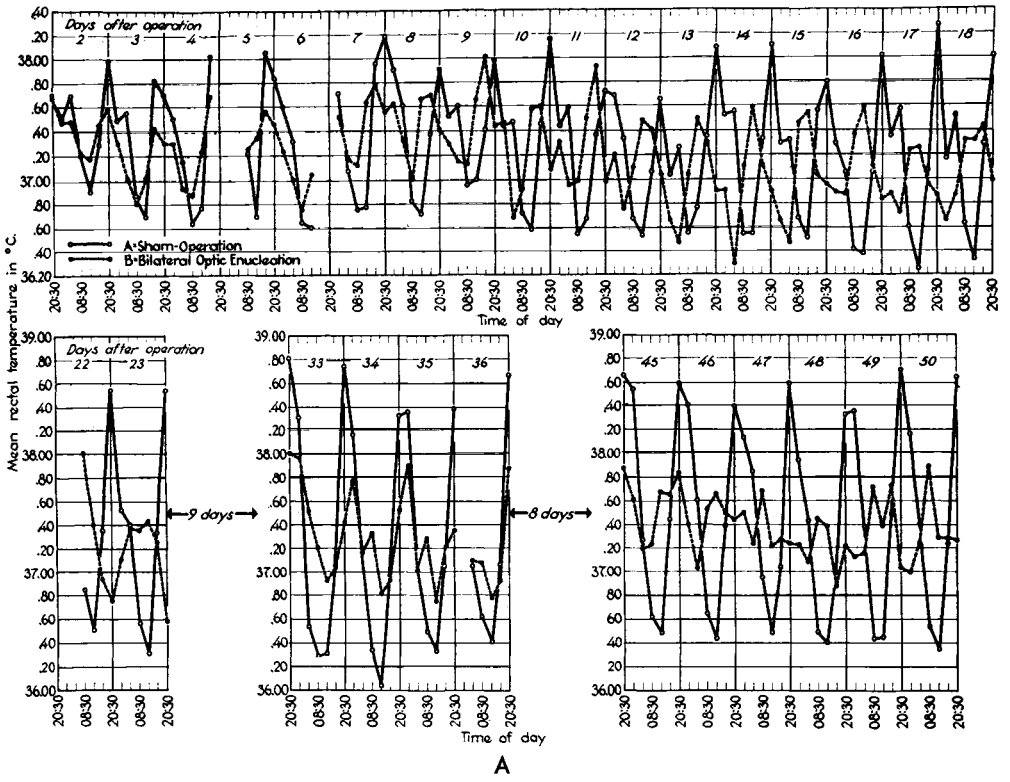


FIGURE 14*a*. Effect of bilateral optic enucleation upon rhythm in rectal temperature of male hybrid mice. Note lead in phase of temperature rhythm in blinded mice; by the 22nd post-operative day it is in temporary antiphase with that of controls. The circadian period in rectal temperature of blinded mice is, on the average, slightly shorter than 24 hours (cf. Fig. 11). Data show "free-running" of temperature rhythm from environmental 24-hour routine. See text, Sect. II E.

feasible the elimination of periodic environmental stimuli. Important studies in continuous light or in constant darkness, with controlled environmental temperature (cf. [1]) are illustrative examples [37, 38].

With non-synchronized analyses, average periods that are close to 24 hours but not exactly that length have been recorded for many rhythms and species [28, 37, 38]. Such periods I have called "free-running from the

24-hour clock" or "free-running" for brevity [11]. Free-running periods may differ from exactly 24 hours by minutes or a few hours.

E. Free-Running

Circadian periods that differ from an exact 24-hour length have implications as to general experimental method [31]; their detection has contributed to analyses of the factors underlying circadian rhythms (cf. [1] and [31]). Free-running periods come to the fore following certain manipulations of either the environment [37, 38] or the organism (Fig. 14) [1, 31, 41–44].

Moreover, in a group of experimental animals treated in the same way [11, 37, 38], free-running periods differ from one individual to the next (Fig. 11). Spotchecks reveal such periods at different levels of organization (Fig. 14) [11]. Environmental counterparts with corresponding frequencies are not now known, and it seems unlikely that all recorded physiologic frequencies will ultimately be found to have geophysical or cosmic counterparts, with exactly corresponding τ.

Free-running periods apparently characterize the physiologic (open) system at least as much as they characterize its environment; with such qualification they may be regarded as largely endogenous. It is important from this viewpoint to raise the question whether organisms with free-running circadian rhythms still use as yet unknown environmental time-cues [45]. Apart from correlations, this problem awaits experimental analyses.

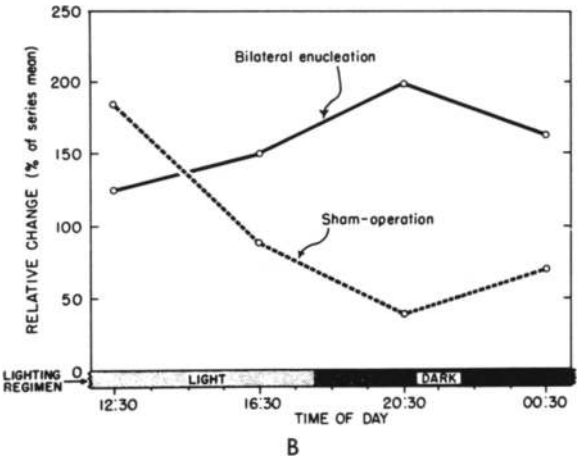


Figure 14*b*. Phase differences in eosinophil rhythm of blinded and sham-operated mice. Temporary antiphase brought about by differences in period of the rhythms compared (τ of sham-operated animals was synchronized with a 24-hour routine, while τ of blinded mice was free-running, i.e., desynchronized from the routine by bilateral optic enucleation.)

F. Beats

Certain circadian rhythms may conceivably result from two distinct components that are additive functions. For instance, effects of a 24-hour routine, y_1, may be superimposed upon a free-running component, y_2, among many other theoretical possibilities. Whenever two frequencies (y_1 and y_2) underlying a rhythmic variable ($y = y_1 + y_2$) differ in period, beats will result.

As a function of the difference in their periods, the two components will tend to reinforce each other or to cancel out in a periodic manner. Maximum reinforcement, when the phase difference is smallest, will be followed by maximum cancellation when the phase difference is greatest.

Figures 15*a*, *b*, and *c* may be used to depict the beat frequency of circadian rhythms with periods differing by 40 minutes, 80 minutes, or 160 minutes, respectively; it can be seen that in these particular cases minimum and maximum amplitude will alternate in a predictable fashion at intervals of 17½ days, 8½ days, and 4 days, respectively (cf. [46]).

The abstract Fig. 15 was computed in an attempt to

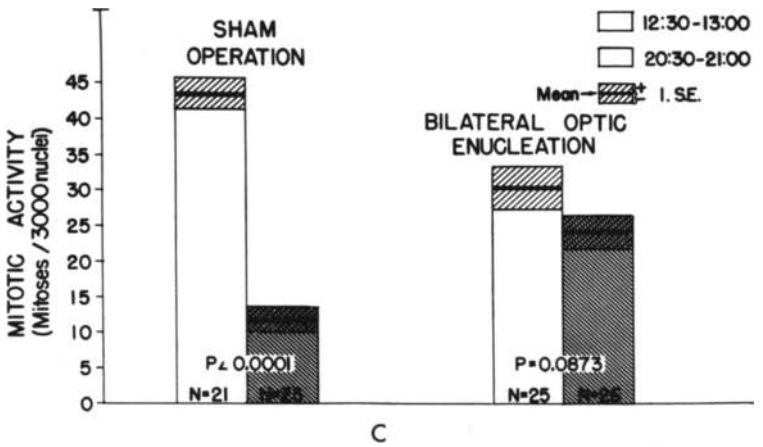


Figure 14*c*. Spot check of mitotic rhythm in pinnal mitoses of blinded and sham-operated mice. In view of the data of Fig. 14*a* and 14*b* these results are interpreted as free-running of mitotic rhythm, particularly since sham-operated controls showed a significant within-day difference, with predicted t_e [11].

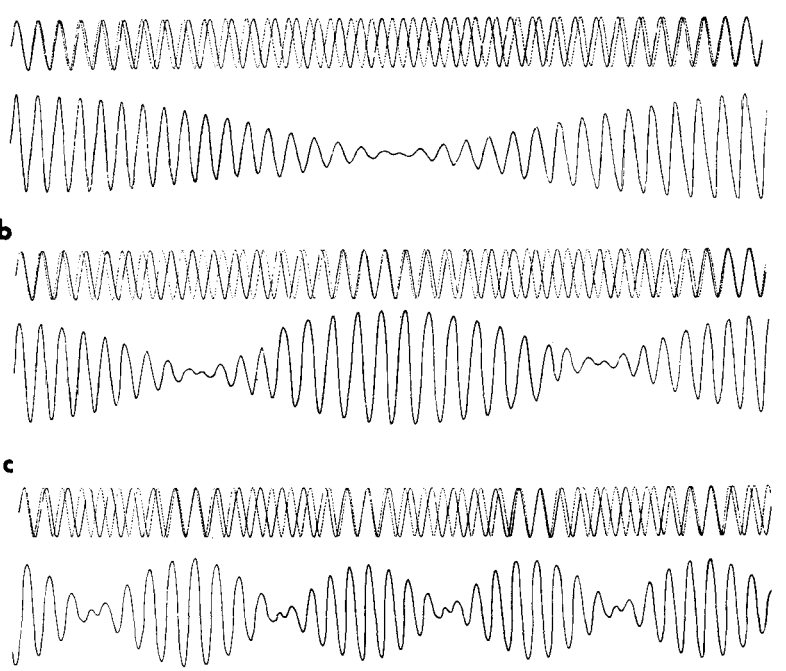


FIGURE 15 *a–c*. Beat frequency as a function of the difference in period of two hypothetical component frequencies underlying a numerical valued observation. See text, Sect. II F.

interpret beats on the rectal temperature curve of certain institutionalized patients. Surprisingly similar to Fig. 15*c*, however, are the water excretion data of Lewis and Lobban in Fig. 16 [47, 48].

The observations summarized in Fig. 16 were made in an isolated community in Spitzbergen, under conditions of relatively far-reaching environmental control. Most unusual and valuable for the present discussion is another aspect of the same data: the subjects lived on a 21-hour routine; they adhered strictly to a schedule dictated by specially-adjusted wrist watches showing 12 hours during 10½ ordinary hours. If these water excretion data are interpreted as resulting from the interference of two periodic components, it seems most plausible that the artificial routine of the "21-hour day" was one such component. The other component(s) could be the transiently continuing effect of the 24-hour schedule on which the subjects had previously lived, or a free-running function with a period close to 24 hours, and/or synchronizer interaction, discussed elsewhere [11, 47, 48].

III. FACTORS UNDERLYING TEMPORAL COORDINATION

A. Internal Factors

Circadian sequences of cellular events, their adrenal pacemaker in mammals, as well as superimposed and juxtaposed humoral and neural controls of rhythms, are sketched in Fig. 17. Underlying this scheme are metabolic studies, approaches by "removal and replacement," recording of spontaneous and induced potentials, and other work. Except for renewed emphasis on the effects of adrenalectomy upon certain rhythms

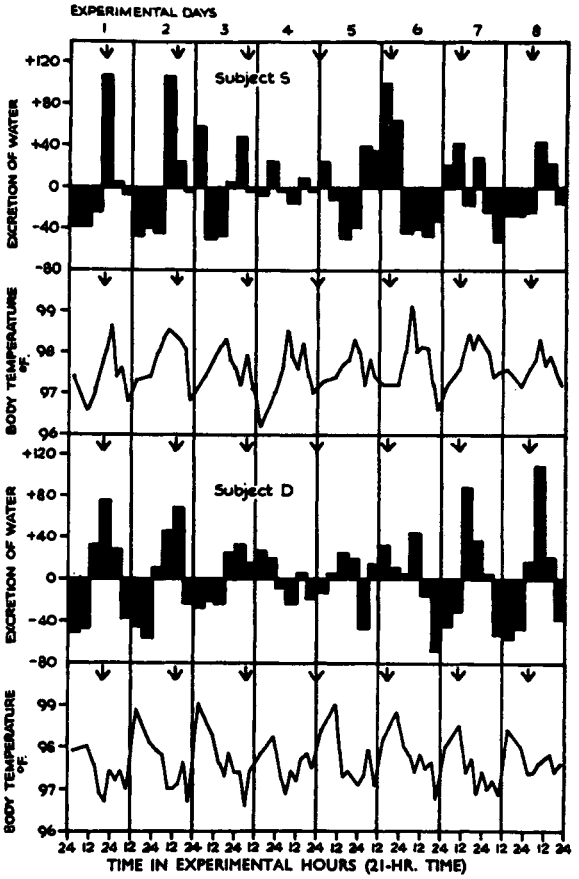


FIGURE 16. Beats in physiologic circadian data of Lewis and Lobban [47, 48]. Compare, for instance, the water excretion data for subject D in this figure with the abstract Fig. 15c.

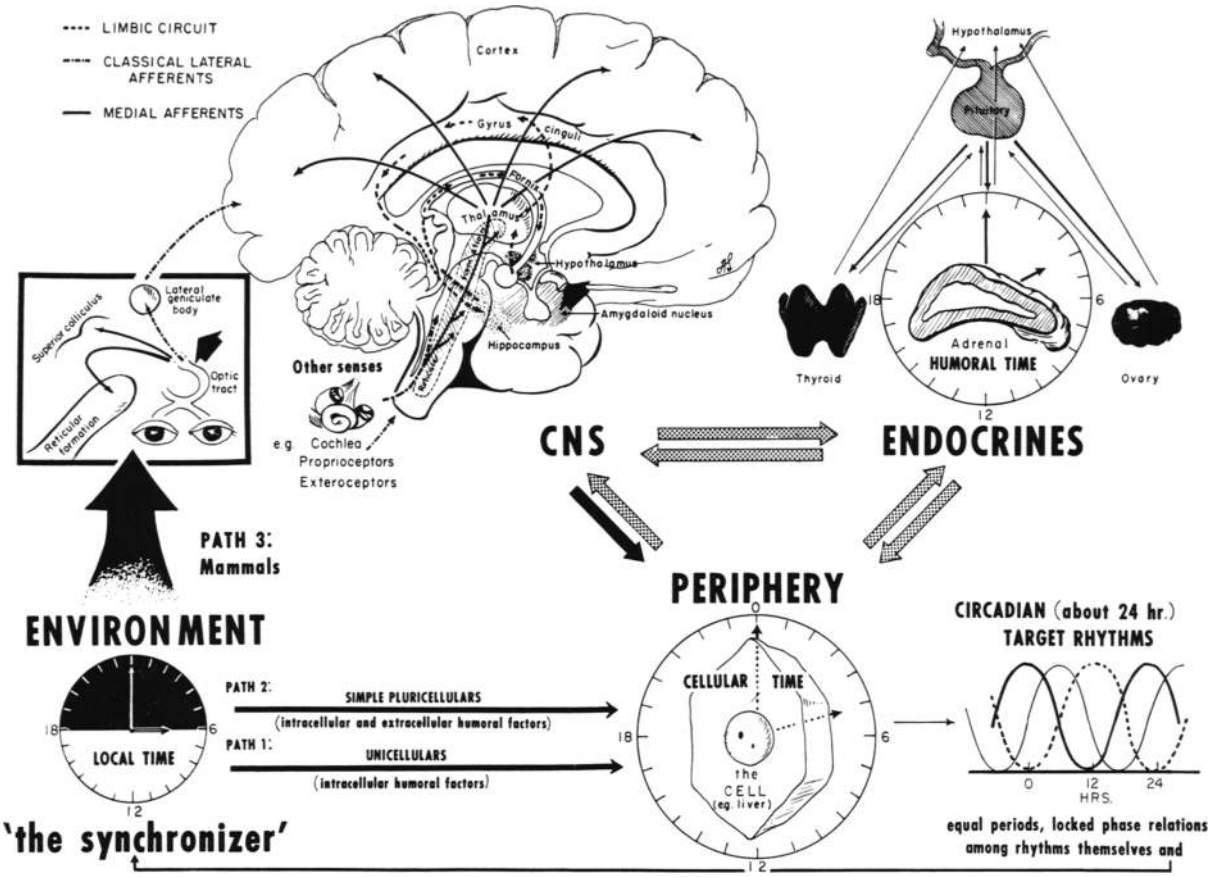


FIGURE 17. Sketch of factors and pathways known or hypothesized to participate in synchronization *among* circadian rhythms themselves, as well as in synchronization *between* rhythm(s) and environmental synchronizer(s).

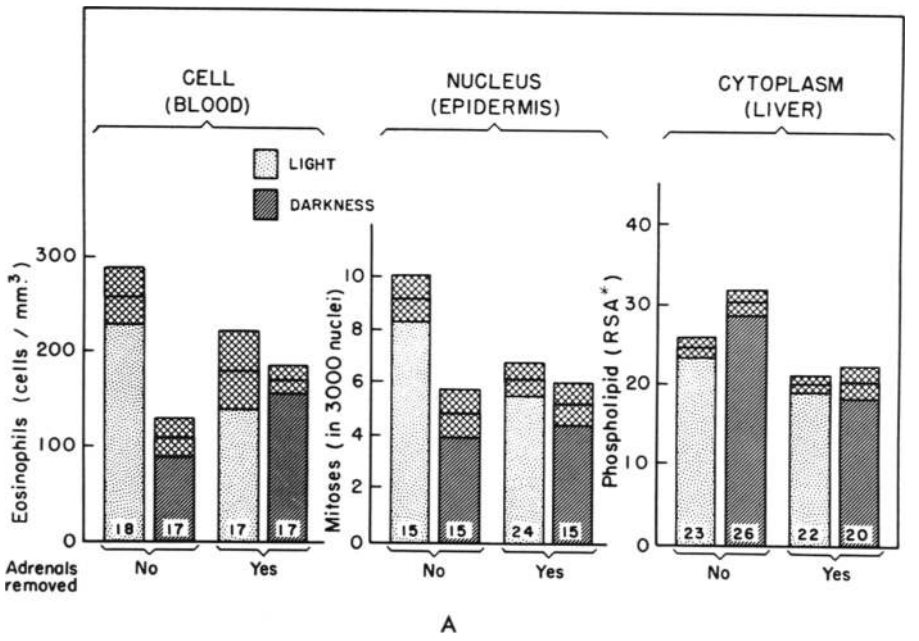


FIGURE 18*a*. Alteration of rhythms of blood eosinophils, epidermal mitoses, and hepatic phospholipid metabolism in verified adrenocortical insufficiency. (*RSA = relative specific activity.)

in mice and man (Fig. 18), the reader is referred to available reviews ([31], cf. also [1, 11, 50]), so that some repetition be avoided.

B. External Factors

Circadian rhythms can be reset by a shift of the synchronizer, as has been amply documented, analyzed, and discussed [1, 11, 36, 37, 51]. Resetting is feasible at various levels of physiologic organization (Fig. 19); some general rules [31] seem to apply in species of varying complexity [3, 28, 52].

C. Phase Shifting

By manipulating the environment, essential steps in a sequence of periodically integrated metabolic events may be shortened or lengthened, rather than eliminated or deranged at random. The exigencies of the temporal coordination within a mammal temporarily override

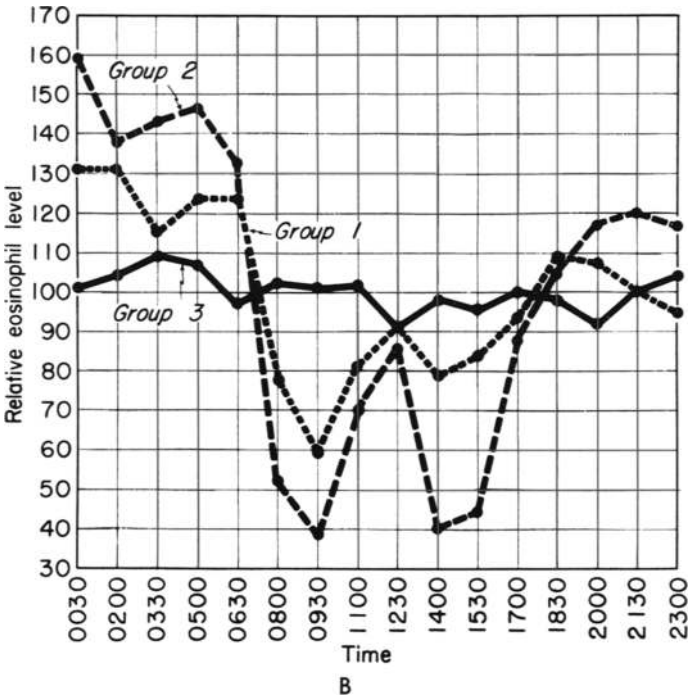


Figure 18b. Obliteration of eosinophil rhythm in man with verified adrenocortical insufficiency. Group 2, unlimited activity (adrenal sufficiency); group 1, limited activity (adrenal sufficiency); group 3, limited activity (adrenal insufficiency) [49].

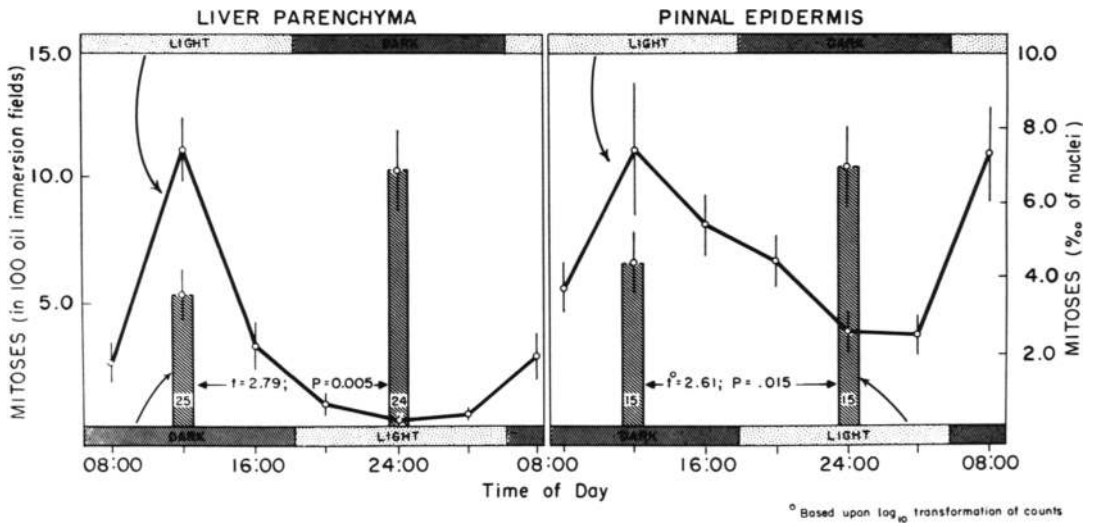


Figure 19A

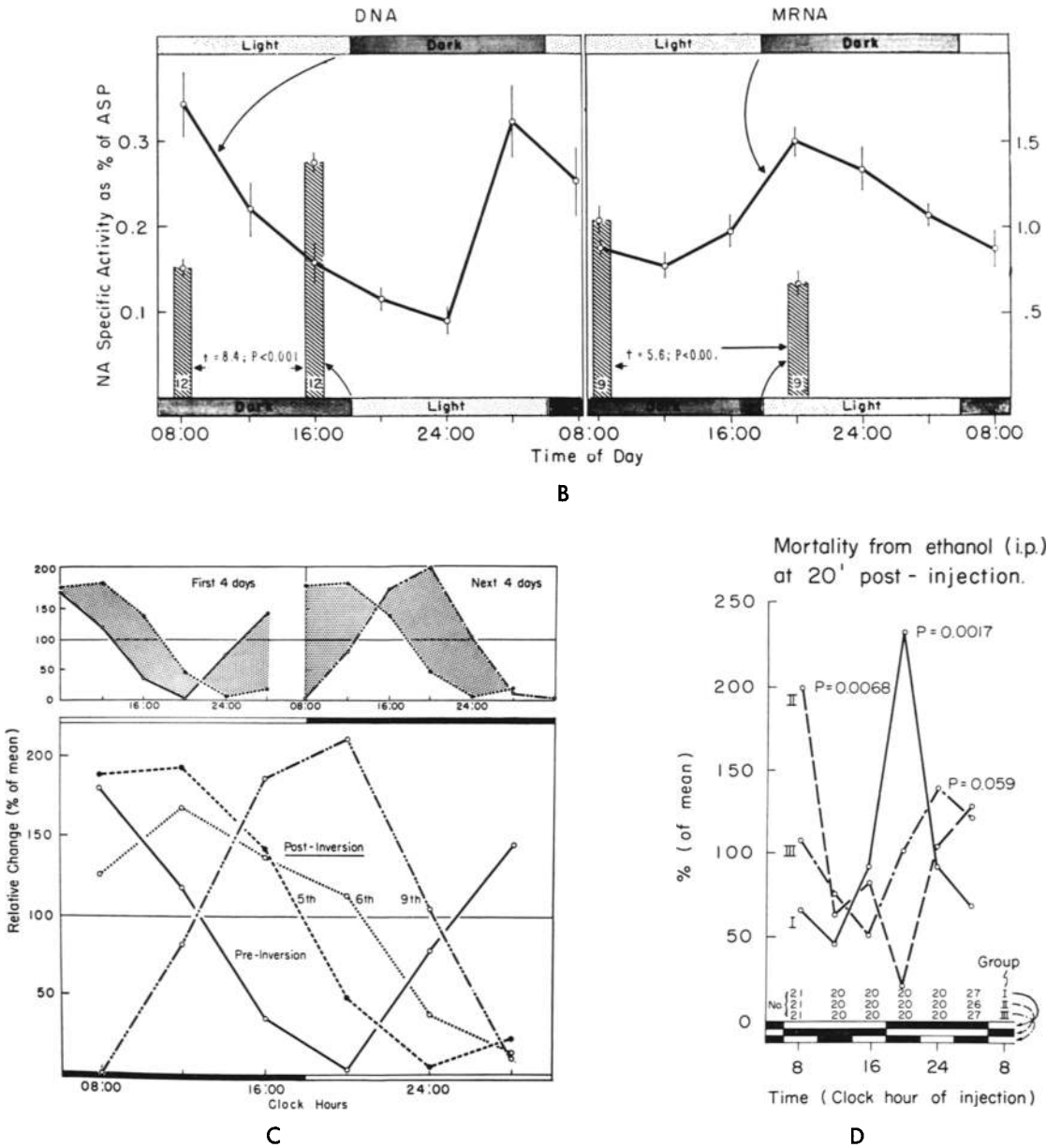


FIGURE 19 *a-d*. Phase shifting of circadian rhythms at different levels of physiologic organization by manipulation of lighting: Mitosis in different tissues (19a), metabolism of different nucleic acids (19b), liver glycogen (19c), susceptibility to ethanol (19d). Mouse. Data in Figure 19d obtained in cooperation with Dr. E. Haus and F. Pass; note differences in external timing between group I in light from 6 A.M. to 6 P.M. and group II, on a reversed lighting schedule. Group III, in turn, was on a schedule providing for the 4-hourly alternation of light and darkness, from which it apparently desynchronized with a free-running period [31, 53].

the claims of new schedules (Fig. 19*c*). Therefore, timing changes only gradually following an abrupt shift of synchronizer. For many higher species and functions the organism's response to drastic temporal changes in the environment does take time: the shift-time or synchronization-time. Only after a lag is the body ultimately able to supply and transport the "old compounds" to the "old places" at the adaptively appropriate "new times" (cf. sect. I C, and [31]); t_e an aspect of adaptation may be changed only as a function of t_i, a parameter of integration. The dependence of external timing upon the internal circadian time structure constitutes a weighty argument against proponents of the unqualified exogenous nature of rhythms.

Following a shift of the dominant synchronizer, internal timing in the mammal undergoes transient changes; shift-times of different functions in the same individuals, studied concomitantly, are not all of equal length. This can apply to the behavior of the same variable in different tissues. Thus, inversion of mitotic

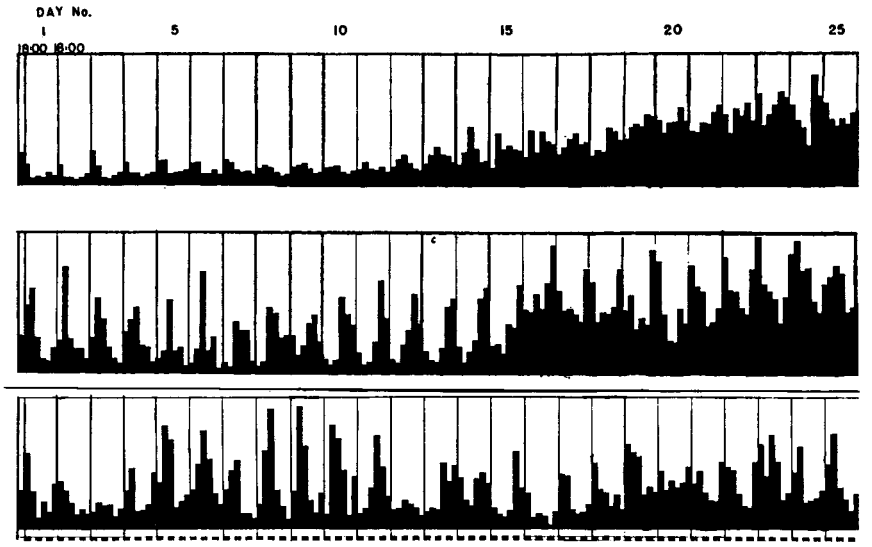


FIGURE 20. Desynchronization of rhythm in gross motor activity of mice from a "4-on/4-off" light-dark schedule. Activity in arbitrary units. Consecutive vertical columns bracket 24 hours. Progressive shift in peak from left to right is apparent, e.g., in the behavior of the mouse at the bottom of the figure. Data obtained in cooperation with Mr. James Anderson.

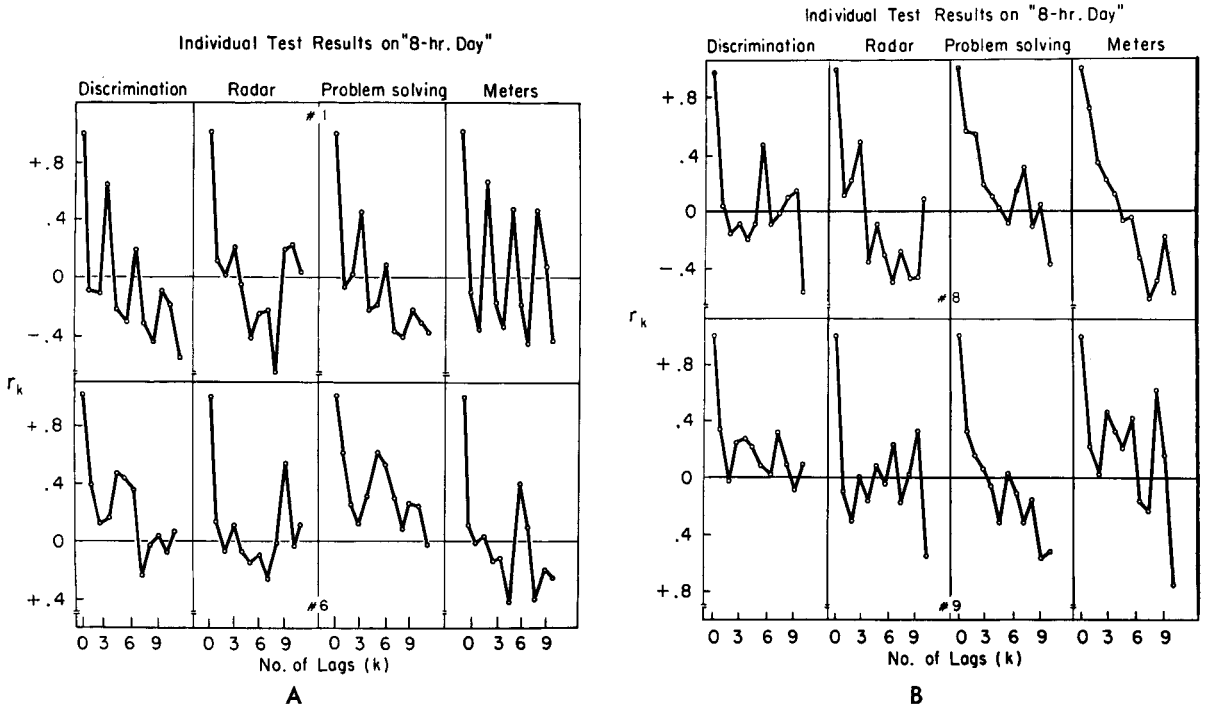


FIGURE 21 *a, b*. Circadian performance rhythms in man adapting to an 8-hour day. One lag = 8 hours. Analysis by Mr. J. Smith, of IBM Research, Minneapolis, Minn. [56]. Circadian periodic components also were found in some heart-rate data of some subjects.

rhythm in liver parenchyma of mice was detected at nine days after lighting reversal, while in the same animals the mitotic rhythm in pinnal epidermis had not yet fully reversed [11]. The shift-time of pinnal mitoses also differs from that of blood eosinophils [11] and liver glycogen [50, 53].

The possible interdependence of periodic physiologic functions can be analyzed by means of phase shifting through a multivariate study of shift-times [31, 53]. Just as one separates different compounds by their rate of travel in paper-chromatography, one may dissociate certain rhythmic functions (in time, rather than space) by their rate of travel following inversion of the dominant synchronizer (e.g., lighting) [31]. Study by phase

shifting of susceptibility rhythms in relation to metabolic and endocrine periodicity might be particularly rewarding.

IV. ALTERED TEMPORAL COORDINATION

A. Limits to Temporal Adaptation

In several forms of life circadian rhythms are no longer amenable to synchronization with an environmental routine, when the cycle length of the latter is lengthened or shortened beyond certain limits [37, 38, 54, 55]. Rhythms in susceptibility of mice to ethanol, as well as in motor activity (Fig. 20), can be desynchronized from the "light-dark" routine, by applying the latter as a "4-on/4-off" regimen (5 days after the change of schedule [Fig. 19*d*] or 9 days thereafter). Free-running periods, seen in Fig. 20 (and, probably, for group III in Fig. 19*d*) indicate such (external) desynchronization of rhythm from routine. The extent to which rhythms also may free-run from each other (internal desynchronization) under such conditions is a critical question.

B. A Problem of Space Medicine

Figures 21*a* and 21*b* depict autocorrelograms on circadian rhythms in performance of men adapting to an 8-hour day [56]. Trained individuals were singly confined by Hauty in a sealed chamber and were committed to alternate 4-hour periods of rest and work provided by a complex operator system. For analysis, mean performance values obtained at 8-hour intervals were used. This wide lag and the short 7-day total period of observation are limitations; only impressions are cited from viewing Fig. 21*a* and 21*b*.

The extent of adaptations apparently differed among subjects. A circadian component was clearly detectable for some functions in subject 1 (e.g., meters) but much less so, if at all, for the same function in some other subjects. Furthermore, extent of adaptations also seemed to differ among the various functions of the same subject (compare meters and discrimination with radar, for subject 1). In subject 1, whose circadian components were more prominent, adaptation to an 8-hour day was more difficult. The extent of adaptation to artificial schedules seems to depend upon the modifiability of our circadian system (t_i).

C. Psychiatric Studies

Three patients, R_1, R_2, and R_3 (in Fig. 22), were subjected to two electroshocks every 12 hours for a week or two [57] until they were regressed. Their rhythms in blood eosinophils and body temperature were only initially and feebly "driven" by the 12-hourly shock schedule; all in all, both rhythms showed a predominating circadian period rather than a 12-hourly period. The shock schedule, although it was drastic in stimulus intensity, did not erase the organism's circadian behavior. These studies constitute further

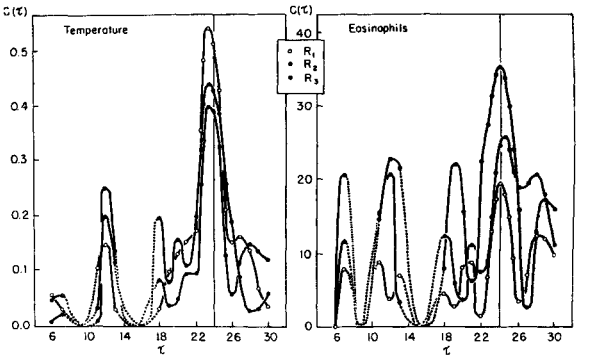


FIGURE 22. Periodograms of rectal temperature and eosinophil variation of three subjects (R_1, R_2, and R_3) receiving (regressive) electric shocks at 12-hour intervals. See [57] and text.

weighty evidence against the rather common belief that circadian rhythms in human beings are all "impressed from without and persisting from within."

It is of further interest (Fig. 22) that in each of the three patients the τ for rectal temperature was shorter than that for blood eosinophils. The significance of the difference in period could not be ascertained; but free-running of one rhythm from another remains an interesting possibility for temporal disturbance.

Interpretation from a physiologic viewpoint of Fig. 22 and 23 is limited by the emotional disorder of these patients who may have had free-running components before the shock schedule was started (available data do not suffice for exploring this possibility). What seems established as of considerable physiologic interest is the persistence of a dominating circadian period, during the administration of a drastic 12-hr. shock schedule and thereafter, during and post-regression (Fig. 23).

D. Convulsive Disorder

Figure 24 shows rectal temperature data on a patient with convulsive disorder. On frequent occasions during a period of several hundred days of observation, the 5 A.M. temperature of this patient was the highest of the day, rather than the lowest. A period following several grand mal seizures (Fig. 24) seems compatible with desynchronization of his temperature rhythm from a 24-hour routine. This aspect of the graphed curve, however, is not characteristic of the rest of the long time-series available on this subject, which reveals only consistent irregularities.

Apart from problems of the temperature rhythm disturbance in some patients with convulsive disorder, there are, of course, the well known "periodicities" in the incidence of the seizures themselves (Figs. 25*a* and 25*b*) [49, 50, 58, 59]. An experimental animal model for periodicity analysis in this field also has become available (Fig. 25*c*) [22, 23].

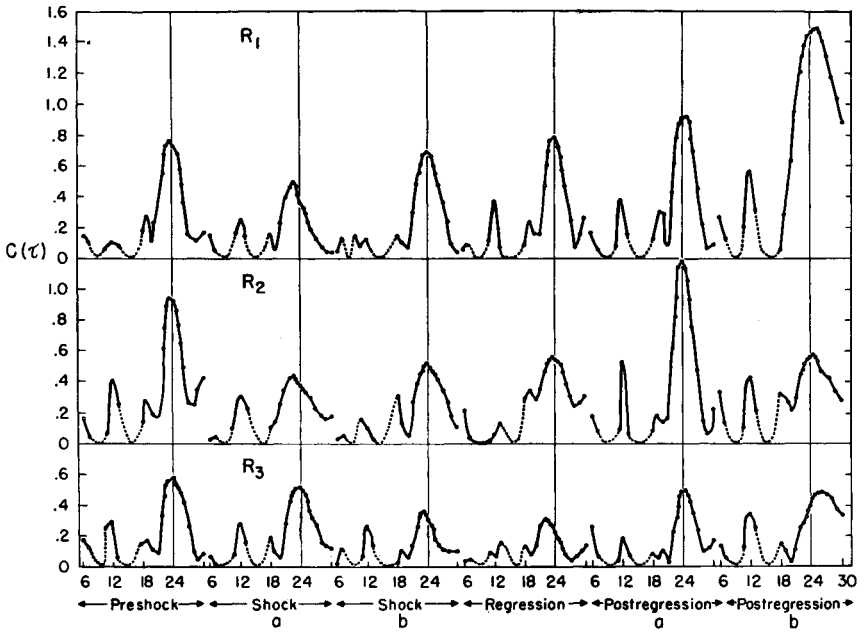


FIGURE 23. Periodograms of rectal temperature variation in R_1, R_2, and R_3; the two halves of shock period are separately analyzed; additional analyses of temperature during periods preceding and following shock treatment also are shown. See text and [57].

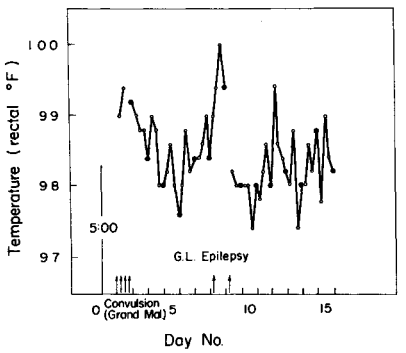


FIGURE 24. Rectal temperatures obtained at 6-hour intervals during interval between two bursts of grand mal seizures (days 4 to 7). 5 A.M. temperatures are black circles. Rectal temperature peaks at ~ 11 A.M. on day 4, on days 5 and 6 at 5 P.M., and at 11 P.M. on day 7 (as well as day 8). Impressions from inspection alone may be misleading, unless restricted to the recognition of frank temperature dysrhythmia.

E. Temperature Dysrhythmia—without Fever

Drastically irregular temperature curves may be a sign of illness, if they are obtained from subjects living on a standardized routine. Temperature rhythm disturbance may occur in the absence of fever in a variety of chronic diseases, ranging from cancer (Figs. 12*a* and *b*), to epilepsy (Fig. 24) and defective mentality (Fig. 26c), but it must not accompany these conditions (e.g., Fig. 12*c* or 26*a*). When present, this disturbance as it is interpreted by inspection alone may be unspecific; yet its diagnostic value may be comparable to that of fever [1]. Fever also is unspecific, yet it is a mainstay of many diagnoses.

For a student of rhythms (rather than clinician) Fig. 12*a* and *b*, 24, and 26*c* are Kind C data (cf. Sect. II B). Rhythms are blurred in these measurements made under circumstances intended to be direct routine-synchronized periodicity analysis.

The same conditions of observation were compatible, however, with the clear demonstration of a circadian temperature rhythm in many other subjects, used to getting up at 6 A.M. and to retiring at 9:30 P.M. Figures 12*c*, 13, and 26*a* and *b* are merely illustrative examples of several hundred cases—each studied at 6-hour intervals for at least 100 days at the Cambridge, Minnesota, State School and Hospital.

This latter population of curves was used as reference standard for the grossly abnormal but afebrile temperatures showing nocturnal peaks in alternation with the usual diurnal maxima (repeatedly over many months). Conceivably, such curves reflect the interaction of two or more distinct components of variation, one of these being the institutional routine of meals and activity, which was rigidly fixed, while another component, perhaps, was free-running (cf. Sect. II F).

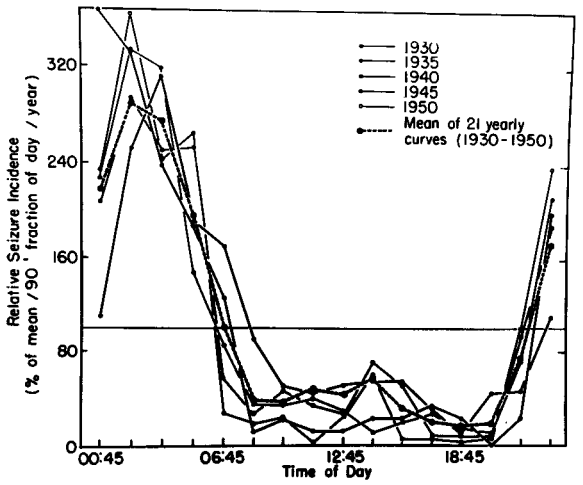
F. Problems of Circadian Desynchronization in Human Beings

Just as the blinding of an experimental animal, intrinsic processes in human disease may desynchronize the body's rhythms from environmental routines. Demonstration of such desynchronization in patients is more complicated than in blinded mice: the latter can be allowed to follow their "own" schedules of running and feeding; the former usually must be studied

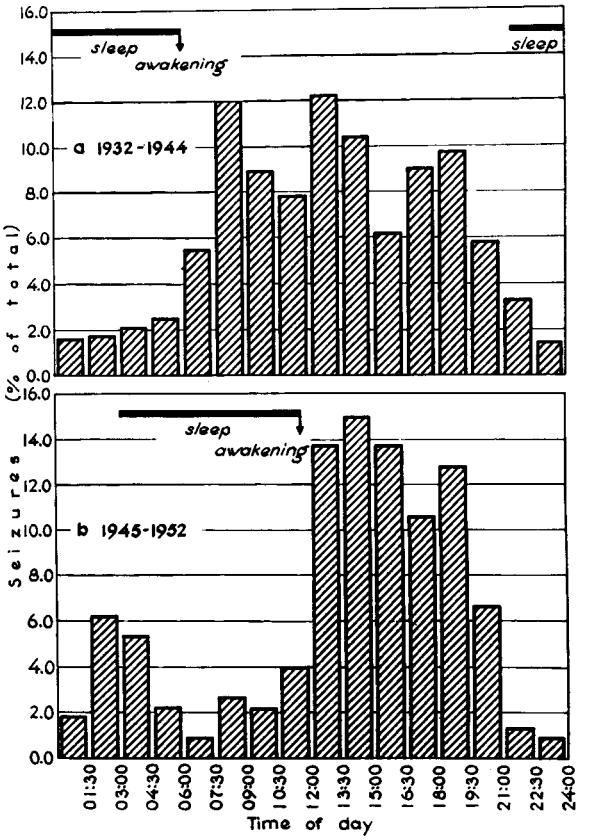
while living on a roughly 24-hour routine of activities and meals.

This introduces complications to periodicity analysis of physiologic time series, rectal temperature, or other. In such cases, power spectra [32] are useful, in this writer's limited recent experience.

Periodicity analysis on a given individual depends, of course, upon instrumentation for obtaining with minimal disturbance, at appropriate intervals, precise measurements over long periods [40]. At least equally important, however, are appropriate computational procedures for objective estimates of the periodicities underlying the data. Such methods now are programmed for electronic computers; their uses gain in significance whenever the possibility has to be considered that several component frequencies may underlie a numerical-valued observation. These considerations apply to medical research, but they seem to be equally germane to several other problems of quantitative biology discussed at this symposium.



A



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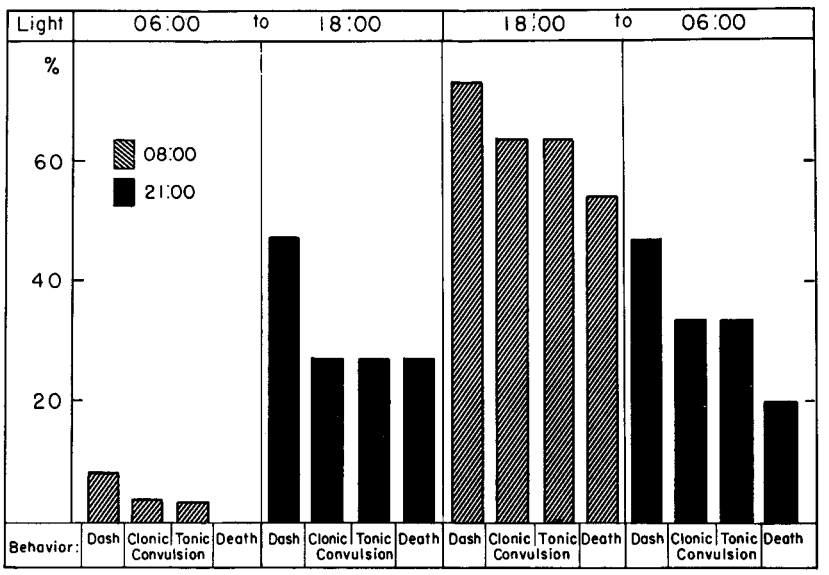
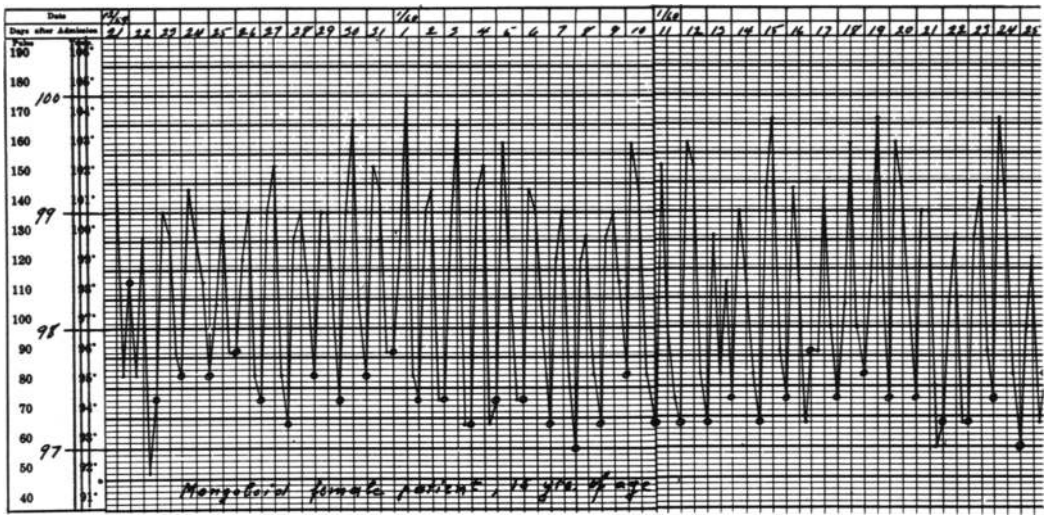
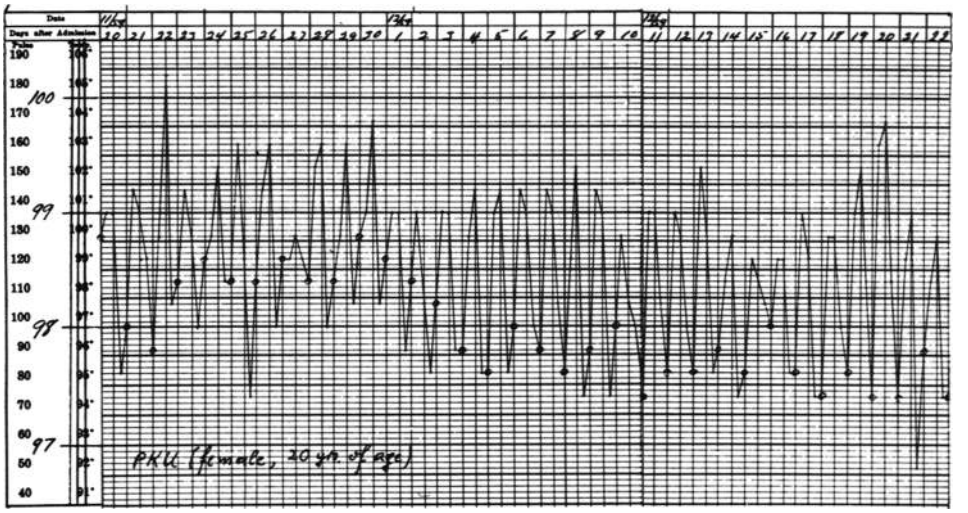


C

FIGURE 25 *a-c*. Problems in timing of convulsive seizures: nocturnal (Fig. 25*a*) or diurnal (Fig. 25*b*, top) clinical seizure distribution. Note also change in seizure distribution following a change in routine (Fig. 25*b*, bottom) and availability of an experimental animal model for one type of convulsive periodicity (Fig. 25*c*).



A



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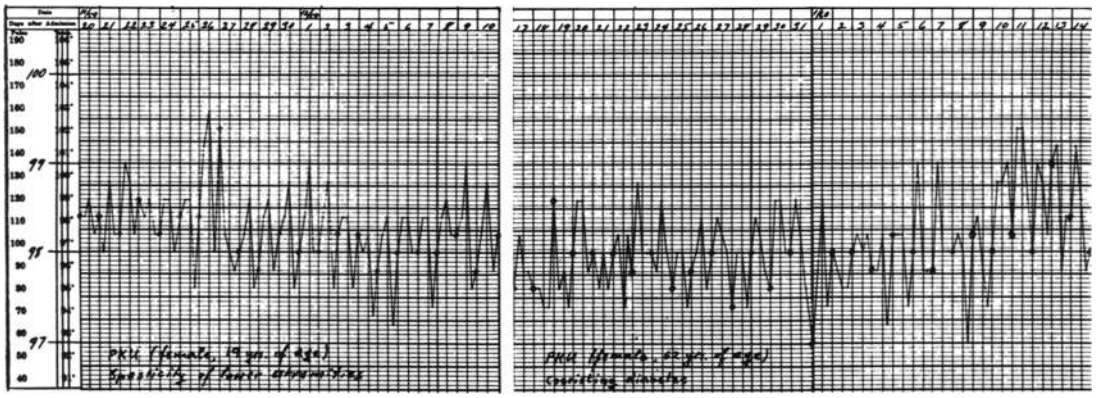

C

FIGURE 26 *a-c*. Rectal temperatures at 6-hour intervals in patients living on an institutional routine providing for diurnal activity and meals and for nocturnal rest. 5 A.M. temperatures are circled. Synchronization of temperature rhythm with routine: in a mongoloid patient (Fig. 26*a*) and in a patient with phenylketonuria (PKU; Fig. 26*b*—beats?). Compare the irregularities of two other PKU-patients, one with spasticity of lower extremities (Fig. 26*c*, left), the other with coexisting diabetes (Fig. 26*c*, right) (See Sect. IV E).

Altered phase relations among the circadian rhythms themselves also await study in health and disease. Several possible anatomical sites for such internal circadian desynchronization could be suggested from Fig. 17. Problems of timing (t_e and t_i) pertain to conditions ranging from central nervous system disease to alteration of periodic processes in growth and repair as in cancer [60, 61].

G. Comment

Temporal organization of physiologic function usually involves a circadian time structure, with great although not unlimited plasticity. The old CONSENSUS PARTIUM is actually a PARTIUM CONSENSUS IN TEMPORE.

The significance of the body's circadian system may be underlined by discussing our ability to withstand injury in the light of the "hours of changing resistance." It can be experimentally demonstrated that a fixed amount of a given agent injected into the same body site is likely to result either in death or in survival, as a function of the changing phase relations among an organism's circadian rhythms (Figs. 3 and 9).

By synchronization with environmental routines integrative circadian systems gain adaptive value. Temporal coordination in physiology has both integrative and adaptive facets. Periodicity analysis provides for resolution of adaptation as a function of integration and vice versa. From this point of view necessarily multivariate studies of the internal timing of rhythms (defined in Sect. I B) seem particularly promising.

Acknowledgements

For permission to reproduce figures or data thanks are due to Dr. E. Charles, Birmingham, England; Dr. G. Hauty, Federal Aviation Agency, Oklahoma; Dr. M. Lobban, Cambridge, England; Dr. Robert Speirs, State University of New York, N. Y.; and particularly to Dr. E. Haus and Mr. F. Pass from the writer's laboratory. Work discussed was supported by the Elsa U. Pardee Foundation, the American Cancer Society, the U. S. P. H. S., and the Department of Public Welfare, State of Minnesota.

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DISCUSSION

FOLK: I would like to call particular attention to Dr. Halberg's brief mention of heart-rate measurements. There has been little mention in this symposium of the heart-rate rhythm, and perhaps he may want to comment upon his data in more detail. It should be noted that in several places the literature includes the state-

ment, "the heart-rate rhythm is entirely under environmental control." From our laboratory we have published data, and have obtained much recent data on heart rates; they permit us to question with confidence this quoted statement. As in other mammalian physiological rhythms, there is probably a day-night setting of cardiovascular activity.

HALBERG: The extreme "sensitivity" of a given function to stimuli of various sorts certainly does not imply that its circadian rhythm per se is exogenous in origin. As Dr. Folk's studies suggest and in keeping also with the work of Professor Menzel in Hamburg, Germany, this generalization can be extended to heart rate, among many other functions.

With reference to the detection of circadian components in heart-rate data from men adapting to an 8-hr. day, the importance of this finding made by Hauty deserves emphasis. If heart-rate variations were purely exogenous, they should lose their circadian components in people living on an 8-hr. day. Autocorrelograms revealed that this was not the case during the days immediately following a change in schedule. Moreover, these autocorrelograms could be based on observations available at 1-hour intervals, by contrast to the 8-hr. lag of the performance figures on the same subjects. The computations were made independently by Mr. J. Smith of IBM research in Minneapolis, Minnesota, and by Mr. Bell of Brooks Air Force Base, San Antonio, Texas, and were interpreted, in addition, in close agreement by Dr. Hauty and some of us at the University of Minnesota, including Professors I. R. Savage, E. Johnson, and B. Brown.

SHAPIRO: The enormous amount of data which Dr. Halberg has presented is extremely convincing especially because of the organized way in which he has presented and evaluated it. I should like to call attention in connection with the data on correlation of EEG and adrenal function in diurnal rhythm to the additional very marked 90 minute EEG cycles which Kleitman has found and others have confirmed during human sleep. Is there any evidence of this rhythm in the data on adrenal function?

The data on varying susceptibility to a "constant" dose of ethyl alcohol on a diurnal cycle again emphasize the spurious nature of environmental constancy in relation to the behavior of living organisms. Perhaps this may account for the impression that clinicians have of the apparent inconstant and often diurnally fluctuating response of patients to medication administered in timed release capsules which in vivo present medication to the patient at a constant rate. If this is so it is probably better in many instances to administer active medication intermittently and with due consideration of diurnal rhythms in susceptibility to minimize side effects and maximize therapeutic effects of active drugs.

HALBERG: About 10 years ago, eosinophil counts and EEG's were obtained at 90-minute intervals for 24 hours on patients with convulsive disorder. Sleep was only occasionally uninterrupted, but interruptions were slight. This work was done by Dr. Rudolf Engel, now at the University of Oregon (Halberg, Engel, Halberg and Gully: Diurnal variation in amount of electroencephalographic paroxysmal discharge and diurnal eosinophil rhythm of epileptics on days with clinical seizure, Fed. Proc., *11:* 63, 1952; cf. also Engel *et al.*, J. Lancet, *72:* 242–248, 1952). Several years later, we determined plasma 17-hydroxycorticosteroids (by the method of Mason) at 90-minute intervals for 24-hour periods on the same patients. Available data thus describe a group of patients rather than normals, and they were collected for study of internal timing along the 24-hour scale of changes in the EEG (obtained just prior to blood withdrawal) and in adrenal cortical hormone level. The 90-minute intervals between successive samples make it difficult to say anything definitive about 90-minute cycles in adrenal function, about which Dr. Shapiro has asked for information. It can be noted, however, that the changes in plasma 17-hydroxysteroid levels of successive samples withdrawn with only slight interruption of sleep were definitely larger than can be accounted for by error of the method. Detection of a periodicity with $\tau = 90'$, however, is further complicated by the fact that there is usually an increasing trend in plasma 17-hydroxysteroid levels during sleep; the reference line for 90′ cycles thus would not be a straight base line but a slope, particularly during the second half or so of the habitual sleep period.

Endogenous eosinopenia and the underlying rise in plasma 17-hydroxycorticosteroids precede (rather than follow) the usual time of awakening—a finding recently extended to normal subjects whose blood samples were withdrawn through indwelling venous catheters and without interruption of sleep, as could be verified by concomitant EEG's. The latter study by Dr. G. Frank, Dr. R. Harner, Dr. J. Mathews, and the writer, as well as the earlier work by Dr. R. Engel (see also A.M.A. Arch. Neurol. & Psych., *69:* 462, 1953), further supports the suggestion that the human circadian adrenal cycle is an endocrine entity, rather than a mere, direct "response" to exogenous stimulation.

Dr. Shapiro's second comment focuses critically upon drug-therapy. In order to adapt medications to the body's periodicity by slow increases and decreases of dosage, Menzel had constructed a special device, by 1953 (Acta Med. Scand. Suppl. 307: 115–116). For certain defined clinical conditions his pioneering approach seems most promising; its ultimate success will depend heavily upon the still unavailable experimental demonstration of significant advantage derived from using such a machine in lieu of conventional procedures. For testing the virtues of timed treatment, the methods of

indirect periodicity analysis have now been recognized as a feasible experimental procedure. These latter methods have already rigorously demonstrated that the timing of drug administration can tip the scale toward death or survival (cf. discussion of susceptibility rhythms). Yet a note of caution seems warranted.

Study of circadian changes in toxic-therapeutic ratios seems indicated before findings on susceptibility rhythms are applied in the clinic. Available data seem to suffice as yet only for suggesting to define a median lethal dose in terms of stage of susceptibility rhythm, determined under conditions of routine-synchronized periodicity analysis. Thus, one may refer to a maximal LD_{50}, at peak time of susceptibility and to a minimal LD_{50}, at trough time of susceptibility. Definition of an LD_{50} test in terms of its timing may then be added to other conventional references such as those as to species, sex, and age of test animal, among others.

Dr. Shapiro's comments on problems of timing medications also bear upon the practice of the "b.i.d," "t.i.d," or "q.i.d." The question whether such prescriptions are optimal deserves study, particularly for drugs known to exert their desired and undesired effects upon different tissues, e.g., upon lymphoid tissue and bone marrow, respectively.

Incidentally, we have circadian charts of white blood cell rhythms in human blood showing that lymphocytes and neutrophils are apparently synchronized with a phase difference. This could reflect production differences in phase as well as different timing in release of these cells.

At any rate, whenever a phase difference can be demonstrated between the circadian peaks of the actually estimated desired and undesired effects of a drug, problems of timing medications will gain critical importance.

There is just one other problem to solve, however, lest our enthusiasm desynchronize from fact. Even when the need for the timing of a given medication will have been rigorously established from animal experiments on rhythms in toxic-therapeutic ratios, the timing of a drug as to a given clock hour will be meaningful only when the patient's circadian system is synchronized or amenable to synchronization with a routine or drug.

In desynchronized patients, in turn, the question may have to be explored whether reference (of drug administration) to an easily and frequently measured rhythm such as that in rectal temperature, may be helpful—since timing as to clock hour almost certainly will be meaningless.