RECENT ADVANCES IN MITOCHONDRIAL MEDICINE AND COENZYME Q_{10}

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This book is intended for general medical practitioners and specialists in various fields of medicine, including neurologists, cardiologists, geneticists, nephrologists, diabetologists, lipidologists, immunologists, oncologists, pharmacists, pharmacologists, biochemists and nutritionists. Pre- and post-graduate education physicians, pharmacists and biochemists will also benefit from materials covered in this monograph.

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RECENT ADVANCES IN MITOCHONDRIAL MEDICINE AND COENZYME Q_{10}

NOVA

Chapter 4

CHRONOBIOLOGY OF MITOCHONDRIA

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ABSTRACT

Biological rhythms are the essence of life. They cover a wide range of frequencies, among which circadian rhythms have gained great interest in view of their ubiquity, partly endogenous nature now firmly documented at the molecular level, and wide-ranged involvement in health and disease. Circadian rhythms in organisms are synchronized by the lighting regimen and the feeding schedule. They are primarily orchestrated by a small brain area of roughly 20,000 neurons situated in the suprachiasmatic nuclei, while clock genes reside in almost every cell. Estimating quantitatively circadian (and other) rhythm characteristics is important in relation to both diagnosis and treatment since changes occurring during the development of a disease condition may affect the amplitude and/or phase as well as the average value and all rhythm parameters may be affected by treatment. After briefly examining circadian rhythms related to mitochondria, notably mitochondria of the heart and brain, we review early work on circadian, ultradian and infradian variation (components with a frequency higher or lower than one cycle per day, respectively). Consideration is given to the role of mitochondria in cellular metabolism, which is tightly coupled to circadian clocks.

Keywords: cellular metabolism, chronobiology, circadian, clock genes, feeding schedule, free-running, infradian, lighting regimen, mitochondria synchronizer, ultradian

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4.1. INTRODUCTION TO CHRONOBIOLOGY

In addition to spatial organization, the temporal organization of all living organisms in time is very important. Biological rhythms are periodically recurring changes in the intensity and nature of biological processes and phenomena [1]. Biological rhythms are inherent to most if not all living organisms in one form or another and are noted at all levels of biological organization, from intracellular processes, tissues, organs and organ systems to the individual, populations, and the biosphere [1, 2].

Biological rhythms also span a broad frequency range, periods varying from milliseconds (activity of single neurons), seconds (heart beat and respiration), hours (about 90-minute basic rest-activity cycle), and days (circadian, circaseptan, and circatrigintan) to years and even decades [2]. Among them, the circadian rhythm plays a special role ever since its partly endogenous nature was placed on a solid molecular basis and core clock genes were shown to be implicated in major disease conditions [3].

For instance, circadian rhythms have been documented for the number of sex hormone receptors in the cytoplasm and the nuclei of hepatocytes in rats [4], the cytoplasmic glycogen volume and the axial ratio in mitochondria of rat hepatocytes [5], the volume of cytoplasm of acinar cells in rats [6], the mitotic index in the epithelium of the esophagus [7], and bioluminescence in Gonyaulax polyedra, known to be controlled by proton transfer from an acidic vacuole system to the scintillons [8].

4.1.1. Definition of Chronobiology

Etymologically, chronobiology comes from the Greek “chronos” (time), “bios” (life), and “logos” (science). Chronobiology is a computer-aided objective quantification, mapping and investigation of mechanisms underlying biological rhythms, the fundamental mechanisms of life [9]. Its primary raison d’être is to replace a homeostatic view of physiology by an approach that accounts for rhythmic variation. In so doing, reference values can be refined by entering inside the conventional physiological range where earlier abnormalities of dynamic changes, such as the amplitude and phase of rhythmic variations, serve as additional endpoints to signal a heightened disease risk. When rhythms are mapped using inferential statistical methods, blunders can also be avoided by recognizing that physiological variables do not assume the same value at all times, and that a given intervention may lead not only to a difference in mean value but also to a difference in amplitude, phase, and/or period [9, 10].

4.1.2. Circadian Rhythms

Circadian rhythms were often referred to as 24-hour periodicities, found as if by accident, when investigators noticed consistent changes throughout the day while testing biological variables in blood or serum and physiological variables such as temperature or blood pressure. Early investigations by Franz Halberg studied the number of blood eosinophils at different times of the day in mice [11] and men [12]. It was not until around 1959 that the word circadian started to replace 24-hour periodicity. Franz Halberg wrote in 1959: "in discussions with Professor William McDonald (and others) of the Department of Classics at the University of Minnesota, the term 'circadian' was derived from 'circa' (about) and 'dies' (day); it may serve to imply that certain physiologic periods are close to 24 hours, if not of exactly that length." [13].

The partly endogenous nature of circadian rhythms was first documented, statistically validated and quantified by objective numerical measures of the uncertainty of its characteristics, by Halberg in blinded mouse models [14, 15]. In blinded C mice and in mice born anophthalmic, the circadian period of rectal temperature deviated slightly but statistically significantly from 24 hours and from the period of control mice. Halberg's experiments on mice established the phenomenon of free-running.

They also documented the critical role of the eyes as transducers for the primary environmental synchronizer, the alternation between light and darkness. In other experiments, Halberg showed that the timing of circadian rhythms could be manipulated by changing the lighting regimen (i.e., the times when lights went ON and OFF in the experimental laboratory) [14]. For this reason, the time of light onset is often used as reference time. Known to many as "Zeitgeber" time, referring to it as "Hours After Light Onset (HALO)" time is preferred since the lighting regimen acts as a mere synchronizer of circadian rhythms rather than as a "Zeitgeber" or "time giver." When animals have free access to food and water *ad libitum*, circadian rhythms are mostly synchronized by the lighting regimen. If food access is restricted during a portion of the day, this information needs to be specified as the feeding schedule is also a strong synchronizer of circadian rhythms [14-18].

The role of the lighting regimen as a synchronizer of circadian rhythms was very useful in many chronobiological studies which relied on staggered lighting regimens of 12 hours of light alternating with 12 hours of darkness (LD12:12), Figures 4.1a-b [14, 16]. Typically, prior to the start of study, a marker variable such as temperature is automatically monitored around the clock from animals in each room to make sure their circadian system has adjusted to their respective LD12:12 regimen. Circadian variation can then be obtained based on measurements taken during regular office hours from animals in each room. Caution needs to be taken, however, not to turn lights on when handling animals during the dark span, as this will disturb the circadian system (introducing phase shifts and reducing melatonin). Study designs based on two, three, or

even six lighting regimens staggered by 12, 8 or 4 hours, respectively, were particularly useful to determine rhythmic changes in the susceptibility-resistance of animals to different anti-cancer drugs and to assess the circadian stage-dependent effect of other interventions [19]. Indeed, apart from the spontaneous rhythms, the response to a given stimulus applied under standardized controlled conditions of the laboratory also changes predictably depending on the circadian stage at which it is administered [15].

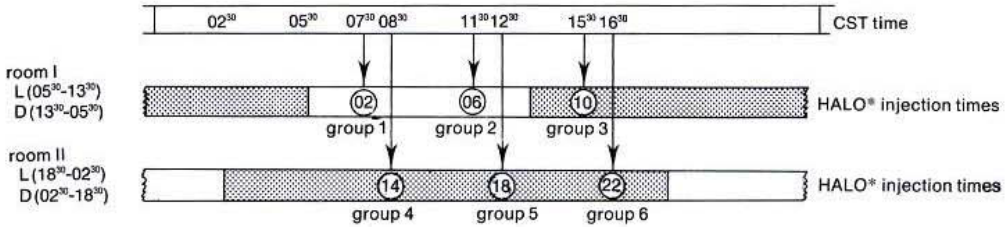

Figure 4.1a. Example of staggered lighting regimens consisting of 8 hours of light alternating with 16 hours of darkness. Six groups of animals are treated between 07:00 and 17:00 at one of 6 different circadian stages (at 2, 6, 10, 14, 18, and 22 Hours After Light Onset, HALO). From [16] (with permission).

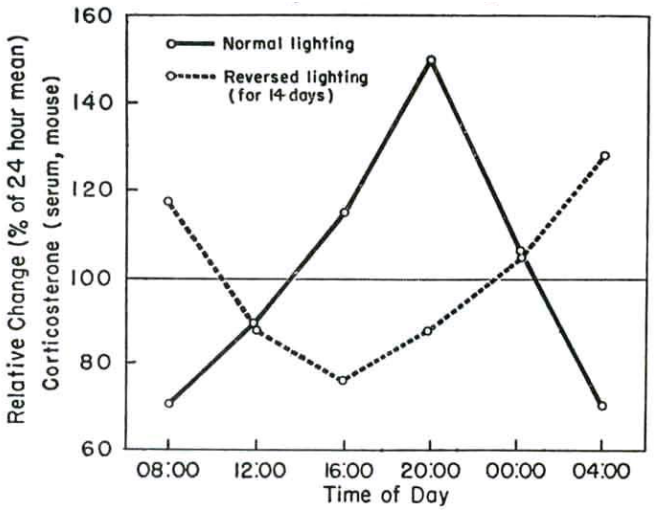

Figure 4.1b. Shift in phase of circadian rhythm in serum corticosterone in female 15-month old D_8 mice after inversion of lighting regimen. From [14] (with permission).

4.1.3. Core Clock Genes Underlying Circadian Rhythms

Circadian rhythms are primarily orchestrated by a small brain area of roughly 20,000 neurons situated in the hypothalamus, directly above the optic chiasm, known as the suprachiasmatic nuclei (SCN). When both suprachiasmatic nuclei are destroyed, the circadian rhythm in telemetered temperature from freely moving animals exhibits a great

amplitude reduction and a circadian acrophase advance [20, 21]. The SCN receive input from specialized photosensitive ganglion cells in the retina via the retinohypothalamic tract. Neurons in the ventrolateral SCN have the ability for light-induced gene expression. Melanopsin-containing ganglion cells in the retina have a direct connection to the ventrolateral SCN via the retinohypothalamic tract, where the signal allows synchronization of an organism's circadian rhythms to the environmental light-dark cycle [22]. The circadian rhythm in the SCN is generated by a gene expression cycle in individual SCN neurons. This cycle has been well conserved through evolution and in essence is similar in cells from many widely different organisms that show circadian rhythms [22].

Core circadian "clock" genes are defined as genes whose protein products are necessary components for the generation and coordination of circadian rhythms. Evidence for a genetic basis of circadian rhythms at the molecular level in higher eukaryotes started with the discovery of the period (per) locus in *Drosophila melanogaster* in 1971 [23]. Analysis of *per* circadian mutants and additional mutations on *Drosophila* clock genes led to a model consisting of positive and negative autoregulatory feedback loops of transcription and translation. Similar models were later described in mammals and other organisms [24-26].

In mammals, including humans, the majority of identified clock components are transcriptional activators or repressors that modulate protein stability and nuclear translocation, and create two interlocking feedback loops [27]. In the primary feedback loop, CLOCK and BMAL1 heterodimerize in the cytoplasm to form a complex that, following translocation to the nucleus, initiates transcription of target genes such as the core clock genes "period" genes (PER1, PER2, and PER3) and two cryptochrome genes (CRY1 and CRY2). Negative feedback is achieved by PER:CRY heterodimers that translocate back to the nucleus to repress their own transcription by inhibiting the activity of the CLOCK:BMAL1 complexes [28]. Modern experimental approaches using systems biology have identified many novel components in biological clocks that suggest an integrative view on how organisms maintain circadian oscillation [29], but this is a topic beyond our scope herein.

Studies in cyanobacteria suggested the presence of an alternative mechanism underlying circadian rhythms, since these single-cell organisms can maintain accurate 24-hour timing in the absence of transcription. In other words, there is no requirement for a transcription-translation autoregulatory feedback loop for circadian rhythms to be manifested [30]. Circadian rhythms in cells lacking a nucleus, such as human red blood cells, also exist in the absence of transcription or genetic circuits, and therefore in the absence of a feedback loop [31]. Importantly, redox oscillations as demonstrated by peroxiredoxin rhythms have been described in different organisms covering the evolutionary tree, from eukaryotes to bacteria and archaea, suggesting that redox "clocks" may have preceded genetic feedback circuits, and that the latter may represent

the major output mechanisms to coordinate cell and tissue physiology and behavior [32, 33].

As noted above, a key feature of circadian rhythms is their ability to synchronize to external stimuli. The presence of cell autonomous oscillators in almost every cell in the body raises the question of how these oscillators are temporally coordinated. Major synchronizers of peripheral clocks in mammals include feeding, temperature, and oxygen. Feeding schedules and temperature cycles can synchronize peripheral clocks and even uncouple them from the SCN [34].

4.1.4. Estimating Circadian Rhythm Characteristics

The simplest model representing changes recurring every day relies on the trigonometric sine and cosine functions, Figure 4.2. Such a model can be fitted to data by least squares, the principle underlying Halberg's cosinor method [35]. Designed for the analysis of short and sparse time series focused on circadian rhythms, the cosine curve was selected so that light onset (0 HALO) or 00:00 could be equated to 0°. The model is characterized by four parameters: the MESOR (M, Midline Estimating Statistic Of Rhythm, a rhythm-adjusted mean), the amplitude (A, a measure of half the extent of predictable change within a cycle), the acrophase (ϕ, a measure of the timing of overall high values recurring in each cycle), and the period (τ, the duration of one cycle).

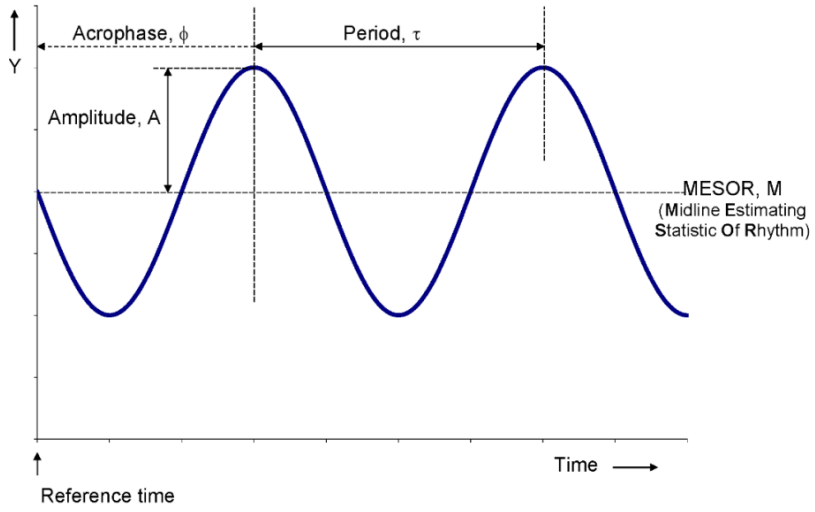

Figure 4.2. Definition of rhythm characteristics. The MESOR is a rhythm-adjusted mean; the double amplitude (2A) is a measure of the extent of predictable change within a cycle; the acrophase is a measure of the timing of overall high values recurring in each cycle, expressed in (negative) degrees in relation to a reference time set to 0°, with 360° equated to the period length; and the period is the duration of one cycle. © Halberg Chronobiology Center.

Often, in the case of circadian studies, the period can be anticipated to be 24 hours (or very close to 24 hours). In this case, regular least squares can be used to test the statistical significance of the rhythm and to estimate M, A and ϕ, with a measure of their uncertainties. In order for the acrophase to move clockwards, it needs to be expressed in negative degrees (with 360° ≡ 24 hours). As the cosinor method is applied to denser data and longer records, more complex signals can be modeled by the addition of harmonic terms, and additional time series analyses can be performed [36, 37].

4.2. Chronobiology of Mitochondria

Mitochondria are double membraned organelles found in the cells of all eukaryotic organisms. Eukaryotic cells (in single-cell and multiple-cell organisms) contain a defined nucleus (with a nuclear membrane surrounding the nucleus) and chromosomes, along with other organelles, each one with specific tasks to be performed in the cell. Mitochondria can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP). ATP is a small molecule used in cells as a coenzyme and a nucleotide used in the formation of DNA and RNA. It is often referred to as the "molecular unit of currency" of intracellular energy transfer that powers the cell's metabolic activities [38]. Much was known about the mitochondrion as an organelle before its time structure was determined. Both circadian and circaseptan (about-weekly) variations in ATP have been documented [39].

In a series of experiments using staggered lighting regimens of 12 hours of light alternating with 12 hours of darkness, Gvozdjáková examined the circadian behavior of mitochondria in different organs [40-42]. Her team focused on the assessment of circadian rhythms of mitochondrial oxidative phosphorylation (OXPHOS) and coenzyme Q_{10} (CoQ_{10}) in isolated mitochondria of the heart, brain and liver of experimental animals. Both 24-hour and 12-hour components were quantified based on samples collected at 6 different circadian stages, 4 hours apart. Two LD12:12 regimens staggered by 12 hours in two separate rooms were used in these experiments. In one room, lights were on from 10:00 to 22:00 and off from 22:00 to 10:00; an inverse lighting regimen was used in the other room.

4.2.1. Chronobiology of Myocardial Mitochondria

Cardiac function relies on a time varying supply of oxidative energy produced by mitochondrial respiration. Mitochondrial dysfunctions in the heart muscle are associated with both structural and functional abnormalities [43]. Ablation of the circadian clock gene *Bmal1* specific to the heart has been shown to result in cardiac mitochondrial

defects, such as reduced enzymatic activities within the respiratory complex. Mice lacking cardiac *Bmal1* function show decreased expression of genes associated with the fatty acid oxidative pathway, the tricarboxylic acid cycle, and the mitochondrial respiratory chain in the heart, and they develop severe progressive heart failure with age [43]. It has also been shown that similar changes in gene expression related to mitochondrial oxidative metabolism occur in C_{57}BL/6J mice subjected to chronic reversal of the light-dark cycle; therefore, they show disrupted circadian rhythmicity. These findings indicate that the circadian clock system plays an important role in coordinating mitochondrial metabolism [43].

4.2.2. Circadian Variation of OXPHOS and CoQ_{10} in Myocardial Mitochondria

ATP production in the mitochondrial respiratory chain in heart muscle and CoQ_{10} concentrations vary along the 24-hour scale in isolated mitochondria of the heart muscle of rats, as shown by Gvozdjáková et al. [40]. Changes in OXPHOS and CoQ_{10} myocardial mitochondria may be involved in the pathophysiology of the heart muscle. A low CoQ_{10} concentration and an impaired OXPHOS in heart mitochondria are considered to be a molecular basis for heart failure and can trigger an acute myocardial infarction, cardiac arrest, or stroke, all of which can end in sudden death [44]. Chest pain associated with acute myocardial infarction is more prevalent in the second quarter of the day [45]. In translating results from the experimental laboratory to humans, it should be noted that most circadian rhythms in diurnally-active humans have an opposite phase as compared to nocturnally-active experimental animals (rats).

Mitochondrial respiration takes place in the inner mitochondrial membrane in heart cells through a series of metabolic reactions using oxygen and nutrients in the cell to produce adenosine triphosphate (ATP), the source of energy in the cells. About 90% of the ATP in the heart is produced from these oxidation-reduction reactions in the mitochondria, with the mitochondria consisting of 20 to 40% of the cardiomyocyte volume [40]. A fat-soluble substance, also in the mitochondria, that participates in the cellular respiration process, generating energy in the form of ATP is CoQ_{10} [46]. The heart, along with the liver and kidney, are three of the organs in the body with the highest energy requirements; therefore, these organs have the highest concentrations of CoQ_{10} [47]. The function of the mitochondrial respiratory process in the heart muscle shows circadian variation in ATP production and in CoQ_{10} concentration [44].

In Gvozdjáková's experiments, focus was placed on the 5 complexes of the mitochondrial respiratory process in the inner mitochondrial membrane. In particular, the circadian time structure of complex I (NADH dehydrogenase-ubiquinone oxidase) and complex II (succinate dehydrogenase-ubiquinone oxidase) was examined. The 24-hour

and 12-hour components of isolated mitochondria in cardiac muscle of rats were estimated. The model for complex I and II shows two maxima and two minima within a 24-hour cycle.

The function of OXPHOS can be evaluated in terms of several parts for complex I and complex II. The parts are S_3 (S_3 state; ADP-stimulated ATP production); S_4 (State S_4; basal respiration of mitochondria); OPR (oxidetive phosphorylation rate; the rate of ATP production); ADP:O (the coefficient of oxidative phosphorylation, which reflects coupling of oxidation and energy production); and RCI (the respiratory control index, which reflects the integrity of the mitochondrial membrane). These parts for complex I and II, as well as CoQ_9 and CoQ_{10} are circadian stage-dependent. The phases of maxima and minima of the fitted model (24-hour and 12-hour components) characterize the "circadian cascade of oxidative phosphorylation" and the "biological clock of CoQ_{10}-CLOCK and Q_{10}-CLOCK."

Gvozdjáková and her team [40-42] reported maximal values of the OXPHOS parts for complex I to occur during rats' inactivity and lasting 5 hours and 20 minutes, whereas during rats' activity, they only lasted for 2 hours and 32 minutes. The opposite was true for complex II: during rats' inactivity, they lasted only 2 hours and 47 minutes, but during their active span, they lasted 6 hours and 12 minutes. These results suggest that the respiratory chain of mitochondria could form super-complexes (complex I and II) in which activity of either complex I or complex II of OXPHOS is almost always high.

A statistically significant circadian variation was reported only for CoQ_{10} (but not for CoQ_9) in myocardial mitochondria of control animals.

Circadian characteristics contribute to the understanding of the pathogenesis of altered cardiac function, such as the triggering of acute myocardial infarction [40, 44]. The "Q_{10}-CLOCK" in the mitochondria may play a key role in the regeneration of the mitochondrial membrane and for re-energizing cardiac mitochondria.

In other studies, a number of components in the electron transport chain/OXPHOS in murine heart mitochondria have been found to exhibit a circadian peak around the mid-daylight hours, during the rats' resting span [43]. When cardiomyopathy was induced with phenylephrine, the circadian rhythms were reportedly diminished in amplitude [43].

4.2.3. Circadian Brain Mitochondrial Cascade of OXPHOS and CoQ_{10}

Circadian variations in mitochondrial OXPHOS cascade parameters were also reported in brain [44]. A two-component model consisting of cosine curves with periods of 24 and 12 hours was used to estimate the timing of the two maxima and two minima occurring along the 24-hour scale for complexes I and II, as reported elsewhere [44].

Circadian variation of the respiratory chain and of the "biological Q_{10}-CLOCK" may contribute to the understanding of the pathogenesis of altered brain function and of

mechanisms underlying the trigger of acute cerebral infarction. Mitochondrial dysfunction has a fundamental role in neurology, as the majority of patients with mitochondrial disease have some degree of neuropathology and this is usually degenerative in nature [48].

Damage to brain mitochondria at the metabolic level may be associated with age. Pathogenesis of mitochondrial disease involves damage of ATP, which is essential for life, apoptosis, production of reactive oxygen species, and for calcium physiology. Damage of brain mitochondrial oxidative phosphorylation and genetic disorders have a major impact on the dynamics of mitochondria, such as mobility, fission and fusion, and distribution. In the nervous system, the dynamics of mitochondria are essential for long-distance energy distribution.

Astrocytes in the SCN display circadian rhythms in clock gene expression and extracellular accumulation of ATP. ATP release from astrocytes is a calcium-dependent process, and intracellular Ca^{++} concentrations fluctuate in an antiphase relationship with rhythmic ATP accumulation in cultures of immortalized rat suprachiasmatic nucleus cells (SCN2.2). Mitochondrial Ca^{++} rhythms were reported to be in almost exact antiphase with the peak in cytosolic Ca^{++}. Given the calcium-dependent nature of ATP release, mitochondrial Ca^{++} seems to be integral to SCN rhythms [49].

Circadian fluctuations in ATP accumulation in the SCN have also been reported [49], exhibiting an average period of 23.7 hours. ATP concentrations in the rat SCN *in vivo* were marked by rhythmic variation during LD12:12 exposure or constant darkness, with peak accumulation occurring during the latter half of the dark phase or subjective night [49].

4.3. CHRONOBIOLOGY: BROAD TIME STRUCTURE CHARACTERIZING MITOCHONDRIA

The importance of circadian rhythms in relation to mitochondria is apparent from experiments by Gvozdjáková and her team, and from studies published by others. Energy metabolism and mitochondria have been discussed with respect to their role in the circadian rhythm mechanism for some time. Chronobiological aspects of mitochondria were studied at least since the 1960s. Both circadians and ultradians (variations with a frequency higher than one cycle in 20 hours) were reported. The prominent circadian rhythms involved in metabolism are important to ensure that development, survival, and reproduction remain synchronized to environmental changes along the 24-hour scale.

4.3.1. Early Work on Circadian Rhythms Related to Mitochondria

Circadian variations in mitochondria were investigated by Heinz von Mayersbach, Karl Philippens, and their team as they used succinate dehydrogenase (SDH) as a marker enzyme to visualize mitochondria histochemically [50]. Using liver preparations, they documented a strong circadian variation in SDH activity, topographical distribution, and reaction-product appearance [50]. Philippens also found that the circadian rhythm in SDH activity was strongest in the periportal region of the liver lobules [51-53]. Circadian rhythms in liver SDH activity, albeit with opposite phase, were found in male and female rats, males peaking during the activity span and females during their rest span [51-53]. In rats fed *ad lib*, Philippens showed that inverting the lighting regimen for 6 weeks shifted the circadian rhythm of α-glycerophosphate dehydrogenase (mGPDH) in isolated mitochondria in line with the shifted lighting regimen, while the circadian rhythm in SDH activity did not phase shift [54].

4.3.2. Ultradian Variations Related to Mitochondria

Ultradian variations were extensively studied by Lloyd and coworkers [55, 56]. Lloyd et al. [55] reported respiratory oscillations in continuous yeast cultures that could be accounted for by cyclic energization of mitochondria, dictated by the demands of a temperature-compensated ultradian clock with a period of about 50 minutes. Electron transport components (NADH and cytochromes c and c oxidase) reportedly showed redox state changes as the organisms cycled between their energized and de-energized phases [55]. Lloyd and Murray [56] conclude that cellular auto-dynamism may be a function of a large ensemble of excitable intracellular components and that self-organization in time and space may encompass mitochondrial, nuclear, transcriptional and metabolic dynamics, coupled by cellular redox state. Their work suggests that ultradians are a widely occurring necessity for the coordination and coherence of living processes, rather than a curiosity only found in yeast [56].

4.3.3. Circadian Clocks and Cellular Metabolism

Circadian rhythms in relation to mitochondria affect all fields of medicine. Their importance is apparent from numerous studies published in the areas of aging [57], brain [48], cardiac function [43], diabetes [58-60], metabolism [61-69], and mood disorders [70], among others.

Circadian clocks are tightly coupled to cellular metabolism [71] and respond to lighting and feeding cycles, as originally shown by Franz Halberg [72]. Meal timing affects both circadian rhythms and metabolism [72, 73]. Caloric restriction achieved by means of intermittent energy restriction or time-restricted feeding reportedly forestalled and even reversed disease processes such as various cancers, cardiovascular conditions, diabetes, and neurodegenerative disorders [74].

Mitochondria are believed to play an integral part in these processes [74]. Nicotinamide adenine dinucleotide (NAD), involved in energy metabolism, may be involved in the interaction between the transcriptional/post-transcriptional delayed feedback loop and a complementary non-transcriptional-transcriptional coordination mechanism underlying the circadian system [75]. Numerous examples of inhibitors that affect the mitochondria of plants and animals and microorganisms are known, which cause large phase shifts in the rhythms of these organisms [76]. Evidence has recently been provided for self-sustained circadian oscillations of the hyperoxidation of the mitochondrial Peroxiredoxin, PrxIII, and cytosolic release of mitochondrial H_2O_2, which may constitute one biochemical output coupling metabolic changes and transcriptional-based core clocks [77].

4.3.4. Circadian Rhythms as Indispensable Control: Methodological Considerations

Establishing circadian rhythm characteristics in control animals is useful since they can then serve as reference for comparison of animals with different disease conditions and for testing the efficacy of different treatments, such as the supplementation with CoQ_{10} [9]. For such comparisons to yield useful information, circadian rhythm characteristics need to be estimated with a measure of uncertainty. The ranges in the timing of maxima and minima of different parts of the electron transport chain listed in studies by Gvozdjáková and her team [40-42, 44], however, are only point estimates. Such timings are associated with a fair amount of uncertainty, which depends on several factors: the rhythm's prominence, the sample size (number of timepoints and number of animals per timepoint), and the amount of "noise." The latter is contributed by several sources, including the amount of rhythm disturbance related to environmental conditions.

In experiments by Gvozdjáková and her team, animals were housed in two different rooms kept on opposite LD12:12 lighting regimens. Lights were on from 10:00 to 22:00 and off from 22:00 to 10:00 in one room, while in the other room, they were on from 22:00 to 10:00. Since animals do not adjust instantaneously to a new lighting regimen, it is recommended to rely on a marker rhythm such as rectal temperature to make sure that animals have fully adjusted to their new routine before starting an experiment. This takes a minmum of one week, but in some experiments, up to 3 weeks were allowed for the

animals to adjust before starting the study. Caution also needs to be taken not to disturb the animals once the study has begun. Turning lights on during the dark span can shift the phase of circadian rhythms. Usual noise from staff can also disturb the animals, and can provide them with unwanted clues about the 24-hour routine of workers in the laboratory. Such disturbances may have contributed to a larger-than-expected 12-hour component in the experiments by Gvozdjáková et al. [40-42, 44].

4.3.5. Infradian Variations Related to Mitochondria

The presence of intact food anticipatory activity in suprachiasmatic nucleus-ablated rodents or those lacking functional circadian oscillator genes [74] points to yet unidentified genes and circuits in eating pattern determination. It has been proposed that circadian coordination may be achieved by means of a distributed, decentralized system of oscillators, with contribution in gain setting by the metabolic hormones ghrelin and leptin [78]. The SCN may also be involved in the coordination of other-than-circadian rhythms, as suggested by a circaseptan amplification in dentin accretion after ablation of the SCN in Wistar rats [79].

Much evidence already supports the partly endogenous nature of circaseptan rhythms, which are most prominent in relation to growth, development, and repair [80]. Pertinent to the topic of this chapter, as demonstrated for the irradiation of four different kinds of tumor cells in culture, the about 7-day changes in relative ATP concentration were much larger than the circadian variation, higher tumor kill being achieved by irradiation at time of maximal β-ATP [39]. These results open a new frontier for the optimization of treatment by timing (chronotherapy) according to circaseptan as well as circadian rhythms. Both components have already been implicated in various disease conditions, such as cardiovascular diseases, diabetes, immunology, and oncology [10].

4.3.6. Broad Chronobiological Applications for Diagnosis and Treatment

Most physiological variables, including those related to mitochondria, are neither constant nor varying randomly. Rather, they follow rhythms with known periods, which render their variation predictable to some extent, the more so the larger their amplitude and the lesser the superimposed random variation. The rhythmic nature of these variables has important implications concerning the definition of reference values for screening and diagnostic purposes. First, the same value may be too high, acceptable, or too low depending on the time of its determination, Figure 4.3. The physiological range can thus be refined by accounting for rhythms. Second, abnormality does not need to be restricted to the mean value, as the amplitude, phase, and/or period of a rhythm may also change in

the presence of disease or pre-disease [2, 3]. Screening for changes in all rhythm parameters is useful to diagnose disease earlier, thereby providing the opportunity to institute countermeasures earlier.

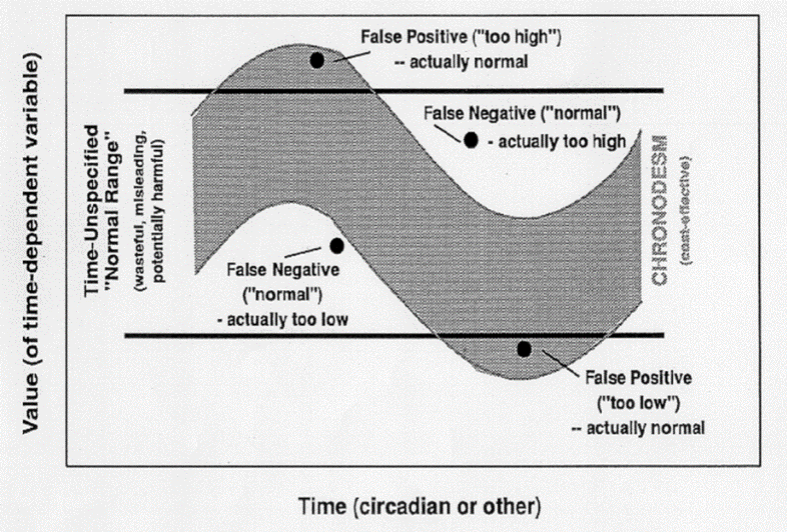

Figure 4.3. The same determination can be too high, acceptable, or too low depending on when it is taken. © Halberg Chronobiology Center.

The effect of external stimuli on physiology is also circadian stage-dependent [15]. This means that treatment can be optimized by timing according to rhythms. The aims of chronotherapy are two-fold: maximizing treatment efficacy and minimizing negative side effects. Chronotherapy targets effects on all rhythm parameters in an attempt to restore healthy rhythmic patterns. For instance, it is common to find disease conditions associated with a dampened circadian variation, while a more robust circadian system reflects good health [3]. Optimal treatment timing is therefore best implemented individually, where chronotherapy is determined based on the chronodiagnosis in an approach known as chronotheranostics [10].

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