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Chronobiology: principles and methods

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Summary. - Chronobiology is a discipline whose principles consider time as an essential dimension of biological phenomena. The reason of this resides in a relativistic vision of biological dynamism for which space, mass, energy and time are properties of dynamic events. Biological time may be linear (chronological time) and cyclical (period time). Chronobiology considers the biological events whose expression is periodic with several kinds of periodicities (biological rhythms). Because of bioperiodic events, a methodology for approaching cycling functions is needed. Chronobiometry is illustrated as an important part for understanding how biological rhythms can be investigated.

Key words: biological clocks, biological rhythms, chronobiology, chronobiometry, desynchronization, synchronization.

Riassunto (*Cronobiologia: principi e metodi*). - La cronobiologia è una disciplina che considera il tempo come una dimensione essenziale della materia vivente. Questa concezione nasce dalla visione relativistica del dinamismo applicata agli eventi biologici. Il tempo biologico è sia lineare (tempo cronologico) che ciclico (tempo periodico). La cronobiologia prende in considerazione gli eventi biologici ciclici con diverse frequenze (ritmi biologici). Per analizzare i ritmi biologici occorre una metodologia specifica di analisi cronobiometrica. La cronobiometria viene illustrata in quanto essa costituisce il mezzo essenziale per comprendere l'approccio temporale ai fenomeni ciclici della materia vivente. .

Parole chiave: cronobiologia, cronobiometria, desincronizzazione, orologi biologici, ritmi biologici, sincronizzazione.

Chronobiology as a scientific discipline

A discipline can be called «scientific» if dealing with a field of natural phenomena that are objective and measurable, possibly not causal but systematic. Chronobiology fulfills these requirements as it explores biological rhythms which are an integral part of the organization of living matter. As a matter of fact, the speculative objective of chronobiological investigation is the rhythmic repetitivity of biological events along both phylogenetic (paleochronobiology) and ontogenetic (neochronobiology) lines.

Chronobiology can be defined as «the scientific discipline that quantifies and explores the mechanisms of biological time structure and their relationship to the rhythmic manifestations in living matter».

It must be noted that since biological rhythms are genetically transmitted, these phenomena necessarily have an inherited character. This implies that the periodic time is a counivocous parameter of living functions, which cannot be neglected, as it is in much of the traditional biomedical thought. Scientists are aware of the fact that living species live and act in time. The concept, therefore, of cyclic biological time is not completely extraneous to scientific doctrine. Traditional biology, however, considers time as an implicit quantity, relegating it a role of implicit factor.

Understanding the message of chronobiology requires renunciation of the concept of «homeostasis», as well as a reformulation of the biological principles dictated by C. Bernard. Chronobiology holds that the physiology of vital functions does not answer to the laws of steady state, invariance, or the unconditional return to the initial equilibrium following a perturbation. Conversely, the rhythmic recurrence of biological processes substantiates the idea that living matter is subjected to a continuous variation in state. Accordingly, biological rhythms represent a syntropic-entropic process with accumulation and dissipation of vital energies. In bioperiodic machineries living matter finds the time necessary for the consumption and the reconstruction of organic materials.

Classification of biological rhythms

Biological rhythms can be classified according to numerous criteria.

Physical classification

This classification is based on the length of the period of oscillation. Table 1 details this repartition.

Table 1. - Temporal classification of biological rhythms

Domain	Period (Tau)
Ultradian	< 20 h
Circadian:	24 ± 4 h
dian	24 ± 0.2 h
Infradian:	> 28 h
circaseptan	7 ± 3 d
circadiseptan	14 ± 3 d
circavigintan	21 ± 3 d
circatrigintan	30 ± 5 d
circannual	1 y ± 2 m

h = hours; d = days; m = months; y = year

The rhythms whose period of oscillation is 24 ± 4 h are defined as «circadian» (from circa dies, i.e., approximately one day). The cyclic events with a period of less than 20 h and more than 28 h are defined respectively as «ultradian» and «infradian».

Functional classification

Besides the physical classification there exists a subdivision based on functional concepts that recognizes four varieties of biological rhythms, i.e., alpha, beta, gamma and delta.

The alpha rhythms coincide with the spontaneous oscillation of biological functions. Alpha rhythms are subdivided into alpha(s) and alpha(f) according to whether they are produced in conditions of «synchronization» or «free-running» (see below). The beta rhythms correspond to the periodicity of the response of biological functions toward stimulations or inhibitions applied at different times. The beta rhythms as well exist in the varieties beta(s) and beta(f) in relation to the presence of either synchronization or free-running conditions. These two varieties are further subdivided into beta(sl) or beta(fl) if the perturbance is physiological, and alpha(s2) or beta(f2) if the perturbance is not due to a physiological event. Gamma rhythms regard the periodic oscillation of biological functions being modulated, perturbed, or influenced by deterministic factors, either physiological, i.e., gamma(sl) or gamma(fl), or non-physiological, i.e., gamma(s2) or gamma(f2). Here again, the differentiation into gamma(s) and gamma(f) varieties depends on the presence of either synchronization or free-running conditions. Lastly, delta rhythms, which are also subdivided into (s) and (f) varieties, correspond to the modification in the periodic oscillation of a given biological function secondary to manipulation of an alpha, beta, or gamma rhythm.

Mathematical classification

The examination of rhythmic phenomena in organic matter reveals that there exist events which repeat themselves after a certain lapse of time as isolated occurrences. These are the «qualitative, punctual, discrete, or episodic rhythms» expressed by a binary condition, i.e., present/absent, event/non-event. For example, the menstrual cycle.

Qualitative rhythms are mathematically describable in terms of finite quantities (0 or 1) and counted as numerical frequencies. Therefore, qualitative rhythms could also be called «frequential rhythms».

In living organisms it can be noted that several phenomena repeat themselves as entities which vary in a «continuum». In other words, the phenomenon is always present and measurable, even though changing as a function of time. Its magnitude reaches the same level following a given period of time. Therefore, the period of these phenomena is given by the space of time (duration) in which the curve reaches the identical level after a complete oscillation. These periodic events are thus a quantitative expression of their variability and can be identified as «analogic or continuous or quantitative rhythms». These rhythms are mathematically expressed by numerical values of a potentially infinite order.

From a classification point of view, there exists a third type of biological rhythm consisting in isolated peaks inscribed on the curve of a quantitative oscillation. Whether these spurts show a cadence in time, they can be defined «episodic rhythms».

Descriptive classification

This classification is used mainly for the description of episodic rhythms or when it is necessary to describe a continuous periodic event in relation to its peak. The rhythms included under this heading are diurnal, nocturnal, serotine, vesperal, morning, daily, weekly, monthly, seasonal, yearly, etc.

Note, however, that these terms define the periodicity only descriptively and do not lend to any inference on the effective duration of the period of the recurring phenomenon. Therefore, a diurnal rhythm is not implicitly circadian; it could be ultradian.

Evolutive classification

Biological rhythms, like all biological phenomena, undergo an evolutive process that tends to modify the periodic properties in function of chronological age. As shown in (Fig. 1), every periodic in function is defined by its mean level, extent of oscillation, and timing of oscillatory crest, these parameters

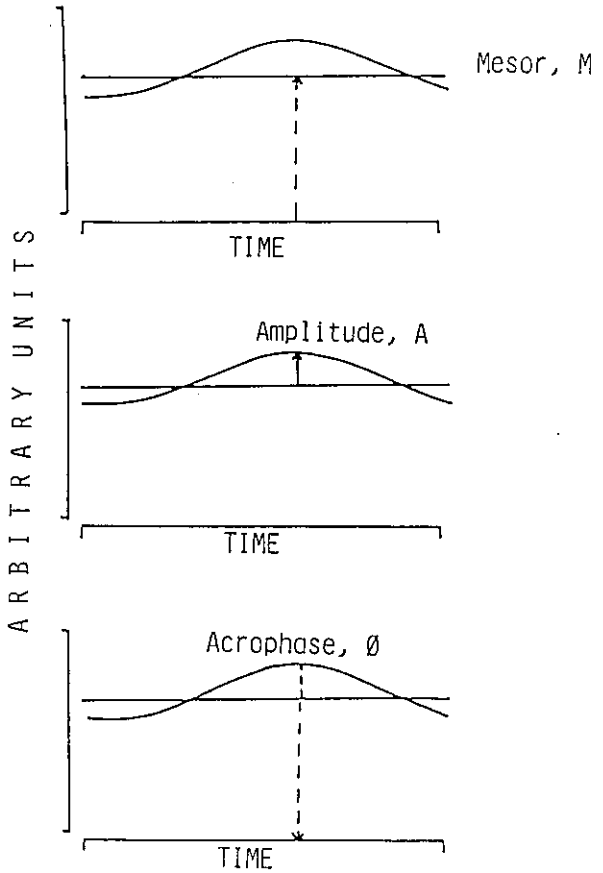


Fig. 1. - The rhythmometric properties of a biological oscillating function.

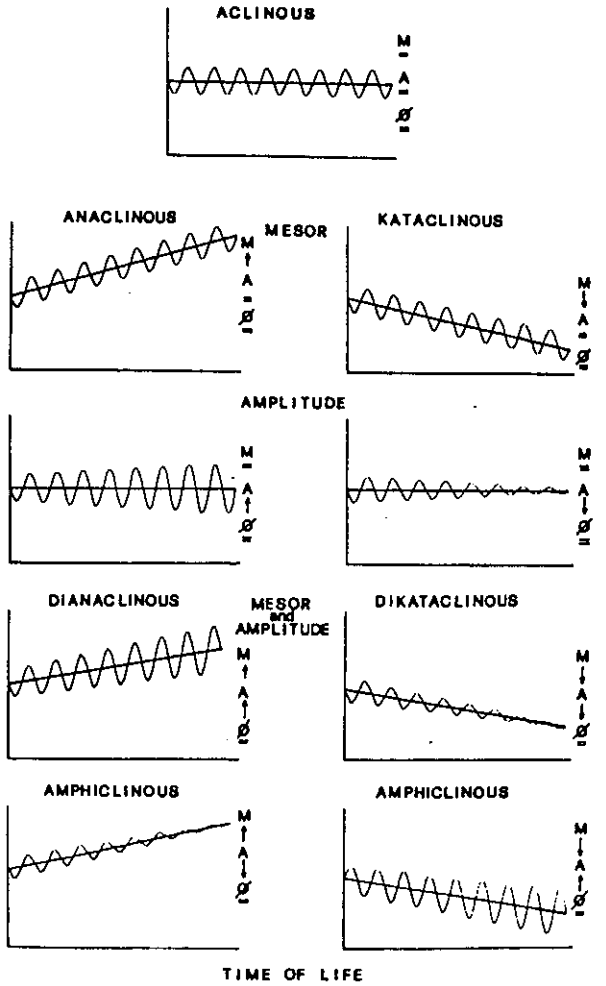


Fig. 2. - Age-related trends in rhythmometric properties of biological rhythms (clinospectroscopy).

being called respectively, mesor (M), amplitude (A) and acrophase (Π or phi).

Utilizing clinospectror analysis (see below), both positive and negative trends (clinous) have been identified for mesor and amplitude as an effect of age (Fig. 2).

Therefore, there are «dianaclinous» or «dikataclinous» rhythms if both properties have a positive or negative trend during the course of life. Rhythms can also be defined as «mesor-anaclinous» or «mesor-kataclinous», and «amplitude-anaclinous» or «amplitude-kataclinous», if the evolution through chronological time involves only one of the parameters in either a positive or negative sense. There is also the possibility of a trend opposite for the two rhythmic properties defined as an «amphiclinous» rhythm. Finally, there can be an «aclinous» rhythm which is a rhythm that shows itself to be stable even though the age increases.

Duration classification

Observing rhythmic biophenomena it can be ascertained that some of these are «permanent or long-lasting» while others are «transitory or temporary». The ovarian cycle is a typical «transient» rhythm because it disappears with menopause. The rhythm of body temperature is instead a permanent rhythm that is found even in the cadaver in the first 24 h following death.

Physiological classification

In considering biological rhythms we cannot neglect the important role they play in the economy of vital functions. In this regard it must be kept in mind that there are rhythms which are «essential» or «vital», and rhythms which are «non-essential». The essential rhythms are the pulsatile activity of heart, respiration, and cerebral electrical activity. The suppression of one of the first two coincides with «physical death». The lack of the electroencephalic rhythm, as is seen in the flat electroencephalogram, defines so-called «clinical death».

It can be derived from the aforesaid that the essential rhythms represent biological life. Death coincides with the abrogation of these fundamental rhythms. From this perspective it is seen that life and biological rhythmicity are a counivocous expression.

Non-essential rhythms are those rhythms whose abolition or desynchronization has no repercussion on vital functions. Their lack can, however, contribute to the development of a primary pathology (protochronopathology). The abolition of non-essential rhythms can be secondary to a given disease (deuterochronopathology).

Biological classification

Examined under the profile of their meaning, biological rhythms may be found in two very important aspects of life, (i.e., the conservative and reproductive functions). The rhythms of the conservative sphere are, in turn, mental and physical. These two categories can be further identified as intellective, affective, endocrine, cardiovascular, metabolic, respiratory, digestive, etc. Reproductive rhythms are, on the other hand, related to sexuality and fertility.

Resistance classification

In biology, the rhythmic manifestation of life have different values with respect to their robustness. There exist, in fact, «resistant» or «permanent» rhythms as well as bioperiodic events that are «weak» or «labile». The resistance of a rhythm depends on its role and to which system it belongs. Basically, resistance is inversely proportional to the susceptibility to be desynchronized following acute perturbations.

The lability of a rhythm is mostly dependent on its spontaneous or forced passage to a different order of periodicity (multiplication or demultiplication of frequency). The rhythm of the heart is said to be labile because it can easily vary in frequency over the 24 h span. Only rarely the lability of a rhythm is due to its abrogation. The abolition of a biological rhythm is an extremely unnatural event which is very improbable to occur. Therefore, the disappearance of a given rhythm must be carefully evaluated and it cannot be established without having verified that the rhythm has just and simply changed its period.

Ontogenetic classification

Biological rhythms are part of the genetic patrimony of living matter. The oscillators are located in each cell, at every level of the biological organization. The rhythms begin to act at the birth of the cell. In metazoes the cellular rhythms take part to a more complex and general rhythmicity whose expression requires coordination and maturation. Some rhythms of very high organized activities, thus, take a certain time for their postnatal ontogeny.

Rhythms in the formative stages are called «immature» rhythms, while those already operant at birth are defined as «mature» rhythms.

Structural classification

Biological rhythms are natural events which recur spontaneously, their periodic component being endogenous. The endogeneous contribution to periodicity can manifest itself freely (free-running rhythms) or it can be conditioned by environmental factors that act cyclically as synchronizers (synchronized or masked rhythms). The free-running rhythms, therefore, may be transformed into synchronized rhythms, and the endpoint of this interplay is a «masking effect» exerted by the exogenous component on the endogenous bioperiodicity. In nature, the overt manifestation for most biological rhythms is the combination of the endogenous component plus the exogenous entrainment. In this case, the masking effect results in a synchronized rhythm, and the external factors of masking can be defined «entraining agents» or «zeitgebers» or «synchronizers».

Importantly, the manipulation of an environmental synchronizer may cause a disturbance of the endogenous periodicity which results in a phenomenon of «external desynchronization». The dyschronic effect must be kept in mind when dealing with a biological rhythm lacking periodicity. This means that the abrogation of a given periodicity may be attributed to an exogenous mechanism being not primarily dependent on an intrinsic defect of internal rhythmicity.

Interestingly, the masking effect may be not only exogenous but also endogenous. The endogenous masking effect can be used to explain the complex conditions of rhythm loss not explainable in terms of cause and effect. For example, the loss of the sleep-waking rhythm produces an endogenous masking effect on numerous other rhythms causing their periodicity to be abrogated (see below).

Consistency classification

There are rhythms that regard one concrete entity, i.e., «real rhythms». Other rhythms are instead the mere expression of a computational parameter, i.e., «virtual rhythms». The nychtohemeral profile of cortisol rhythm, when studied in blood, coincides with the within-day variations of its concentration in plasma or serum. By contrast, the circadian rhythm of pH is caused by the interplay of numerous factors each one characterized by its own rhythm.

Constitutive classification

There are biological rhythms which refer to a single variable (something that is definable by its characteristics), i.e., «elementary rhythms», and others which attain to complex functions, i.e., «composite or factorial rhythms». Examples may be the circadian rhythm of prolactin, on one side, and the circadian rhythm of mood, on the other.

If a rhythm is found to be complex, its eventual abolition could be dependent on an internal desynchronization among the constituent cyclic factors or mechanisms. Sometimes, the aperiodicity is merely due to changes in phase resulting in an antiphasic oscillation of the cycles which contribute to the complex rhythm.

Hierarchical classification

In the magnificent organization of bioperiodic phenomena, it has been found that some rhythms play a prominent role in conditioning other biological cycles. These rhythms are called «guide or primary or independent rhythms» while the driven rhythms are called «guided or secondary or dependent rhythms». Guide rhythms have a strategic importance in the sense that their presence is essential for the dependent periodicity. The lack of a guide rhythm usually produces desynchronizing effects mostly due to the abrogation of the rhythmic interplay. The interruption of the relationship between primary and secondary rhythms causes a phenomenon called «internal desynchronization». The guided rhythms will be absent due to an induced effect. Such a dramatic repercussion is called «endogenous masking effect». The endogenous masking may help us in understanding and interpretating the chronopathology of some biological rhythms.

Genesis of biological rhythms

The capacity to undergo rhythmic oscillations is a characteristic intrinsic to living matter. A fundamental statement of chronobiology states «many rhythms persist even in complete isolation from the major known environmental cycles». This affirmation clarifies that the natural rhythms can be considered to lay outside of the period of the geophysical cycles. This means that living matter has its own time, i.e., the «biological time».

Assuming time as a fourth dimension of biology, one can conceptually and syllogistically argue that a chronome exists into the genome. Besides the physical (physemes) and chemical (chememes) signals, one can assume that the genes provide information also in the form of «chronemes», i.e., signals of periodic type. In such a way the process of clonation is timed by determined periods, and results in a combination of quantal and temporal messages which cause the biological functions to quantitatively change according to a programmed spectrum of periodicities. Speculatively, one could presume that the temporal signals find their periodic genesis within the helicoidal spirals of DNA where the chronome should reside. The DNA double helix could act as a metronome generating a vibration whose length is the period of clonation.

It has been suggested that the gene inherits not only the capacity to clone (ergon) but also the capacity to endure (chronon). The concept of chronon refers to the expression of genes as a function of the chronological time which is linear, irreversible and progressive. The concept of cronome relates to the expression of genes according to the chronobiological time which is cyclical, irreversible but recursive. Accordingly, the chronological time could be seen as the summation of the iterated periods which constitute the time base of biological rhythms.

Biological clocks and control of bioperiodic phenomena

Biological periodicities are driven by a genetic program to run according to a temporal duration (biotemporality) which causes a recursivity in a spectrum of frequencies ranging from milliseconds to years.

The temporal effect of genetic programming, the chronome, is the endogenous component for which the biological rhythms originate as «free-running» bioevents. The free-running rhythms reflect the «time of the body» which is independent from the environmental time measured by the clock, the «physical time». The free-running rhythms reflect the endogenous mechanisms of cyclic temporization whose expression is morphologically seen as an internal clock, a «biological or body clock».

Observing the animals integrated into their environment, it can be noted that the endogenous rhythms are usually not «free-running». The «time of the body» is masked, and the spontaneous biological rhythms are obliged by the exogenous cycles to adjust their period in accordance.

This means that the biological time has innately the capacity to uniform itself with the physical time. Therefore, events that perturbate the environment can modulate the periodic cadence of the genetically determined endogenous rhythms. The strongest interferences are those provided by systematic events having a cyclical character in their manifestation. The light-dark alternation, meal timing schedule, social routines, including work shifts, etc. (see below) are deterministic as entraining agents.

In the entrainment of endogenous rhythms many structural entities intervene with a role of mediation (Table 2).

Table 2. - Central nervous structures involved in the chronoregulation of biological functions

Suprachiasmatic nuclei
Olfactory bulbs
Fornix
Septum
Limbic structure
Hippocampus
Preoptic area
Retino-hypothalamic connection
Midbrain raphe nuclei
Ventromedial hypothalamus
Dorsomedial hypothalamus
Locus coeruleus
Brain stem
Autonomic nervous system
Superior cervical ganglic
Pineal gland

The most important determinants of biological timing are the endogenous oscillators, structures of the organism that function as rhythmic «pacemakers». Other machineries of synchronization are the «pace-resetters», elements of the organism that regulate the temporal structure of one or more rhythms in response to one or more environmental synchronizers. The informative relationships between pacemakers and pace-resetters are determined by special connecting structures called «transducers» that translate the exogenous stimuli to the internal clocks. Transducers may have either negative or positive effects on the oscillators. The series can be integrated by the «modifiers» and the «logic controllers» which act, respectively, in modifying and controlling the exogenous and endogenous stimuli.

With regard to biological clocks there exists an eternal diatribe between positivists and negativists. The prevalent opinion is positivistic in the sense that the biological clocks are accepted as identifiable entities which reside inside tissues and organs. Those who believe in the existence of biological clocks assert that these structures of self-sustaining timing play a primary role in coordinating the miriad of peripheral biological rhythms. Such a coordinative capacity presupposes a leadership with which the biological clocks drive the phase of the rhythms provided by each cell of the organism. This implies that the biological clocks are formally equipped to ubiquitarily interact with all the cells by means of neural, physical and chemical messages. For this reason they are prominently located inside the non-mitotic structures of the nervous system, both encephalic and spinal.

The structural organization of biological clocks is difficult to be deciphered. An attempt will be made here by presenting the principal models. The model I, the simplest, is made by an oscillator that times a second oscillator, and so forth. This primordial model proposes a linear cybernetic control. The model II describes a primary oscillator followed by a series of oscillators in succession. The model III proposes an interaction between various oscillators of equal hierarchical importance arranged in a cybernetic network. The control by nodal clocks explains the occurrence of collateral interactions conditioning the mechanisms of positive or negative feedback. This interactive mechanism of chronoregulation has been called «feed-sideward».

Chronoanatomic research has brought to light a series of structures responsible for rhythmic programming and chronobiological integration of the organism with the environment. Information on the neuroanatomical structures involved in the central regulation of biological rhythms derive essentially from animal studies. Table 3 lists the structures which are presently recognized to play a rhythmogenic role as oscillators.

Table 3. - Central structures involved in the coordination of oscillating biological functions

Pacemakers
Paceresetters
Modifiers
Transducers
Logic-controllers
Synchronizers

Desynchronization, resynchronization, chronization

Chronobiological studies provided evidence that various environmental factors act hierarchically as synchronizers of biological rhythms. The most powerful synchronizer is the light-dark alternation. Isolated from geophysical temporality, human beings progressively tend to delay the resting time. This phenomenon occurs even in conditions of perennial light or darkness. A rapid change in time zones (passing through three or more time zones), as occurs in transmeridian flights, gives rise to a psychophysical disturbance commonly known as «jet lag syndrome» prominently due to the dyschronism between biological time and physical time. The resynchronization following geographical dyschronism occurs with a phase shift of about 90 min every 24 h. It is, however, necessary to keep in mind that the direction of time zone transiction is crucial. In east-west bound flights, travellers must recuperate a time span equal to the temporal difference between the time zones. In west-east bound flights, subjects must recuperate 24 h minus the difference in time zones, i.e., the physical time already passed in that zone which was not biologically «lived» by the travellers. This implies that the resynchronization takes much more time. The resynchronization can be, however, accelerated or delayed by numerous factors (Table 4).

Table 4. - Factors affecting resynchronization rate

Acceleration	Retardation
Extroversion	Introversion
Serality	Manility
Phase advance	Phase delay
Delay shift	Advance shift
Labile rhythms	Stabile rhythms
Strong temporal pressures	Weak temporal pressures
Higher performance task	Lower temporal task
Yought	Ageing
Low neuroticism	High neuroticism
Low pulse/respiration ratio	High pulse/respiration ratio

The meal schedule is also a robust synchronizer. Subjects eating a complete meal only once a day will show a phase shift for many biological rhythms toward the hour that the meal is given. Social routines (sociotemporality) are also important, especially shift work. A random shift can produce desynchronizing effects for many periodic functions, especially those related to physical and mental performance. Other environmental agents causing dyschronism are stress, fasting, fatigue, etc., if abnormally prolonged in time and/or cyclically repeated.

Several drugs can induce desynchronization as well. The lists of these drugs should compose a new chapter of pharmacology to be used in pharmacological surveillance.

Interestingly, some drugs may be used for resynchronizing the biological rhythms disturbed by exogenous interferences. These drugs are called «chronizing agents or chronizers». A list of the pharmacological molecules promoting chronization is reported in Table 5.

Table 5. - Pharmacological agents used as chronobiotic drugs

ACTH
Barbiturates
Tricyclic antidepressants
Lithium
Nomifensine
Xantine derivantes
Levodopa
5-hydroxytryptamine depletors
Indomethacin
Melatonin

Ontorhythmogenesis and gerorhythmoclinia

As an innate expression of vital dynamism, biological rhythms originate at the time of fecondation, the so called «ontogenic zero point». In order to show overt expression and integration, some rhythms require a central coordination which is controlled by biological clocks whose development is related to the maturation of the central nervous system.

The need of maturation indicates that the ontogenesis of biological rhythms results from the recruitment, organization and phase synchronization of the myriad of cycles generated by cells at the peripheral level.

It is important, however, to remark that some rhythms which require a high degree of integration can be already found in fetuses. The fetal synchronization reinforces the idea that maternal influences of cyclical nature are active on the temporal organization of bioperiodic functions in utero.

After the period of maturation, what is the destiny of biological rhythms as a function of chronological age? In other words, what is the effect of aging on biological rhythms?

In traditional biology, the aging process is thought to consist in a phenomenon of linear loss for biological functions (Fig. 3).

Chronobiology considers aging in the context of cyclicity which characterizes biological functions. Studies done in this regard have demonstrated that senescence is associated with a decline which affects the mean level (gerontologic decline of tonic activity), and the extent (gerontologic decline of phasic

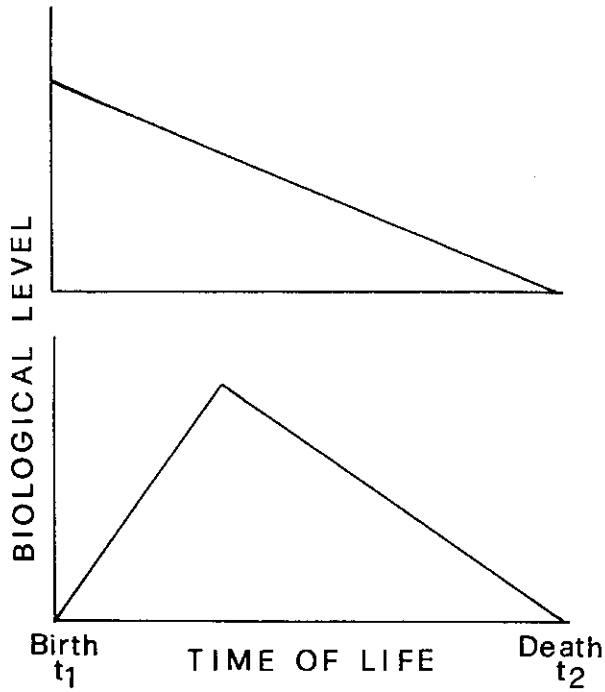


Fig. 3. - Diagrammatic representation of the traditional concept of aging as a process of linear decay.

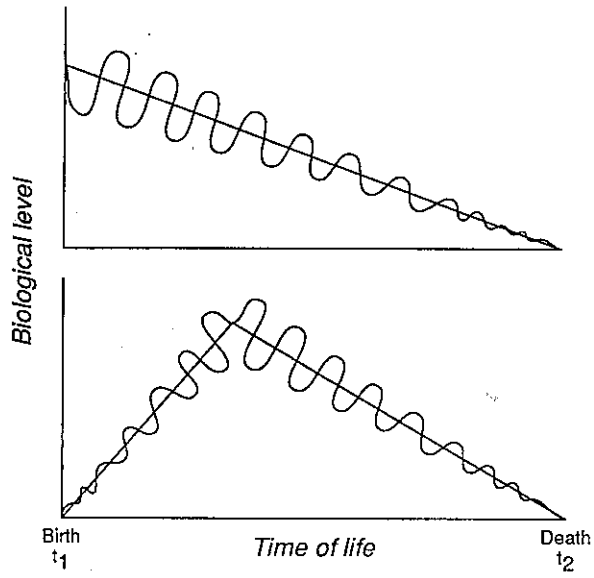


Fig. 4. - Diagrammatic representation of the chronobiological concept of aging as a process of rhythmometric decay (gerorhythmoclinia or clinospectroscopy).

activity) of oscillation, as well as a shift in the phase (gerontologic rephasement) and/or a change in the period (gerontologic demultiplication/multiplication) of biological rhythms. Accordingly, it can be said that aging is a process associated with a trend (clinous) which is negative for height and oscillatory extent of bioperiodic functions. This implies that the effects of senescence consist in a model of periodic-linear regression, in which one can see the combined decline of the oscillation in its mean level and amplitude, i.e., a phenomenon of gerorhythmoclinia (Fig. 4).

Presently, the periodic-linear regression analysis of gerontological trends of biological rhythms is feasible by means of the clinospectror method. This procedure, developed by this author, combines the estimation of the oscillatory changes, period by period, as a function of linear advancing age. The model of the gerontological destiny of biological rhythms is represented by a curve, i.e., the gerorhythmoclinogram (Fig. 2).

Interestingly, the clinorhythmometric study of senescence has documented that some biological rhythms may show a decline (kataclinous rhythms or katarhythms), some others may exhibit an amplification (anaclinous rhythms or anarhythms), in either the mean or amplitude of oscillation, and, finally, some others may appear not to change at all (aclinous rhythms or isorhythms), with advancing age. The gerorhythmoclinograms of these trends have been already illustrated in Fig. 2.

The kataclinous trend is a typical expression of rhythms «causative» of senescence. The anaclinous trend, by contrast, represents the periodic-linear model of rhythms «adaptive» to aging.

Because of the gerorhythmoclinia the concept of «homeorhythomostasis or rhythmostasis» has to be seriously criticized unless limited to a very short period of life cycle.

Definition of biological rhythm

In the preceding paragraphs, bioperiodic phenomena have been treated as temporal signals whose

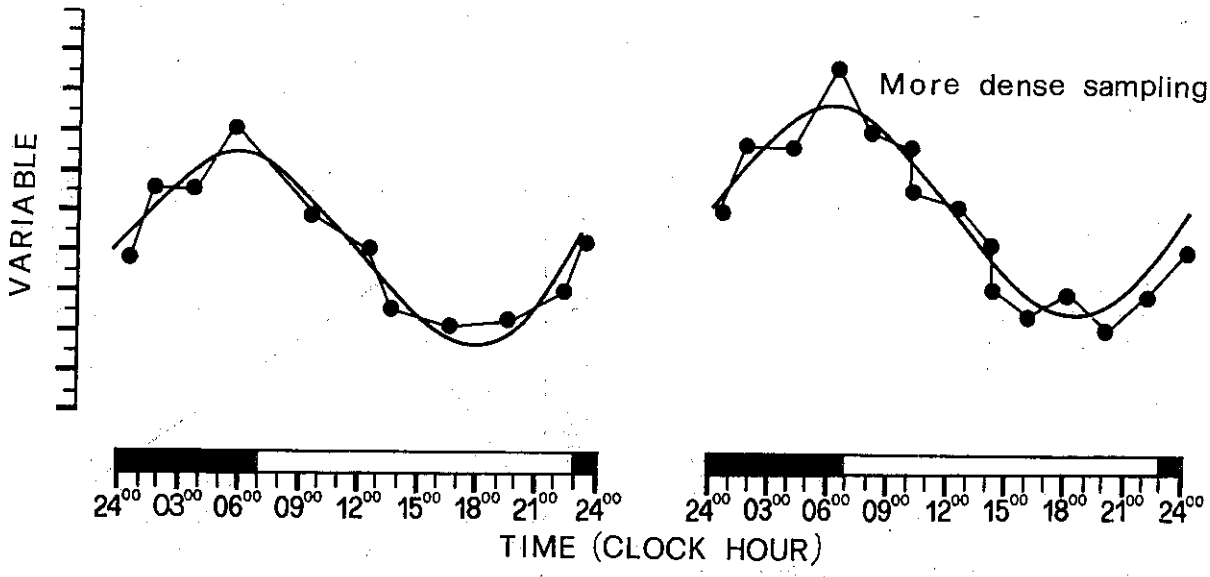


Fig. 5. - Diagram illustrating how both sampling time and interval cause the same discrete series to show different values.

variation may be expressed by discrete values coincinding with the sampling time.

It must be stressed that a discrete time series is not a faithful reconstruction of the signal as the approximation depends on timing and density of collected data (Fig. 5).

In the discretization of bioperiodic phenomena there is the risk of systematic errors, any temporal series being constituted by an intrinsic component which is superimposed by occasional or random elements. The discrete series can, therefore, be contaminated by aberrant values, the so called «biological noise», not belonging to the systematic component of the periodic variation (Fig. 6).

In order to remove the biological noise due to aberrant values, the so called «outliers», the periodic signal has to be geometrically approximated by a curve adjusted to the raw series of data (Fig. 7).

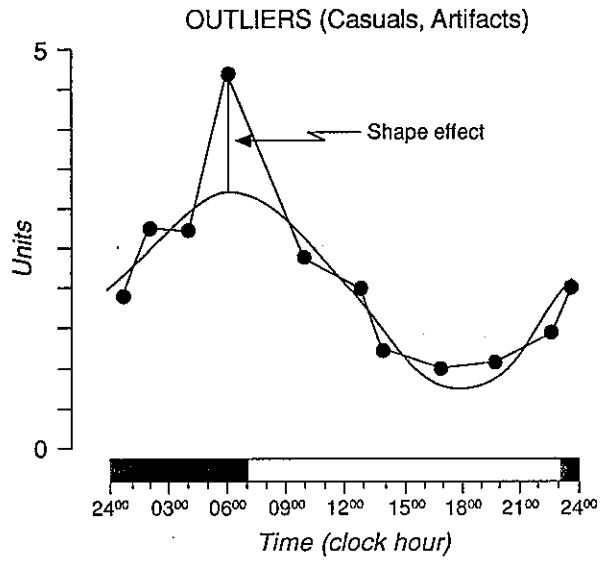


Fig. 6. - Abnormal values can be included within temporal data due to casual effects.

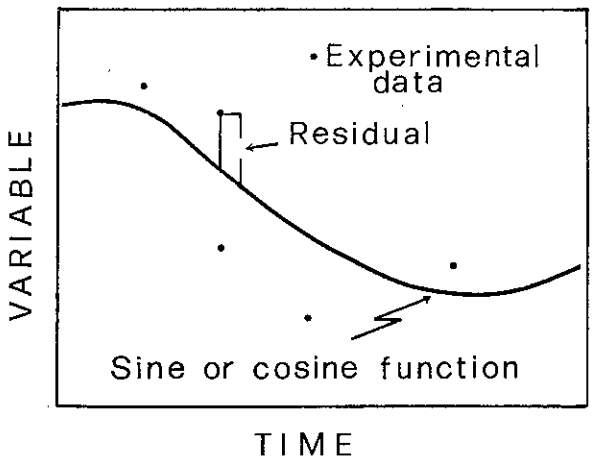


Fig. 7. - Sine or cosine function fitting a time series of discrete data.

As the signal is periodic, the analytical curve has to be representative of the harmonic oscillation. Therefore, one can assume that a biological rhythm is better expressed by the oscillating wave which optimally fits the discrete signal in its periodic fluctuation. In line with this thought, one can suggest that a biological rhythm is by definition «*the periodic component of a temporal series of biological data whose waveform profile has been analytically validated*». In other words, a biological rhythm is «*a biosignal whose period of oscillation has been validated by analytical models of periodic regression using one or more harmonic components*». Assuming such a definition, one can argue that a discrete variation of temporal biodata is not implicitly a pattern demonstrating a biological rhythm. Obviously, the analytical resolution of a discrete biosignal doesn't imply that the biological rhythm is arranged in a perfect sinusoidal shape. The sinusoidalization is just a model for removing the aleatory components which causes biological noise and, thus, biometric disturbance.

Furthermore, there are other valid biostatistical reasons that recognize the harmonic representation as the ideal signal of biological cycles. As a variation occurring over the 24 h scale is not an implicit demonstration that the pattern varies according to a 24 h period, there is the need to statistically validate the oscillation in its periodicity. The rhythm detection level may be statistically verified assuming an «alpha» probability (P) of less than 5% for the null-hypothesis of non-oscillation (zero-amplitude). Using the harmonic model, the biological rhythmicity may be, thus, statistically validated in its significance against the P probability less than 0.05 that the oscillation is due to a casual phenomenon.

The periodic regression methods used in the analytical detection and sinusoidal testing of discrete time series of biodata are discussed in the following chapter.

Chronobiological methodology or chronobiometry

In biometrically processing temporal series of biodata, time (T) may be regarded as a systematic parameter which is independent on the biological variable under scrutiny. Time illustrates how the biovariable changes, but it is not the causal determinant of that change, which, in turn, depends on the dynamic properties of living matter.

Statistically speaking, phenomenon Y and time T are associated, but time is not a random variable, its progression being numerically predictable.

In general, the level of Y (Yt) at a given time (t) is related to the antecedent value. For this reason, any temporal series may be said to be autoregressive and historical. If the relationship

between Y and T maintains itself unchanged, we will have a linear temporal phenomenon. If the relationship changes spontaneously we will have a non-linear temporal event. If the relationship resumes the same value at t time we will have a cyclic phenomenon.

In descriptive terms, temporal data of a cyclic phenomenon, based on a given unit of time can be divided into various components: *a*) a fundamental harmonic component that expresses the true periodic structure of the rhythmic phenomenon; *b*) one or more subharmonic components whose period is a submultiple of the principal wave; *c*) a random component that corresponds to the noise eliminated by the oscillatory curve.

Thus, biological noise is typical in a discrete series, and it influences the biometrical estimates of numerical statistics. However, biological noise is not accounted for in the continuous series, and it has very little influence on analytical statistics. In biometrically analyzing time data series, chronobiology applies both methodologies, i.e., *a*) numerical or non-inferential or macroscopic chronobiometry; *b*) analytical or inferential or microscopic chronobiometry.

Non-inferential chronobiometry (macroscopic analysis)

Non-inferential chronobiometry is principally based on measures of central location and dispersion of data sampled at each time point (Fig. 8).

The within-day profile of mean values that results from this analysis may be called the «mean-chronogram». Importantly, in each point the dispersion around the mean may be measured in terms of standard deviation (SD), standard error of the mean (SE or SEM), or 95% confidence limits (95% CL).

The true effect of time on temporal distribution of data may be ascertained by means of the «one-way variance analysis» (one-way ANOVA). The homogeneity of variance in each time point (homoschedasticity) may be verified by means of Bartlett's test or Duncan's test.

The estimates on the chronograms are as follows: *a*) overall mean level; *b*) peak level; *c*) peak time (zenith); *d*) trough level; *e*) trough time (nadir); *f*) difference between peak and trough levels; *g*) coefficient of variation for time-qualified mean levels; *h*) diurnal mean; *i*) nocturnal mean.

Inferential chronobiometry (microscopic analysis)

The analytical approach to time data series includes various methods.

Inferential chronobiometry with unknown period

This type of inferential chronobiometry is based on the principle that every quantifiable temporal variation (signal) can be represented by a sinusoidal-

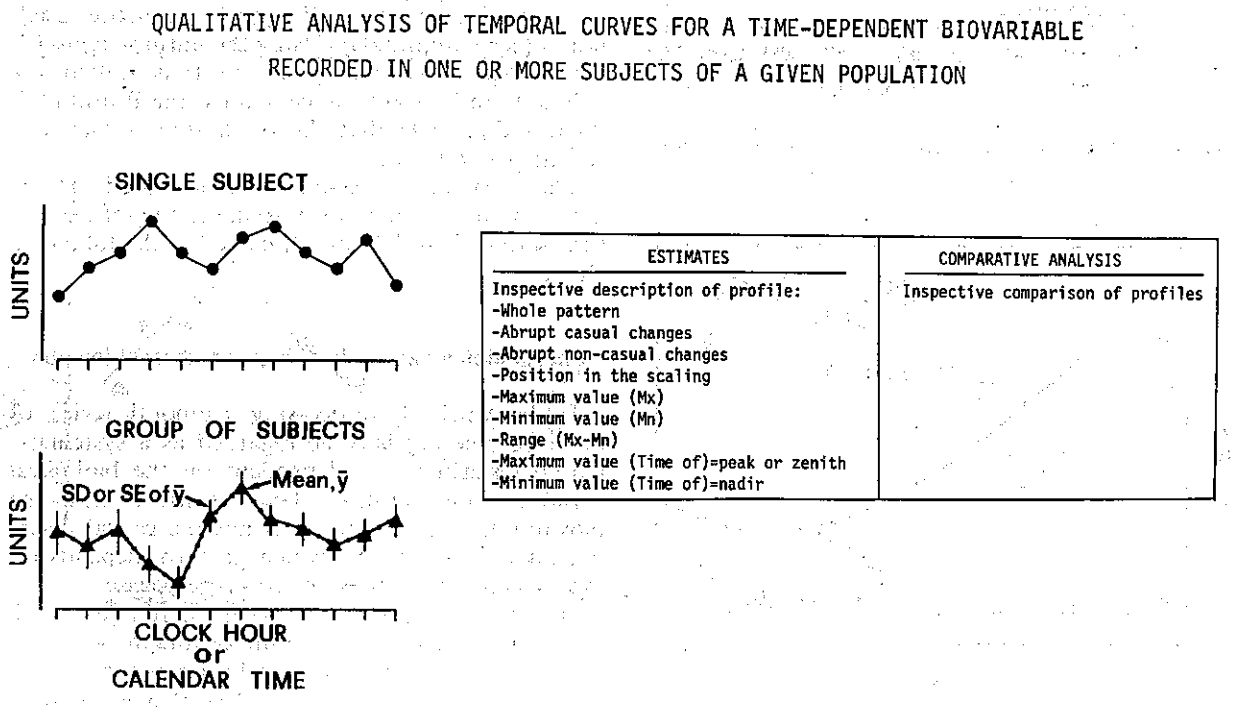


Fig. 8. - The bidimensional representation of time-qualified raw data constitutes the chronogram in which the temporal curve relates to single values (single-chronogram) or to mean values with their dispersion (mean-chronogram).

wave (fundamental harmonic wave). The analysis is mainly used for two purposes: *a*) to find the period that represents the signal; *b*) to recognize a signal by incans of its frequency spectrum.

Analysis of the best fitting period (periodogram). - In this type of analysis the best fitting waveform profile is found using the function

$$Yt = Cl + C2 * \cos (C3 * X)$$

The most important coefficient in this formula is C3 which corresponds to $2\pi/TAU$ where TAU is the period which optimally approximates the raw data time series. TAU is determined as $2\pi \times C_3$.

Spectral analysis. - The analysis of resolution in harmonics (spectral analysis or Fourier analysis) is one of the most important methods of numeric elaboration of signals whether they are discrete (digital signals) or continuous (analogic signals).

A digital signal is a series of x values arranged in a given interval of time to give a sequence of discrete numbers which are the instantaneous expression of a continuous function. An analogic signal is a continuous series being a function of time (t).

The discrete signal for the resolution in a harmonic series (discrete Fourier transform) must be represented in terms of x values equispaced in time. Therefore, the formula is

$$f(x) = a(o) + a_1 \times \cos(n) + b_1 \times \sin(x) + a_2 \times \cos(2x) + b_2 \times \sin(2x) + \ldots$$

A relationship exists between the sampling numerosity (n) and time (t)

$$t = nI$$

where I is the interval of sampling. Given the equidistance of x values, t will correspond to the time cycle or period (TAU) or duration of the signal itself.

Spectral analysis consists in the transformation of the sequential signal, i.e., a temporal entity, into a frequency spectrum, i.e., a frequential entity. The spectrum of the resolved frequencies is the microstructure of the decomposed signal (Fig. 9).

Assuming the period of revolution 2π to be equal to TAU, the formula for spectral analysis can be rewritten as

$$f(t) = a(o) + \sum_{i=1}^{+\infty} a(i) * \cos\left(\frac{i\ 2\pi}{TAU} \times t\right) + b(i) * \sin\left(\frac{i\ 2\pi}{TAU} \times t\right)$$

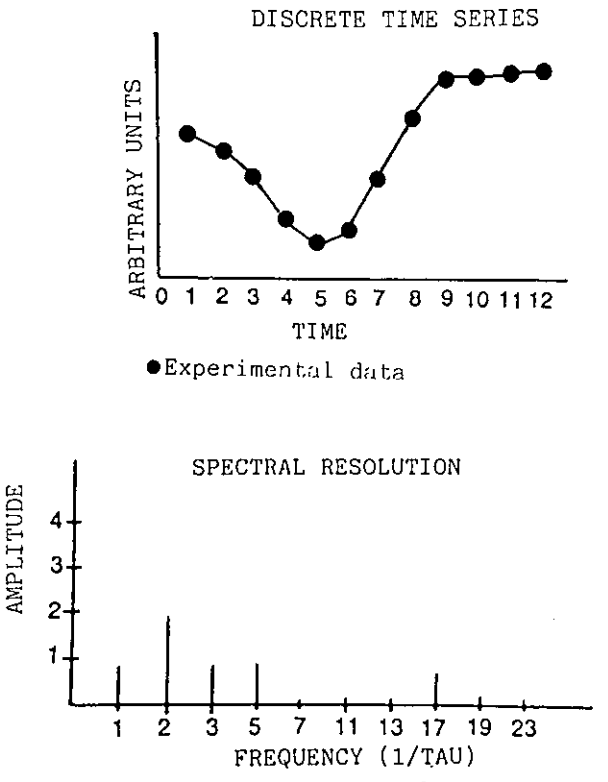


Fig. 9. - Representation of a signal in the domain of time (top panel) and in the domain of frequency (botton panel).

which demonstrates that spectral analysis is linear in frequency.

The frequential resolution describes the harmonic components assuming that every variation of a given duration can be described by a sinusoidal wave of equal period (fundamental harmonic) and by even or odd submultiples of this wave (secondary harmonics). The harmonics constitute the so called «formants» according to which the signal can be analytically reconstructed as the sum of sine waves (Fig. 7).

Therefore, a frequential resolution is a way of recognizing a signal by its intrinsic structure, as it is probabilistically impossible that the oscillatory properties of the formants, which are expressed by the parameters amplitude and phase, might belong to a different pattern of discrete or continuous values.

Accordingly, the fundamental use of spectral analysis is «pattern recognition». It is, however, important to stress that Fourier analysis makes no testing for the statistical significance of the fitted harmonic waves.

Inferential chronobiometry with pre-fixed known periods

Chronobiology, in its incessant methodologic development, has been oriented mainly towards the

methods of periodic regression analysis in which the period of oscillation is known *a priori*. The reasons for this methodological preference depend on the fact that in biology and medicine it is necessary to know whether or not biophysical or biochemical functions maintain the physiological periodicity within the recognized spectrum of biological rhythmicities (see Table 1). Obviously, in this validation one must know *a priori* the period to be validated. Accordingly, the methods of inferential chronobiometry with a «pre-fixed» period all propose the probabilistic validation of the null hypothesis of zero-amplitude for that a given period of oscillation. In this manner, all the methods show the advantage of estimating the statistical significance of the rhythm under scrutiny (sinusoidality testing) which is not methodologically detectable by using the methods cited above.

Importantly, the sinusoidality testing is based on the P level of statistical probability (rhythm detection level) according to which the zero-amplitude assumption is rejected. A rhythm is said to be «significant» or «not significant» whether P is respectively < 0.05 or > 0.05. The postulated zero-amplitude is checked by computing the «Percent Rhythm (PR)» which is the percentage of variability accounted for by the harmonic function with reference to total variability of experimental data made equal to 100%.

The methods of inferential analysis with a known period give rise to descriptive, integrative and evolutive chronobiometry or, rhythmometry.

Descriptive inferential chronobiometry

The descriptive inferential chronobiometry encompasses various methods.

Cosinor analysis by Halberg et al. (1967, 1972). - In descriptive rhythmometry the most important method of periodic regression with a fixed period is called «Cosinor analysis». The Cosinor procedure can be separated into two main methods: Single Cosinor and Population-Mean Cosinor. Both methods are based on a sinusoidal approximation using a cosine function. However, Single Cosinor deals with the analysis of a single time series (single-chronogram) regarding a single subject or a group of individuals. The Population-Mean Cosinor elaborates by summarization the rhythmometric data obtained by Single Cosinor from several individuals of a given group.

Cosinor analysis applies as a trigonometric operand the cosine because zero degrees correspond to the reference time or zero time of the rhythm. Zero time coincides with midnight for circadian rhythms, winter solstice (22 December in the northern hemisphere, 21 June in the southern hemisphere) for circannual rhythms.

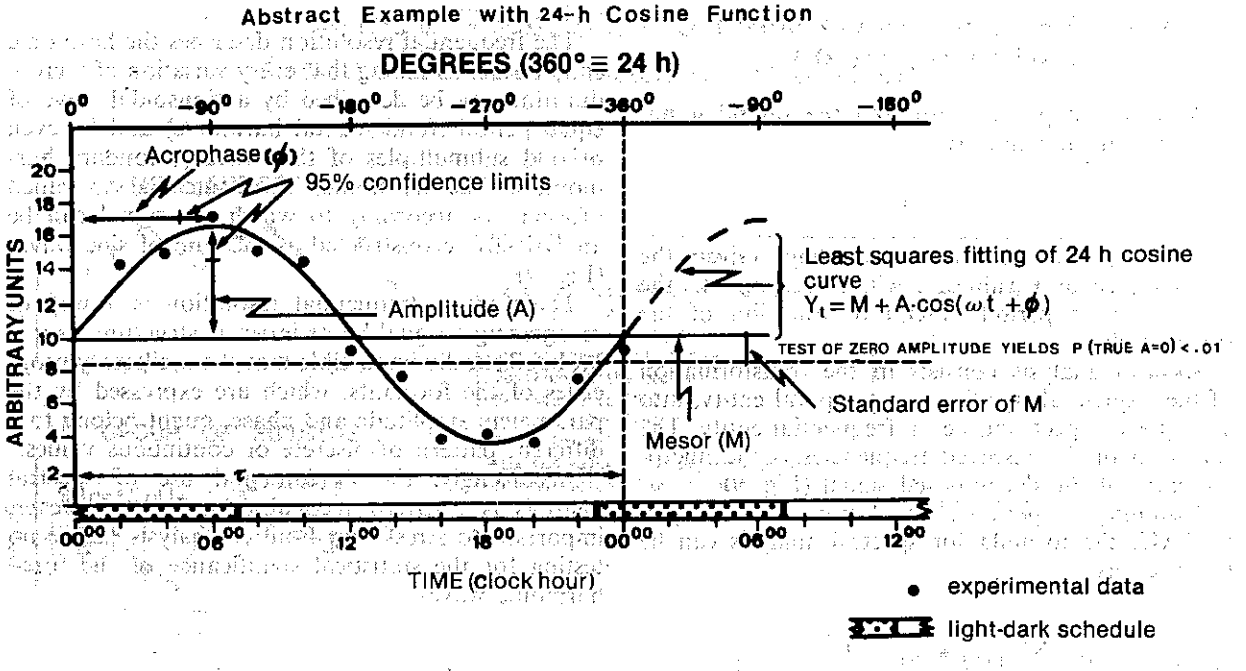


Fig. 10. - The waveform profile provided by cosinor analysis (cosinorgram) represents the best harmonic wave for fitting the time-qualified data experimentally investigated. The waveform profile expresses, with the vertical position, both mesor and amplitude, and, with its horizontal distance, the acrophase.

Cosinor analysis approximates the following equation to experimental data using the least squares method for minimization

$$Yt = M + A * \cos\left(\frac{2\pi}{TAU} * t + phi\right)$$

where M is the mesor (acronym for midline estimating statistic of rhythm), the mean level of oscillation, A is the amplitude, the extent of oscillation from the mesor or half of the total oscillation, π is 3.1415926536, TAU is the chosen period, t is a temporal fraction of the cycle, an instant of the whole revolution, and phi is the acrophase, timing of the crest of waveform profile (W).

Therefore, Cosinor analysis quantifies the best fitting sinusoidal wave in three parameters: M, A and phi that represent the properties of the rhythm for that a given ultradian, circadian or infradian period.

Cosinor analysis quantifies further estimates such as the «percent rhythm, (PR)» and the «rhythm detection level (P)». The waveform profile can be, thus, validated whether or not it rejects the null hypothesis of zero-amplitude at a statistically significant level of probability. This means that the rhythm is validated as statistically significant for that a given period of oscillation tested by Cosinor.

In the cosine formula, phi is expressed in negative sexagesimal degrees in order to represent the parameters amplitude and acrophase on a polar diagram by means of a single vector turning in a clockwise fashion (see below).

Cosinor method expresses in a graphical form on cartesian axes the optimal waveform profile, the so-called «cosinorgram» (Fig. 10).

Cosinor procedure additionally expresses both amplitude and acrophase in polar coordinates within a circle whose 360 sexagesimal degrees correspond to the period of oscillation of that given bioperiodic event. Such a chronobiologic quadrant is called «polargram» (Fig. 11).

The polargram is a very practical and immediate description of the cosinor-derived rhythmometric parameters. Amplitude and acrophase are treated in a bivariate fashion and represented by a single vector starting from the center. The length of vector is proportional to the amplitude, while its direction indicates on the border the temporal localization of acrophase in negative sexagesimal degrees or in physical time. Importantly, the polargram allows a bivariate representation of confidence limits for the amplitude-acrophase pair in form of an ellipse of confidence centered at the tip of the vector. The confidence ellipse immediately shows whether or not a rhythm is significant, respectively by its eccentric or concentric location with reference to the pole of the circumference. To note that the statistical limits are given by Single-Cosinor as the standard error of the mean, and by Population Mean-Cosinor as 95% confidence limits.

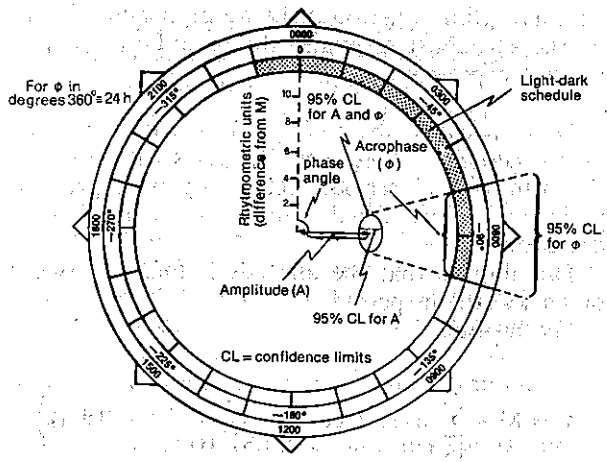


Fig. 11. - The polar representation of rhythmometric parameters is made using the circumference of a circle as a place for estimating the acrophase timing of a given rhythmic oscillation assuming the entire period to be equal to 360 sexagesimal degrees. The oscillatory amplitude is represented by a vector starting from the pole whose lenght is proportional to its numerical value. The vector is orientated with respect to the sexagesimal degrees by a given angle corresponding to acrophase timing. Because of the polar representation, the dispersion limits of the amplitude-acrophase pair are given as an elliptical region of confidence centered at the tip of the vector.

Cosinor analysis has several fundamental prerequisites with respect to the macroscopic analysis of temporal series.

The waveform resolution eliminates the spurious or aberrant data belonging to «biological noise». The sinusoidal wave provides rhythmometric data that are relatively independent from the density and equidistance of sampled data which is critical in numerical analysis of raw time data series. Cosinor methodology has shown that any waveform profile may be defined by only three parameters, mesor, amplitude and acrophase, which simplify the description of biological rhythms, and, additionally, facilitate the statistical comparison among oscillating bioevents. As a matter of fact, the enormous variability of the chronogram makes the raw data series diffult to be compared and described in their periodic shape. It must be added that the rhythmometric parameters provide information on important functional attributes of oscillating bioevents. The waveform profile, if validated as significant, demonstrates that the temporal variability of a given bioevent is systematic and predictable in its periodicity. The mesor provides an objective measure of the tonic level which sustains the rhythm. The amplitude, in turn, quantifies the phasic component which characterizes

any oscillating function of living organisms. Finally, the acrophase estimates the periodic activity in its best expression.

Least squares spectral analysis with pre-fixed known periods. - This type of analysis is based on the principle of periodic regression with prefixed periods that are fitted unitarly in increasing or decreasing order.

This implies that the analysis is linear (forward or backward) in period.

The formula is

$$Y = M + \sum_{i=1}^{N} a(i) * \cos\left(\frac{2\pi}{TAU(i)} * t + Phi(i)\right)$$

where N is the number of approximated periods and i is the unitary increment from the lowest period to the period of the fundamental harmonic. For example, a raw time data series that covers 24 h can be studied in its harmonic components by validating whether the periodic regression rejects the null hypothesis of zero-amplitude at a significant level of probability ($p < 0.05$) for each fitted period starting from one cycle per hour to one cycle every 28 hours. In this way, the unitary harmonic components of the ultraradian and circadian domain are examined in their amplitude and statistical significance.

Expressing the extent of oscillation as «percent rhythm», the spectrum is given by a cartesian diagram of each amplitude, on the ordinate, versus each fitted period, on the abscissa, i.e., the so called «power spectrum» (Fig. 12).

Observing the power spectrum one can immediately decipher the significant harmonics (formants), including the most significant (MSH) and the least significant (LSH) one.

Using the formants one can construct a «harmonic model» of the signal (Fig. 13).

The harmonic model allows the reconstruction of the original signal, uncontaminated by biological noise, through the determination of the «resultant wave» (Fig. 14).

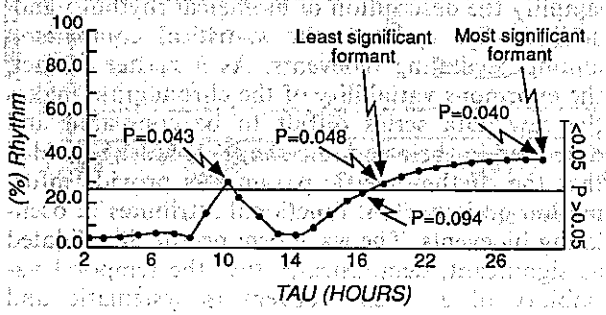


Fig. 12. - Power spectrum representing each fitted harmonic wave in its oscillatory amplitude (ordinate) and period (abscissa).

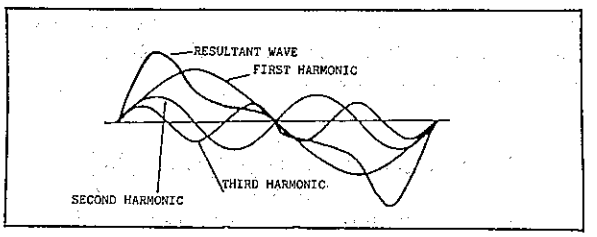


Fig. 13. - Harmonicogram representing the oscillatory profile of each fitted harmonic wave. The resultant wave is computed point by point as the mean of values provided by each wave.

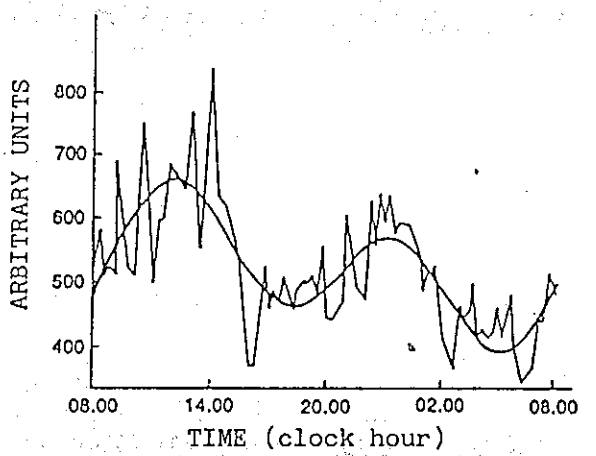


Fig. 14. - The resultant wave provided by least squares spectral analysis is the best wave fitted to the experimental chronogram.

Serial section analysis. - This is another method of descriptive inferential chronobiometry derived from Cosinor analysis. The procedure consists in iterating the cosine function on a longitudinal series of biodata that covers a time span longer than one period of revolution.

Basically, the method applies the Single Cosinor analysis to a portion of sequential data (serial section) which is included in the prefixed period of analysis (chronobiological window). The periodic regression analysis is repeated shifting across a given number of time data points within the chronobiological window. The serial section is, therefore, composed of a constant number of data in each iteration. The analytical approach is progressive, and ends when the chronobiological window includes the last portion of data within a complete serial section.

Every iteraction computes the rhythmometric parameters, mesor, amplitude, acrophase, as well as the percent rhythm and rhythm detection level. As a consequence, serial section analysis allows the validation of the significant continuity of rhythmometric properties over the time in which the sampling was performed (Fig. 15).

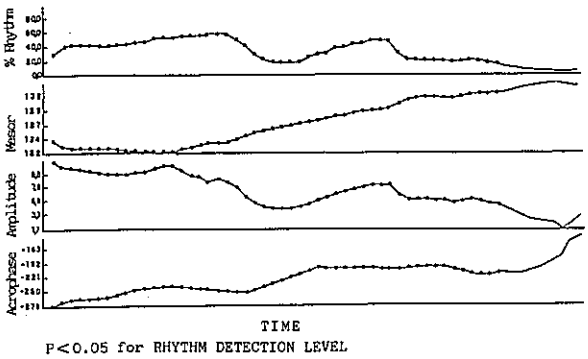


Fig. 15. - Percent rhythm, mesor amplitude and acrophase of each iteration of the periodic analysis by moving the chronobiological window with a given period along the time series in which the data cover a time longer than the fitted period. P value of rhythm detection level in each iteration is expressed by a symbol.

In detail, «the analysis estimates whether or not *a*) mesor and/or amplitude tend to vary; *b*) acrophase undergoes an anticipatory or a posticipatory shift as in the presence of free-running or desynchronizing conditions.

Least squares spectral analysis with pre-fixed known frequencies. - Cosinor analysis may be regarded as mono-component model of periodic regression as it fits a single sinusoidal wave into the raw time data series.

Some discrete signals in biomedicine are asymmetrical in their duty-cycle in the sense that they show a different portion of time in which the measured values fall below and above their mean level. In this case the best fitting wave is found by periodic regression models which use more than one harmonic component for approximating the discrete time data series. The multiple-component model is usually given by the fundamental wave of period TAU and N subharmonics, each one (i) having a frequency multiple of the fundamental one (2π).

Therefore, the multiple-component periodic regression analysis is linear in frequency.

The formula is

$$Yt = M + \sum_{i=1}^{N} A(i) * \cos\left(\frac{i2\pi}{TAU} * t + phi(i)\right)$$

Integrative inferential chronobiometry

The integrative rhythmometry presently consists in a method called «Cosint analysis». This procedure is used for estimating bioperiodic events as integral functions within two temporal extremes of their period of revolution.

Cosint analysis by Cugini (1987). - The Cosint method consists in a periodic integration analysis which estimates the area covered by the best fitting waveform profile of a given biological rhythm. The extremes t1 and t2 may be equal to the period being examined (t2 – t1 = TAU) or to a fraction of it (t2 – t1 <TAU). Importantly the cosint analysis can be performed knowing the periodic regression which optimally approximates the raw time data series. Therefore, Cosint analysis can be applied to cosine models either mono-component or multiple-component. In the first example, Cosint analysis is performed for a single armonic wave (Fig. 16).

In the other example, Cosint analysis is applied to a profile found by a two-component harmonic model (Fig. 17).

Importantly, the integral over time of the oscillatory curve gives rise to the parameter aesor (acronym for area estimating statistic of rhythm).

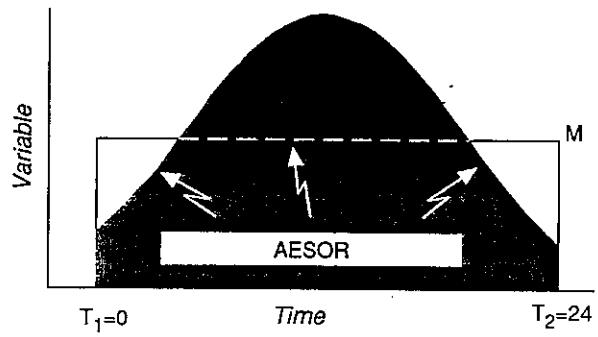


Fig. 16. - A biological rhythm can be estimated as an integral function by means of the rhythmometric parameter aesor which corresponds to the area covered by the best fitting waveform profile provided by a mono component harmonic model.

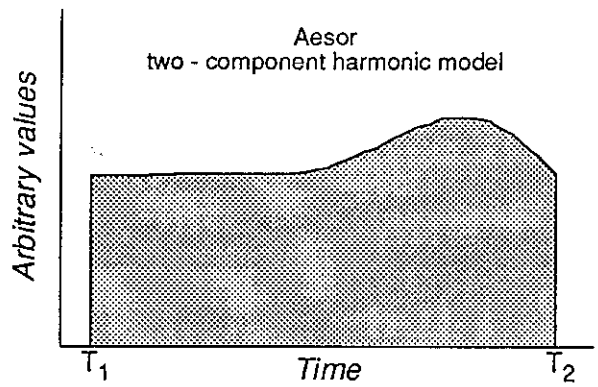


Fig. 17. - The aesor corresponding to a waveform profile of a biological rhythm provided by a two-component harmonic model.

The aesor is determined by the following formulas

$$\text{AESOR} = \int_{t1}^{t2} \left\{ M + \sum_{i=1}^{N} A(i) * \cos\left(\frac{2\pi}{TAU(i)} * t + \right. \right.$$

$$\left. \left. + \text{phi}(i) \right) \right\} dt$$

for a model linear in period, or

$$\text{AESOR} = \int_{t1}^{t2} \left\{ M + \sum_{i=1}^{N} A(i) \times \cos\left[\frac{i2\pi}{TAU} \times t + \right. \right.$$

$$\left. \left. + \text{phi}(i) \right] \right\} dt$$

for a model linear in frequency.

The integral area is calculated by summing the subareas in which the surface can be divided using as unitary distance the time base (time unit) of to the period of the scrutinized rhythm (for example, the minute for circadian rhythms, the day for circaseptan rhythms, the month for circannual rhythms). The unitary distance of time corresponds to the h interval. The subareas are given by quadratic surfaces whose sides correspond to the two Y values calculated by the periodic regression function in the h interval of time base (Fig. 18).

The surface of two contiguous subareas will therefore be given by the formula

$$\text{Area} = (Y1 + 4Y2 + Y3) * h/3$$

The total area will result from the sum of all the unitary subareas (Fig. 19).

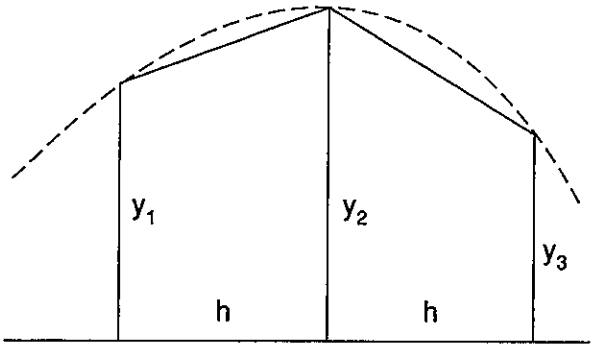
The estimation of whole area will therefore be given by the formula

$$\text{Area} = Y1 + 4Y2 + 2Y3 + 4Y4 + 2Y5 + \ldots$$
$$\ldots + 4Y(X-1) * h/3$$

As the whole area coincides with the surface underlying the oscillatory curve of a given biological rhythm, its estimate represents a chronobiometric parameter which can be called aesor from the acronym of area estimating statistic of rhythms.

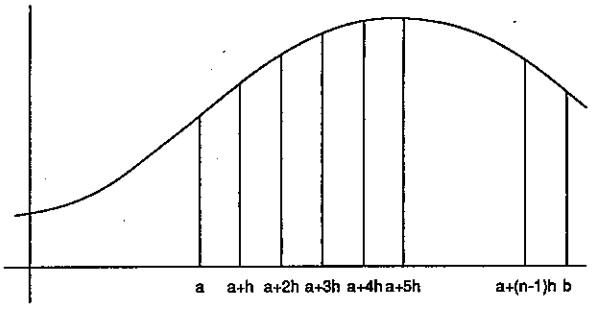
Since the values of Y1, Y2, Y3 ... are given by the periodic regression function F(Y), the formula for calculating the area, say the aesor, can be rewritten as

$$\text{AESOR} = \{F(Y) + 4F(Y + h) + F(Y + 2h) +$$
$$+ F(Y + 3h) + F(Y + 4h) + F[Y + (n-2)h] + \ldots$$
$$\ldots + 4F[Y + (n-1)h] + 4F[Y + (n-1)h] +$$
$$+ FX(Y + nh)\} * h/3.$$



$$(y_1 + 4y_2 + y_3) \times \frac{h}{3}$$

Fig. 18. - Two contiguous subareas constitute the mininum for calculating the area under the oscillatory curve of a biological rhythm.



$$A = (f(a) + 4f(a+h) + f(a+2h) + f(a+2h) + 4f(a+3h) + f(a+4h) + \ldots + f(a+(n-2)h) + 4f(a+(n-1)h) + f(a+nh)) \times \frac{h}{3}$$

Fig. 19. - The whole area covered by the oscillatory curve of biological rhythm results by the sum of each trapezoidal subarea.

Evolutive inferential chronobiometry

The evolutive rhythmometry or clinorhythmometry is composed by two methods which respectively analyze the age-related changes in mesor, amplitude and acrophase, on one side, or period, on the other side.

Clinospectror analysis by Cugini (1991). - This type of analysis considers the changes in mesor, amplitude and acrophase (but not period) of biological rhythms with respect to the chronological age.

So far, the inferential methods which have been discussed appear to be capable of examinating and describing a rhythmic bioevent at a given time of the life cycle. It must be, however, remarked once again that the majority of biological rhythms tend to vary in their oscillatory properties with advancing

age. The rhythmometric trend related to chronological age involves the mesor, amplitude and acrophase (gerorhythmoclinia). Therefore, there is the need of biometrically investigating such a complex of changes by means of appropriate procedures (clinorhythmometry). The approach to age-related changes in rhythmometric parameters requires a periodic-linear regression analysis in which the trend in periodic parameters is analyzed as a linear function of age. Such a method is called «Clinospectror analysis». Its formula is given below

$$Y(age) = [a(M) + b(M) * age] + [a(A) + b(A) * age] ... \ ... \cos \left\{ \frac{2\pi}{TAU} * t + [a(phi) + b(phi) * age] \right\}$$

where a is the intercept, i.e., the value of rhythm parameters at zero age, and b is the angular coefficient of the trend, and M, A and phi are the rhythmometric parameters mesor, amplitude and acrophase, t is a fractional time of the period in use.

The optimal periodic-linear regression determined by Clinospectror analysis gives rise to a clinorhythmogram, a planegraph whose ordinate and abscissa express respectively the oscillation around the mesor and the chronological age. Examples of clinorhythmograms are given in Fig. 2.

According to clinorhythmometry, several models of aging can be identified as already discussed in previous chapters. Additionally, Clinospectror analysis may be used for predicting the evolution of a biological rhythm as a function of life span. It is possible therefore to make projections to future times as well as extrapolations to past times down to birth. It is also possible to predict biological age (BA) with respect to chronological age (CA), and to compute the senility index (SI) from the formula SI = BA/CA.

Complex demodulation method. - Biological rhythms may vary in period as a function of chronological age. The age-related changes in frequency can be estimated by the model of complex demodulation according to the following equation

$$y(t) = M + A(t) * \cos \{2\pi * [t - phi(t)] / TAU\}$$

Conclusions

It is important to stress that the chronobiologic methodology is composed by other procedures for other specific analyses. In addition, chronobiology uses reference standards which are time-qualified, i.e., chronodesms, cosinordesms, aesordesms. It is, however, beyond the informative scope of this article to discuss the chronobiologic methodology in all its aspects. Readers can find a stimulus in reviewing the literature cited below if interested in more details.

This article is a concise attempt to outline the principles and methods of chronobiology. Further information will be provided by the articles which compose this book. These reports can be regarded as a «summa chronobiologica» considering that they have been written by some of the most distinguished chronobiologists in the world.

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