1

Introduction to Chronobiology

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Recorded recognition of the importance of biological rhythms in plants and animals dates back at least to 5000 B.C. Over the years, the understanding of rhythmic phenomena has grown, but the acceptance of chronobiology as a science has been slow nonetheless. This chapter reviews the development of modern chronobiology by recounting the milestone events that have contributed to the current knowledge in this field, and discusses why chronobiology has developed slowly as a science.

Acquisition of Facts and Concepts in Chronobiology

Chronobiology is the study of mechanisms and alterations of each organism's temporal structure under various situations (Halberg et al. 1977). Such a definition, while clear and useful for chronobiologists, requires amplification for those unfamiliar with the concept of biological time structure. In actuality, this concept is not new. It is stated in Genesis that the first task of God was to create light and then the alternation of light and darkness. Today, it is well recognized that the light–dark cycle of the ambient and laboratory environments serves to synchronize the phasing of 24-hr and perhaps yearly physiochemical rhythms of organisms. Direct evidence that the importance of temporal factors was recognized by mankind even in biblical times comes from Ecclesiastes: "To every thing there is a season and a time to every purpose under the heaven: a time to be born and a time to die; a time to plant, and a time to pluck up that which is planted. . . ." Apart from the obvious wisdom of this recommendation, the precise reference to time of year conveys the early knowledge of the critical importance of cyclical environmental changes for the survival of species. Such a recognition also is present in the aphorisms of Hippocrates (1961) with specific reference to seasonal differences in the occurrence of human diseases.

As a matter of fact, biological systems possess a very prominent temporal structure. Major periodic components of biological rhythms are found around 24 hr and 1
year.* Other bioperiodicities (\(\tau\)) such as those less than 24 hr (termed ultradian rhythms) or approximately equaling 7 days, 20 days, 30 days, etc. (called infradian rhythms), are exhibited by certain biological functions of many species. However, circadian† (\(\tau \approx 24\) hr) and circannual (\(\tau \approx 1\) year) biological rhythms, having been widely documented for a multitude of physiological variables of both plant and animal species, including ours, are most familiar to scientists. Obviously, with respect to evolution, circadian rhythms appear to be related to the rotation of the Earth around its axis (Fig. 1); similarly, circannual rhythms appear to be related to the rotation of the Earth around the Sun.

It is likely that circannual rhythms represent an adaptive phenomenon from the perspective of the reproduction and survival of species. Man learned rapidly that nutrients were not continually available in quality and quantity; this is true of prehistoric man—the hunter, fisherman, and gatherer—and thereafter of modern man—the farmer, peasant, and animal breeder. There was, and remains, a time to plant and a time to harvest. This cannot be ignored nor altered appreciably.

In connection with this, it is widely accepted that astronomical clocks were built long ago in Carnac (Brittany), in Stonehenge (near London), in Chichén Itzá (Yucatán), etc., serving both religious and practical agricultural purposes. The answers to the critical questions such as when to plant and when to harvest domesticated plant species were obtained by consulting astronomical clocks as well as other "signs" (signals), such as the precise timing of matings of many animal species, the flowering of certain plants growing in the wild, and bird migrations, among others. Seasons of planting and harvesting were of such importance that they often were celebrated as religious feasts. Thus, even early man monitored time, at least on a yearly basis, with regard to predictable annual changes in the reproduction of both edible animals and plants. With this in mind, it is not surprising that a set of bioperiodic phenomena were reported by several Greek and Roman scholars including Aristotle, Pliny, and Galen in connection with the reproductive patterns of sea animals, among others (Aschoff 1974; Fox 1923).

For centuries it was believed that cyclic changes in organisms represented exclusively the simultaneous effects of cyclic changes in environmental factors, such as the alternation of light with darkness and/or of heat with cold over periods of both 24 hr and 1 year. In ancient times, the Sun was given the status of an omnipresent and omnipotent God: the Egyptian Ra, the Greek Apollo, the Roman-Heliopolitan Jove, the Aztec Tonatiuh, etc. Not until 1729 was the accepted tenet of an "exclusively exogenous origin" of circadian rhythms in plants questioned. The French astronomer J. J. de Mairan reported in 1729 that the circadian changes in the position of appendages of the heliotrope persisted in constant darkness. This was the beginning of a wide variety of research. However, as far as plants are concerned, it was not until the work of Pfeffer (1875, 1915) started at the end of the nineteenth century that convincing experimental evidence was produced indicating that circadian rhythms persist in complete and constant darkness. Using a set of specially designed devices enabling the continuous mechanical recording of changes in plant limb and leaf positions, Pfeffer tested the hypothesis that the light–dark (LD) alternation over 24 hr plays

* The term "biorhythm" is used infrequently by chronobiologists, since it has been given a completely invalid and unacceptable definition by proponents of the astrological forecasting business. Among many scientifically valid objections, it is not possible to predict attributes of biologic time structure by one datum only, i.e., date of birth. A thorough and unbiased review of many scientific investigations of the popularized concept reveals a clear lack of support for biorhythm forecasting (Klein and Wegmann 1979, AGARD Lecture Series 105:2.10–2.12).

† The use of the Latin root *circa* (about) in the term "circadian" (about 24 hr) was proposed by Halberg et al. (1977) to specify with respect to both statistical and biological considerations that the period is not necessarily exactly 24 hr.
Fig. 1. The “Flower-Clock,” designed by K. Linné in 1745, relies on the knowledge that at relatively precise (sun-related) clock hours flowers of certain plant species are open while those of others are closed. The half-circle to the left presents those plant species for which the flower openings occur between 0600 and 1200; the half-circle to the right shows plants for which the flower closing occurs in the afternoon, between 1300 and 1800. According to Linné, a trained botanist walking through the country without a watch should be able to estimate the time by noting the pattern of flower closings and openings of selected plants. For example, the water lily opens between 0600 and 0700; the St. John’s wort opens between 0700 and 0800; the scarlet pimpernel opens between 0800 and 0900 and closes between 1300 and 1400; the oenothera, or evening primrose, closes between 1700 and 1800. (Drawing by U. Schleicher-Benz in Lindauer Bilderbogen. Published by Friedrich Boër and J. Thorbecke, Lindau, Badensee, West Germany. Reproduced with permission of the publishers.)
the major role in inducing plant movement. Despite the experimental attempts of Pfef-
fer, the hypothesis that leaf and limb move-
ments are caused by the LD alternation
never fitted the data. Instead, the pioneer
work of Pfeffer demonstrated the endoge-
nous origin of circadian changes; the per-
sistence of biological rhythms in organisms
maintained in constant environmental con-
ditions has been widely confirmed for many
species, from the eukaryote to man (As-
choff 1963; Boissin and Assenmacher 1969;
Bünning 1963; Halberg 1960b; Halberg and
Reinberg 1967; Mills 1964, 1966; Pitten-
drigh 1960; Reinberg 1974; Schweiger et al.
1964; Sweeny 1969; Vanden Driessche
1973; Weitzman et al. 1979).

It was not until many years later, around
1950, that Pfeffer's findings were clearly
understood and appreciated. Yet results of
investigations on temporal factors in the or-
ganization of biological functions were, in
general, slow to be recognized by the scien-
tific community. Several other examples of
the long latency in the development of scien-
tific interest in biological rhythms follow;
these were selected from experimental
studies that emphasize reported data and
findings, rather than from philosophical
considerations of the "cyclicity of time"
without supporting data, as is the case for
ancient Chinese medicine (Reinberg 1974)
or as presented in The Art of Prolonging
Life (Hufeland 1789) at the end of the eigh-
teenth century.

As a first example, we cite the work of
Sanctorius, who in 1657 constructed a monu-
mental balance with a huge tray on which
a completely furnished room was set. San-
torius resided for several consecutive
months on his balance tray (Fig. 2). A ser-
vant provided food and assistance and
made readings on the balance scale. By
means of this self study, Sanctorius was the
first to design a "laboratory for chronol-
ogy" and to use an "autorhythmic
metric method"—repeated self-measurements
of physiological variables as a function of
time—to document bioperiodic phenomena
(Halberg et al. 1977). Sanctorius reported a
monthly rhythm (circamensual variation
with period, $\tau \approx 30$ days) both in his body
weight and in the turbidity of his urine. Later Seguin and Lavoisier (1790, 1797)
reported a circadian rhythm in the body
weight of the healthy human male. In fact,
they state in their paper that the period was
about 24 hr ("... a peu près de 24 heures
...") and that a subject who does not ex-
hibit a circadian rhythm in his body weight
should be suspected to be ill.

The Briton J. Davy (1845) was the first
to report the existence of both circadian and
circannual rhythms in his own body
core temperature. Previously, in 1773, Mar-
tin reported some "effects of sleep on the
body heat" (Martin 1773). Again, the use of
an autorhythmic procedure allowed
Davy to demonstrate that these bioperiodi-
cities were closely related neither to physi-
ical activity, for example the riding of a
horse or running, nor to environmental
temperature, which was actually measured
and compared with that of Davy's body
temperature. The fact that the same vari-
able could undergo rhythms of different pe-
riods was demonstrated later by Halberg
and his colleagues (1965) through reanaly-
zation of data collected by Hamburger (1954).
Hamburger collected each of his daily urine
voidings for 16 years, measuring the vol-
ume and 17-ketosteroid concentration. The
statistical technique of spectral analysis
clearly revealed prominent periods approx-
imately equaling 24 hr, 7 days, 30 days, and
1 year. As a result of these findings, empha-
sis must be given to two facts thus discov-
ered: (1) the existence of both a circadian
and a circannual periodicity, among others,
in the same variable, for example, in body
temperature as well as in the urinary vol-
ume and 17-ketosteroid concentration and
(2) the persistence of biological rhythms in-
dependent of internal (exercise) or external
(environmental temperature) factors.

During the nineteenth century, addi-
tional data were accumulated to evaluate
the nature and origin of biological rhythms.
Some experiments were designed so that
measurements of phenomena were done at
Fig. 2. Sanctorius is depicted sitting upon the tray of his room-scale balance. This scientist, an original pioneer of experimental chronobiology, was by 1711 already performing self-measurements several times daily—a method which is now termed "autorhythmyometry." Sanctorius found evidence for a circadian rhythm of body weight related to perspiration. In addition, he reported the existence of 30-day rhythms in body weight in the human adult male. (From Kayser 1952, reproduced with permission.)
regular intervals while the organism—animal or plant—was maintained under constant laboratory conditions. Other research involved investigations conducted under circumstances allowing for random changes in the experimental environment. Results of these types of investigations provided important new information about the role of environmental influences upon bioperiodicities. For example, the fact that circadian rhythms persist in constant conditions with a period differing from 24 hr was first reported by de Candolle (1832). According to this Genevan plant physiologist, the leaf movements of Mimosa pudica persist in darkness with a period of 22–23 hr. This is precisely the type of result Pfeffer obtained, although he thought this was due to light leaks in the darkroom until he confirmed the persistence of the rhythm and change in period length in leaf and limb movement under constant conditions.

Two additional major breakthroughs in chronobiology were derived from the research of Büning before 1950. The first, in 1935, was the direct demonstration of the genetic origin of circadian rhythm characteristics in the beanplant Phaseolus (Büning 1935). For example, the circadian rhythm of stem and leaf movements in this plant differs between two genetically distinct stocks exhibiting periodicities of about 23 and 27 hr, respectively. Hybridization experiments produced plants with hybrid circadian rhythm characteristics (r ≈ 25 hr). Thus, Büning demonstrated that biological periodicities, i.e., circadian rhythms, are transmitted from generation to generation according to genetic rules. Evidence exists to support the theory that the controlling elements which transcribe the circadian rhythms reside in the nuclear genetic material. Recent work on Drosophila has identified several genes responsible for certain biological rhythm characteristics. Using circadian rhythm mutants of Drosophila to build a genetic mosaic of mutant and nonmutant parts, Konopka and Benzer (1971) showed the rhythm of the composite flies resembled the genotype of the head. This is also the case, for example, when circadian rhythm patterns of a female Drosophila (XX) differ from that of the male (XY) (Rensing 1973).

The second major finding of Büning (1963) was that an organism, such as a plant, is able to “measure” time. A sophisticated manipulation of the lighting regimen over 24-hr durations allowed Büning to demonstrate that a time-restricted short exposure to light induced flowering when given at a critical clock hour for several days. Büning found the same quantity and quality of light when given at other clock hours to be ineffective in inducing flowering. In other words, light serves not only as a source of energy but also as a signal. Whether or not a light signal is effective in inducing a biological response, in this case flowering, varies according to the (biological) time it is presented. Further research by Büning contributed to developing the concept of the “biological clock.” The message was that organisms possess built-in and inborn mechanisms enabling the measurement of time, at least over 24 hr and 1 year. This message is still valid (Ehret 1974; Queiroz 1974), although it is presented in a different manner. Modern chronobiologists (Ehret 1974; von Mayersbach 1978; von Mayersbach and Leske 1961) consider a cell to be genetically programmed for performance of a given task at a specific biological time, or to reach the maximum of a certain activity at a precise circadian phase. When organisms are synchronized to environmental conditions (see Chap. 2), the biological times and phases coincide with specified clock hours in the 24-hr scale.

The concept of how organisms use biosystems to measure time has changed in complexity and in the depth of understanding from the initial one posed by Büning in the thirties. The photofraction (duration of light in each 24-hr span) or the scotofraction (duration of darkness in each 24-hr span) varies with the time of year. Because of their precise predictability, photoperiodic phenomena appear to be “the most
appropriate references for precisely measuring time" for an organism (Queiroz 1974). In fact, the appropriate photoperiod is strictly delineated for each species with regard to programming activities such as reproduction, migration, diapause, dormancy, etc., in plants and animals. For example, according to Büning, over the 24-hr span there is an "external coincidence" between the external alternation of light and darkness and the alternation of phase during which a plant is either sensitive or insensitive to light with regard to the induction of flowering. Taking into consideration experimental data obtained later by Aschoff (1960, 1963), Hamner and Takimoto (1964), and Pittendrigh (1960, 1961), the complementary hypothesis of an "internal coincidence" among temporal patterns in both the internal and external environments was derived. This newer concept takes into consideration the existence of several circadian oscillators driven either by a signal such as sunrise (light-on) and/or sunset (light-off). Therefore with regard to the photofraction, the interval of time between the two signals—the phase relationship of sunrise and sunset—determines whether or not the phenomenon, flowering, occurs. In addition, as Pittendrigh (1960, 1961) emphasized the light signals can be replaced by a system capable of shifting the phase of the oscillators, e.g., by causing change in the crest time of the 24-hr cycle of external temperature, hormonal secretions, or other biological functions. The capabilities of biological systems to measure time or to program activities represent two sides of the same coin. For instance, a biological system's ability to respond and the efficiency of its response to a signal can depend upon the phase of the bioperiod.

The concept of biological clocks developed by Büning represented a significant step forward. Unfortunately, the expression "biological clock" has been and continues to be misused. It is tempting, especially for some animal biologists, to search for a master clock somewhere within the body. Initially, the proposed master clock was envisioned as being located within the pituitary, although many experiments showed this not to be the case (Reinberg 1974). Then, the search for the home of the wandering master clock was relocated to the hypothalamus. Again, dissection and inspection of this structure proved fruitless. As was the case for the legendary phoenix, the master clock reborn from experimental ashes tries to find a place today in neuroendocrine structures, either the epiphysis or the suprachiasmatic nucleus (SCN). The latter is likely to be the circadian oscillator system which drives certain neuroendocrine circadian rhythms such as those of adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), and prolactin (PRL). Physical destruction or neurotoxic inhibition of the SCN results in the cessation of these neurohormonal circadian rhythms. However, their ultradian rhythms continue to persist, as do circadian rhythms in other physiochemical functions of the body including rest and activity, eating and drinking, body temperature, and corticosterone hormone secretion from the adrenal cortex (Fuller et al. 1981; Moore-Ede et al. 1980; Moore 1980; Krieger 1979; Mörnex and Jordan 1980; Suda et al. 1979; Szafreczyk et al. 1981).

With evidence for an endogenous, i.e., genetic, basis of biological rhythms, the role of exogenous factors upon rhythmic systems was not immediately understood. It was not until 1954 that Halberg et al. (1954) and Aschoff (1954) almost simultaneously developed the idea that cyclic variations in environmental factors are capable of influencing the expression of circadian rhythms. Aschoff (1954) coined the word "Zeitgeber" (time giver); Halberg et al. (1967) coined the word "synchronizer," and Pittendrigh (1960) proposed "entraining agent." Despite the fact that the exact definitions of the terms given by these three scientists differ, there is general consensus among chronobiologists that "synchronizer," "Zeitgeber," and "entraining agent" are synonymous. One of the most powerful synchronizers for a large variety
of plants and animals is the LD alternation over 24 hr. But other cyclic changes in environmental factors, such as temperature, noise, social interaction, etc., also must be considered as having synchronizer potential. The respective strength of each one as an influence upon biological rhythms depends upon the experimental circumstances and the investigated species. What must be kept in mind is that a synchronizer does not create a rhythm; it is capable only of influencing its expression, for example, by forcing an alteration in period length and/or timing of the circadian crest with respect to clock hour. It was Jürgen Aschoff and his group in Erling-Andechs, Germany (1959, 1960, 1974) who demonstrated that the circadian period can be manipulated or entrained by the period of the Zeitgeber only within certain narrow limits (e.g., 24 ± 2 hr).* Beyond these limits the organism's circadian rhythms resist entrainment and instead become free-running; they oscillate, exhibiting their spontaneous period as is the case when known synchronizers are suppressed, for example, when constant laboratory conditions are experimentally instituted. Aschoff (1960, 1974) also demonstrated that without time clue and cue the period of free-running circadian rhythms is shorter than 24 hr for nocturnally active species (e.g., rodents), while it is longer than 24 hr for diurnally active species (e.g., man). Another important basic contribution from the Erling-Andechs group was the demonstration that newborn lizards (Hoffman 1957) and chickens (Aschoff and Meyer-Lohmann 1954) exhibit circadian rhythms even if the mother and, thereafter, the eggs during incubation are maintained in constant environmental conditions (for example, continuous darkness and fixed temperature and humidity).

With regard to human beings, Halberg and his group in Minnesota (Halberg et al. 1959a) demonstrated that the most powerful synchronizer of circadian rhythms is cyclic changes in socioecological factors. For our species this means that the alternation of rest and activity related to our social interactions constitutes the major synchronizer for most functions. Usually we are active during the daytime, the span associated with natural or artificial light. Yet this time span coincides also with relatively higher levels of light, temperature, noise, and odors among others in our ecological niche. Chronobiologists recognize that the mere consideration or statement of clock hours, per se, need have no meaning relative to biological time, especially in modern society in which millions of persons worldwide are involved in night or shift work and transmeridian flight (Klein and Wegmann 1979). Specifically, the hours given by the clock have no synchronizing effect if there is no obligation for adherence to a given rest–activity routine defined by the specific scheduling of work, rest, and social interaction. This was clearly demonstrated by Mills (1964, 1966) in an underground cave experiment during which an isolated subject was found to disregard time cues provided by his own wrist watch. The subject exhibited so called free-running (non-24-hr) circadian rhythms.

Although interest in biological time structure increased during the first half of the twentieth century, it was not until 1960 that an objective, statistical approach for detecting and quantifying such was fully appreciated. In 1960 Colin Pittendrigh organized at Cold Spring Harbor in Massachusetts a meeting during which two major topics were addressed (Aschoff 1960; Halberg 1960b; Pittendrigh 1960). The first topic was practical as well as critical for the subsequent development of the field and dealt with the requirements for modern chronobiology as a quantitative biological science, i.e. the utilization of rigorous methods for data sampling, gathering, and statistical analysis. The second topic emphasized at this meeting dealt with the find-
ings of specially designed experiments relying upon specific and accurate physical and chemical measurements (see Chap. 2) by Aschoff (1959, 1960), Halberg (1960a, b), and Pittendrigh (1960) producing evidence for the temporal structure of organisms.

Circadian rhythms in a large variety of physiological functions and biological phenomena have now been demonstrated. The respective peaks and troughs of such rhythms are not randomly distributed over 24 hr. On the contrary, their timings represent a morphology, an anatomy in time, with more or less strict temporal relationships among processes.

Knowledge about the temporal organization of biological functions, especially in mammals, has developed since 1960 (Bartter et al. 1962). Thus, new and obviously well-documented illustrative examples can be given (Bartter et al. 1962; Halberg and Reinberg 1967; Halberg et al. 1967; Krieger 1974; Weinberg 1974; Weitzman 1974). Let us consider (Fig. 3) a healthy human adult synchronized with diurnal activity and nocturnal rest. The peak time of plasma ACTH occurs during nightly sleep. At this time, there is almost no adrenal hormone secretion. Subsequently, after a certain phase delay with reference to the ACTH circadian peak, the plasma cortisol level rises to its peak around the time of awakening. The crest time of the urinary 17-OHCS rhythm occurs approximately 4 hr later with reference to the plasma cortisol peak. Plasma cortisol as well as other corticosteroids influence certain circadian patterns of other physiological variables such as the urinary excretion of potassium, grip strength, and airway patency. The peak of these variables usually occurs 4 to 6 hr after that of plasma cortisol (Fig. 3). The concept of biological time structure is so important to the field of chronobiology that it is included in the definition of chronobiology given at the beginning of this chapter.

Returning to the historical perspective and the acquisition of new concepts in chronobiology, it is not surprising that ultradian rhythms, with \( \tau \) ranging from a fraction of a second to a fraction of a day, were not discovered until the development of appropriate monitoring equipment and analytical techniques. The discovery of ultradian rhythms enhanced the understanding of biological time structure. In connection with this, the continuous recording of electric potentials (EEG, eye movements, etc.) during sleep enabled Kleitman (1963) to describe electrical stages of sleep associated with the ultradian periodicity of rapid eye movement (REM). New methods for hormone determinations in plasma samples as small as 0.1 ml enabled the withdrawal of blood at intervals of a few minutes, instead of a few hours, and led to the detection of both ultradian and circadian rhythms in endocrine activity (Weitzman 1974; Krieger 1974). Ultradian rhythms in neural and muscular activity had already been explored in the 1930's by neurophysiologists using electrophysiological methods, among others. Both Fessard (1936) and Cardot (1933) were experimentally justified in stating that "... rhythmic activity is a basic property of excitable systems." ("L'activité rythmique est une propriété fondamentale des systèmes excitables.") However, these authors were concerned with ultradian rhythms of only neuromuscular and related systems. Reinberg and Ghata (1957) generalized this concept to other biological systems and to circadian and infradian (rhythms with period lengths greater than 28 hr) spectral domains. It is now well accepted that rhythmic activity is a fundamental property of living matter (Halberg et al. 1977; Halberg and Reinberg 1967; Reinberg 1974).

In retrospect, the Sixties can be considered the golden age of molecular biology. It was of mutual interest for both molecular biologists and chronobiologists to study cellular bioperiodicities. The major question was and remains: What are the basic mechanisms underlying circadian and other biological rhythms in the cell? Barnum (Barnum and Halberg 1953; Barnum et al. 1958), Hastings (Hastings 1959; Hastings and Keyman 1965), Sweeney (1969), Ehret
Fig. 3. Aspects of the human temporal structure. The acrophase, $\phi$ (crest time of the best-fitting cosine function approximating all data as determined by the least squares method, see Chap. 2) is given for each variable with 95% confidence limits. Subjects' synchronization was approximately 16 hr of diurnal activity and 8 hr of nocturnal rest. $\phi$'s are not randomly distributed over the 24-hr scale. On the contrary, they represent physiologically validated temporal relationships. The circadian $\phi$ of plasma ACTH leads that of plasma cortisol in phase. The $\phi$ of blood eosinophils coincides roughly in phase opposition with that of cortisol. This latter leads in phase the $\phi$'s of physiologically related variables such as urinary 17-OHCS and $K^+$, grip strength, and peak expiratory flow (bronchial patency). The $\phi$ both for systolic and diastolic blood pressure as well as for heart rate are roughly in phase with the $\phi$ of urinary catecholamine and aldosterone concentration as well as plasma renin activity. A larger dose of a vagolytic agent—SCH1000 (ipropium bromide)—is required during the rest than during the activity span to be effective on bronchial tone. The temporal organization of these and other variables can be viewed as representative of adaptative phenomena. Since man is a diurnally active animal, it is not surprising that both the activity of his adrenal glands and sympathetic nervous system predominate during the day. (This chart utilizes data gathered from studies of F. Bartter, C. Gaultier, J. Ghata, F. Halberg, M. Lagoguey, A. Reinberg, L. Scheving, M. Smolensky, and E. Weitzman.)

(1974), Schweiger (Schwieger et al. 1964), Queiroz (1974), Vanden Driessche (1973, 1975), and Ashkenazi (Ashkenazi et al. 1973) were pioneers who initially contributed to the current understanding of the temporal organization of molecular activities. This topic is treated in detail from the point of view of cellular morphology and mitosis in Chaps. 3 and 4 of this book.

Those aspects of chronobiology presently applied to medicine—which include chronopathology, chronotoxicology, chrono-
nopharmacology, chrononutrition, and chronotherapy (Halberg et al. 1977; Halberg and Reinberg 1967; Reinberg 1974; Reinberg and Halberg 1971)—also have historical background. Although circadian and/or circannual changes in physiological and pathological phenomena were considered many centuries ago by Hippocrates (1961), such rhythms have been studied rigorously only since the nineteenth century (Beau 1836; Féré 1888). For example, a circannual rhythm in human birth was reported by Quetelet in 1826. Circadian rhythms in epileptic seizures were reported by Beau in 1836 and by Féré in 1888. Manson in 1880 reported the circadian rhythm of microfilaria. The circannual rhythmicity of suicide in 1897, with a crest in spring, was reported by Durkheim (1952). Pincus (1943) reported a diurnal rhythm in the excretion of urinary ketosteroids by young men. It has to be emphasized that the existence and the importance of endogenous bioperiodicities were either ignored or underestimated until the birth of modern chronobiology (Ghata and Reinberg 1954; Reinberg et al. 1973; Smolensky et al. 1972; Smolensky 1980). In human beings, among other animal species, there is an alternation in one's tolerance and susceptibility to potentially noxious agents as a function of the time of day, month, and/or year (Halberg 1960a,b; Halberg et al. 1960; Reinberg et al. 1973; Reinberg and Halberg 1971; Smolensky et al. 1972, 1980). In other words, there must be an appreciation for an old tenet dating back to Hippocrates—the organism's temporal structure has to be taken into consideration to better understand circadian and circannual changes in the incidence of disease as mirrored by both morbidity and mortality statistics. This topic is discussed further in Chap. 5.

In 1814, Virey in earning his Doctor of Médecine degree from the University of Paris devoted his entire thesis to chronobiology and chronopharmacology. The first to gain his medical diploma by researching chronobiology, Virey considered the significance of human circadian rhythms in relation to both the risk and treatment of disease. According to Thomas Sydenham, as quoted by Virey, laudanum (opium) must be taken in the evening while purgatives and enema must be administered in the morning to be fully effective. In the conclusion of his thesis, Virey suggested that priority be given to researching the importance of timing in therapeutic intervention. Yet Virey's recommendations were not heeded until a century later when reports were published by other pioneers such as Möllerström (1953), Jöres (1935a,b), and Menzel (1944, 1958). The experimental attempt to determine at what time insulin must be given to a diabetic (Möllerström 1953) or to escape from the "stupidity of a three times a day" drug administration (Jöres 1935a,b) has still to receive appropriate attention by the medical community.

The dramatic experiments of Halberg (Halberg 1960a,b; Halberg et al. 1959b, 1960) on the "hours of changing resistance of mice" were the beginning of modern chronotoxicology (Fig. 4). A fixed dose of a potentially noxious agent (E. coli endotoxin or ouabain, for example) might kill 80% of the mice treated at a certain clock hour but only 20% of the mice treated 12 hr earlier or later. Other investigations performed on laboratory animals by Halberg et al. (1959b, 1960), Scheving et al. (1974), Pauly and Scheving (1964), and von Mayersbach (1978) further demonstrated that the effect(s) of a drug varies as a function of the biological time of administration. These same studies also showed that the timing of medications can alter the characteristics of rhythms (Chap. 6). Systematic investigations of human chronopharmacology actually commenced with the studies of Reinberg et al. (Reinberg 1965; Reinberg and Sidi 1966; Reinberg et al. 1964, 1965, 1967), Halberg et al. (1967), and Rutenfranz and Singer (1967). Aspects of these as well as other chronopharmacologic studies are provided in Chap. 6.

Finally, it must be recognized that the
**Fig. 4.** Chronotoxicology was first considered as the hours of diminished resistance. Experimental results reported by Halberg (1960b) demonstrated the existence of circadian rhythms in the susceptibility of mice exposed to various potentially noxious agents, for example, a fixed "dose" of *E. coli* endotoxin, ouabain, or white noise. The end point of response in the studies on mice herein summarized was the number of deaths per treatment group per time of testing. In addition to circadian variability, susceptibility rhythms of about 7 days, 30 days, and 1 year, using other indices, are now known for a large variety of agents, animals, and plant species (see text). (From Halberg 1960b, reproduced with permission.)

The current motivation for chronobiologic studies of nutrient metabolism dates back to Charles Chossat in 1832. Chossat demonstrated that the circadian rhythm of the cloacal temperature of pigeons that had been deprived of food and water persisted with a large amplitude even until death. The circadian rhythm in temperature was independent of 24-hr changes in the environmental temperature. This phenomenon was re-demonstrated in mice 130 years later by Galicich et al. (1963) for other physiological variables. The persistence of circadian rhythms during fasting conditions is, therefore, known today as Chossat’s phenomenon and, of course, pertains to chrononutrition, which is presented in Chap. 7.

Let us consider now, still from an historical point of view, an interesting question which constitutes the second part of this introductory chapter:

**Why the Importance of Chronobiology Was Not Recognized Earlier**

To understand why the importance of chronobiology was not recognized earlier, it is necessary to consider the environmental and educational milieu of early scientists, including their educational background, exposure to misleading hypotheses, and limited access to research tools, techniques, and methods for investigating biological rhythms.

**Inadequate Educational Background**

Most scientists since the seventeenth century grew up and resided in cities. Childhood and youth were spent far from a rural environment. The experiences of children
raised in an urban setting differed widely from those of children raised in a rural setting. Most of the knowledge that inhabitants of cities possessed about plant and animal life was acquired from school lessons and readings. The personal experiences of rural children, especially with regard to the behavior of wild and domesticated animals, plant cycles, etc., were replaced for urban children by second-hand information, with the possibility of numerous distortions. During the nineteenth and the first half of the twentieth centuries, circadian and circannual changes were either ignored or regarded as a "curiosity of nature" by city-bred scientists. To recognize the crucial importance and the major interest of bioperiodic phenomena, the student would have had to experience as a young person how widespread and generalized they are among observable species.

In addition to this common lack of personal experience with the manifestations of overt biological rhythms, the education of young students of western countries provided an exclusively linear representation of time. The Western model of time as the sand clock (or the candle-clock) with its continuous and constant flow envisions life as a simple and constant addition of hours, days, and years from birth until death. In opposition to this model, the oriental (Chinese, Indian, etc.) representation of time is more subtle, since cyclic and periodic changes are taken into consideration. A spiral representation of time results from the combination of both linear and nonlinear changes. From one morning to another we are neither exactly the same person, nor a very different one. An understanding of this point of view is trivial for the oriental culture. On the other hand for the occidental culture of western scientists, which takes for granted that time is linear, consideration of the significance of time from any other point of view—in terms of period, phase, and waveform—requires reorientation.

Yet another related set of circumstances helps to explain why chronobiology did not develop at a faster pace. Medical disciplines advance through teaching and research. The discipline of chronobiology has been rather slow to advance for a variety of practical reasons.

First, none of the currently active chronobiologists entered undergraduate, graduate, or medical school with the primary intent of biological rhythm study. As a matter of fact, until very recently chronobiology was not taught in colleges or medical schools, since no chairs or departments of chronobiology existed. Many became aware of biological rhythms only by accident, discovering them through repeated trials of their research protocols at different clock hours. It is in this manner that most scientists were introduced to chronobiology; it is these experimenters who discovered for themselves the practical significance of the temporal organization of physiochemical processes; it is they—those strictly schooled in "homeostatic" theory—who are responsible for the current advances in chronobiology.

Second, for any new philosophy to gain attention and be influential, there must exist a large enough nucleus of academically well-respected and well-organized individuals to make an impact on scientific thought. Until the last decade, the number of active and well-trained chronobiologists was quite small. A review of the quantity of citations dealing with chronobiology in Index Medicus under the entries of "Periodicity," "Diurnal," or "Circadian" reveals only a moderate number, even until 1955–1960. In 1983, several pages of citations are devoted to publications of chronobiologic research. This reflects the elevated prominence of the field and the increased number of scientists conducting research on biological rhythms.

Third, a scientific society serves as a catalyst for the development of a field of specialization. The International Society for the Study of Biological Rhythms was initiated in 1939. Among the founders were Hjalmar Holmgren, Jacob Möllerström, and Arthur Jöres. After World War II the Society formally resumed its international
activities in 1953. In 1970, the Society became known as the International Society for Chronobiology.

Fourth, findings are communicated primarily through the publication of research. Earlier, it was not easy to publish findings dealing with biological rhythms, since without the availability of a convenient methodology and appropriate quantitative statistical procedures for time series analysis (Chap. 2), editors were reluctant to accept manuscripts. This is no longer the case, with improvements in both experimental design and statistical techniques for time series analyses. Let us point out that many classically trained scientists—editors of respected journals—viewed biological rhythms more as a scientific curiosity than as an important and undeniable component of biology. Prior to 1970, no journal was devoted solely to the field of chronobiology. Now there are two: The International Journal of Chronobiology/Biological Rhythm Research and Chronobiologia.

In summary, from the perspective of education the slow progress of chronobiology as a science resulted from a lack of (1) appropriate numbers of adequately trained chronobiologists to teach its methods, (2) departments or chairs in academic institutions dedicated to developing and teaching this science, (3) national and international societies to transfer findings and methods, (4) communication through the publication of relevant manuscripts, and (5) research grants and long-term research programs for chronobiology.

**Misleading Hypotheses**

In addition to the existence of inadequate educational opportunities, prevailing theory slowed or inhibited the advance of chronobiologic hypotheses and concepts. Several generations of students, including ours, have been taught that a set of regulatory mechanisms maintain the constancy of biological systems and physiological functions in a "homeostatic" manner. The constancy of the internal milieu (milieu intérieur) is presented as law. Tables of so-called biological constants are given in many medical textbooks for reference. Regulatory mechanisms are usually considered exclusively as feedback processes. A change in biological function is supposed to be immediately counteracted or balanced in such a way that the so-called "equilibrium," "steady-state," or "constant level" is maintained. Change is interpreted as resulting from stress, an explanation which is usually well accepted even if the term stress has never been defined adequately.

One has to realize that at the end of the nineteenth century, during the time of Claude Bernard (Bernard 1926), and through the time of Cannon (Cannon 1929) in the 1920’s, the quality of biological measurements and determinations as well as the quantity of experimental data were very limited. Although the homeostatic theory (Cannon 1929) represented a considerable step forward in biology and medicine, it unfortunately has been overlooked that Claude Bernard himself emphasized the existence and importance of biological variability in the internal milieu (see Halberg 1967). It also has been forgotten that even for Cannon homeostatic regulation represented no more than a theory, certainly not a law. As advocated by Bernard, the theory must be changed when it no longer fits experimental facts.

In conventional biological tables, the value of a variable is given as a mean (X) ± 95% according to sex, age, and health status. The margin of error, which is typically quite large, includes all types of "noise" such as (1) instrumental noise related to the imprecision of experimental laboratory techniques, (2) methodological noise related to difficulties in standardizing individuals and experimental conditions, and (3) biological noise related to interindividual variability. But the margin of error also includes predictable variabilities related to biological rhythms. With the homeostatic approach, these latter are simply included as additional sources of noise, even if the amplitudes of the rhythms create an unaccept-
ably large range of values, as is the case for such variables as plasma cortisol and testosterone, circulating lymphocytes, and blood pressure, to mention a few.

The homeostatic “law” is associated with a set of misleading concepts such as “biological equilibrium” (the only state of equilibrium in biology is death, since exchanges are the rule), “steady state,” etc. The feedback model was put forth to explain fluctuations around a proposed “constant level” of function. According to this argument, biological rhythms thus represent a non-meaningful and insignificant component of such fluctuations. By 1957 (Reinberg and Ghata 1957), it was obvious that this model could not explain why the fluctuation in a variable exhibits a precise period in a given species (e.g., the period of the ovulatory cycle in mammals) and why the fluctuation persists when environmental factors are kept constant. The feedback model, proposed as an explanation of biological rhythms in order to fit homeostatic theory, represented nothing more than a kind of intellectual regression, a blindfold. It inhibited both deeper discussion of existing experimental evidence as well as detailed research on mechanisms of biological rhythms (Halberg and Reinberg 1967).

The homeostatic theory fails to fit the experimental findings that a fixed dose of a potentially noxious agent will kill 100% of a group of mice at one phase of a circadian rhythm, while 100% of a comparable group of mice treated identically 12 hr earlier or later will survive. The “stress” in these experiments is rigorously standardized. While a given toxicant is totally inefficient when presented at one time, it leads to the complete destruction of a sample of animals when presented at another. These effects are both predictable and reproducible. Moreover, the homeostatic theory does not fit the fact that in persons adhering to diurnal activity and nocturnal rest, the plasma cortisol concentration can be as high as 20 μg/100 ml in the morning just before arising from sleep, and be nearly undetectable (≈ 0 μg/1) during a 6-hr span of time during nightly rest (Krieger 1974; Weitzman 1974). The homeostatic theory cannot explain why in the same patient who has been rigidly standardized for chronobiologic study a glucose tolerance test yields different responses depending upon whether it is performed in the morning or in the evening (Jarrett and Keen 1969), or in April or in September (Méjean et al. 1977).

Obviously, the concept of homeostasis has been both overgeneralized and misused in biology and medicine by the epigones of Bernard and Cannon. Predictable cyclic variations with several homeostases can be considered (e.g., from a theoretical point of view as a circadian rhythm of “set points”). It has to be emphasized again that precise end points or limits must be interpreted as being variable in time due to periodic fluctuations (biological rhythms). Even if one wishes to explain why plasma potassium or blood glucose are almost constant (in fact they exhibit small-amplitude circadian rhythms), one must take into consideration large-amplitude circadian rhythms in the involved control systems (e.g., secretions of insulin, glucagon, and cortisol).

In actuality, the relative constancy of variables (for example, plasma glucose, electrolytes, hormones, proteins, and water, blood pressure, and body temperature, among others) appears to be critical for survival. Organisms are confronted with both periodic (predictable) and aperiodic (non-predictable and random) changes in their environments. Regulatory mechanisms, presumably involving feedback loops, are responsive to aperiodic changes in the environment over the time domain of seconds, minutes, and hours. On the other hand, genetically programed biological rhythms constitute the basis for which organisms respond to periodic environmental changes having prominent τs of approximately 24 hr, 7 days, 30 days, and 1 year. For example, large-amplitude circadian rhythms of both neuroendocrine and endocrine secretions control the variables of plasma glucose, electrolytes, proteins, etc., so that
only small-amplitude circadian changes result even though the environmental inputs, prominently exemplified by the intake of food, vary randomly in time, quality, and quantity. Short-term regulatory mechanisms also are subject to influence by circadian and other bioperiodicities.

One must admit that the homeostatic theory was a powerful braking force in the development of chronobiology, slowing the appreciation and understanding of clean experimental evidence documenting periodicity. In the name of the scientifically canonized "Saint Homeostasia" (Reinberg 1974), objective demonstrations of chronobiologic facts either were simply ignored or denied. In addition, papers submitted for publication in journals of high regard were rejected unanimously by "experts" (up to now, only some of them have offered their mea culpa). The homeostatic theory was a dogma, an article of faith. As it happened chronobiologists were asked: "Do you really believe in biological rhythms?" with a touch of condescending irony. Fortunately, there was no witch-hunt of chronobiologists, despite the fact they are often working at the "witching time of night": Hamlet, III, ii). With respect to scientific discovery and advancement, some scientists behave as if they were blind and deaf when confronted with facts that are incompatible with their education.

The best manner of overcoming the retarding effects of obsolete theoretical considerations is to gather a critical mass of experimental evidence. This was the task of chronobiologists in the Sixties. One of the present tasks, as far as medicine is concerned, is to evaluate whether or not the chronobiologic approach for diagnosing and treating disease is more accurate, more effective, and more powerful than the homeostatic one. Since physiological variables such as blood pressure (Halberg and Reinberg 1967; Halberg et al. 1967), plasma cortisol (Krieger 1974; Weitzman 1974), and plasma testosterone (Dray et al. 1965), vary as a function of both the time of day and year, the definitions of hyper- or hypocorticision, etc., require revision. Similarly, the experimental treatment of certain diseases such as cancer based upon an animal model (see Chap. 4) shows that the chronobiologic approach proposed by Scheving and Halberg (sinusoidal change in the administration of carciostatic agents with the highest dose given at the circadian time of best drug tolerance) is both less dangerous (lower risk of injury or death due to drug toxicity) and more effective (up to 10 times greater survival at a given time) than the homeostatic approach (same amount of agent given in equal doses at equidistant time intervals).

Inadequate Tools, Techniques, and Investigative Methods

In order to collect data for the study and description of biological rhythms, special equipment and methods of investigation are necessary. Relatively precise instruments are needed to record biological events with regard to month, day, hour, minute, and second. Earlier in this chapter it was mentioned that astronomical clocks and related calendars were constructed during the stone age to serve both religious and practical purposes. The critical chronobiologic answers to questions such as when to plant, when to hunt, and when to harvest were given by astronomical clocks and calendars as well as natural signals from bird migrations, plant flowering, etc. Although primitive measures of the time of day (solar clocks) and time of night (water clocks) were used in ancient Egypt (the water clock of Ramses II), reliable mechanical clocks did not become available until the seventeenth and eighteenth centuries due to the genius of Huyghens and Harrison, who developed the pendulum spring, and marine clocks. Obviously, precise reference to time of day for biological events could only be provided when accurate, reliable, and inexpensive time pieces were widely marketed.

Chronobiologic studies of body weight and body temperature became possible
only with the availability of accurate balances and thermometers (Sanctorius 1711; Davy 1845). Blood glucose levels can now be determined every minute continuously during 24-hr spans by entirely automated instruments, while Claude Bernard had to be satisfied with only a single (nonspecific) glucose determination per day, requiring a large amount of blood.

The development of modern chronobiology is closely related to the development of tools, instruments, and techniques permitting study of variables over relatively long spans of time (Kaysner and Heusner 1967). Precision, reliability, and reproducibility are required, as is miniaturization. It is not surprising that radioimmunoassay methods enabled a step forward in chronocrinology (Krieger 1974; Weitzman 1974). Nowadays plasma cortisol can be determined every minute during 24 hr using only minute quantities of blood. When Pincus first demonstrated in 1943 a circadian rhythm in the urinary excretion of 17-ketosteroids, he utilized only 3 samples per 24 hr. Today measurements of physiological variables such as EKG, EEG, rectal temperature, wrist movements, and respiratory movements can be recorded continuously for many days from normally ambulatory subjects using portable and miniaturized instruments.

Just as histology and pathology enable separation of anatomical components into spatial serial sections, with careful planning and adequate experimental protocols and sampling procedures chronobiology enables separation of biological variables into temporal serial sections (Reinberg 1974). Chronobiologic methodology involving a minimal set of requirements is needed to design and conduct meaningful experiments. Reducing the "thickness" of the "slices of time" does not solve the problems related to rhythm detection, quantification, and interpretation. It is necessary to know how the subjects were synchronized and how they were standardized for chronobiologic research. In addition to these particulars, other pertinent information such as time of year, nutritional status and regimen, age, sex, and weight is required as well. When these minimal methodological requirements are neglected, rhythms may not be detectable, as was the case when some authors reported an absence of circadian rhythmicity in plasma calcium in healthy adult men, while others reported its existence (Halberg and Reinberg 1967). Moreover, when a proper chronobiologic methodology is not used, authors studying the same rhythm may report different peak times and amplitudes (Halberg and Reinberg 1967). This apparent lack of reproducibility of periodicity in, for example, calcium and some other studied variables, did not favor the scientific credibility of chronobiology.

Today it is also well recognized that the appropriate statistical analysis of time series comprises an indispensable aspect of chronobiologic methodology. Problems of data gathering and analysis are closely related in chronobiology. When specific methods of statistical analyses were not available, the quantification of rhythms constituted a problem that was not easily solved, especially when the temporal variations were of small amplitude. Discussion of methods pertinent to all aspects of chronobiology—chronopharmacology, chrononutrition, chronopathology, chronotoxicology, etc.—is presented in great detail in the following chapters. With rare exception, it is no longer appropriate to subjectively evaluate the parameters of rhythms through the use of chronograms (graphs of data over time) alone. Several appropriate, objective statistical techniques specifically designed for chronobiologic experimentation are readily available.

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