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Time and Life: Applications of Modern Chronobiology

Lawrence E. Scheving
University of Arkansas for Medical Sciences

John E. Pauly
University of Arkansas for Medical Sciences

TIME AND LIFE: APPLICATIONS OF MODERN CHRONOBIOLOGY

LAWRENCE E. SCHEVING[1] and JOHN E. PAULY[1,2]
Department of Anatomy[1] and Department
of Physiology and Biophysics[2]
University of Arkansas for Medical Sciences
Little Rock, Arkansas 72205

ABSTRACT

Chronobiology is that branch of science which objectively quantifies and explores mechanisms of biological time structure. It is an integrating discipline that impacts on all forms of life.

When physiological functions are plotted along a time scale, they appear as regularly repetitive wave forms with means, amplitudes, phasing and periods. In nature these rhythms are found to have many frequencies, from a fraction of a second (ultradian) to a year or more (infradian or circannual); and those with periods of about one day (circadian) have been explored extensively.

Examples of several circadian rhythms are given for experimental animals and man. Evidence is presented to show that it is particularly important to consider biological rhythmicity when interpreting experimental results or attempting to extrapolate from one species to another. An organism is indeed a different biochemical and morphological entity at different times, and it may be expected to react differently to a stimulus at different circadian phases. By taking advantage of natural rhythms in the susceptability to drugs, it is possible to optimize chemotherapy and radiotherapy for cancer and other diseases.

THE RHYTHMIC NATURE OF LIFE

Chronobiology is that branch of science that explores mechanisms of biological time structure (Halberg and Katinas, 1973). Although it is considered a comparatively young science, the writers of ancient times, including the poets, were fascinated with rhythmic phenomena, particularly as they pertained to plants (Scheving, 1976); and many of the important early scientific investigations of rhythmic behavior were performed by botanists. In 1963 E. Bunning summarized the work that had been accomplished, including his own important contributions, and Cumming and Wagner (1968) did a more recent review on plants.

During the past 30 years a great number of publications on rhythms in lower animals and humans have appeared. Rhythms with many frequencies at all levels of biological organization have been demonstrated. Because of the regularity of these rhythms, some refer to them as biological or physiological clocks. Oscillation has been firmly established as a fundamental property of life (Scheving, 1976). Ehert, (1979) considers chronobiology the newest of the four integrating disciplines of biology, ranking in importance with genetics (developmental biology and evolution are the other two).

At the same time that chronobiology was developing at an almost exponential rate, the concept of "homeostasis" continued to be taught in biology classes. Homeostasis, introduced in 1878 by Claude Bernard and championed by Walter Cannon, claims that an organism has capabilities of self-regulation which maintain body fluids and hormones in a rather narrow range by negative feedback, preventing sensitive cells from damage that might be caused by strong variations, including those in the environment. This "steady-state" concept, as taught up to the present time, has governed the thinking of generations of biologists, despite the fact that 40 years ago it already was known that neither body fluids, hormones, organs nor cells exhibit a constant composition.

The range of frequencies that has been found in living systems extends from less than a second to a year or more. It is noteworthy that many correspond to frequencies found in the physical environment such as the approximate 24 hr light-dark cycle brought about by the rotation of the earth on its axis. The rhythms themselves, however, are endogenous, innate and coded in the genome. They will freerun in the absence of a synchronizing force (Scheving, 1976). There is strong evidence that many rhythms are adaptive and serve to adjust organisms in advance to the periodic changes in the environment (Scheving, 1976).

This paper will concentrate on circadian rhythms which have frequencies that correspond to the 24-hr day (circa, about; dies, day). The adjective "diurnal" is sometimes used synonymously with circadian, but it is more appropriate to use this term to describe animals that are active during the day as opposed to nocturnal animals that are active by night. Circadian rhythms are ubiquitous in eukaryotic unicellular and multicellular organisms. Recent data on growth rate of bacteria suggested that circadian as well as rhythms with higher frequencies (ultradian) also may characterize the prokaryotic cell (Sturtevant, 1973); it should be kept in mind, however, that controversy presently exists as to whether the prokaryotic organism is characterized by circadian variation.

Most fluctuations in physiological and biochemical variables are not apparent in the same sense that the pulse, respiratory cycle or menstrual rhythm are; they become overt only when properly measured at frequent intervals along a 24 hr time-scale. Because of their somewhat "invisible" nature, there has been a tendency on the part of some investigators to slight or ignore them in experimental design. In spite of all that is known, they simply have not been accorded the attention they deserve. This undoubtedly is due in large part to the fact that the science is young (Scheving, 1974).

Illustrative Examples: The rhythm in serum steroids was one of the first to be documented and has been studied extensively (Pincus, 1943). This rhythm, illustrated in Fig. 1 for both rat and man, will be used to describe some of the basic properties of rhythms and especially the terminology commonly employed.

In diurnally active man, the adrenal cortex secretes increased amounts of cortisol before awakening, and peak titers are reached shortly after arising. In the nocturnally active rat, the peak of serum cortisterone (predominate steroid of the rodent) occurs shortly before the period of activity begins (Scheving, et al., 1974). The four-fold or greater change in the level of the steroid seen along the 24 hr time-scale (amplitude) clearly shows that these variations are not minor fluctuations around the 24 hr mean, and they cannot be ignored in experimental design (Scheving, 1974). It should be realized that fluctuations with higher than circadian frequencies (ultradian; Weitzman and Hellman, 1974) and lower frequencies (infradian or circannual; Haus and Halbert, 1970) also characterize the rhythm in serum steroid as well as in many other variables. Notice in Fig. 1 that the rhythm in steroids of the nocturnally active rodent is 180° out of phase with the one for diurnally active man. It should be stressed that such a dramatic difference is not always the case, because some of

the other rhythms are not so far out of phase between the two species. Figure 2 illustrates just such a situation where the rhythmic variations in serum prolactin of the rodent and man certainly are not 180° out of phase (Scheving and Dunn, 1974). These observations are important, because they demonstrate that one must be careful when extrapolating from data obtained on the rodent to man. Figure 3 demonstrates the rhythmic variation in the mitotic index of human skin; the maximum cell division in skin takes place at night. Figure 4 depicts the rhythm in DNA synthesis in the bone marrow of the rodent (Scheving and Pauly, 1973). A similar rhythm has been described for the mitotic index in human bone marrow (Killman et al., 1962) (Fig. 5). The rhythmic variations in DNA synthesis or the mitotic index in bone marrow or gut become important considerations when attempting to manage the treatment of a cancer such as leukemia by chronotherapy or radiotherapy.

Figure 6 shows that the histological pattern of glycogen activity in the liver of the rat is dependent on the temporal organization of the organism. In short, even morphology reflects circadian biochemical or physiological changes; however few morphologists consider structural changes with reference to time when interpreting their results (Scheving et al., 1974). Illustrated in Fig. 7 is the reproducibility of rhythms over a 72 hr span in a group of young men. Variables

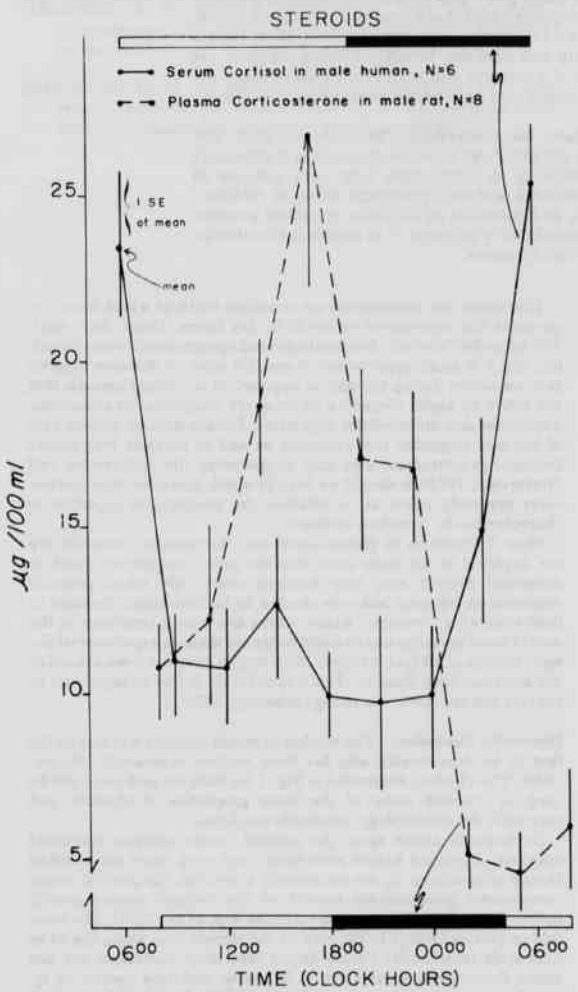

Figure 1. Prominent circadian fluctuation of the predominant serum steroids of rat and man. The rats were standardized to a light-dark cycle (14 hours of light alternating with 10 hours of darkness) and fed *ad libitum* for two weeks prior to the study. For man, the meal times were 0700, 1245 and 1645 hours; rest or sleep time was 2100-0600. The subjects were awakened, however, for sampling at 2400 and 0300. (Scheving, Mayersbach and Pauly, 1974)

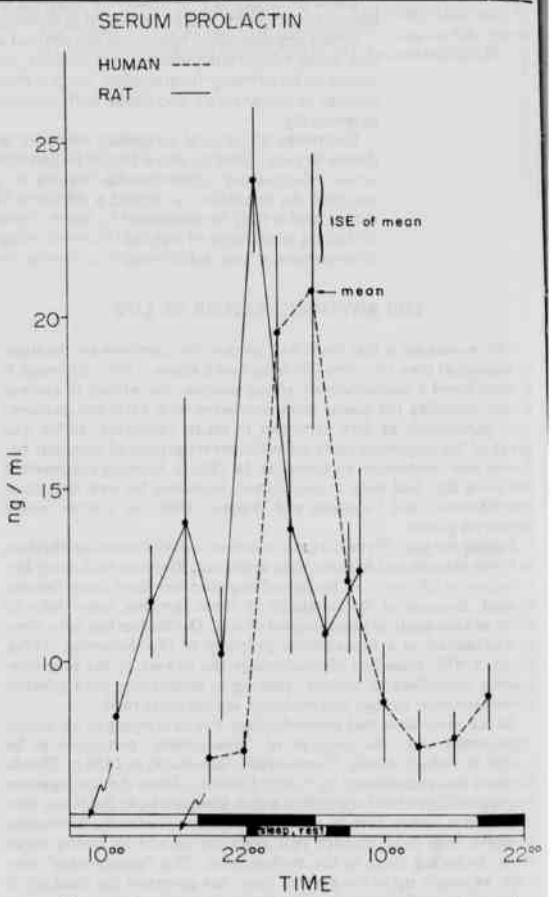

Figure 2. The two chronograms depict the serum prolactin rhythm for man and the rat. Note that the rhythms in the two species are not 180° out of phase as was the case for the serum steroid rhythms illustrated in Fig. 1. Such data caution against extrapolating from data obtained from the nocturnally active rodent to the diurnally active man without knowledge of the rhythmic variation of the variables under consideration. For man, meal times were 0700, 1330 and 1630 hours; rest or sleep time was 2215 to 0700. The subjects were awakened, however, for sampling at 0100 and 0400. N = 13. Rats were fed *ad libitum* and were standardized to 14 hours of light alternating with 10 hours of darkness. N = 8/time point. (Scheving and Dunn, 1974)

measured ranged from oral temperature to the ability to perform mental and physical tasks (Kanabrocki et al., 1973; Scheving et al., 1977) Note that the crest of the rhythm in performance corresponds to the time of poorest performance. Mood and vigor ratings, depicted as chronograms, were determined on a scale of 1-7 by the subjects themselves. It has been shown repeatedly that with minimal training, individuals can accurately monitor their own circadian rhythms for many diverse behavioral and physiological variables, including blood pressure. Halberg has advanced the concept of self-measurement or autorhythmometry (Halberg et al., 1972; Halberg, 1973). Such a concept has already been applied satisfactorily in the monitoring of health and disease (for example, in hypertension). Autorhythmometry promises to have even greater application, especially if it is taught early in life, preferably no later than high school (Halberg et al., 1972).

Figure 8 shows the same data as Fig. 7, but they are depicted after having first been analyzed by an inferential statistical method commonly referred to as the "cosinor". The cosinor technique is one of several objective methods by which time-series data can be analyzed. Essentially the data were fitted to a 24 hr cosine curve by the method of least squares, and the rhythmic parameters were determined; this is readily done by a computer. The rhythmic parameters include "mesor" (overall 24 hr mean if the data are equidistant), amplitude, and acrophase (Halberg et al., 1972). The computer-determined acrophase (point estimate, illustrated by a dot) represents the time when the crest occurs in relation to the rest-activity cycle. The confidence limits also are shown (horizontal bars). Again, it is important to point out that the acrophase for performance corresponds with the poorest performance. The percentage range of change, shown in

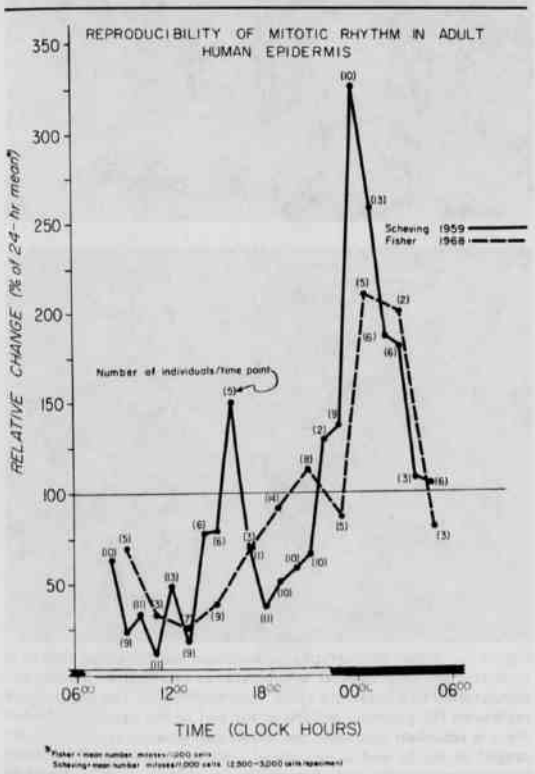

Figure 3. The rhythm in the mitotic index in the adult human epidermis. A majority of the cells divided at a predictable phase of the circadian system. Remarkable reproducibility has been demonstrated in studies done many miles (London and Chicago) and many years apart. (Scheving, Mayersbach and Pauly, 1974)

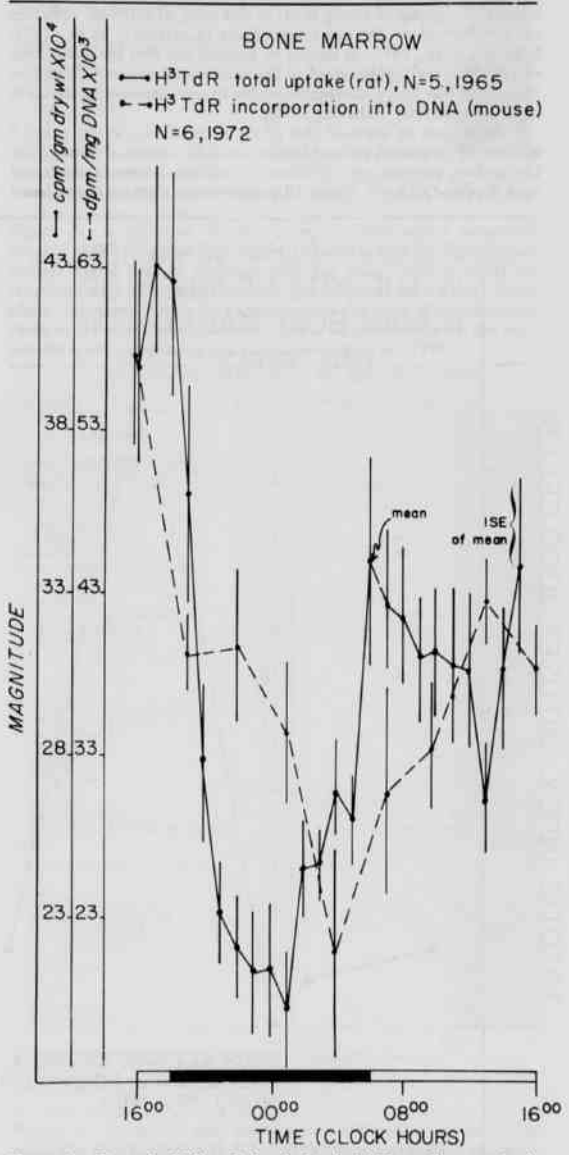

Figure 4. Reproducibility of the rhythm in ^{3}H-thymidine uptake in the bone marrow of rodents. The isotope rhythms were determined by injecting subgroups of animals with ^{3}H-thymidine during a single 24-hour period at the intervals shown on the chronograms. The animals were sacrificed one hour after injection, and the tissues were collected and analyzed by scintillation-counting techniques. (Scheving, 1976)

column 2, is the average difference between the lowest and highest values over the three-day period; temperature, however, is an exception, because the actual change is shown in degrees rather than percentage. Figure 9 is another acrophase map compiled from more extensive data obtained from a different study, two years earlier, on a comparable group of young men. In this case, 41 different variables were measured on the same individuals (Kanabrocki et al., 1973; Scheving et al., 1977). It should be pointed out that the individuals essentially were synchronized to the same social routines. It can be concluded that every variable amenable to measurement oscillates in a rhythmic manner (Scheving, 1976).

It should not be assumed that all variations shown in Fig. 8 and 9 are merely responses to food intake, because certain of these (catecholamines, steroids, etc.) continue to oscillate in lower animals and man deprived of food. Figure 10 compares the rhythms of the heart rate and norepinephrine in subjects fed regular, three-meals-per-day diet, with those rhythms in subjects that fasted for 12 hrs prior to and throughout the sampling. Of course some variables, such as glucose, are strongly influenced by diet (Scheving and Pauly, 1977). Under certain circumstances food-intake can override the strong synchronizing force of the light-dark cycle in animals (Pauly et al., 1977). This can be done by restricting food intake to precise periods for the day, for example to 4-hr spans for rodents or to one meal per day for human beings. Several rhythmic variables can be synchronized in this way, but others show evidence of being synchronized to both the restricted feeding schedules and the light-dark cycle, the net result being a rhythmic waveform demonstrating an interaction between the two potential synchronizing forces (Philippens et al., 1977). Interestingly, other variables remain strongly synchronized to the light-dark cycle in spite of food manipulation (Scheving et al., 1974b).

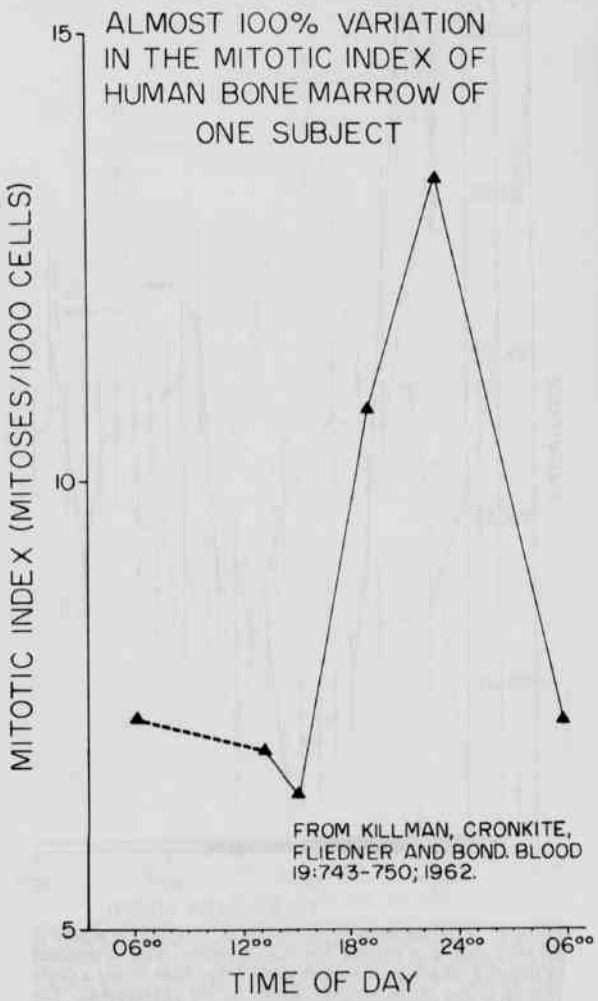

Figure 5. Circadian variation in the mitotic index of bone-marrow cells in a single subject.

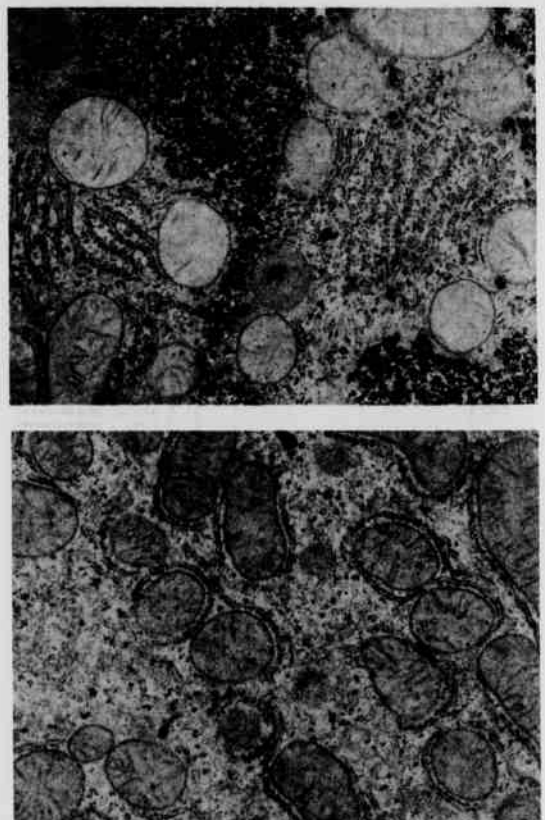

Figure 6. These photographs demonstrate the circadian change in ultrastructure of periportal hepatocytes in rats (fed *ad libitum* and standardized to a light-dark cycle, light 0600-1800). The upper figure represents the glycogen pattern at the end of the dark period when there is abundant glycogen; the rough endoplasmic reticulum is arranged in stacks and is associated with mitochondria. The lower figure represents the end of the light phase when there is almost no glycogen present; the rough endoplasmic reticulum is more evenly dispersed in the cytoplasm surrounding individual mitochondria. Smooth reticulum and free ribosome are clearly visible. x20,000. (Courtesy of H. v. Mayersbach, Hannover, German.)

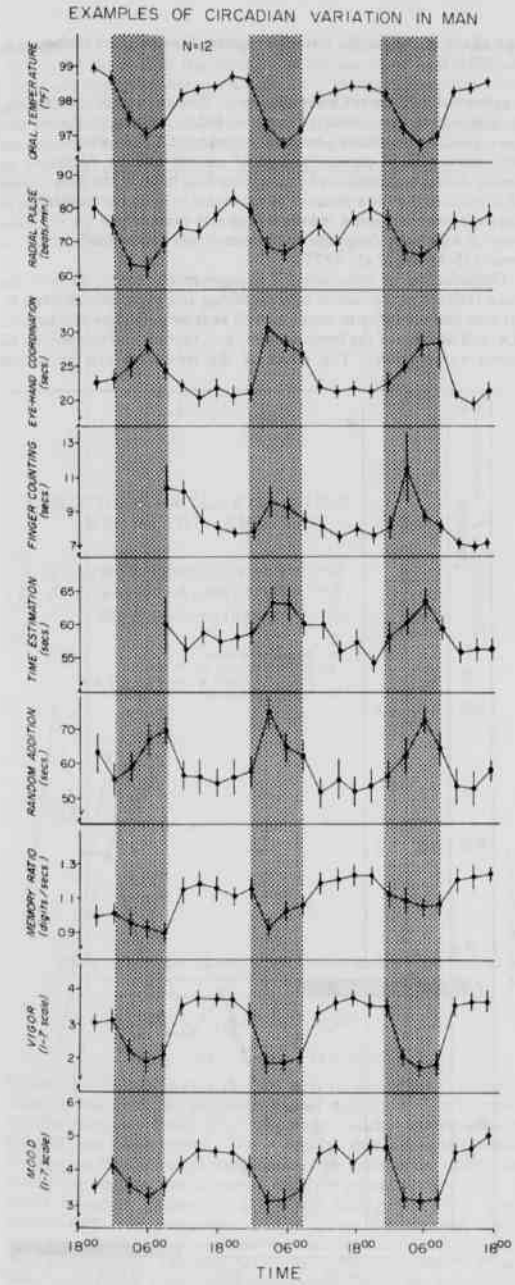

Figure 7. Rhythmic variation in diverse variables in a group of 12 presumably healthy young men over a 72-hour period (sampled at 3-hour intervals). Note that the time of poorest performance represents the crest of the rhythm. Meal times: 0615, 1215 and 1630 hr; rest or sleep time; 2100-0600, however subjects were awakened for sampling at 2400 and 0300 hours. (Scheving, 1977)

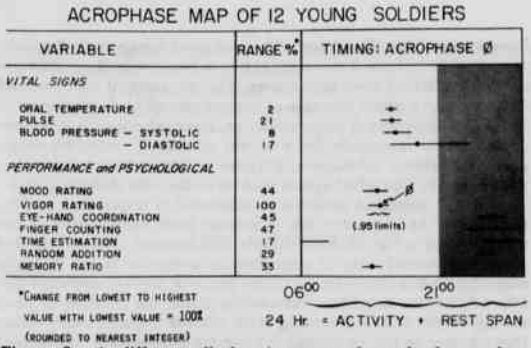

Figure 8. A different display (computer-determined acrophase map) of all the data shown in Figure 7 as well as data on diastolic and systolic blood presure obtained over the same 72-hour span. All measurements were performed on the subjects themselves. Acrophase (represented by a dot) approximates the peak of the circadian cycle in the variables measured, shown with reference to the rest-activity schedule of the subjects. (Kanabrocki et al., 1973)

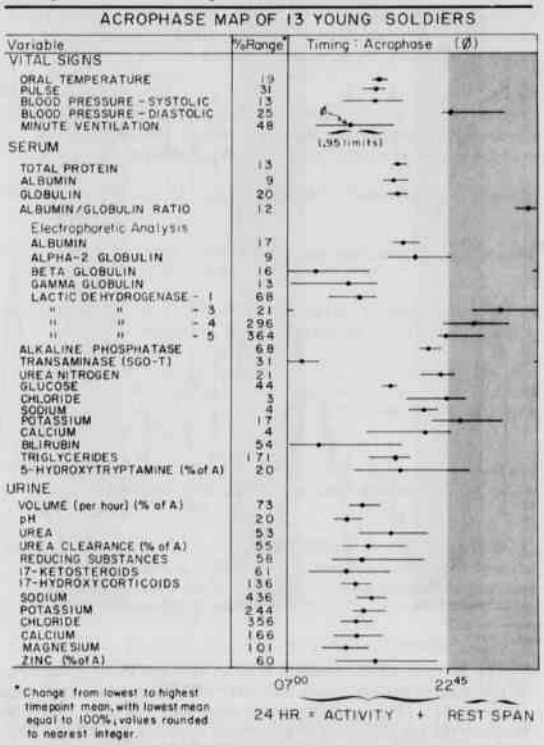

Figure 9. Acrophase map showing data obtained from studies on man. The map illustrates 41 different rhythmic variables in vital signs and in constituents of serum and of urine. Meal times were 0830, 1430 and 1630; rest or sleep time was 2245-0700. The dot represents the time when the crest of the rhythm occurs in relation to the rest-activity cycle. The horizontal bars represent the confidence interval. The center column gives the average 24-hour range of change for the group, that is, the percent difference between the highest recorded means. (Kanabrocki et al., 1973)

Drug Susceptibility Rhythms: The biological system is rhythmically changing; it follows that an organism is biochemically a different entity at different circadian phases. Consequently, it may respond differently to a given stimulus at various circadian stages (times of day). This differential response to an identical stimulus has been demonstrated repeatedly for a variety of stimuli, including drugs, poisons, chemical substances, physical agents such as noise and x-radiation, and biological agents such as endotoxins (Scheving et al., 1974a). The circadian variation as measured in response to various stimuli may be dramatic; this is evident from examination of the chronograms in Fig. 11. For example, the duration of sleep resulting from an identical dose of pentobarbital sodium averages 104 min when the dose is administered to one phase of the rat's circadian system; when it is administered at another phase, the duration of sleep averages only 43 min (Scheving et al., 1968a). Figure 11 also shows that whether or not an animal will survive a potentially lethal fixed dose of amphetamine may depend on the circadian phase at which it is administered. When the dose was given at one phase, 76.6% of the animals survived; whereas at another phase, only 6.6% survived (Scheving et al., 1968b). The third example demonstrates that a carcinostatic drug, cytosine arabinoside (ara-C), is far more toxic at one phase of the mouse circadian system than another (Scheving et al., 1974b).

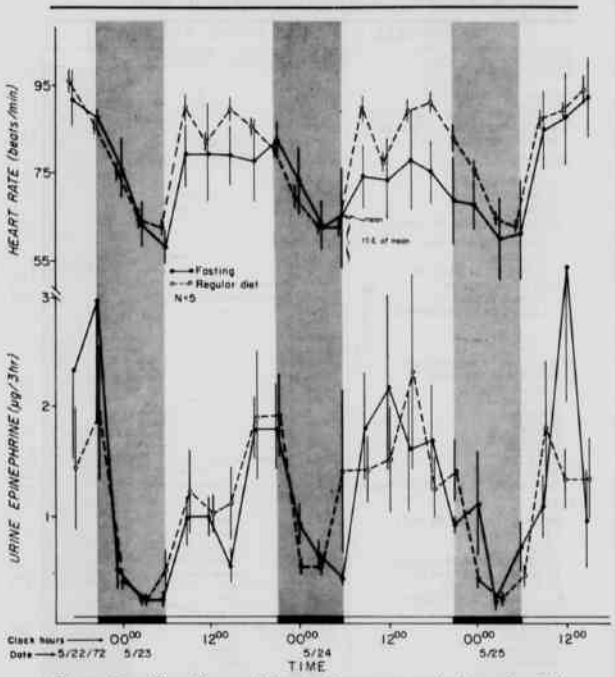

Figure 10. Circadian variation in heart rate and urine epinephrine in presumably healthy young men over a 72-hour span. Meals were eaten at 0615, 1215 and 0630; rest or sleep time was from 2100-0600, however the subjects were awakened for sampling at 2400 and 0300. Note that the group designated as fasting had been subjected to the regular three meal/day schedule through the evening meal of 23 May; after this meal, they did not eat until after the 0600 sampling on 25 May. The only effect noted from fasting was a reduction in the amplitude of the heart-beat rhythm. A third group of subjects all ate a fixed amount of food every three hours over the same period that the one group fasted; and for this group this feeding schedule had no dramatic effect on either variable. The data of the third group are not shown simply to avoid an overly cluttered graph.

Application to Cancer Chemotherapy: Recognition of the variation in response to carcinostatic drugs has led to a series of studies that have produced a critical mass of experimental data which suggests that conventional chronotherapy of cancer can be optimized by timing the administration of drugs according to body rhythms. Figure 12 illustrates one of a number of examples of such optiminization in the experimental mouse, where it is clearly evident that the circadian stage at which the drugs are administered can dramatically affect the results (Scheving et al., 1977).

Optimization of treatment of experimental cancer, in fact, has been realized in the rodent by quantifying and exploiting rhythms in: (1) host susceptibility to drugs as well as their underline mechanisms (i.e. cell division of the bone marrow, gut, thymus and spleen) and (2) tumor susceptibility. The effect of the treatment can be gauged

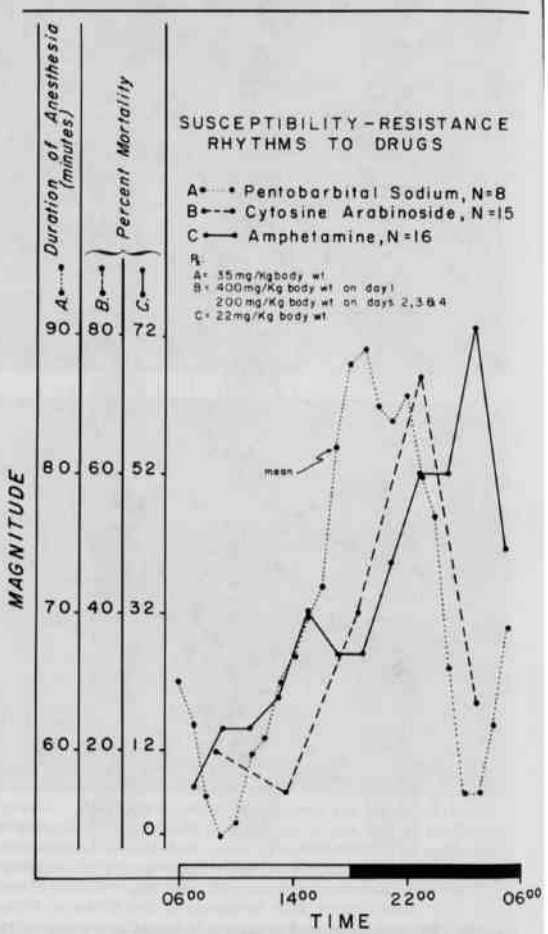

Figure 11. Circadian variation in susceptibility of rodents to pentobarbital sodium, cytosine arabinoside and amphetamine. (For details of each see Scheving et al., 1968a, 1968b, and Scheving et al., 1974b, respectively.)

directly by tumor size, mitotic activity or DNA formation, and indirectly by rhythms in temperature of the tumor or excretory products such as polyamines, certain amino acids and light-chains in the case of immunocytoma in LOU rats (Halbert et al., 1977).

It is concluded that consideration of time structure of organisms as revealed by their rhythms, may lead to the elucidation of many unexplained biological mechanisms. First, however, the "dogma" of a "constancy of the internal environment" either has to be abandoned or modified. Biologists must think in terms of all life being a composite of highly organized rhythmic events. When this is widely recognized, there will follow a new era of progress in biology and medicine.

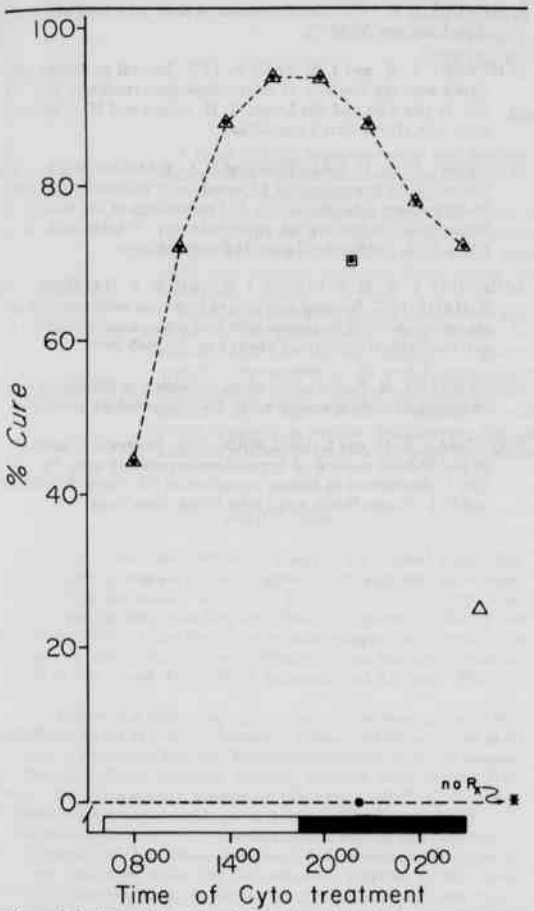

Figure 12. The △ implies that the best sinusoidal ara-C treatment schedule was used (the chronobiological approach). The □ implies that the reference schedule of treatment (non-chronobiological approach) was administered. The • implies that cyclophosphamide (cyto) was administered in combination with ara-C once/course (four courses) to each mouse; however, different groups received it at different circadian phases ◬. Horizontal scale, time when cyclophosphamide was administered. The group that did not receive cyclophosphamide is shown just the right of the time scale. N for each group was 20. The important point is that cure rate (% of mice alive 75 days after tumor inoculation) ranged along the 24-hour time scale from 44% to 94% and **none** of the animals receiving the chronobiological approach died of acute drug toxicity whereas **30%** of the animals receiving the non-chronobiological treatment died from acute drug toxicity. Only 25% of the animals receiving ara-C alone were cured, △, and none were cured that had received cyto alone, •. For details of this study, see Scheving et al. (1977).

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