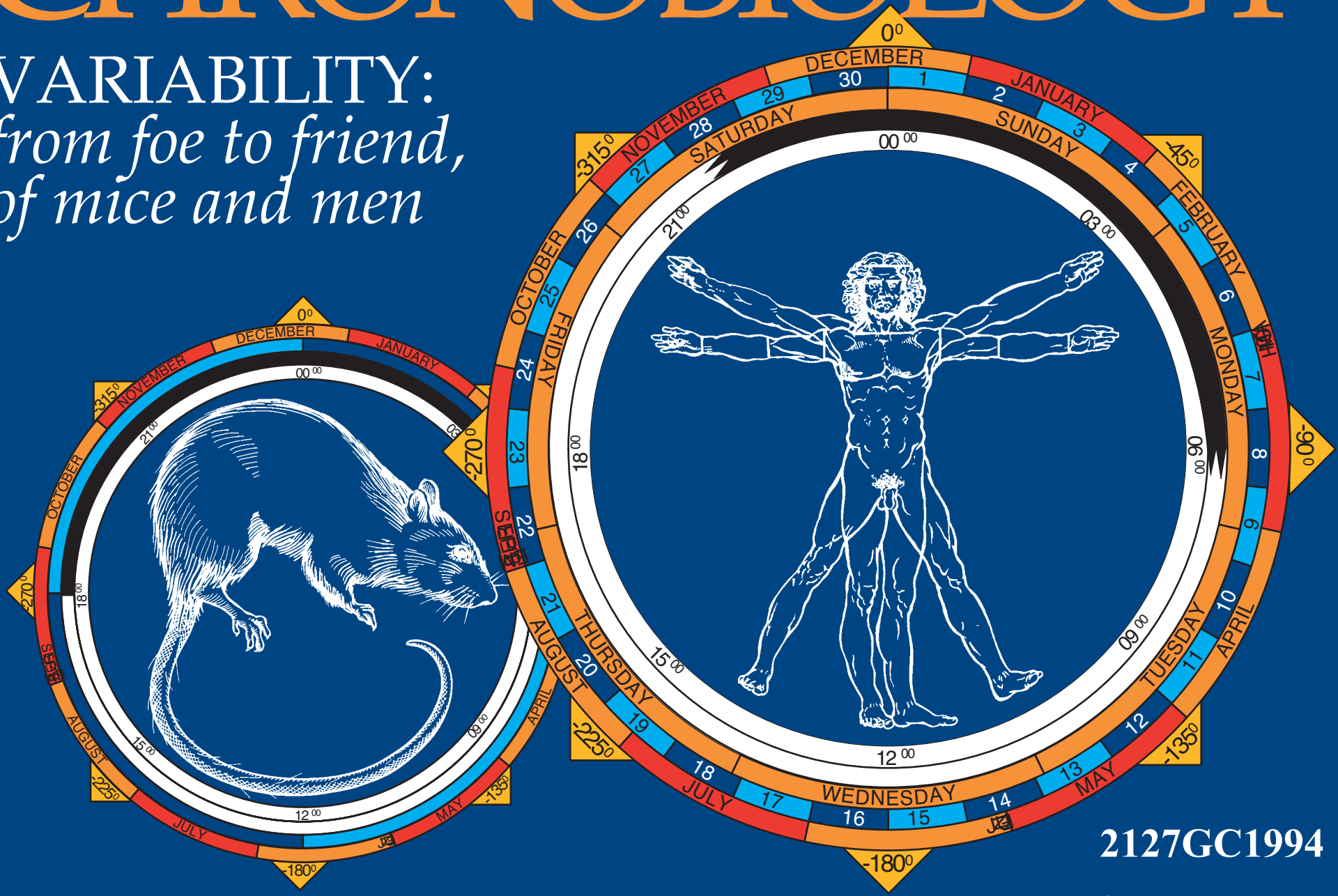
INTRODUCTION TO CHRONOBIOLOGY

VARIABILITY:
from foe to friend, of mice and men



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Medtronic Chronobiology Seminar, #7, By Germaine Cornélissen and Franz Halberg, University of Minnesota

"STATE OF THE ART" FOR RATS AWAITS TECHNOLOGY TRANSFER TO HUMANS

Beat-to-beat recording of blood pressure and heart rate is now widely used by the pharmaceutical industry but is as yet unavailable for humans. Thus, telemetry from the untethered, freely moving rat yields the diastolic blood pressure values shown as dots in the top row. Chronobiometric software documents internal phase drifts within the circadian cardiovascular system, under conditions of 12 hours of light alternating with 12 hours of darkness, as shown for diastolic blood pressure and heart rate in the middle and bottom rows, respectively. The data are analyzed by chronobiologic serial section, a procedure similar to a moving average that yields local estimates of the circadian rhythm characteristics as they change as a function of time. The acrophases represent the timing of overall high values recurring each day. They are expressed in (negative) degrees, with 360° equated to 24 hours and 0° set to a selected reference time. Since they assume a value close to 0° (or -360°), they are doubly plotted to help convey the continuity of changes as a function of time. Whereas the acrophases of heart rate remain close to the reference time (0° or -360°), the acrophases of diastolic blood pressure slowly drift to earlier and earlier times. Since the rat was kept in a 24-hour synchronized environment, the drift in acrophase also indicates an external desynchronization of diastolic blood pressure. Such unusual circadian (or other) behavior cannot be identified on the basis of casual measurements but requires both longitudinal monitoring and a chronobiologic analysis of the data. This is now possible even for small rodents by telemetry. When alterations of the human cardiovascular chronomes have been related to an increased risk of developing adverse vascular events, it is critical that similar unobtrusive monitoring devices capable of on-line data analysis and interpretation be developed for human use as well. This is the more important since in dealing with blood pressure today there is an uncertainty, if not error, underlying decisions to treat or not to treat well in excess of 40%. It is also important for the case of various kinds of cardiac arrhythmias that all exhibit a chronome awaiting quantification and the detection of changes within the physiologic range of variation that signal an elevated risk before the occurrence of catastrophic events. With respect to blood pressure, the elevation of this variable is currently recognized to be the major risk factor of heart attacks and strokes that may kill more individuals than all other diseases combined.

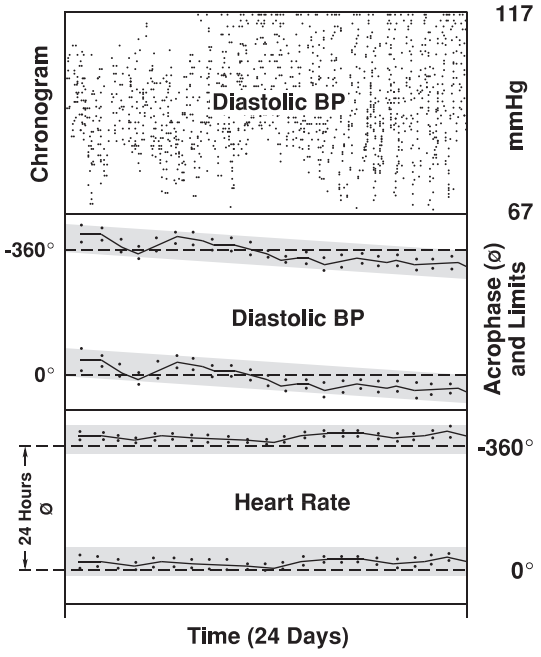
Physiologica exercitatio, cum refutationibus homeostaseōs, ad chronobiologiam, commune vinculum omnibus artibus, omnibusque scientiis ac disciplinis

Ex officina Medtronica Patricii Delmorensis Minneapolios, Minnestae USA, 1993*

Physiologic exercise, with refutation of homeostasis, toward chronobiology, the common bond of all the arts, sciences and other disciplined endeavors

From the studio of Patrick Delmore Medtronic, Inc., Minneapolis, Minnesota, 1993

RAT TELEMETRY ILLUSTRATES DIFFERENCE BETWEEN THE CHRONOMES OF BLOOD PRESSURE (BP) AND HEART RATE



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AGOSTINO CARANDENTE, 70 YEARS OF AGE

Chronobiology: 'the common bond of all the arts [, sciences and disciplines']

Commune vinculum omnibus artibus [, omnibus scientiis, omnibus disciplinis]

In a day and age when invitations to lecture even at the clinics of Catholic University in Rome are extended with the condition that no Latin, but English be spoken, it seems mandatory to provide the English translation of the above, slightly paraphrased motto and seal of the University of Minnesota. The quotation is abbreviated from the jurist, philosopher and politician Marcus Tullius Cicero (106 BC-43 BC): *'Etenim omnes artes, quæ ad humanitatem pertinent, habent quoddam commune vinculum, et quasi cognatione quadam inter se continentur' ('Indeed, all the arts which pertain to humanity have a certain common bond and are held together by a certain kinship among themselves').* In this quotation (from *Pro Aulo Licinio Archia Poeta Oratio*), Cicero asks his audience to realize that while he is a lawyer by profession, he is also most interested in, and has benefitted from, the very broad culture *(humanitas)* of the poet Aulus Licinius Archias, who eulogized the broad values intrinsic to everything alive. Like Archias, Agostino Carandente, by the thoughts and deeds of his life and his publishing house, appropriately named 'The Bridge' *(Il Ponte),* has tied together a community of scholars in the science which is so pertinent to all of the arts: the discipline revolving around life in time, chronobiology. Chronobiologists share a quantitative mathematical, an inferential statistical and a humanistic kinship. Accordingly, this seventh entry in the series of University of Minnesota/ Medtronic Seminars is dedicated to Agostino Carandente, family man, renaissance man, top industrial manager, publisher, physician and chronobiologist *par excellence.*

This Festschrift aims to sketch, in condensed form, a few of the highlights of chronobiology, in order to honor Agostino Carandente, M.D., who for the last two decades has used the resources available to him as manager of a major pharmaceutical company in a nearly full-time endeavor to consistently foster chronobiology. Agostino Carandente has also inspired his children to follow the trail he helped blaze and contributes his familial, intellectual and financial means to the science of the body's time structure.

As a professional assembly, chronobiologists met Dr. Carandente for the first time in 1973 in Hannover, Germany, at the biennial meeting of the International Society for Chronobiology. There he pleasantly surprised us with the gift of Chronobiologia, now in its 21st year of publication. He came with his close friend and professional associate, the late Franco Ceresa, the professor of internal medicine at the University of Turin, Italy, a master of endocrinology. (Franco was also a master oenologist who, in his home, produced and bottled a perfect Barolo, 'the wine of kings and the king of wines'.) The late Norberto Montalbetti (1), the dynamo of chronobiologic laboratory medicine (2), had introduced Franco to the Minnesotan chronobiometry (3). Franco in turn acquainted Agostino with the ways to quantify the large extent of change in the circadian adrenocortical cycle.

Agostino then implemented the first multicentric chronobiologic Italy-wide clinical trial of a corticosteroid drug combination. His brainchild was eventually marketed with a recommendation of timing in its name. The numbers in Dutimelan 8 15 indicated that the drug is to be taken at 08:00 and 15:00 (4). The drug was a marketing success for Hoechst Italy and for Dr. Carandente, the manager, who had become a leader in the pharmaceutical industry of his milieu and beyond (5).

Agostino Carandente believes in the promise of chronopharmaceuticals, not only for the treatment of after-the-fact disease, but as a new category of drugs broadly for disease prevention (6). His philosophy is further to build bridges between many aspects of health and humanism, as discussed in printed detail in 1989 when 'Il Ponte' celebrated its 30th year (7). His penchant for the mathematical aspects of health care is one factor leading him to chronobiology; the other related factor is his recognition that life in the usual range of variation must be resolved by analytical statistics.

The motto of Chronobiologia, 'Measure what is measurable and render measurable in time what as yet is not' *(Omnia metire quæcumque licet et immensa ad mensuram* **tempestive** *redige),* is as much or more his motto as it may also have been that of Galileo, to whom it was attributed by Giovanni dell'Acqua, the late professor of medicine at Catholic University in Rome (8), and by the undersigned (9). Agostino welcomes the new endpoints of rhythms and trends; he recognizes them as objective and quantitative and also as meaningful and critical new tools for predicting clinical outcomes, since they are in the broadest sense timed and timely *(tempestive).* Current medicine, aiming at the cure of overt disease, may broaden its scope to become literally and figuratively individually quantified *health* care, rather than population-based *illness* care. Toward this goal, Agostino Carandente the industrialist has made the critical next step by becoming a worldwide mentor of the discipline as well as its publisher.

In sponsoring research on drug timing, Agostino Carandente supported chronobiology as a much broader science (the 'commune vinculum'). He did not want to restrict the scope of Chronobiologia to a utilitarian clinical trial forum, but had it serve all arts, all sciences, all disciplined human endeavors. He organized chronobiologic meetings in Capri for endocrinologists and for diabetologists, revolving around drugs that he marketed, yet he also considered broader chronobiologic topics at conferences he organized in L'Aquila, Como and Pavia, Italy.

Agostino saw to it that magistral lectures on chronobiology were given at international meetings of groups such as surgeons or cardiologists. In order to convince leaders of the World Health Organization, he invited them to mix with chronobiologists in Stresa, Italy. He contributed to meetings by chronobiologists elsewhere in Italy as well as in Hannover, Germany, and in Bethesda, Maryland; Washington, D.C.; Little Rock, Arkansas; Copper Mountain, Colorado, and to the first Gordon Conferences on Chronobiology in Plymouth and Andover, New Hampshire, all in the USA. He sent his country's delegations to these events and others, such as regular meetings of the International Society for Chronobiology. The programs of these conferences, including plant physiologists and scientists in animal husbandry as well as medicine, best demonstrate that chronobiology is of very broad scope as the common interdisciplinary tie of many fields and many concerns, agriculture as well as education and the cosmos as well as *terra firma* and the humanities.

Agostino built the first laboratory totally dedicated to chronobiology in L'Aquila, with special facilities for human isolation and for animal monitoring (by vital sign telemetry and by an automatic rodent urine collector, with provisions for the immediate freezing of the urine at the end of preset intervals). His physician daughter Franca became Italy's first regular professor of chronobiology, first in L'Aquila and then in Milan. In L'Aquila, postgraduate courses were given to the winners of a national competition by an international faculty of chronobiologists. The Hoechst Foundation paid all expenses of the selected postdoctoral scholars (and teachers), and through them Agostino spread the new science in his country and beyond.

As Secretary-General of the Hoechst Foundation, he realized that the sensor technology was and remains available to measure vital signs automatically for long spans, even lifetimes. Hence, he explored a relation of his firm with another multinational giant, Siemens. He started the development of the Polychronor, a multivariable physiologic monitor. Much of the chronobiologic software had been developed over the previous decades, largely in preparation for a $100 million U.S. Biosatellite study. What was (and still is) needed was to combine hardware and software into a readily-acceptable, unobtrusive unit that would both collect the data and provide, as-one-goes, a chronobiologic interpretation thereof. At any time, the whole past history of vital signs would become available and could be recalled in a moment by the interested chronoliterate individual and/or at any visit to the physician or other health professional. This longitudinal vital sign history would drastically improve the already-invaluable history. Indeed, a history is important, since among physicians of comparable skill, those allowed only a physical examination were less often correct in the diagnosis of the same patients than those who were only given the history. This is hardly surprising, since the single 'physical' is a snapshot of a roller coaster, when, for instance, blood pressure usually varies by over 50 mm Hg in 24 hours. The best of two worlds is to make available at the time of a physical examination an as-one-goes 'digested' summary (history) of at least some of the vital signs aligned with any symptoms also recorded as-one-goes, along with subjectively rated and coded measures of well-being. Such an instrument for resolving the physiologic dynamics within the normal range could indeed also record, concomitantly and as-one-goes, events and activities, including exercise and emotions (along with the vital signs and any symptoms). Thus, one could, for the first time, amalgamate and improve both the classical history and the status quo. The latter would now represent an as-one-goes 'history' as a start, at least for vital sign measurements. This Diapolychronor would immensely improve the current history, which is often based solely on an imperfect memory. Automatically, one would obtain a 'historical physical examination.'

The availability of this 'history' from physiologic monitoring and its ongoing analyses, aligned with a diary, for the resolution of the organism's time structure, could immensely improve curative health care, yet its major value would be in disease prevention. The multifrequency rhythms and trends of each pertinent variable, the chronomes, could thus be continuously updated and any of their alterations scrutinized in lieu of exclusive reliance on the 'signs' and 'symptoms' at the moment of a visit to health care personnel. Timing could then be added to dosing in such a device for any therapy, whether by electrical, other physical means or drug delivery devices, as needed. The Polychronor was an interesting, critical if as yet only halting, step toward the foregoing long-range goal of a Diapolychronor for the recognition of chronome alteration and the institution of corrective action, as soon as possible, and preferably automatically.

Agostino joined his faith in the future of chronobiology to his love of his family by guiding his physician children into the field. His daughter Franca Carandente, now holder of the chair of chronobiology as a full professor at Italy's major university in Milan, is a foremost teacher of the discipline and the author of a text in the field; she also manages Chronobiologia and coordinates research. Investigations with her at different levels of organization document, in Chronobiologia, the time-dependence of the effects of ACTH 1-17, an analogue of the natural hormone, and the importance of the timing of cefodizime, a drug tested for use as both antibiotic and immunomodulator. Drugs which help host defense as well as attack an invader will be particularly important for the treatment of cancer and AIDS.

Agostino's son Orazio, a clinical cardiologist in Milan, has also contributed to a better understanding of our time dimension, whether it be in relation to transplants of the pancreas in the laboratory or to a budding chronocardiology in the clinic. He contributed a review of the many body functions coordinating blood pressure dynamics introducing 'homeostatic' results with initial chronobiologic qualifications. His name can be found on the metachronanalysis of an invaluable longitudinal ECG record demonstrating time-microscopically free-running in data that seem to be 24-hour synchronized and also on a review of the time structure of cardiac arrhythmia, i.e., ventricular and supraventricular arrhythmia vs. atrial fibrillations. These findings are authenticated responses to the intuition of his father Agostino.

Most recently, Chronobiologia provided statistics based on millions of cases that reveal the importance of about 7-day morbidity patterns worldwide. In the same year, Chronobiologia also provided an inferential statistical basis for carrying out chronobiologic study designs cost-effectively and with high power.

In professional and in personal relations, Agostino's magnanimity remains unsurpassed. Only ingrates among those very many who were so often invited to his gourmet table could forget his intuitive genius, the best aspect of his hospitality. 'Keep going' (in German: **"Gehen Sie weiter"**) is his consistent advice. Although he is formally retired, he still does all he can in maintaining his role as mentor as well as an active publisher. The quality of his publications remains exquisite in form, to say nothing of content. His greater family consists of chronobiologists worldwide: Brazil, China, France, Germany, Italy, Japan, Mexico, Poland, Spain and the USA are the homes of some of those honored by his many Chronobiologia Awards.

Even if chronobiologists publish also, as they must, in 'regular' journals of their original formal specialization, Chronobiologia fulfills a need. As a minimum, it saves the definitions of terms, if not an entire glossary, with each paper. As an optimum, it constitutes an outlet for eminently chronobiologic findings such as circadian amplitude-hypertension and the associated increase in left ventricular mass. Chronobiologic publications open avenues for new modalities to diagnosis and prevention. The journal is a forum for many in addition to the more than 100 participants in the international chronome endeavor, some with teams of their own, throughout his beloved Italy, as well as in other countries worldwide. The chronome initiative from womb to tomb, aiming at medical chronobioethics, with focus upon the priorities in early life, is also a dynamic monument to Agostino.

AD CHRONOBIOETHICA MEDICA: Propositiones chronobiomedicæ tardæ (Prima ad Tertiam) in agenda tempestiva praeventiva (Quarta ad Sexta) transformandae

Primum *nil nocere*
Secundum *nil tormentare extendendo vitam, a medicis tempestivis desperatam, patiente neque gaudente neque volente*
Tertium, *quæ cum ita sint, pro clementia ac misericordia, nil prohibere quin vita lege celeriter tamen conficiatur patientis confici desiderantissimi desiderantissimæve*
Quartum *ad mensuram redigere chronomata: periodicitates vel inclinationes, non unas sed maximas vitæ proprietates*
Quintum *his numeris chronobiologicis uti ad crescendam salutem viventium totiusque mundi integritatem*
Sextum, *per examinationem chronomatõn laborare ut periculum morbi eliminetur et finis vitæ adveniat sua sponte et sine dolore totiusque mundi pollutio cognoscatur ac eliminetur*

TOWARD MEDICAL CHRONOBIOETHICS: Emphasis upon late chronobiomedical ethics (First to Third) must be transformed (Fourth to Sixth) into a timely preventive agenda

First: Do no harm
Second: Do not torture a patient who neither enjoys nor wishes to continue a life for which chronomedicine can provide no hope
Third: Under the foregoing circumstances, in the name of mercy, do not prevent the legal yet speedy termination of the life of those demanding it
Fourth: Render measurable chronomes—the characteristics, chrones, of rhythms and trends, that are not **some** but **the principal** features of life
Fifth: Use these new chronobiologic quantified characteristics, the chrones, for optimizing the health of the living and the integrity of the environment as a whole
Sixth: By examining chronomes in health, work toward eliminating undue disease risk so that, for the individual, the end of life occurs painlessly and spontaneously and, for the environment, early pollution is recognized and eliminated

Address to the International Society for Research on Civilization Diseases and the Environment, in the house of the European Union Brussels, December 10, 1993

MEDIZINISCHE CHRONOBIOETHIK IN STATU NASCENDI:
Ein Schwerpunkt verspäteter chronobiomedizinischer Vorschläge (Vom Ersten zum Dritten Punkt) sollte auf eine zeitgerechte und zeitlich gezielte Vorbeugung (Vom Vierten zum Sechsten Punkt) vorverlegt werden

Erstens *nicht schaden*
Zweitens *nicht quälen diejenigen die weder das Leben geniessen noch es weiterführen wollen, sobald eine Chronomedizin keine Hoffnung ermöglicht*
Drittens, *im Sinne der Barmherzigkeit, unter den vorangehenden Umständen, verhindere nicht die rechtliche und schnelle Beendigung des Lebens derer die dies wünschen*
Viertens *mache messbar Chronome—die Kennzeichen, Chrone, von Rhythmen und Tendenzen welche die fundamentalsten Eigenschaften des Lebens erfassen*
Fünftens *nutze diese quantifizierten chronobiologischen Kennzeichen zur Optimisierung der Gesundheit alles Lebendigen und der Integrität der gesamten Umwelt*
Sechstens *schaffe durch Chronomuntersuchung an der Abschaffung gesteigerter Risiken, so dass für das Individuum das Lebensende spontan und schmerzlos eintritt und für die Umwelt das Frühstadium der Verschmutzung erkannt und beseitigt wird*

It is a great pleasure, in behalf of outstanding scholars in the field of biologic clocks whom Agostino also regularly invited to his courses and meetings, and in the name of many fellow students of quantitative chronobiology, to salute him with deep gratitude and even deeper affection. To paraphrase Winston Churchill, never in the brief history of chronobiology is so much being done so promptly for so many than it is now by a concerned paterfamilias, dear friend and visionary chronobiologist, Agostino Carandente:

'per aspera et per amicitiam ad astra et ad chronobiologiam,

commune vinculum

omnibus artibus

omnibus scientiis

omnibus disciplinis

*salutis gratia totorum viventium mundique totius.'**

Franz Halberg

*'Through hardship and friendship to the stars and chronobiology, the common bond of all arts, sciences and disciplines, subserving the 'health' of all life and its cosmos.'

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INTRODUCTION TO CHRONOBIOLOGY

Variability: from foe to friend

A summary of over 40 years of studies building a new, originally Minnesotan science with emphasis beyond circadian systems (a concept coined and documented at the University of Minnesota) upon resolving the chronome, the lawfulness within the physiologic range in which healthy function occurs.*

**Like* genome, *derived from* gene *and chromo*some, *omitting part of the root words,* chronome *is derived from* chronos *(time),* nomos *(rule) and chromo*some *to describe the structure of rhythms and trends characterizing every biologic variable in its physiologic range of variation. Chronobiology splits this otherwise neglected seemingly indivisible range into rhythms, as does fission to the 'indivisible' atom.*

While counting eosinophils, a white cell, in mouse blood, Franz confronted a confusing variability: without any apparent reason, a count of a hundred or more cells per mm^3 of blood was followed by one near zero; or, in a different inbred strain of mice, a count of a thousand or more cells was followed by one of one hundred or so. Several kinds of blood cell counts were high at one time of day and low at another. These large changes persisted and even increased in extent when extra precautions were taken not to disturb the animals by handling them as little as possible. With one time-unspecified count of blood eosinophils, no conclusions could be drawn. The same enormous variability characterized the content of glycogen in liver or the number of cells in the state of division in this organ and other tissues. The variability remained unpredictable until Franz pursued the patterns of variation in time of these and very many other variables. Statistically, the changes became predictable.

The underlying about-24-hour cycles were uncovered. It became apparent that some of these rhythms, not strictly but approximately periodic phenomena, were of large amplitude and high reproducibility. The application of relatively simple statistical procedures to the data allowed the quantification of the underlying patterns. Thus, a heretofore vexing variability was rendered predictable. The about-daily changes were named 'circadian' in order to emphasize by 'about' *(circa)* their statistical rather than purely deterministic causality and, equally important, their built-in nature: the spontaneous rhythms had periods near *(circa)* 24 hours as they recurred in similar *(circa)* but not identical sequences and at similar *(circa)* intervals which, on a statistical basis, differed from their precise environmental, societal or geophysical match. This finding applied not only to the circadian system, but also to about-7-day (circaseptan) changes and their multiples and submultiples, the multiseptans, to components with still other periods, all of which changed further with age and disease. Since all these components intermodulated, it was of immediate importance to broaden focus from a single rhythmic change to various rhythms with different periods in the presence of trends. Thus, the critical lawfulness of biologic structures in time, the chronomes, was unraveled, not only for the eosinophil counts, but for the activity of the body as a whole and its resistance to various stimuli, for mitosis in different organs and tissues, and for different liver functions, related to different stages of the cell cycle.

Applications to cancer treatment followed. The fields of chronopharmacology and chronotherapy developed, based on the demonstration of the hours of changing resistance to noise, bacterial endotoxins, radiation and drugs. While originally rhythms appeared to be the exception and regulation for homeostasis the rule, the ubiquity of rhythms was documented for practically any and all variables examined, along with their crit-

ical importance. For this purpose, an emerging science developed rigorous and objective methods for the test of rhythmicity and superimposed trends and for the quantification of the characteristics (the chrones) of rhythms and trends, that constitute the chronome of a given physiologic function or system. These procedures had to be applicable even when the data were not collected at regular intervals. The methodology served not only for the analysis of data but also proved to be of great value for optimizing the design of experiments. Human studies that involve thousands of people and cost hundreds of millions of US dollars can now be preceded by a chronobiologic pilot investigation on very few subjects, so that a large investment for the detection of rare effects is carried out at the right time rather than at a time when the effect may be altogether missed.

When the studies on blood eosinophil counts were repeated in mice kept under different experimental conditions such as different lighting regimens, Franz was again confronted with new sources of variability. In order to resolve this new problem, he decided to repeat the sampling on the same animals at different circadian stages in a longitudinal fashion, before and after the reversal of the temporal location of a regimen of light and darkness alternating at 12-hour intervals. He found that the timing of rhythms along the scale of 24 hours could be moved to any desirable location in time by manipulating the lighting regimen. This is how the role of the environment as a synchronizer became understood for variables such as the formation of ribonucleic acid (in liver), hormones in blood and the tolerance of potentially harmful stimuli impinging upon the body as a whole. Not only the timing of the alternation between light and darkness, but also meal timing could affect the timing of rhythms. The interactions among two or more synchronizers were clarified and social and ecologic synchronizers discovered.

The mechanism through which the alternation of light and darkness could affect rhythms was then sought. The study of blinded mice provided an answer and led to the discovery of the phenomenon of free-running. This finding in turn provided a key element to the development of chronobiology as a science *sui generis.* It meant that rhythms were not only an immediate response to the cyclic changes in the environment; they were not even learned in a given individual's lifetime, but they were an internal feature of organisms. The endogenicity of rhythms and their ubiquity rendered them a basic, fundamental property of life itself. These properties, first uncovered for the circadian system, were soon extended to other spectral components: the circasemiseptans, with a period of about 3.5 days; the circaseptans, with a period of about a week; the circatrigintans with a period of about a month; the circannuals, with a period of about a year; and others.

The intermodulations among rhythms and their critical importance from an applied viewpoint as well led to the concept of the chronome, the set of multifrequency rhythms and trends with development, maturation and aging. In health, during most of adulthood, changes as a function of rhythms' stages can exceed by far changes as a function of age.

Some investigators have taken the view that rhythms are controlled by a master oscillator or pacemaker which they localize in the suprachiasmatic nuclei. Claims are made that all circadian rhythms are obliterated after bilateral suprachiasmatic enucleation. Early confirmed work by Franz had shown the critical role of the adrenal cortex in coordinating circadian rhythms in blood eosinophils, but not in serum iron. Evidence here gathered shows that some circadian rhythms can persist in one or the other system after removal of either the suprachiasmatic nuclei (and even of the brain as a whole) or of both the adrenals. Neuroendocrine and peripheral systems intermodulate by means of feedsidewards, interactions among three or more *a priori* periodic entities. The modulation by the pineal of pituitary-adrenal interactions is a case in point. Periodically recurring sequences of stimulation, no effect and inhibition by a modulator, such as the pineal, upon the interaction of an actor such as pituitary ACTH with a reactor such as the adrenal, are the results of such feedsidewards. These intermodulations occur at several frequencies, as documented for the circadian and the circaseptan ones, and play an important role in the coordination of rhythms within the organism. They coordinate the manifestation of all rhythmic

behavior by the organism. Feedsidewards constitute the temporal matrix of life. Feedsidewards replace as concept and documented fact putative time-invariant feedbacks by the predictably time-variant interactions at several frequencies among *a priori* periodic features of the organism, Table 1.

This point of view leads to the proposition that there is merit in mapping the chronome as a way of quantifying health. To cite but one example, blood pressure or heart rate rhythms have been interpreted as a result of motor and other activity, meals, rest and/or sleep and emotions. All of these factors influence these variables but do not account for the persistence of their rhythmicity in bedrest or in weightlessness or for the fact that a blood pressure increase precedes rather than follows the daily increase of motor activity. The basic spontaneous preparation by neuroendocrine and cardiovascular activation in anticipation of daily activities must not be mistaken for a mere response to external stimuli. In this sense, certain blood pressure or other rhythm alterations resolved by the methods of chronobiometry can be interpreted as an elevation of the risk of developing one or the other civilization disease. Such blood pressure or other chronome disorder may occur within the currently 'acceptable physiologic range' before disease becomes overt. There is hence an opportunity to intervene early and prophylactically in response to chronome alteration and the potential to reduce health care costs while improving the quality of care with emphasis on prevention by self-responsibility and self-help. Chronotherapy is then placed into the service of both primary and secondary prevention, while it is also useful for timing the treatment of overt disease.

Table 1
TWO VIEWS OF MEDICINE AND BIOLOGY

View	Homeostatic	Chronobiologic
1. Reality	Set Points	Rhythms and Trends
2. Endpoints	Mean ± SE	MESOR Period(s) Amplitude(s) Acrophase(s) (Waveform) — Coefficients of Polynomials
3. Physiologic Range of Variation	Broad Random	Narrow Structured by Rhythms and Trends Predictable
4. Sources of Variation	Exogenous Response to External Stimuli	Endogenous and Exogenous Genetic Program, Including Spectral Structure, Allowing for Anticipation of Stimuli, Preparation for Them, and Prevention of Harm
5. Hierarchy	Up/Down	Collateral and Up/Down
6. Teleonomy	Time-Unspecified Righting Regulation	Lead-Timed and Time-Specified Preparation and Correction Coordination
7. Mechanism	Feedbacks (Erratic Modulation) Between Paired Entities Along Axes of Networks	Feedsidewards (Predictable Chronomodulation) Among More Than Two Concomitantly Interacting Entities in Networks
8. Simplified Analogy	Thermostat	Pendulum
9. Biologic Implications	Darwinism (Based on Natural Selection of Random Mutations That Are Externally Adaptive)	Internally Integrative As Well As Externally Adaptive Evolution
10. Medical Applications	Mainly Limited to Diagnosis Of Overt Disease by Values Outside The Physiologic Range Chronome-Unspecified Treatment*	Diagnosis and Treatment Refined by Narrowed Reference Range And Assessment Within That Range of Chronorisk Leading to Prevention and Timely and Timed Treatment
11. Value	Wasteful	Cost-Effective
12. Personal Satisfaction	Frustrating Work	Sheer Fun

*Notable exceptions such as vaccinations notwithstanding.

The following are some annotated illustrations of data underlying major concepts, facts and methods in the field of chronobiology.

1. From confusing variability to the ubiquitous and critical lawfulness of murine chronomes

Time plots of original values (dots) of variables such as blood eosinophils (E) (Figure 1/I, left) or glycogen content (Figure 1/III, left) reveal great variability, confusing at first, until the data are processed by relatively simple statistical techniques such as averaging and stacking over an idealized 24-hour span corresponding to an anticipated periodicity. Once this is done and the results are displayed as a function of time, they show the time-macroscopic ubiquity of circadians. The data averaging for different hours of the day also reveals differences in the time course (in phase) of different functions of a given organ such as the liver (Figure 1/VC; see also Figure 2/III), of cell division (mitosis) in different organs and tissues (Figure 1/VB) and of different variables at the level of the body as a whole (Figure 1/VA and D). The structure of the circadian system becomes apparent by the application of the methods of chronobiometry: the circadian variation in blood eosinophils determined years apart in two laboratories as far apart as Minnesota and Maine is closely reproduced (Figure 1/I, right). The lawfulness of the circadian variation yielded by the application of chronobiologic techniques is also revealed for the drastic changes in liver glycogen content (Figure 1/III, middle). In this case, a circadian rhythm in the liver's glycogen is seen to persist under conditions of starvation and dehydration, with little if any alteration in the dynamic rhythm characteristics, as compared to usual *ad lib* conditions, once the data are expressed as a percentage of the overall series mean, Figure 1/III, right.

After a prominent circadian rhythmicity was found at different levels of organization, several series of experiments were carried out under rigorously standardized laboratory conditions in order to investigate the effect of a single physical stimulus such as the exposure to noise. Outcomes were as different as no response, convulsion or even death, as a function of the circadian stage at which the organism was exposed to noise. Whether the stimulus was audiogenic or the exposure to an endotoxin, or to a drug such as ouabain, or to whole-body irradiation, predictable changes were found as a function of the circadian stage at which the stimulus was applied, albeit with differences in the timing of these susceptibility-resistance rhythms to different agents. The *hours of changing resistance* were thus uncovered, and the times of overall largest response by the organism to a fixed stimulus applied at different rhythm stages mapped (Figure 1/VD). Applications followed (Figure 1/IV). Prominent susceptibility rhythms were documented in the experimental laboratory, as illustrated here for the case of the mortality from agents affecting the central nervous system and for the case of the survival from (tolerance of) toxic doses of anticancer drugs. In each case, the nonoverlap by the elliptical 95% confidence region of the center of the circular plot (pole) can be interpreted as the presence of a statistically significant circadian rhythm in the susceptibility of the organism to each of these different agents. The orientation of the directed line (vector) indicates the time of acrophase, that is the time of the largest anticipated response. Such charts are helpful in guiding the timing of the administration of the various agents so mapped. The chronotherapy of cancer is one critical application resulting from this work.

FROM CONFUSING VARIABILITY TO UBIQUITOUS AND CRITICAL LAWFULNESS OF MURINE CHRONOMES

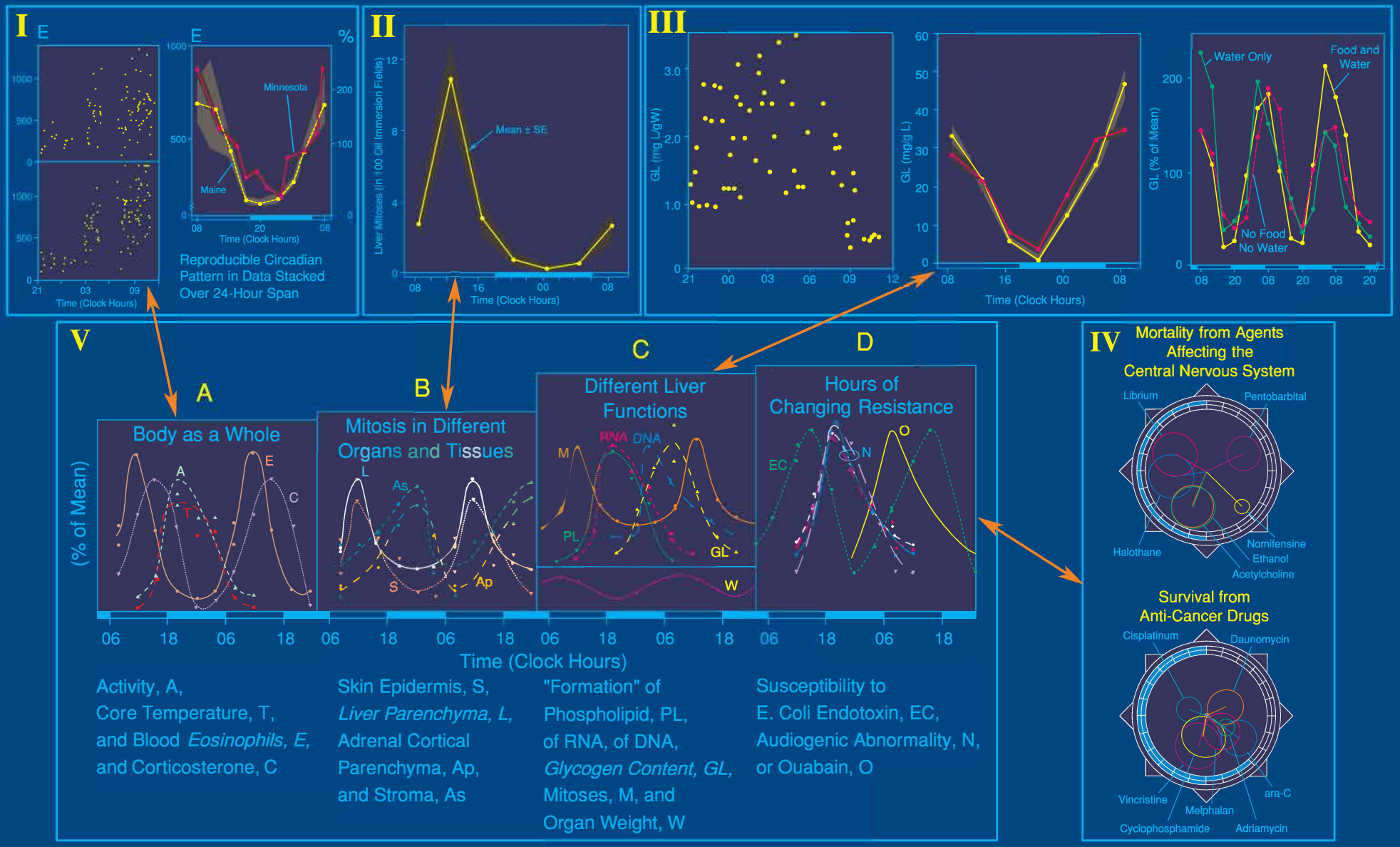


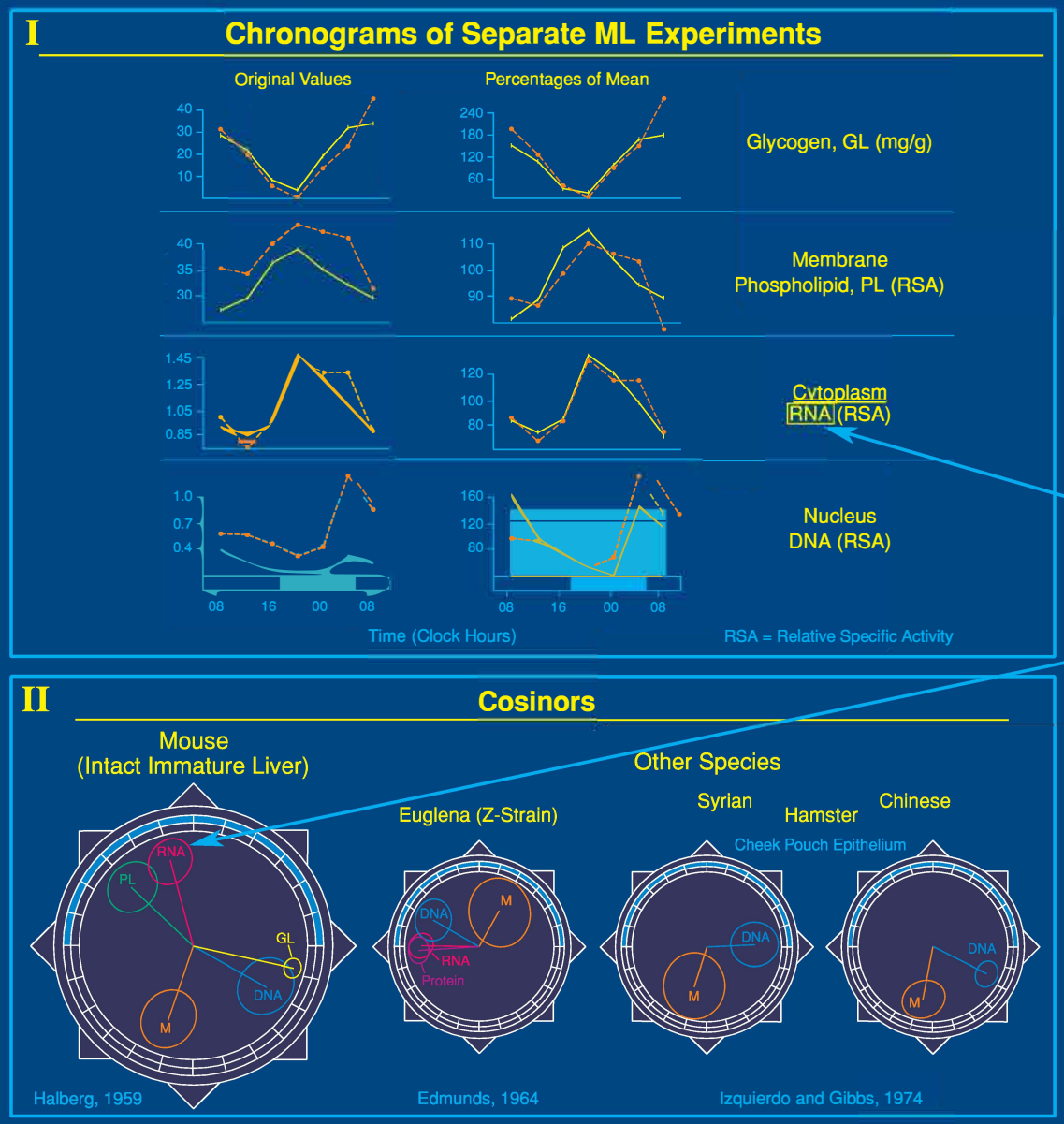
Fig. 1

2. Circadian cell cycle

The marker rhythms of an *in vivo* circadian cell cycle (Figure 2/III) time-specify the classical cell cycle and fill in some of its gaps (G). Circadians are revealed by a combination of histology for mitotic counts, differential centrifugation for cellularly localized wet chemistry and tracer studies for the determination of the relative specific activity of phospholipid, RNA and DNA phosphorus. In growing mouse liver, as in the organ regenerating from partial hepatectomy, daily recurring RNA formation precedes DNA formation, at variance with the former dogma postulating information flow from DNA to RNA to protein without any consideration of rhythmicity and its timing. The circadian rhythmicity of each variable and the sequence of acrophases, that is the timing of overall high values recurring in each cycle, are reproduced in separate experiments (Figure 2/I). The reproducibility improves when the data are expressed as a percentage of the mean, whereby modulations of the mean by rhythms other than circadian may be reduced. These bioperiodicities can be quantified by the cosinor method, which describes the 24-hour data set as a whole rather than by the inspection of a single value, the peak in a plot of the data as a function of time. Indeed, a polar cosinor plot (Figure 2/II) shows the sequence of events that start at the level of the membrane by phospholipid labelling. RNA formation in the cytoplasm follows promptly, and, with a lag, DNA formation and eventually mitosis.

The sequence from RNA formation to DNA formation to mitosis here demonstrated in the intact immature mouse liver is also found in regenerating liver (not shown) and in *Euglena.* The time relation between DNA and mitosis is also mapped in hamsters (and humans, not shown). These findings attest to the generality of the rhythmicity in DNA formation, whereas earlier DNA was regarded as the most constant feature of organisms. The critical importance of a circadian cell cycle lies in its eventual use for cancer therapy targeted in time.

24-H SYNCHRONIZED CIRCADIAN CELL CYCLE IN GROWING MOUSE LIVER (ML) AND ELSEWHERE



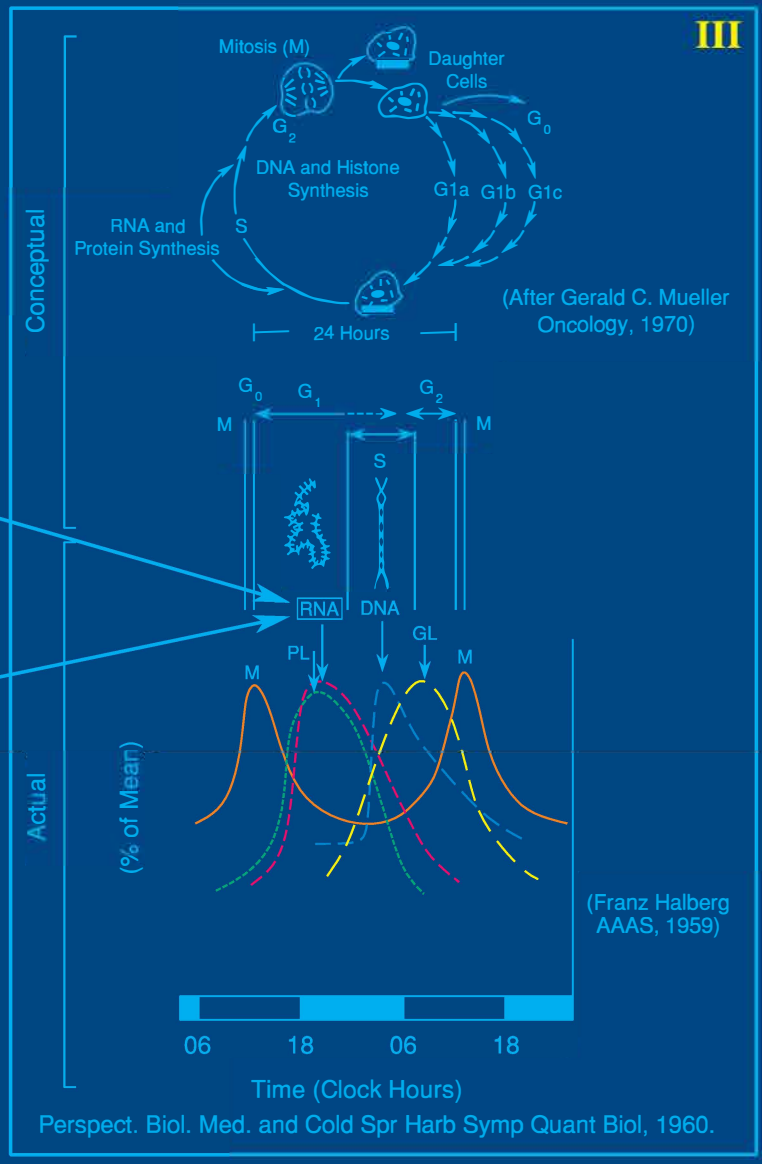


Fig. 2

3. Applications to cancer chronotherapy

The treatment of cancer is usually scheduled by the convenience of the health care system first, according to the availability of the clinical facilities and of the physician or nurse. Chemotherapy being usually highly toxic, the drug may cause harm to various organs, from the gut and kidney to the heart and/or bone marrow. The cancer (shown as a crab) may also be hit, but the treatment by convenience may not be optimally planned. As a consequence, before killing the cancer, the treatment may kill the patient (Figure 3/I, #1). Charts such as those shown in Figure 1/IV are helpful for determining when a given agent is less toxic to the host; they make it possible to time treatment to minimize the undesired toxic effects of the treatment. This targeting for the times of the optimal tolerances constitutes an important improvement, but in itself it is not a sufficient advance. If the scheduling of the treatment takes into consideration only toxicity to the host, treatment may not be optimal in terms of killing the cancer. As a result, targeting by host markers lessens the impairment of life quality by debilitating and nauseating drugs, but it does not necessarily increase the treatment's efficacy (Figure 3/I, #2). The possibility of minimizing toxicity by timing is illustrated for the case of adriamycin (Figure 3/II). Results from five separate studies involving a total of 858 mice show that mortality reaches 80% when adriamycin is administered in the middle of the daily dark (active) span, whereas it is only 30% when given in the daily light (rest) span. Overall, the time when this drug is least tolerated is late during the dark (active) span. Translating these results to humans, some usual clinic hours may correspond to a time when the host is most susceptible to the toxicity of this drug. It seems reasonable to seek the time of best tolerance for treatment unless the best time for killing the cancer can be determined.

Large-amplitude circadian (and other) rhythms have been mapped for some tumor markers in saliva and urine, where they may not or at best only indirectly reflect tumor burden. The non-invasive assessability in serial samples of urinary or salivary rhythms, and any immediate decrease as an index of the time of drug activity could render these markers suitable for guiding treatment timing so as to optimize efficacy. Marker rhythm-guided chronotherapy was carried out with prednisolone on the LOU rat bearing a transplanted immunocytoma: the excretion of light chains by this tumor is circadian periodic for a large part of the lifespan remaining after tumor inoculation. In this very attractive model, a therapeutic gain of about 70% is associated with optimal timing in relation to the circadian rhythm in urinary light chains excretion used as a tumor marker (Figure 3/III, left).

In the clinic, a relatively unspecific marker, namely cancer temperature, served for guiding the radiotherapy of patients with large tumors of the oral cavity (Figure 3/III, middle, right and bottom left). Radiation was applied for five weeks. Patients were randomly assigned to receive daily treatment at one of five circadian stages, either at the time of their daily peak tumor temperature (shown by a star) or 4 or 8 hours before or after that time. Peak tumor temperature was determined by assessing the circadian variation from repeated measurements of the tumor temperature, taken several times a day for a few days prior to the start of treatment. Tumor regression rate was largest for those patients receiving the treatment at the time of their peak tumor temperature. Patients treated at that time also had the largest percentage of disease-free survival at a two-year follow-up. Both in the experimental laboratory and in the clinic, chronotherapy is feasible and accompanied by large therapeutic gains. While targeting treatment in time by tumor markers may increase the chances of killing the cancer, the treatment may still be accompanied by great toxicity (Figure 3/I, #3).

The longitudinal assessment of proliferation markers in a patient (EH, 72 y) with a müllerian duct adenocarcinoma involving the ovary reveals circadian, infradian (notably circaseptan) and ultradian components, notably with periods of about 14 to 16.8 hours, as illustrated for the salivary concentration of CA130 and for the urinary excretion rate of macrophage-colony stimulating factor (M-CSF) (Figure 3/IV). When, apart from their circadians and infradians, some tumor markers exhibit ultradian variations, and thus more than one peak per day, the best time can be sought to administer the treatment to optimize its efficacy first while also, as a secondary consideration, attempting to optimally shield the host from the treatment's toxicity (Figure 3/I, #4).

Those not statistically inclined may proceed directly to chapter 5.

TARGETING CANCER (♋) TREATMENT IN TIME WITH PRIORITY FOR EFFICACY (AND TOLERANCE THEREAFTER)

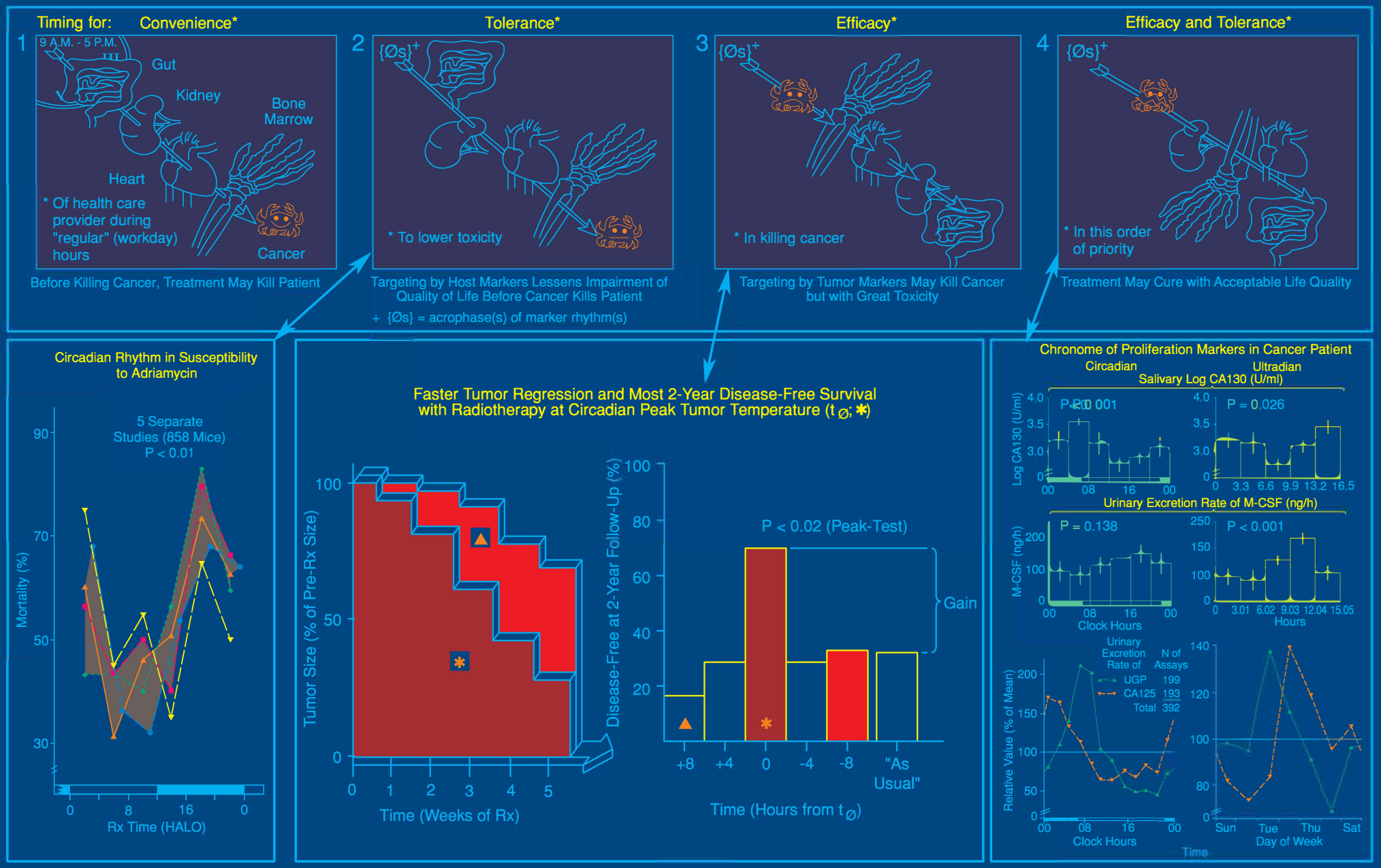


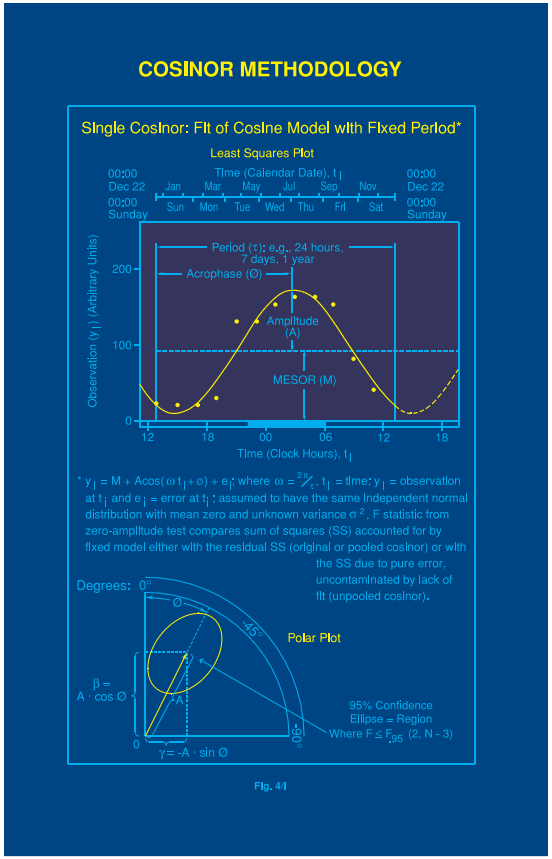
Fig. 3

4. Methods of chronobiometry

The single cosinor method involves the least-squares fit to the data of a model consisting of one or several cosine curves with one or several periods anticipated to characterize the data, with or without the inclusion of polynomial trends. To obtain estimates of the MESOR (a rhythm-adjusted mean), the amplitude and acrophase (measures of extent and timing of change within a cycle) of each cosine term entering the equation, as a first approximation, the error term is assumed to have an independent normal distribution with mean 0 and unknown constant variance σ^2. When the periods can be anticipated on the basis of prior biologic information, the equation can be linearized in its parameters, so that linear least-squares techniques can be used (Figure 4/I); otherwise the adjustment needs to be done nonlinearly, in which case point-and-interval estimates are also obtained for the period(s) in addition to the MESOR, amplitude(s) and acrophase(s). An F-statistic is used to perform a zero-amplitude test by comparing the sum of squares accounted for by the model either with the residual sum of squares (original or pooled cosinor), or with the sum of squares due to the pure error, uncontaminated by lack of fit (unpooled cosinor). For each cosine term entering the equation, a polar representation can be used to illustrate its characteristics. The amplitude-acrophase pair is represented by a directed line (vector); the length of the vector represents the amplitude and its orientation along the circular scale in relation to the selected reference time (0°), the acrophase.

For circadian rhythms, the reference time is usually midnight, mid-sleep or the time of the acrophase of another physiologic variable such as body core temperature; circaseptan rhythms are usually referred to midnight between Saturday and Sunday, and circannual rhythms to midnight on December 22 preceding the start of data collection. The 360° of the circular scale are equated to one full cycle (i.e., to the period length). By using a cosine (rather than a sine) function, 0° can be placed on top of the circle; by expressing the acrophase in negative degrees, angles vary clockwise. The ellipse shown around the tip of the vector represents the 95% confidence region for the joint estimation of the amplitude and acrophase. From this error ellipse, conservative confidence intervals can be derived for the amplitude and acrophase separately by taking concentric circles tangent to the error ellipse and by drawing the tangents from the center of the graph (pole) to the error ellipse, respectively.

The advantages of rhythmometry (Figure 4/II) are: 1) a more accurate and more precise estimate of the overall mean value provided by the MESOR. When data are non-equidistant, the estimate of the arithmetic mean but not of the MESOR may be biased, for instance, when most of the data are collected near the acrophase of a rhythm and few data are collected near its bathyphase; when data are equidistant, the estimate of the MESOR usually has a



smaller standard error (not shown); 2) the double amplitude represents the extent of predictable change within a cycle whereas the range may include outlying values that may but need not be biologically meaningful (in the case of technical blunders); 3) the acrophase represents a more robust measure of timing of overall high values than the time of a single value-based maximum.

Once estimates of the MESOR, amplitude and acrophase of a given periodic component are obtained for several series on a given individual, or on several different

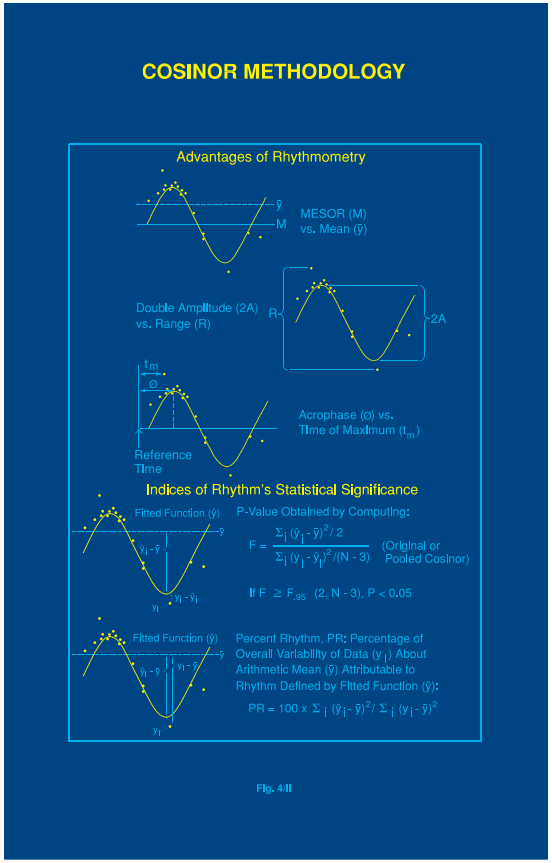


Fig. 4/II

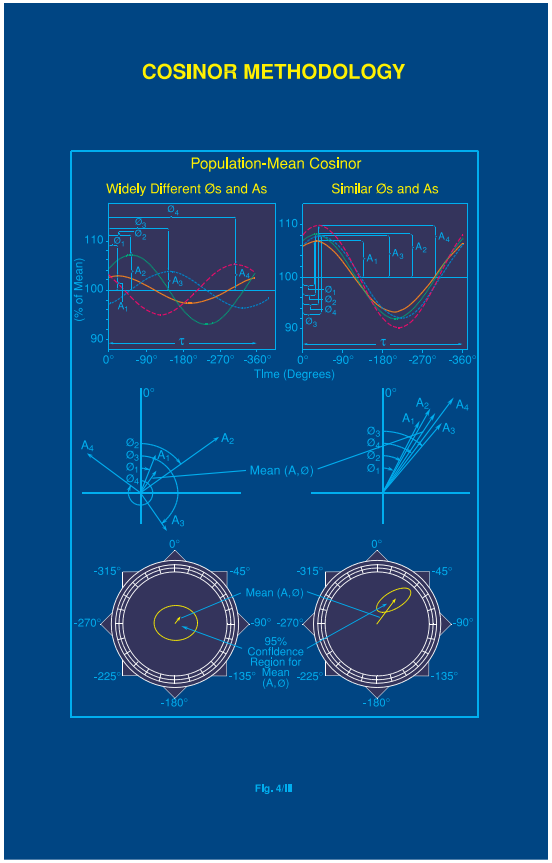


Fig. 4/III

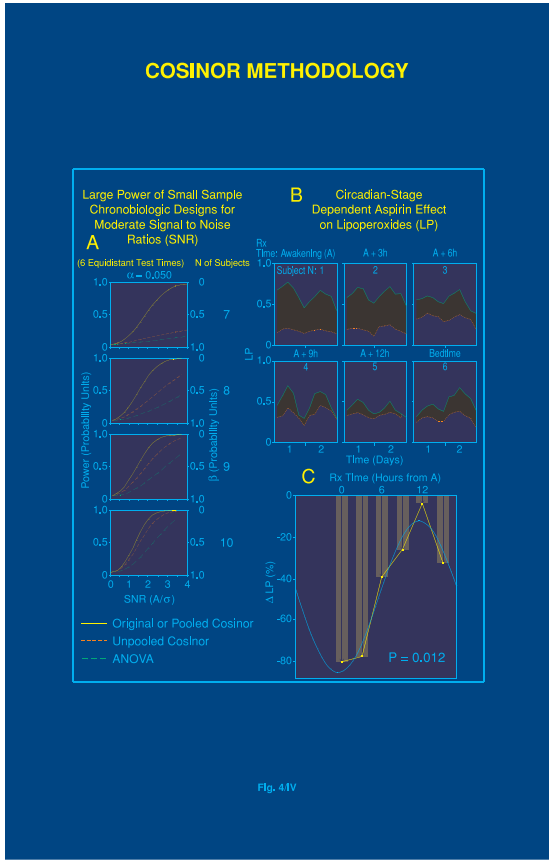


Fig. 4/IV

individuals for a given variable, the extent of clustering or similarity of these amplitude-acrophase pairs can be estimated by the population-mean cosinor method, which can be applied to multiple series from a given individual as well as from a population. Two abstract examples are shown (Figure 4/III). On the left, the case of 4 series with widely different acrophases and amplitudes is shown, whereas on the right, the case of 4 series with similar acrophases and amplitudes is illustrated. The fitted cosine functions are shown on top, their vectorial representation in the middle, and the cosinor representation at the bottom. Even though the curves are perfectly sinusoidal, when the amplitudes and acrophases are widely different, the error ellipse covers the center (pole) of the plot so that the zero-amplitude (no-rhythm) assumption cannot be rejected. When the amplitudes and acrophases are similar and they cluster in a given region of the plane, the error ellipse does not cover the pole. Thus, a rhythm can be documented by rejection of the zero-amplitude hypothesis; inferences can then be drawn for the 'population' as a whole, i.e., for a set of time series from one or several individuals.

The merit of using cosinor rhythmometry in designing experiments (Figure 4/IV) is illustrated theoretically by power considerations (IVA) and practically by actual data on the effect of daily low doses of aspirin on circulating lipoperoxides based on a sample of only 6 women (IVB and C). By assigning subjects more or less evenly along a full cycle of an anticipated periodicity (in this case between awakening and bedtime), it is shown that relatively large power (~80%) can be reached to resolve the rhythmic structure of the data with chronobiologic designs based on a relatively small sample (N=8) for

POWER OF COSINOR APPROACH ENABLING SMALL SIZE (N) CHRONOBIOLOGIC DESIGNS

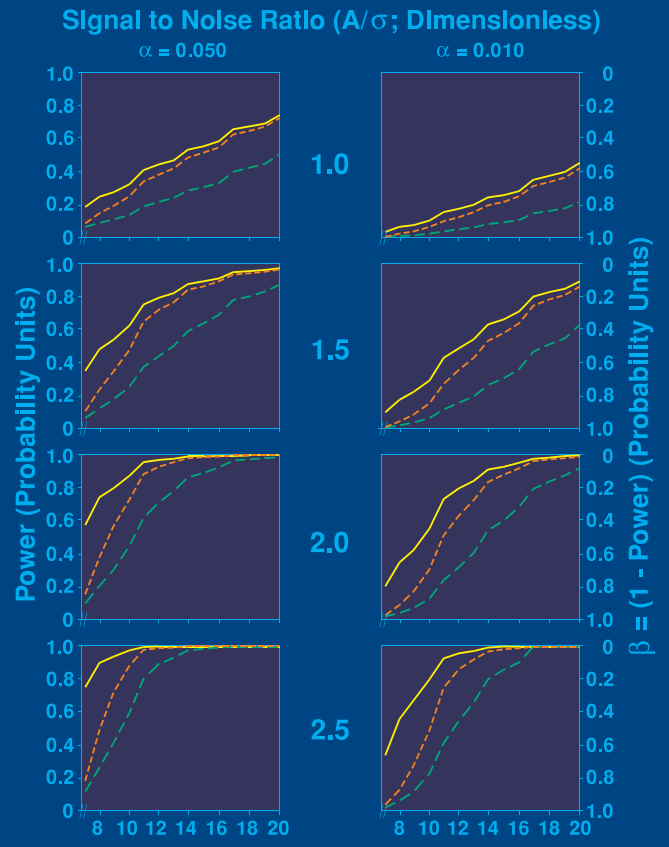
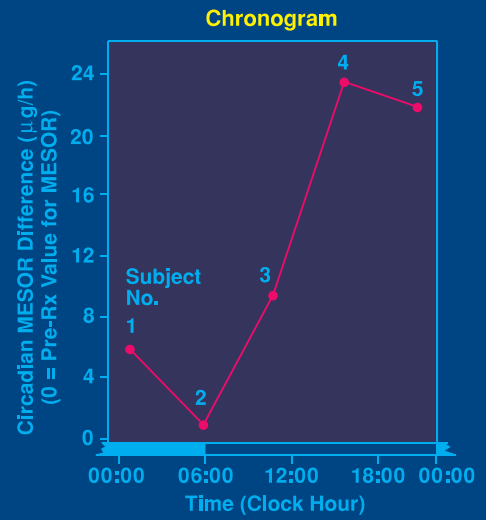
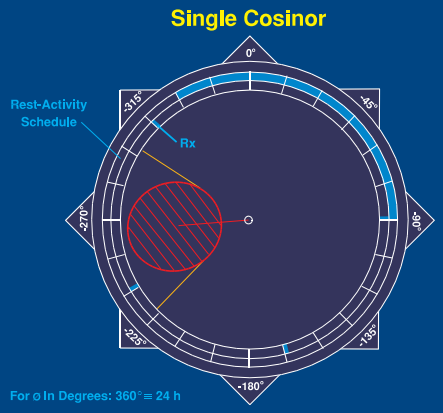


Fig. 5

N-OF-5 STUDY: CIRCADIAN RHYTHMIC URINARY FREE CORTISOL RESPONSE TO ACTH 1-17 BY HETEROGENEOUS SMALL GROUP OF PATIENTS WITH RHEUMATOID ARTHRITIS*

Response Criterion: Circadian MESOR Difference (from Before to After Rx)





P	Percent Rhythm	MESOR ± SE	Amplitude	Acrophase (ø) (95% C.L.)
0.021	98	12 ± 1	13 (5, 21)	-265° (-227, -303)

CL = Confidence Limits

* 5 men, 45-75 years of age, with rheumatoid arthritis, each provided 6 urine samples in unequal daily fractions (19:30-06:00, 06:00-08:30, 08:30-11:30, 11:30-13:15, 13:15-17:30 and 17:30-19:30) for 24h before and after Rx. MESOR determined from fit of 24-h cosine curve to each man's data before and after Rx. PR = Percent Rhythm; P = P-value from zero amplitude test. Günther et al., 1980.

Fig. 6

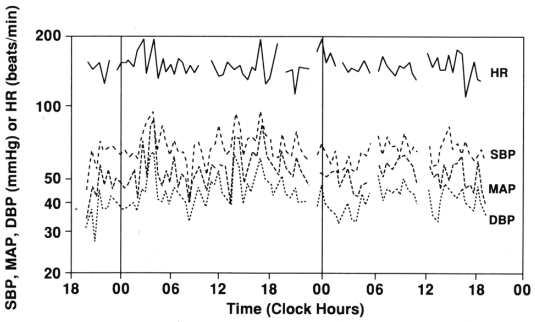
moderate signal-to-noise ratios (~2) when the data are analyzed by cosinor and, to a lesser extent, when the more conventional analysis of variance is used (see also Figure 5).

In practice, a chronobiologic design allowed, for instance, the demonstration of a circadian stage-dependent effect of aspirin on lipoperoxides on the basis of only 6 subjects, each assigned randomly to a given treatment time. Each took 100 mg of aspirin each day for one week and provided blood samples at 4-hour intervals for the determination of lipoperoxides in platelet-rich plasma for two days prior to the start of treatment and during the last two days of the aspirin test span. Whereas the effect is quite pronounced in the morning, it is barely demonstrable 12 hours later.

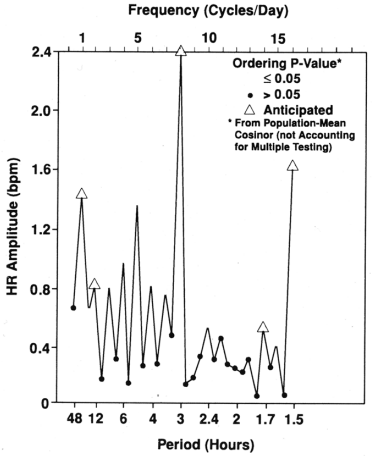
For the case of an ACTH analogue as well, a chronobiologic pilot design based on only 5 subjects serves to demonstrate that an effect clearly apparent at one circadian stage can be missed at another circadian stage, Figure 6.

Microscopy in time: How to resolve a ubiquitous rhythm and trend (chronome) structure in noisy data?

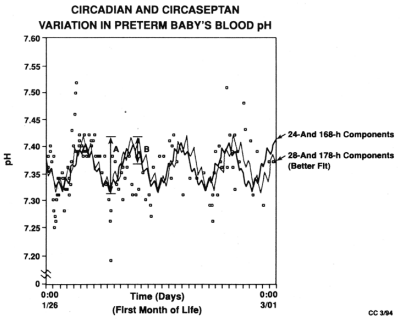
1. The naked eye discerns variation but no regular cycles in 48-hour blood pressures (BP) and heart rates (HR) during the first week of life. [Data of B. Tarquini and G. Mainardi]



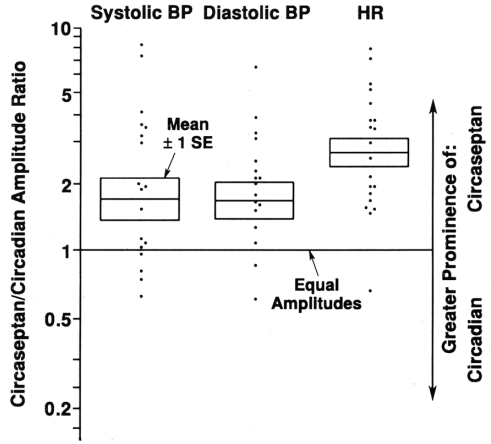
2. Time-microscopy (a least-squares spectrum) transforms records of a large number of babies to display the extent of change (amplitude) as a function of the frequency (of change): peaks represent circadian and ultradian rhythms, the largest peak at 1 cycle in about 3 hours probably related to feedings.



3. A premature baby boy is monitored for the first month of life. A circadian and an even more prominent circaseptan can be demonstrated for blood pH as well as for other vital signs such as blood pressure and heart rate.
[Data of J. Rigatuso]

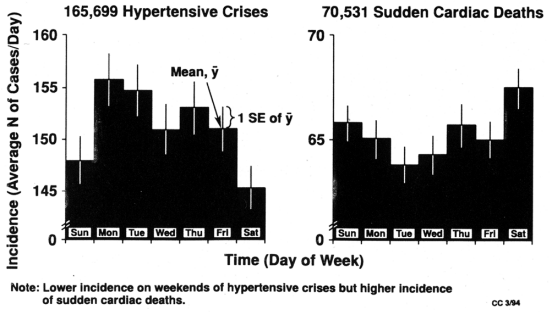


4. How general is the circaseptan over circadian prominence in neonatal blood pressure and heart rate?



Premature babies monitored around the clock for several weeks show longitudinally and on a group basis the larger prominence of the circaseptan over the circadian variation in blood pressure and heart rate (each dot represents one baby monitored around the clock for at least one week).
[Data of D. Johnson]

5. How critical are circaseptan rhythms and their harmonics or subharmonics, the multiseptans?

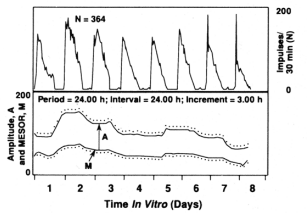


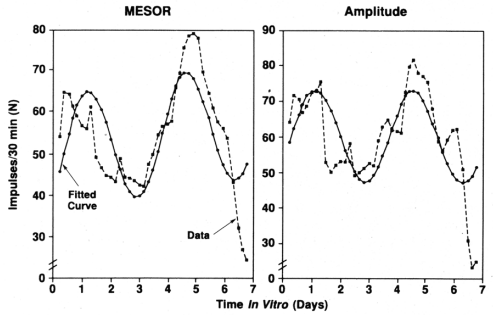
Circaseptans and circasemiseptans characterize morbidity and mortality statistics of over 6,000,000 emergencies, including myocardial infarctions, strokes, hypertensive crises and sudden cardiac deaths. [Data of T. Breus]

6. How fundamental are circaseptan or circasemiseptan components?

They are found in fossils and unicells, on earth for millions of years, in the beating of isolated cardiac cells, and they modulate the MESOR and circadian amplitude of neuronal firing in cultured snail retinas. [Data of S. Michel]

On longitudinally collected data, analyses at one (circadian) frequency can be carried out pergressively, like a moving average, to uncover changes occurring along the scale of other anticipated (lower) frequencies.





Summary

Among different procedures for resolving a statistically deterministic time structure, a sample illustrated herein shows the extraction of information from noise, in the blood pressure of neonates, or in the presence of a prominent circadian component hiding the circasemiseptan modulation. The fact that rhythms can be anticipated with some frequencies, that are usually synchronized by the socioecologic environment, contributes great power to chronobiologic designs and analyses, recommended as *the* control in biology, notably medicine.

5. Multifrequency chronome components

A. Circadian

The components of the chronome are internally coordinated through feedsidewards, in a network of spontaneous, reactive and modulatory rhythms. The endogenicity of a chronome component was first demonstrated, statistically validated and quantified by objective numerical measures of the uncertainty of its characteristics for the case of circadians in the blinded mouse models (Figure 7/I). In studies on the effect of the lighting regimen as a synchronizer, the question as to the transducer arose. Do the eyes mediate the effect? To answer this question, two models were studied: the blinded C mouse and the mouse born anophthalmic. In the study on blinded mice, the controls were sham-operated and had consistently on the average high blood eosinophil counts in the middle of the daily light span and low counts during the dark span. By contrast, the blinded mice showed the same result in one study and the opposite effect a few weeks later. It was postulated that the rhythm of the blinded mice may have a circadian period slightly different from that of the 24-hour synchronized control mice. Since it was not practical to bleed a mouse every 4 hours over a long span, rectal temperature was measured around the clock, in some studies for the lifetime of the groups of mice investigated. This work led to the discovery of free-running rhythms with a period that differed invariably from 24 hours, and also differed among some of the mice. It can be seen from the average rectal temperature curves of two groups of mice (Figure 7/IA) that the daily peaks occurred, on the average, about every 24 hours in the sham-operated mice. These temperature peaks in the blinded mice occurred earlier and earlier each day. Accordingly, a plot of the circadian acrophases as a function of time post-operation (Figure 7/IB) shows a downward drift to earlier and earlier clock-hours for the blinded mice but not for the control mice. A histogram of the estimated circadian periods (Figure 7/IC) shows that the sham-operated animals have a 24-hour synchronized circadian rhythm (the periods cluster very tightly around 24 hours; light bars), whereas the mice that had a bilateral optic enucleation have a circadian period shorter than 24 hours (dark bars). A deviant circadian period notwithstanding (the free-running circadian period of inbred C mice being shorter and that of a woman isolated from society longer than 24 hours), the internal timing can be preserved, as shown for three variables in Figure 7/ID. These experiments on mice establish, on an inferential statistical basis, the phenomenon of free-running of several variables of a circadian system with some degree of maintained internal synchronization after removal of the eyes (transducers for the primary environmental synchronizer, the 12-hourly alternation of light and darkness). Similar studies of the free-running of human as well as murine and other systems with circadian and also with other frequencies constituted an indirect demonstration of the endogenicity (i.e., of the genetic basis) of the chronome, now amply validated by chemical mutagenesis and gene transfer.

Rhythms being a fundamental feature of life, found at all levels of organization, it is important to recognize their coordinating role. Apart from the spontaneous rhythms characterizing functions such as serum corticosterone or melatonin (Figure 7/IIA and B), reactive rhythms are found in response to a given stimulus applied under standardized controlled conditions of the laboratory: the adrenal response to ACTH is a case in point (broken line in Figure 7/IIA). Such response rhythms have been named β-rhythms, the spontaneous rhythms being called α-rhythms, whether or not they are 24-hour or otherwise synchronized.

Much controversy can be resolved by studying the effect of the interaction by more than two variables at different rhythm stages; a third entity may modulate, in a predictable insofar as rhythmic fashion, the effect of one entity upon the second. Predictable sequences of attenuation, no-effect and amplification can then be found. A case in point is corticosterone production by bisected adrenals stimulated by ACTH 1-17 in the presence vs. absence of pineal homogenate (Figure 7/IIC). Such chronomodulation is also observed for the effect of ACTH 1-17 upon the metaphyseal bone DNA labelling in the rat (Figure 7/IID). Some of these multiple entity interactions involve more than one frequency; this is the case for the effect of the immunostimulator cefodizime (HR221) on corticos-

ENDOGENOUS TIME STRUCTURE (CHRONOME) OF INTERNALLY COORDINATED FREE-RUNNING RHYTHMS (TOP) THROUGH FEEDSIDEWARDS IN NETWORK OF SPONTANEOUS (α), REACTIVE (β) AND MODULATORY (γ, δ) RHYTHMS (BOTTOM)

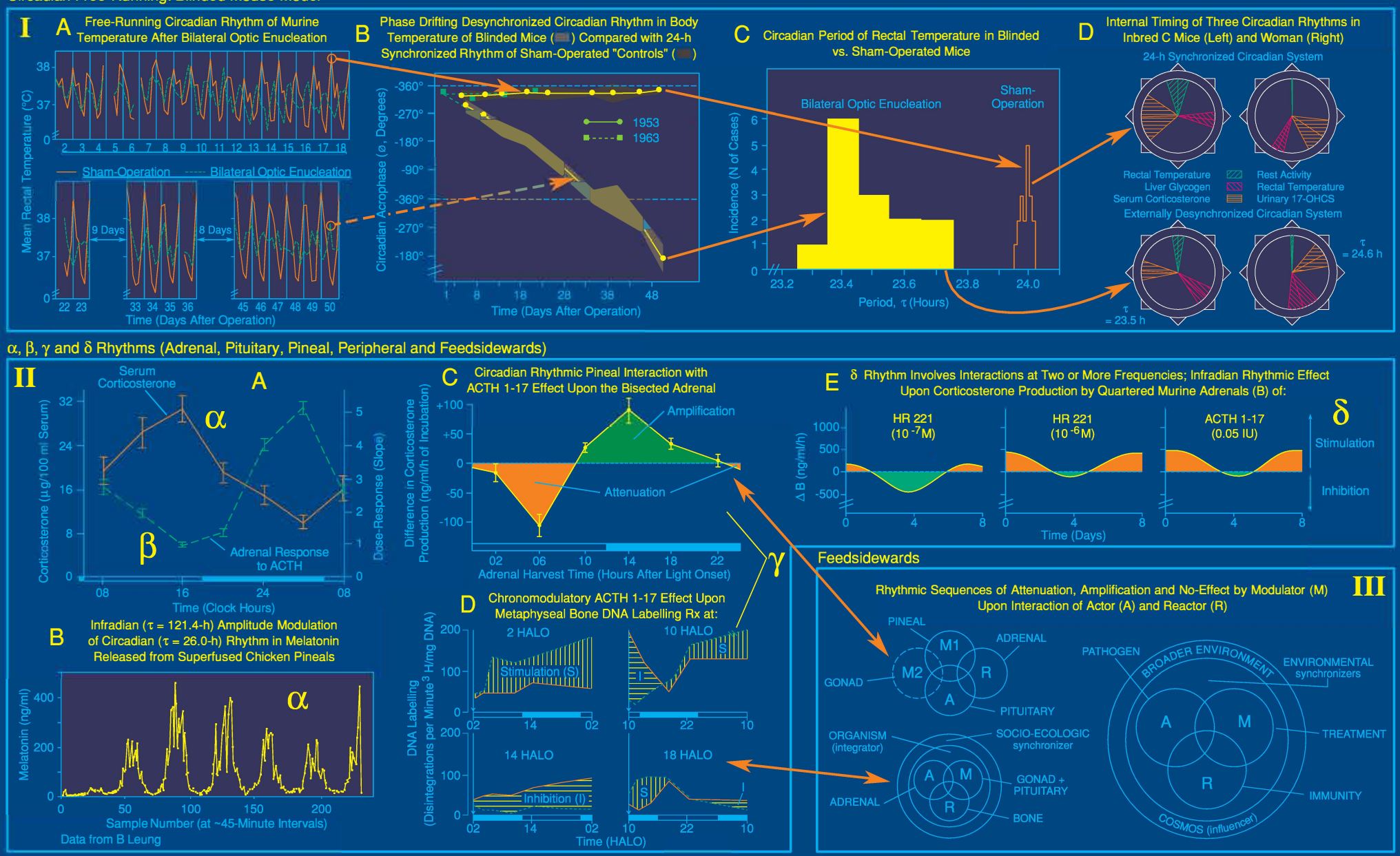


Fig. 7

terone production by the adrenals stimulated by ACTH 1-17 (Figure 7/IIE). Chronomodulations involving one or several frequencies are known as γ- or δ-rhythms, respectively; they are part of feedsidewards, i.e., rhythmic sequences of attenuation, amplification and no-effect by a modulator upon the interaction of an actor and a reactor (Figure 7/III).

The above and a wealth of other evidence refutes the assumption of homeostasis as a regulation for a putative constancy, which presumes, at all times, a similar response to a given stimulus until time-unspecified feedbacks come into play, Table 1. The alternative is the recognition of the reality of chronobiologic dynamics by feedsidewards. Thus one does not deal with *a posteriori* rhythms, as the result of feedbacks, but with intermodulations among *a priori* genetically coded rhythms, the *sine qua non* of life. The characteristics of rhythms and trends and the coordination resulting from relations within chronomes serve as a definition of health. Chronome alterations provide harbingers of increased risk of developing disease. Chronome mapping can also be used for guiding the timing of treatment when needed.

B. Circaseptans (about 7-day features)

A 'human' biologic week, as a feature of nature, not only culture, can be found in data from antiquity. In a histogram prepared by Hildebrandt and Bandt-Reges of the spans elapsed between the onset of symptoms and the 'critical day' of fever recorded in their populations of patients by Hippocrates (c. 460 BC-c. 370 BC), Galen (c. 129-c. 200) and Avicenna (980-1037), peaks appear at 7 days and multiples of 7 days; we validate by χ^2 the non-randomness of the patterns (Figure 8/I; left). There is more modern evidence as well documenting the need to consider and to optimize concomitantly circaseptan and circadian drug administration patterns. It stems from studies on the immunomodulation of malignant growth (Figure 8/V) in LOU rats bearing an immunocytoma and kept on a schedule of light (L) and darkness (D) alternating at 12-hour intervals (LD 12:12); the effect of a 7-day pre-treatment with lentinan (Rx) was compared to that of pre-treatment with saline (S): the growth of the malignant tumor was inhibited and survival time was lengthened when this immunomodulator was administered daily during L, the rat's resting span, in doses varying sinusoidally from day to day as a circadian-circaseptan chronotherapy (Chr); the rest span, however, is not the usual treatment time for humans, and a systematic sinusoidal variation of doses from day to day also is not the standard practice. When treatment was given (as would be convenient for humans) during the rat's usual activity span (D) according to the habitual equal daily doses (of many conventional human treatment schedules) i.e., homeostatically (H), tumor growth was accelerated and survival shortened.

Outcomes of immunotherapy, such as that by lentinan, depend on the chronome. Circadian and circaseptan components, and also circannual ones (not shown here), should underlie the design of treatment administration schedules. Many added developments contributed to the formulation of the chronome, a multifactorial multifrequency rhythm and trend structure. These included 1) the need to treat according to a broader-than-circadian schedule; 2) the practicability of assessing the other components of a rhythm spectrum automatically by modern recorders; 3) the demonstration of the genetic basis of these rhythms; 4) the interdigitation of rhythms with a) trends of growth, development, maturation, as well as aging, and b) with trends accompanying an elevation of disease risk or illness and treatment; and 5) the response of rhythms and trends to influences from the solar system and from beyond.

The endogenicity of circaseptans is supported by their manifestation after a single stimulus. During the regeneration of the kidney after unilateral nephrectomy or contralateral ischemia studied by Hübner (not shown), there are sharp peaks occurring about every 7 days in DNA labelling and mitotic activity; cosinor analyses validate the circaseptans

ENDOGENICITY OF CIRCASEPTANS SUPPORTED BY SINGLE STIMULUS MANIFESTATION, IF NOT INDUCTION (I, II) AND FREE-RUNNING UNDER SYNCHRONIZED (III - CH) AND DESYNCHRONIZED CONDITIONS IN HEALTH (III - SF) AND DISEASE (IV - EH); IMPORTANCE REVEALED BY EFFECT OF TIMING LENTINAN TREATMENT (Rx; V)

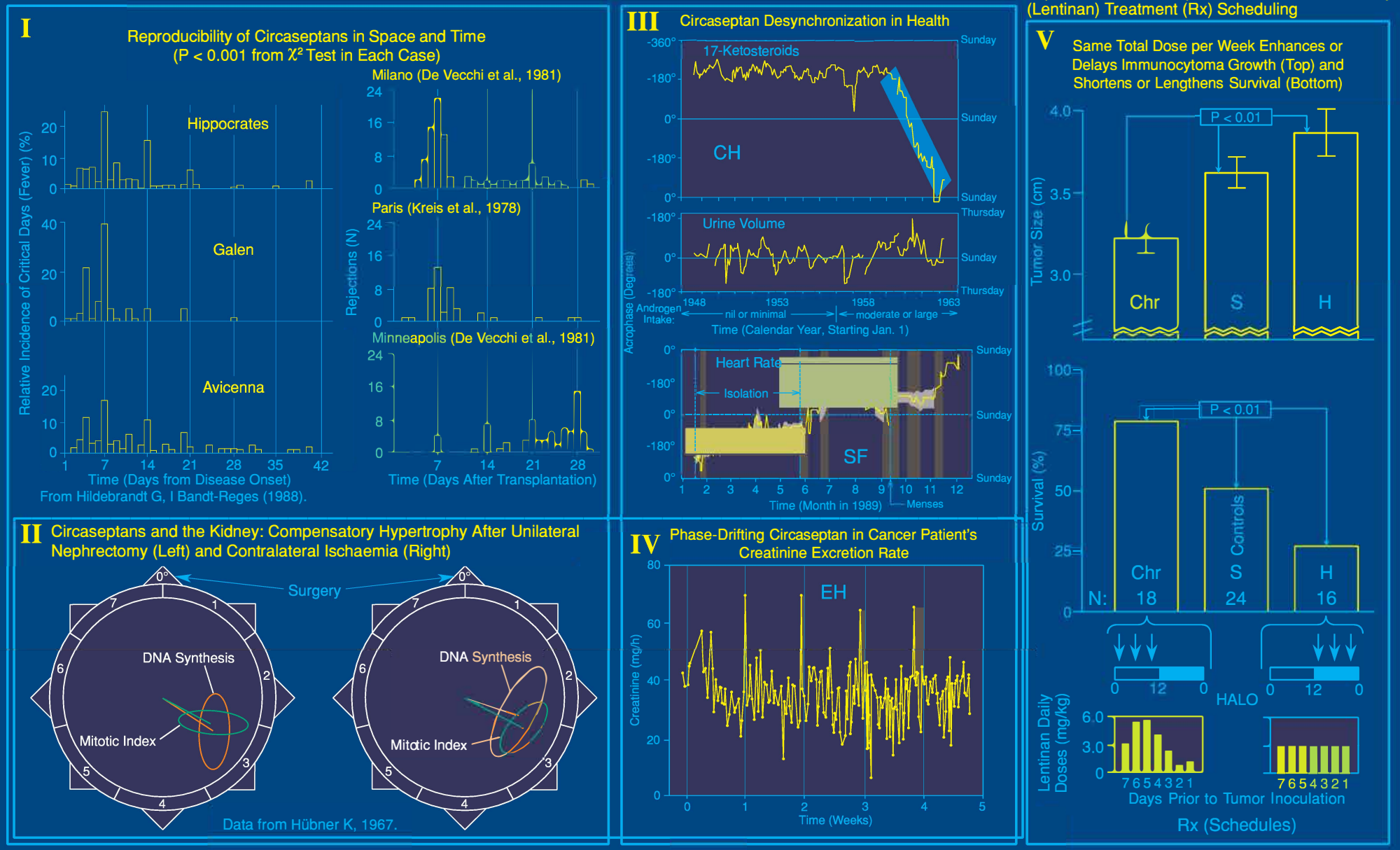


Fig. 8

(Figure 8/II). An about-7-day pattern also characterizes the rejection episodes following the allografting of a kidney, a heart or a pancreas in rodents, or of the human kidney in different institutions and continents (Figure 8/I, right), irrespective of the day of the week when the surgery is performed.

The endogenicity of circaseptans is also supported by their free-running from the social 7-day routine. The urinary 17-ketosteroid excretion by a clinically healthy man (CH) living on a precise 7-day social routine is circaseptan-rhythmic for a decade, with a one-week synchronized period and a peak on Wednesdays or Thursdays, until massive doses of testosterone are self-administered, when the circaseptan acrophase starts drifting, scanning more than a full week over the ensuing several years: the circaseptan rhythm is then characterized by a period close to but statistically significantly shorter than precisely one week (Figure 8/III, top). The circaseptan advancing free-run is found in the presence of a delaying circasemiseptan, with a period slightly longer than 3.5 days (not shown). Supporting the endogenicity of circaseptans and circasemiseptans is their free-run. The observation that the circasemiseptan lengthens while the circaseptan shortens suggests some extent of independence of these two components from each other and certainly from urine volume that remains 7-day synchronized (Figure 8/III, second row).

Circaseptan (and circadian) components characterize the blood pressure and heart rate of a woman who became amenorrheic while spending over 100 days isolated in a cave without time cues (SF). Upon return to a societal routine, her circadians quickly resynchronized to the societal day (not shown), but her circaseptans failed to do so for weeks, until menstruation was reinduced by hormones (Figure 8/III, third row). That menstruation may synchronize circaseptans is also apparent in psychophysiologic data in health and in pre- or overt pathology. A phase-drifting, if not free-running, circaseptan is also found in the creatinine excretion rate of a patient with a müllerian-duct adenocarcinoma involving the ovary (EH) (Figure 8/IV).

Circaseptans are prominent in the blood pressure of babies at term (Figure 9/I, top). Circadian and circaseptan changes in several vital signs such as systolic and diastolic blood pressure of very pre-term babies (with a gestational age at birth less than 33 weeks), assessed during the first 3 weeks post-partum, show a circaseptan-over-circadian prominence. The circaseptan component of systolic blood pressure of a very pre-term baby predominates over the circadian one (Figure 9/I, second row) during his first 4 months of life. Ontogeny may reveal the basic nature of circaseptans both in terms of the prominence of the circaseptan amplitude (e.g., in respiratory rate; Figure 9/I, bottom) and the admittedly wobbly phase drift compatible with a free-run of the circaseptan period. Circaseptans and circasemiseptans also characterize the incidence of sudden infant death syndrome.

A large prominence of circaseptans is observed in morbidity/mortality statistics of all ages. Circannuals, circaseptans and circasemiseptans are found (along with circadians; not shown) in morbidity/mortality statistics of human sudden adult death, myocardial infarctions and strokes, among millions of other emergency situations (Figure 9/III). These circaseptans and circasemiseptans retain stable phase characteristics over time (Figure 9/II, middle) and represent a rather general feature, as suggested by a worldwide summary of the incidence of myocardial infarctions (Figure 9/II, bottom). Circaseptans and circasemiseptans are also prominent features of likely underlying mechanisms such as the glutathione content of human platelet-rich plasma *in vitro* (Figure 9/IV).

C. Circatrigintans (about 30-day features)

By 1657, Santorio's aphorisms comment on changes in body weight of one or two pounds, recurring about once a month in a mature man in a perfect state of health, who observed the utmost moderation in living. An extremely unusual case of a man who bled from his thumb about once a month is recorded, as is a purpura of the calf recurring for six years at intervals of about four weeks in a 60-year-old man with Morbus maculosus Werlhofii. Circatrigintans characterize the frequency of neutrophil leukocytes with 'androgen-induced' nuclear appendages. In a variety of life forms, variables related not only to the reproductive tract but also to other organ systems of individuals of

CHRONOBIOMETRY OF CIRCASEPTANS IN HUMAN NEONATES (I) AND ADULTS (II - IV) FOR RECOGNITION OF RISK ELEVATION LEADING TO PRIMARY AND/OR SECONDARY PREVENTION

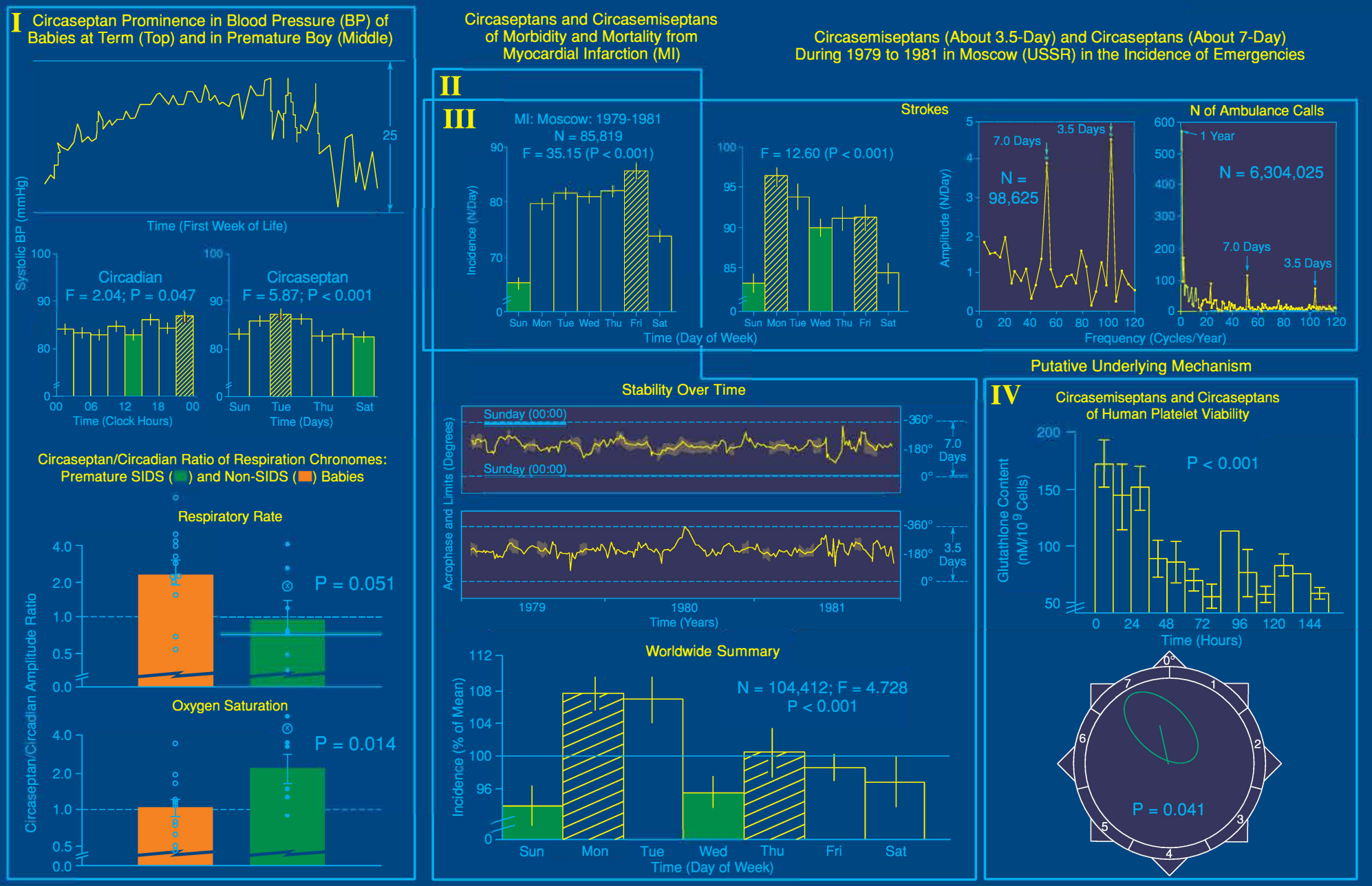


Fig. 9

both genders show overt or covert cycles in the range of about 30 days. These changes can be demonstrated and validated, e.g., by the nonlinear least squares fit of a cosine curve with a 30-day period or (in cycling women) with a period corresponding to that of the menstrual cycle length (without or with the concomitant fit of harmonics).

Human circatrigintans are ubiquitous: an acrophase chart shows the timing of overall high values and its 95% confidence interval for each variable investigated (Figure 10/I). The relatively tight horizontal 95% confidence intervals attest to the statistical significance of the circatrigintan component of the chronome in a variety of physiological variables. In this chart, 360 degrees represent one menstrual cycle and 0° the first day of menses. The results serve, among others, as a map for the sequencing of events during the menstrual cycle. This sequence of events can be quite different on the circatrigintan scale as compared to that along the circadian scale, as seen for the case of the systolic blood pressure and heart rate of a clinically healthy woman (Figure 10/IV): whereas systolic blood pressure peaks during the follicular stage of the menstrual cycle, heart rate peaks later, perhaps shortly after ovulation. This is revealed by the nearly opposite direction of the vectors that indicate a nearly antiphasic time relation validated by non-overlapping elliptical 95% confidence regions around their tips. This circatrigintan systolic blood pressure vs. heart rate allophase contrasts with the relatively close, albeit different, phase relation existing between these two variables along the circadian scale, as also indicated by the cosinor plots (Figure 10/IV, bottom). A circatrigintan rhythm of systolic blood pressure persists during pregnancy (Figure 10/II), as does a desynchronized circaseptan, pointing perhaps to interrelations between these two chronome components.

A circatrigintan was found in the urinary excretion of 17-ketosteroids by a healthy man (Figure 10/III), consistently during 15 years, persisting when the subject's wife was in menopause. An increase in the circatrigintan amplitude of 17-ketosteroid excretion by a healthy man coincides with the start of a rather massive androgen self-administration and might relate to a male sex gland cycle. Circatrigintans can be the major chronome components in human prematurity, as in the case of the blood pressure of a boy in whom they can exceed the prominence of circadians and circaseptans.

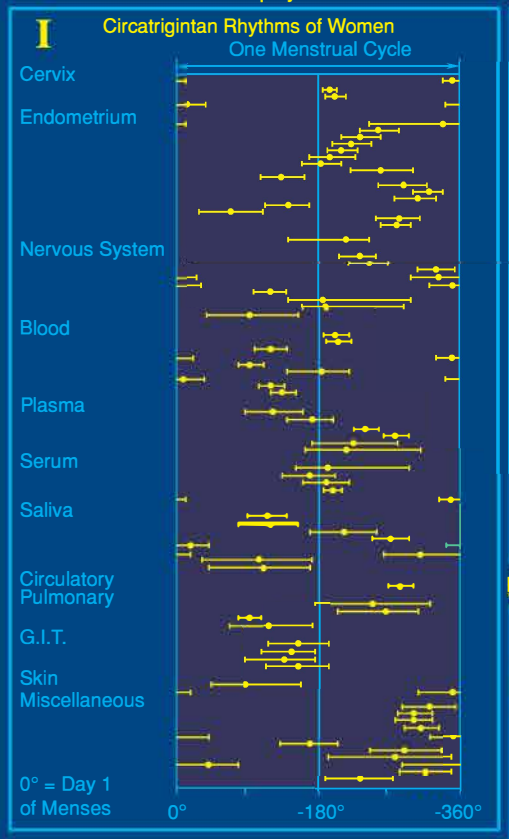
Circatrigintan alterations are associated with the presence of disease and risk elevation, as revealed by the least-squares spectrum of breast surface temperature of a woman with breast cancer (Figure 10/VA). The amplitudes at different frequencies of breast surface temperature characterizing the cancerous right breast are shown below those of the healthy left breast. The large peak around 24 hours in the healthy breast surface temperature is not detected in the cancerous breast temperature: a much smaller peak is found at a shorter (21-hour) circadian period. The result could be interpreted as a circadian desynchronization associated with breast cancer, an oversimplification since the infradian domain of the breast surface temperature spectrum is also altered. Another interpretation recognizes that an infradian component with an about-28-day and one with an about-7-day period characterize the healthy breast but not the breast with cancer. Instead of the circatrigintan and the circaseptan peaks, two other infradian peaks have appeared, one at a period of about 84 hours and another at one of 42 hours. These results could be interpreted as a spectral change due to a circaseptan frequency multiplication; this too would be an oversimplified restriction of focus only on the infradian domain of the mathematical rhythm spectrum.

From the viewpoint of an integrated chronome, a third hypothesis can be formulated, based on the recognition that the 84- and 42-hour components are not only harmonics of the 7-day synchronized (168-hour) circaseptan, but also subharmonics of the desynchronized (21-hour) circadian. Accordingly, the spectral change in surface temperature of the cancerous breast involves a concomitant infradian frequency multiplication and a desynchronized circadian frequency division ('demultiplication'), thereby achieving a spectral compromise yielding new infradian and circadian components bearing a harmonic relation to each other and to the environmentally synchronized circaseptan component of the healthy breast in the same patient. An inter-rela-

CIRCATRIGINTAN (ABOUT-30-DAY) RHYTHMS IN THE BROADER SPECTRUM

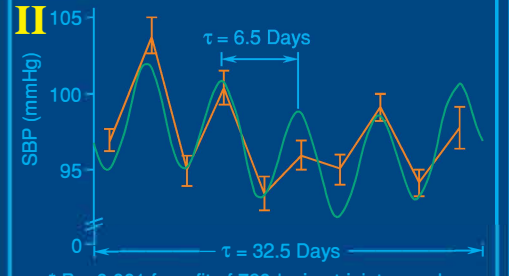
Ubiquity

I Circatrigintan Rhythms of Women



Persistent Systolic Blood Pressure (SBP) Circatrigintan During Pregnancy*

II

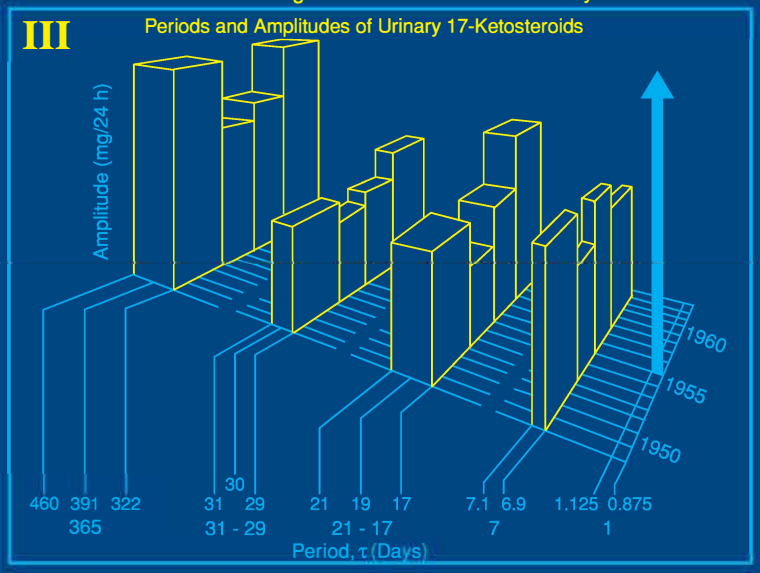


* P < 0.001 from fit of 780-h circatrigintan and 156-h circaseptan components.

Data from D Ayala.

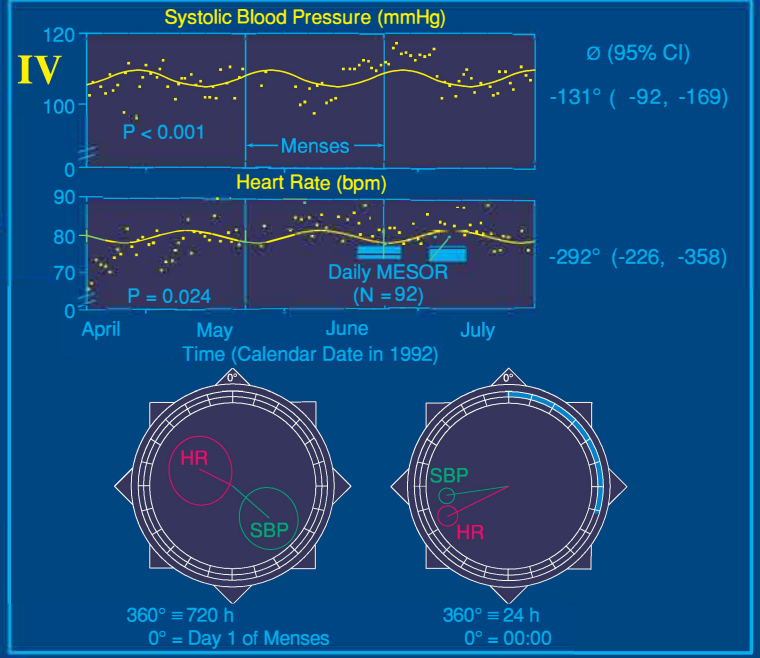
Persistence of Circatrigintan Over 15 Years in Healthy Man

III Periods and Amplitudes of Urinary 17-Ketosteroids



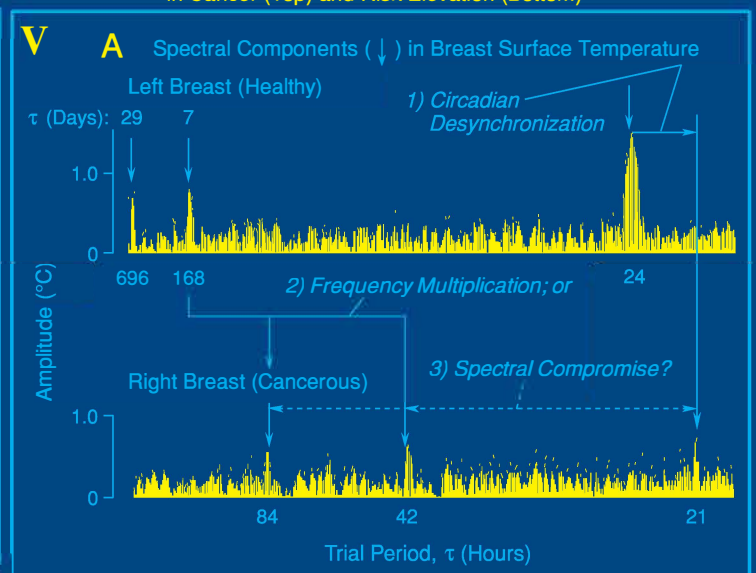
Larger Circatrigintan than Circadian Allophase (Clinically Healthy Woman; AP, 28y)

IV



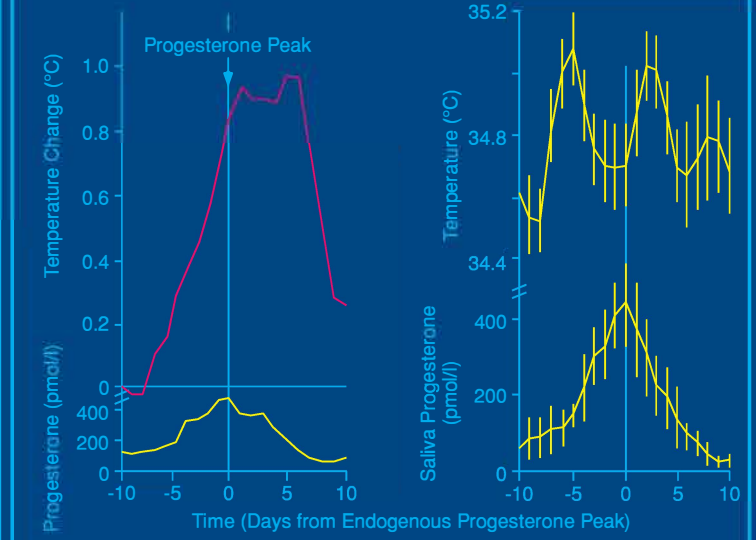
Chronome Alteration of Breast Surface Temperature in Cancer (Top) and Risk Elevation (Bottom)

V **A** Spectral Components (↓) in Breast Surface Temperature



Adapted from Gautherie and Gros, 1977.

B Circatrigintan to Circaseptan Variance Transposition from Healthy to High Breast Cancer Risk



From H Simpson et al., 1989.

Fig. 10

tion of several components of the chronome is further corroborated by the results on breast surface temperature showing a circatrigintan-to-circaseptan variance transposition from healthy breasts to breasts at a high risk for developing cancer (Figure 10/VB), supporting the foregoing hypothesis of a coordination among several chronome components.

D. Circannuals

About-yearly features characterize biology broadly in plants and animals. Human growth rate, body weight, metabolic rate, the intake of carbohydrates, lipids and calories, core temperature, blood pressure, peak expiratory flow, urinary 17-ketosteroid excretion and behavioral variables such as the onset of menarche, sexual outlets and pineal gland function and weight are circannual periodic (Figure 11/I). The importance of human circannuals is seen in morbidity and mortality statistics: the incidence of cardiovascular and respiratory diseases is eminently circannual periodic, as is the incidence of cancer and suicide.

Some circannuals persist and phase-drift under constant environmental conditions (Figure 11/IV). When the deviation of the natural period from the calendar year is small, a full-cycle scan by the circannual phase would take longer than the individual's life span. In natural lighting, in continuous light, in continuous darkness or on a fixed 12-hourly alternation of light and darkness, a circannual of large amplitude with little or no damping from year to year characterizes the ovarian weight of the Jamuna River catfish (Figure 11/IV). Under the particular natural lighting and other conditions studied, the best-fitting circannual period was very close to the exact 365-day calendar year, namely 371 days; under constant environmental conditions the best-fitting circannual period was longer than exactly one year (393 days in continuous light and 400 days in continuous darkness) (Figure 11/IV). These findings suggest endogenicity.

Physiologic monitoring combined with chronobiologic analysis can recognize the presence of a heightened risk. The circannual amplitude of diastolic blood pressure, assessed longitudinally by several 24-hour profiles of clinically healthy women in North America and Japan, correlates negatively with the familial and personal risk of developing high blood pressure or related diseases later in life, as does the amplitude of the hormone aldosterone (Figure 11/III). The circannual amplitude of aldosterone correlates negatively with the circadian MESOR of diastolic blood pressure as well as with a questionnaire-derived cardiovascular disease risk index. Discrimination and classification techniques applied to these data have singled out aldosterone as a classifier of cardiovascular disease risk while corroborating the circannual stage-dependence of the discriminating power of aldosterone as a classifier of this condition.

For breast cancer risk assessment, circadian profiles are less discriminating than circannuals. Group means of the 24-hour MESORs of circulating prolactin of clinically healthy Minnesotan women, at high or low risk of developing breast cancer, and of Japanese women, at a low such risk, are plotted for each season (Figure 11/IIA); a circannual variation becomes apparent (Figure 11/IIB); its amplitude is larger for the low-risk than for the high-risk Minnesotan population (not shown) and is largest in a Japanese population, at the lowest breast cancer risk when the study was carried out. The individual circannual prolactin amplitude correlates negatively with the total risk load assessed by questionnaire ($P=0.025$), Figure 11/IIC. The circannual amplitude of the concomitantly assessed TSH correlates positively rather than negatively with the total risk load for developing breast cancer ($P=0.019$), Figure 11/IIC. The circannual amplitudes of these same two hormones also correlate with the risk of developing prostatic cancer.

CIRCANNUAL RHYTHMS

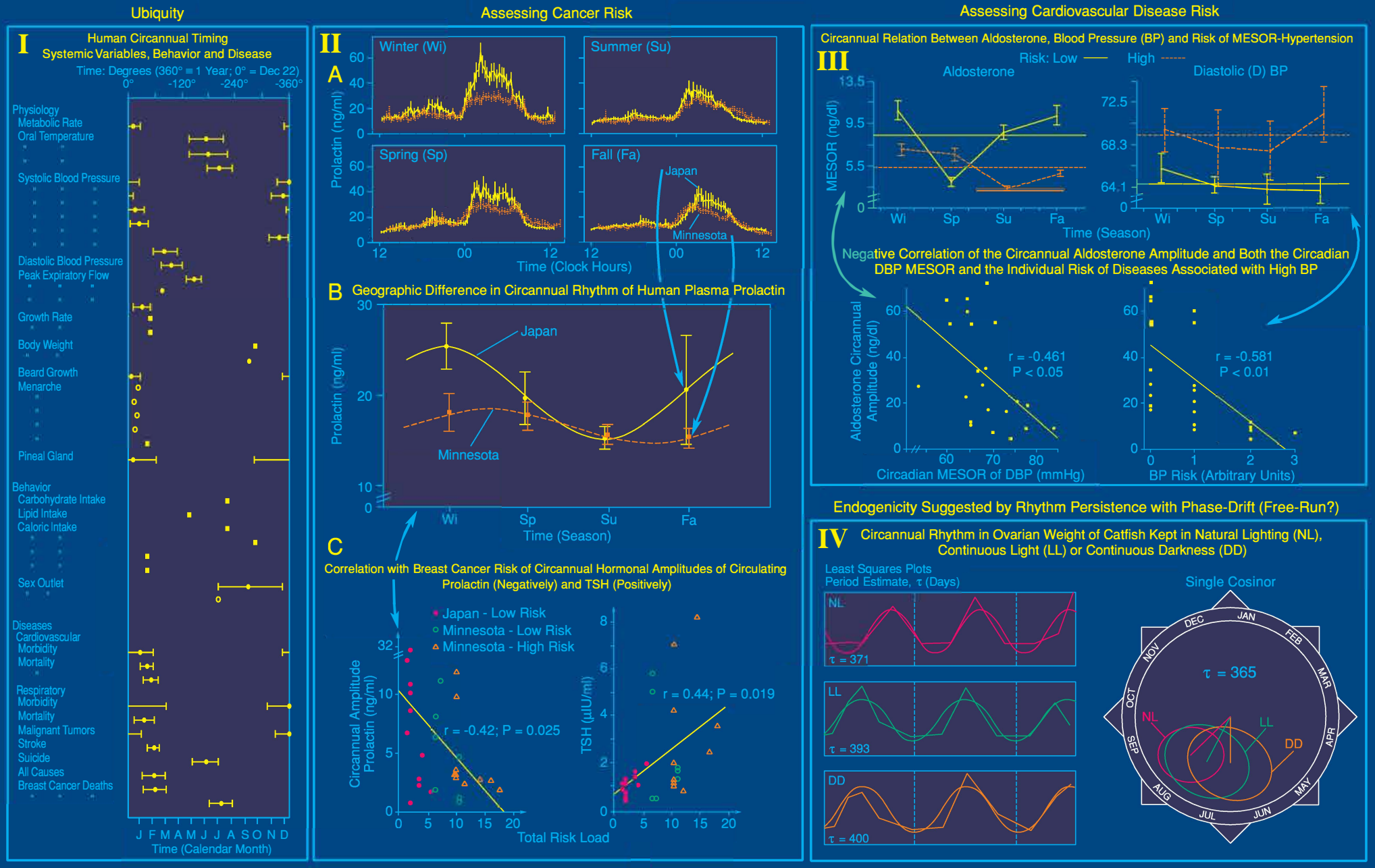


Fig. 11

6. The chronome and the cosmos

A framework of (i) genetically anchored rhythms, (ii) trends and (iii) noise, with (i) amenable to synchronization by the socioecologic environment is influenced, but not necessarily synchronized by planetary/interplanetary phenomena (Figure 12/I), notably by the sun's effects upon the interplanetary magnetic field (IMF) and thus upon the geomagnetic disturbance index, Kp.

A. Similar peaks in physiologic and physical spectra and cross-spectral coherence at other frequencies. Only a small circatrigintan difference is found between the time structure of Kp and a very premature boy's physiology (Figure 12/III). Spectra such as those of heart rate shown during the first 4 months of life and around 2 years of age, in parallel with the spectra of Kp, were computed. When the circatrigintan period of Kp is equated to 100%, the deviations in period of different physiologic variables of this boy are only of the order of 2%.

Cross-spectral coherence with Bz (the vertical component of the IMF induction vector) is found at a trial period of about 5 days (P=0.049) for this boy's systolic blood pressure and at a trial period near, yet different from, 3.5 days (P=0.008) for his diastolic blood pressure. Cross-spectral coherence between Kp and the blood pressure and heart rate of a clinically healthy cardiologist (YW) at high risk of developing cardiovascular disease is found at a trial period of 27.7 days (P<0.05) with a secondary peak at about 4 days (P<0.10). In the case of a woman (VLG) who spent over 3 months in isolation without time cues, cross-spectral coherence at a trial period of about 3.5 days is found between the disturbance of cosmic ray intensity (gauged by the standard deviation) and the micturition intervals and water excretion (P<0.01).

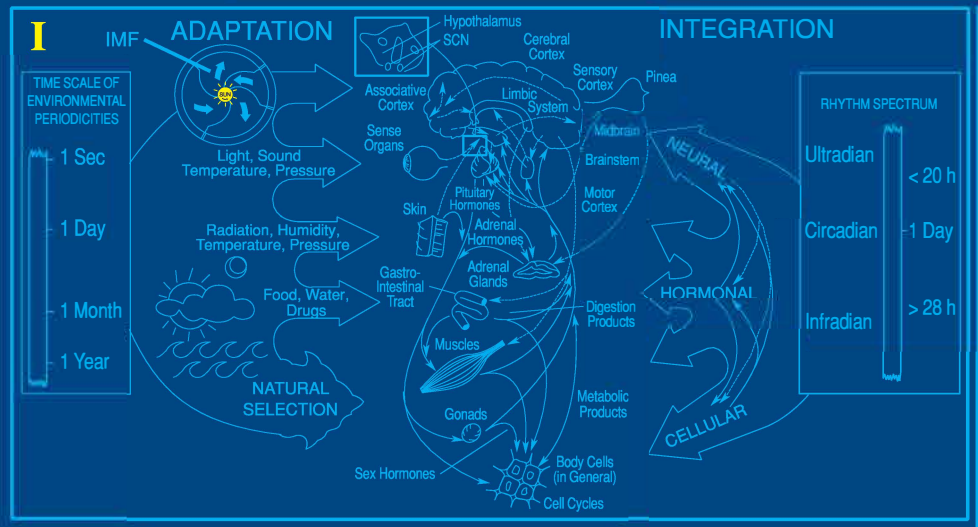
B. Cross-spectral coherence between the daily incidence of myocardial infarctions and Kp occurs at periods near, yet different from 7.0 and 3.5 days. This result suggests, as one of many possibilities, the influence of a variable sector structure of the IMF, consisting, e.g., of 3 spans of ~7.6 days and one of ~3.7 days during which the IMF is alternatively oriented toward or away from the sun. A strong coherence of myocardial infarctions with Bz and Kp is found at nearly identical periods. The adaptation of evolving life to such a sector structure may have contributed to the current endogenicity of the now primarily societally rather than sectorially synchronized circaseptan system, that prominently characterizes the incidence of emergencies (Figure 12/V, left).

C. Desynchronization (or rather free-run) of societally synchronized biologic rhythms from Kp and Bz. Morbidity/mortality statistics are 7-day synchronized, yet the phases of the near 168-hour component of the geomagnetic Kp index and of Bz are wobbly and drifting. That magnetic disturbance is at best an influencer is supported by the systolic blood pressure of a clinically healthy woman (Figure 12/V, right), which 'free-runs' from a 164.4-hour component showing some stability for Bz.

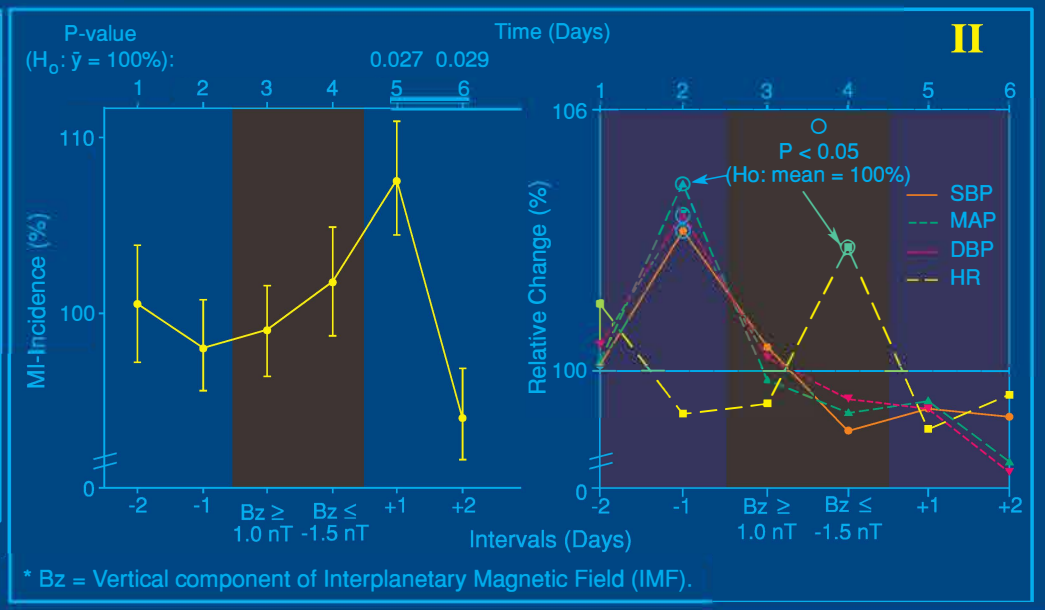
D. Perturbations related to southward Bz turns. Southward turns of Bz have been associated with auroras and magnetic storms. A biologically relevant southward Bz turn was defined as a change between the daily average of Bz of ≥1 nanoTesla (nT) to one of ≤ -1.5 nT. Once such an event is identified, the daily incidence of myocardial infarctions is recorded for these two days as well as for the two days preceding the day when Bz is ≥1.0 nT (days -1 and -2) and the two days following the day when Bz is ≤ -1.5 nT (days +1 and +2). The daily incidence of myocardial infarctions for these six days has been averaged over all events identified during this 3-year span. After a southward Bz turn, there is an increase in myocardial infarctions (P=0.027), Figure 12/II. During such an event, there is an increase in heart rate of a man (FH) who monitored this variable and blood pressure around the clock 6-10 years after a cardiac bypass operation. There may also be biologically effective events in the IMF that precede a southward Bz turn since the blood pressure of FH is elevated before a southward Bz turn (P<0.05), Figure 12/II.

E. 'Remove and replace' experiments. The circaseptan component of heart rate of a man (RBS) is less prominent during a span identified in Walsh spectra as lacking circaseptan features in solar activity.

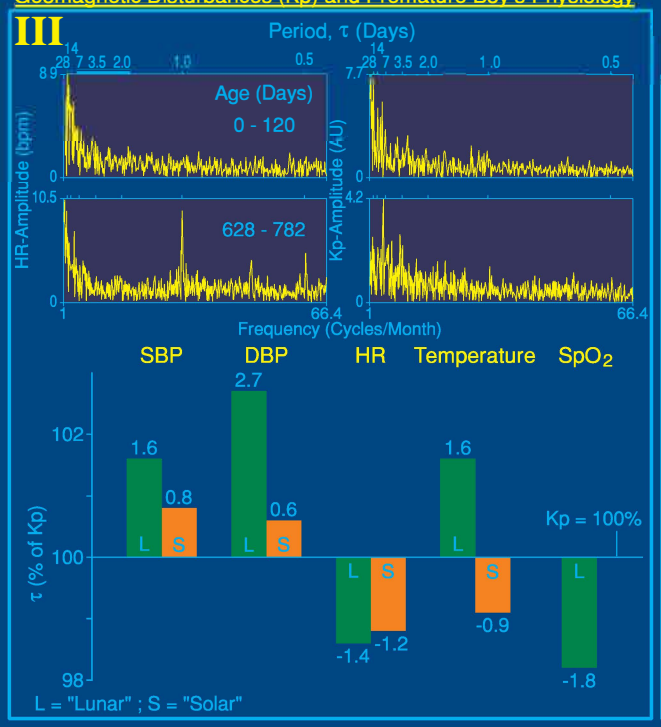
Evolving Mechanisms of Human Time Structure

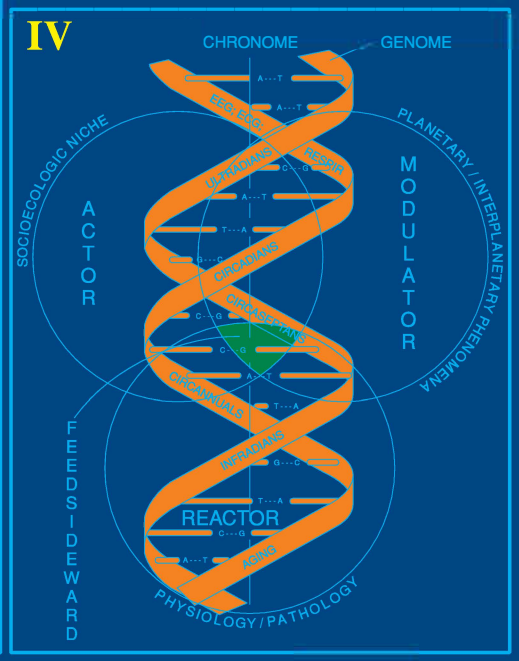


Increase in Myocardial Infarction (MI) After, in Human Heart Rate (HR) During and in Blood Pressure (BP) Before Southward Turn of Bz*



Spectra Reveal Small Circatrigintan (Bottom) Difference Between Geomagnetic Disturbances (Kp) and Premature Boy's Physiology





Circaseptan Human Morbidity and Mortality Societally Synchronized (Left) and Human Blood Pressure Not Synchronized with Solar Wind (Right)

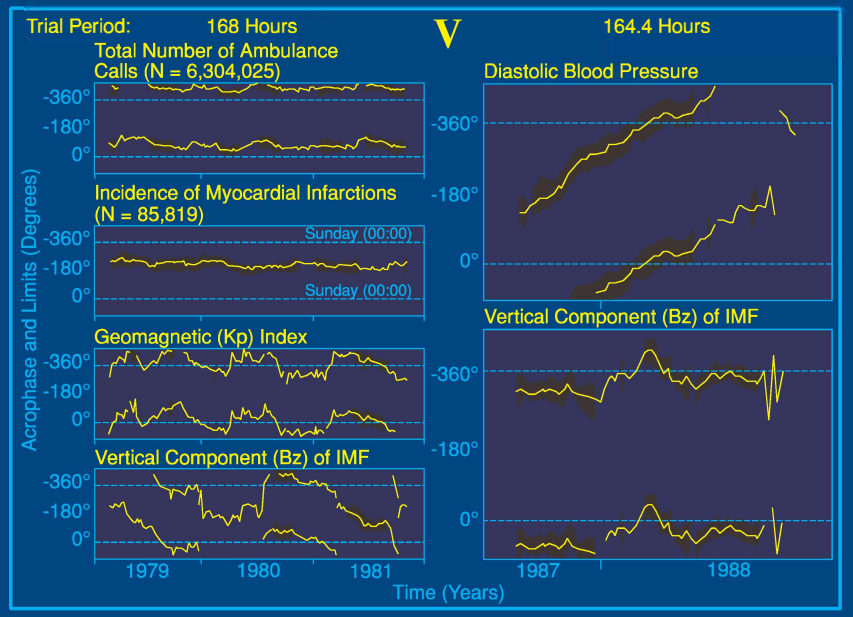


Fig. 12

7. Persisting rhythmicity during transient cortical or lasting functional 'brain ablation' and after removal of the suprachiasmatic nuclei

Much controversy has revolved around the circadian rhythm alteration after lesioning of the suprachiasmatic nuclei (SCN). Several investigators have claimed that all circadian rhythms are obliterated after bilateral lesioning of the SCN; others including ourselves have shown rhythm alteration to a differing extent for different variables rather than an overall obliteration after such intervention. Figures 13-15 provide evidence supporting the persistence of rhythms after bilateral lesioning of the SCN and illustrate the merits of chronobiometric analyses to obtain a valid interpretation of the data. Figure 13 shows the circadian temperature patterns in Fischer rats, without or with unilateral or bilateral lesions of the SCN. A visual inspection of the data (Figure 13/IIA) may erroneously convey the impression that rhythmicity is abolished. The use of chronobiologic techniques such as a plexogram (that is, the stacking of the data over an idealized anticipated cycle of 24 hours) shows that there is a persisting circadian pattern, albeit an altered one, with a reduced amplitude and an advanced phase, Figure 13/IIB and C. This is also shown in curves obtained from several different animals (Figure 13/I). Whereas the circadian temperature amplitude of rats with a histologically validated bilateral lesioning of the SCN is much reduced, it is noteworthy that in rats with a unilateral lesion of the SCN, the amplitude is actually larger than that of sham-operated rats. Further evidence supporting the influence of planetary-interplanetary phenomena is provided by the fact that sham-operated rats and rats with a unilateral lesion of the SCN kept in continuous light of low intensity have a circadian period of core temperature that is desynchronized from 24 hours, with an average period close to the 'lunar' day; this is not the case for rats with a bilateral lesioning of the SCN which can have either a shortened or lengthened circadian period (Figure 13/III).

Persistence of the circadian rhythmicity of the ^{3}H-TdR incorporation into DNA of different organs and of the mitotic index of the corneal epithelium of BD_2F_1 female mice after bilateral lesioning of the SCN has also been extensively demonstrated by Scheving (Figure 14/I-VI). For animals with a bilateral lesion of the SCN, Pasley could not validate a circadian rhythm in the drinking of water, but demonstrated it ($P=0.002$) for the drinking of 5% ethanol (Figure 14/VII and VIII). The SCN is not the sole or primary pacemaker of the entire circadian system.

Circadians have been shown to persist even during transient cortical (Figure 15/I) or lasting functional (Figure 15/II) brain ablation. Rectal temperature and blood eosinophils have been measured longitudinally on three patients with emotional disease (one catatonic and two paranoid schizophrenic adult women). In data from these patients, periodograms show the major peak at a trial period of 24 hours before treatment and near 24 hours rather than at 12 hours during 12-hourly electroshock, regression, and post-regression (Figure 15/IB and C).

A circadian rhythm in systolic and diastolic blood pressure, urinary excretion of sodium and potassium, as well as urine volume, is also shown to persist in a 4-year-old comatose girl (Figure 15/II), indicating that even the brain as a whole may not be necessary for the persistence of a circadian system in some physiologic variables.

The adrenal glands are also an important source of circadian rhythmicity. For instance, a circadian component of variation in the circulating eosinophil count cannot be demonstrated for patients with adrenocortical insufficiency (Figure 16/I, bottom), whereas in healthy people with either restricted or unrestricted activity, this rhythmicity is not only demonstrable, but amplified by enhanced motor activity. Whereas for patients with Addison's disease a circadian rhythm in eosinophil counts cannot be statistically validated, circadians persist, however, for serum iron ($P<0.001$). Whereas in intact rats a circadian rhythm can invariably be detected for pregnenolone, corticosterone and dehydroepiandrosterone, in adrenalectomized and orchidectomized rats, a circadian rhythm remains demonstrable for plasma pregnenolone and for brain dehydroepiandrosterone (Figure 16/II). Circadians of adrenal corticosterone also persist on the day after stepwise cerebral ablation (Figure 16/III).

CIRCADIAN RHYTHM ALTERATION RATHER THAN OBLITERATION AFTER LESIONING OF SUPRACHIASMATIC NUCLEI (SCN)

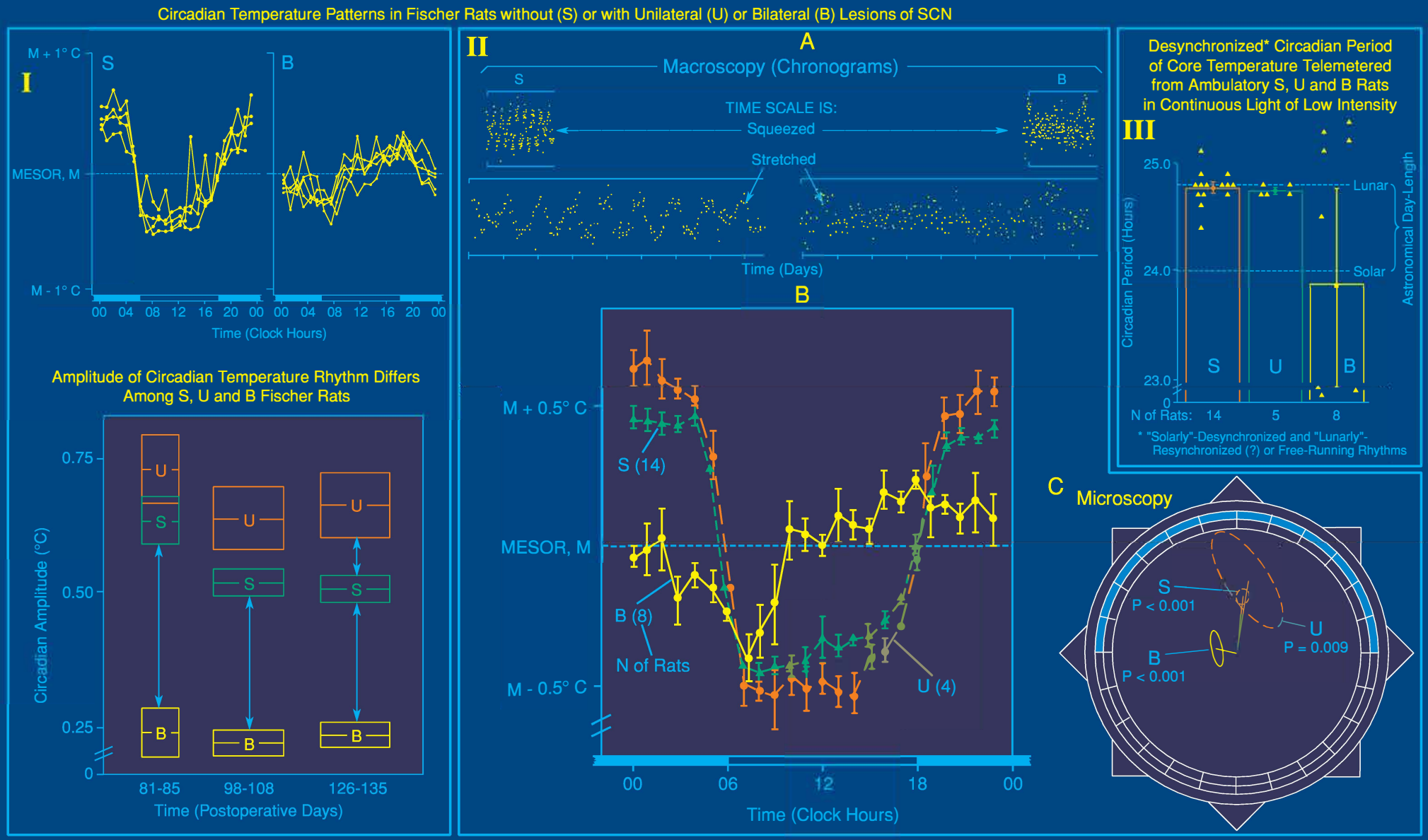
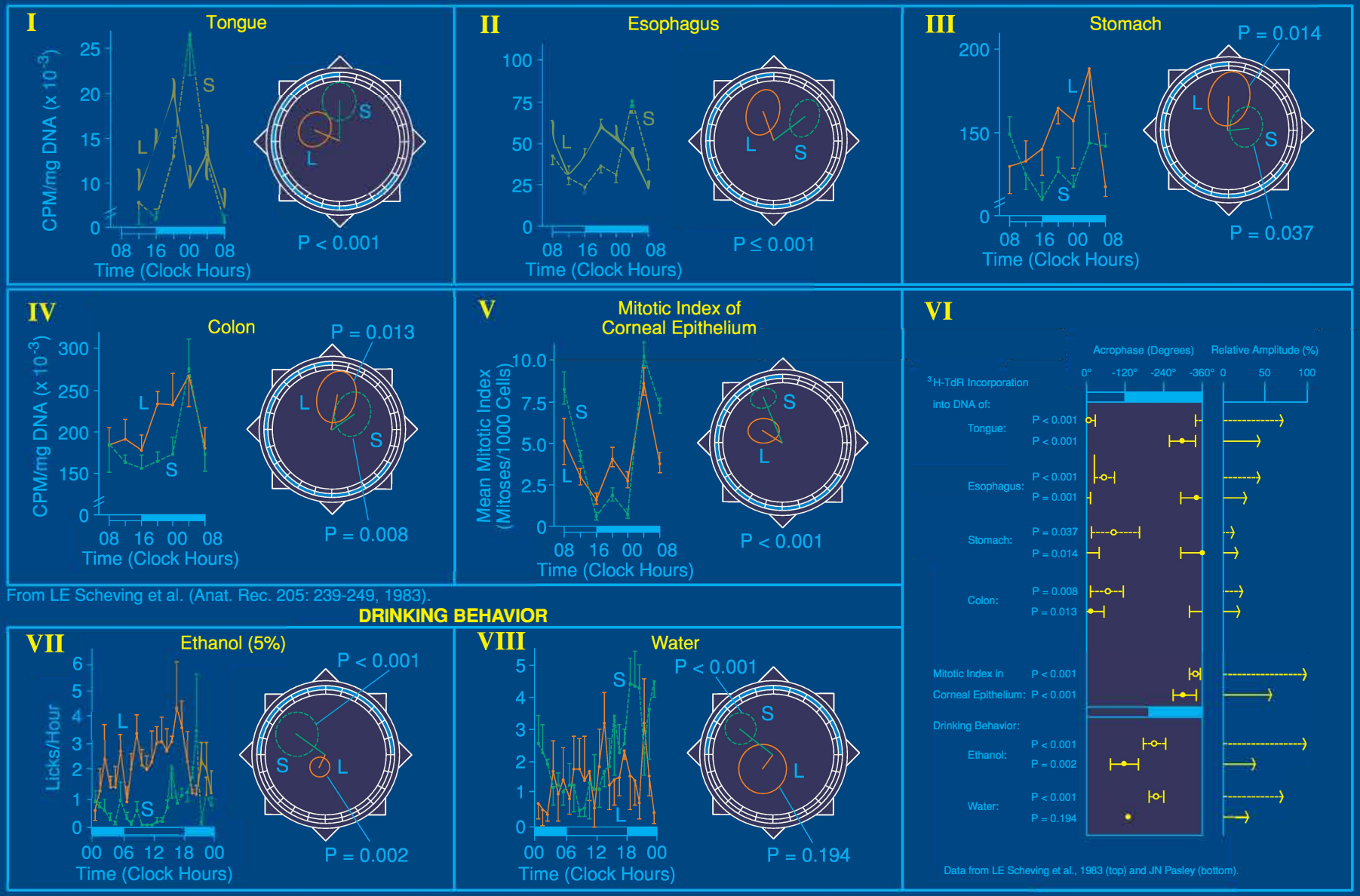


Fig. 13

PERSISTENT, ALBEIT ALTERED, CIRCADIAN RHYTHMICITY OF ^{3}H-TdR INCORPORATION INTO DNA OF DIFFERENT ORGANS AND OF MITOTIC INDEX OF CORNEAL EPITHELIUM OF BD_2F_1 FEMALE MICE AFTER BILATERAL LESIONING OF SUPRACHIASMATIC NUCLEI (SCN) (I - VI)
PERSISTENCE OF ALTERED RHYTHM SEEN IN ETHANOL (VII) BUT NOT WATER (VIII) CONSUMPTION AFTER BILATERAL LESIONING



From LE Scheving et al. (Anat. Rec. 205: 239-249, 1983).

From JN Pasley. (Advances in Chronobiology, JE Pauly and LE Scheving (eds), Alan R. Liss, Inc., New York, Part B. pp. 467-471, 1987).

S = Sham-operated; L = SCN lesioned.

Fig. 14

PERSISTING CIRCADIANS DURING TRANSIENT CORTICAL (TOP) OR LASTING FUNCTIONAL (BOTTOM) "BRAIN ABLATION"

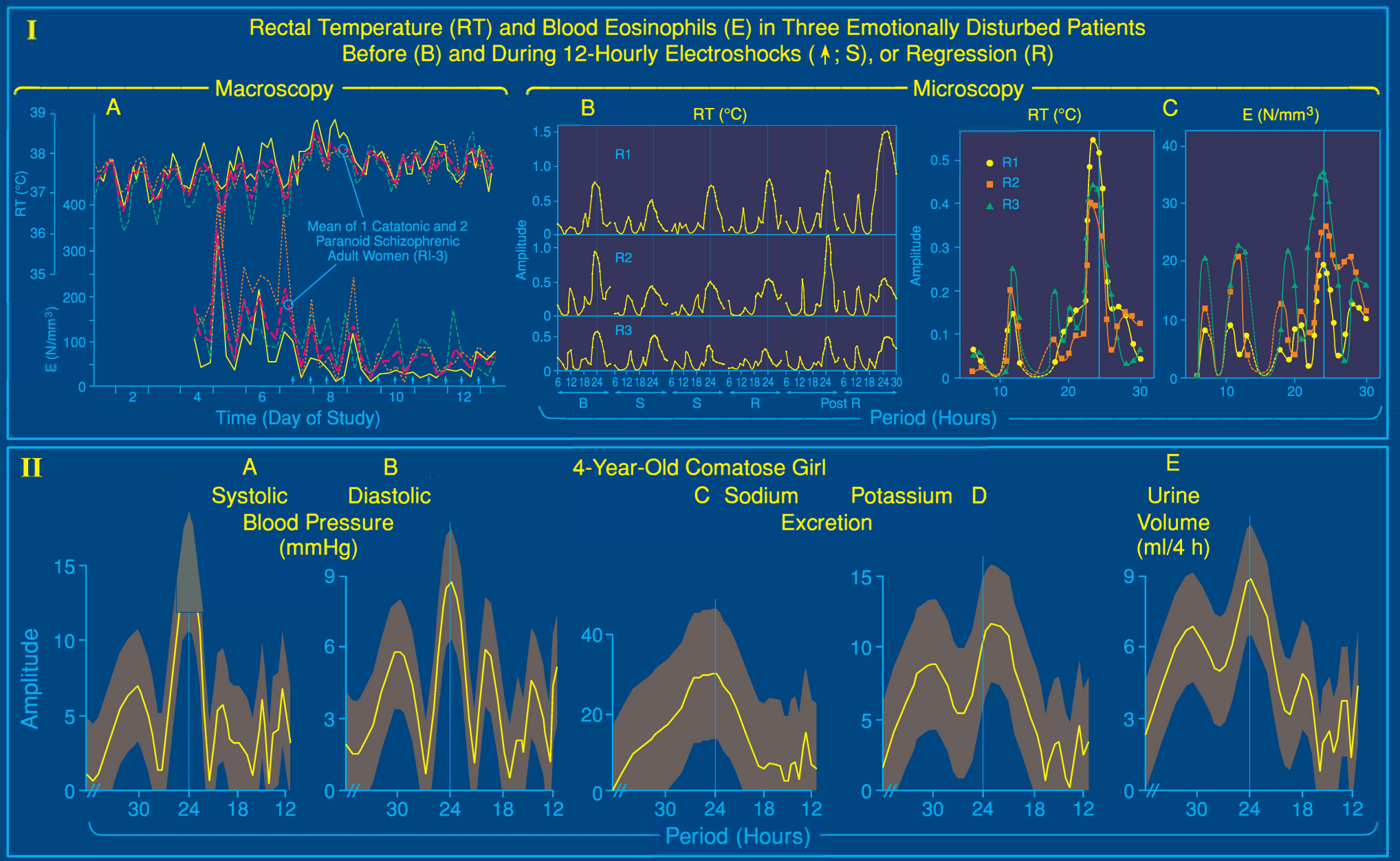


Fig. 15

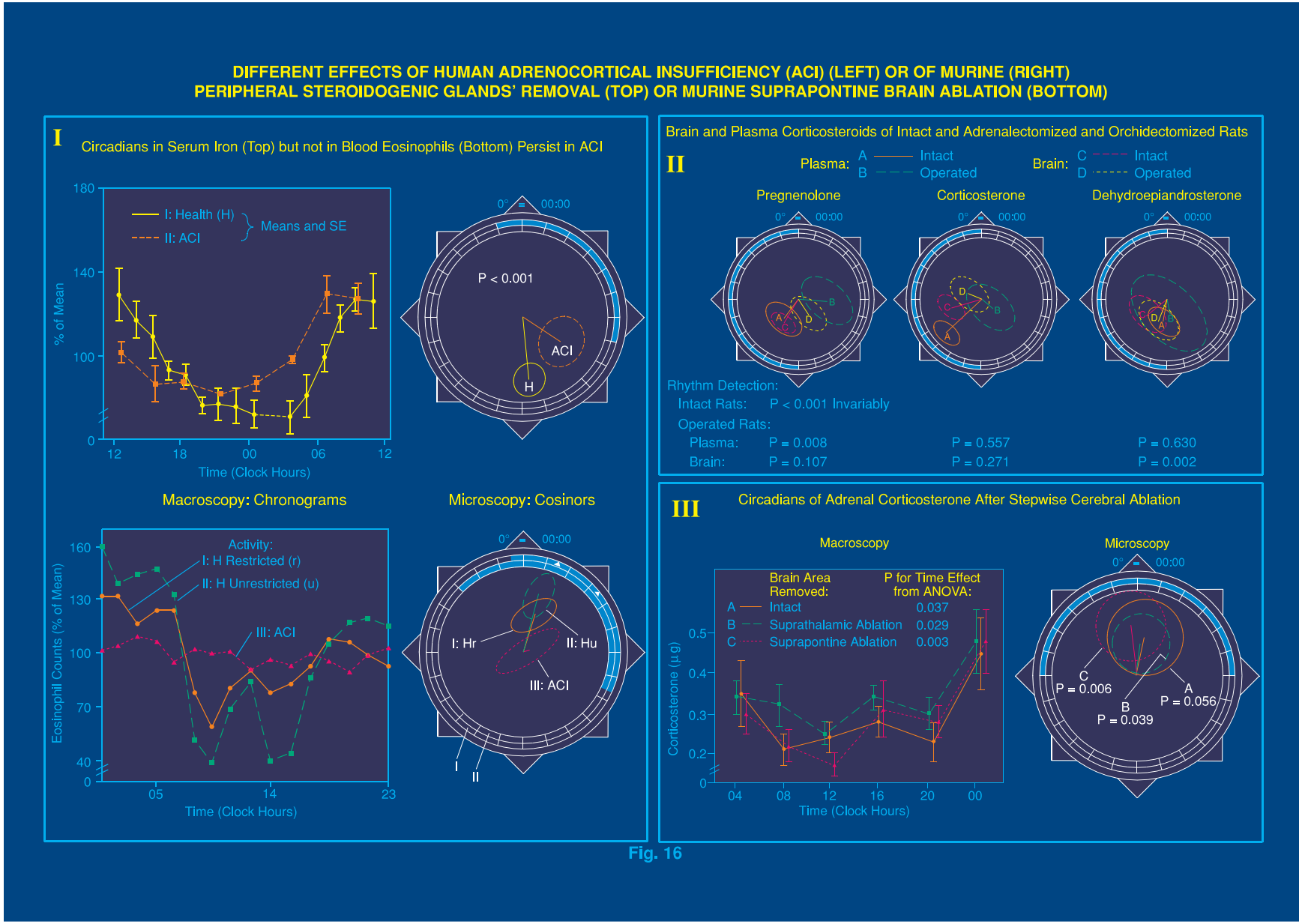
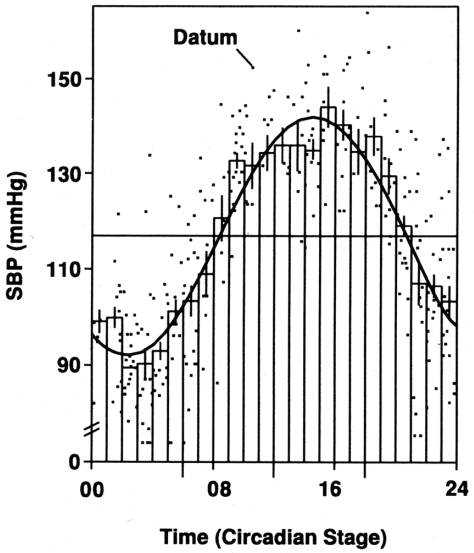


Fig. 16

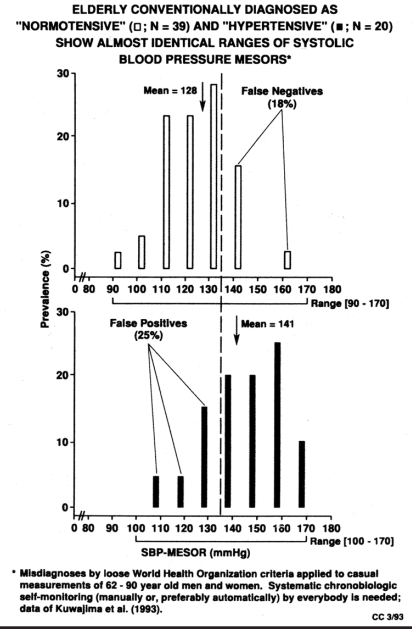
Figures 13-16 indicate that both the adrenals and the brain are important organs for maintaining an unimpaired and coordinated circadian system, yet some circadian rhythmicity persists even after removal of either one of these organs, adding support to the concept of a cellular circadian component in the genetically anchored chronome.

Did you know?

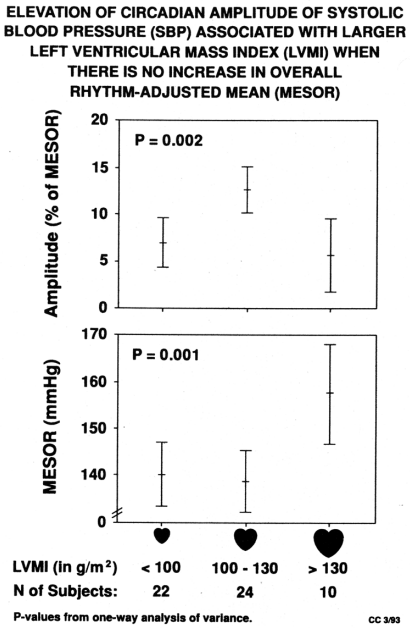
1. In most healthy adults, blood pressure varies by more than 50 mm Hg each day.



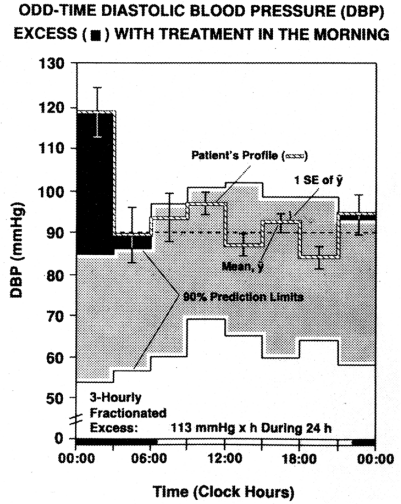
2. There is over 40% discrepancy between a diagnosis based on casual blood pressure readings and one based on around-the-clock ambulatory blood pressure monitoring.



3. Too large a circadian blood pressure amplitude usually precedes the onset of an overall elevation of blood pressure (MESOR-hypertension). [Data of Y. Kumagai]



4. Blood pressure can be acceptable during part of the day and elevated at times that are unexpected and unlikely to be monitored.



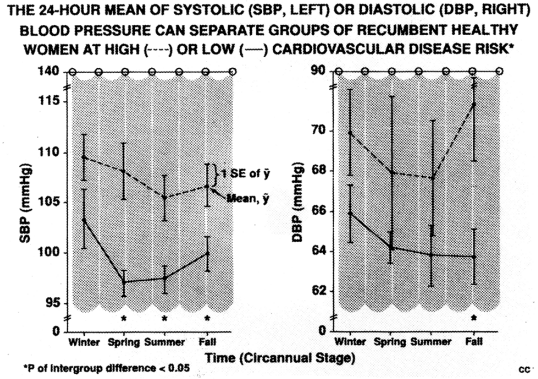
5. A chronobiologic systems approach assesses rhythmic changes in blood pressure and compares an individual's blood pressure with rhythm-qualified peer-group limits derived from actual data provided by clinically healthy gender- and age-matched peers.

See Figure 17

6. By timing treatment according to each individual's blood pressure profile, less medication is needed for more of desired and less of undesired effects.

See Figure 18

7. Well within the current range of acceptability, sizeable differences in the 24-hour blood pressure mean are found, already in the first trimester, between women with or without adverse pregnancy outcome (see Figure 17/IA) and between healthy women at high or low familial risk of developing vascular disease.



Conclusions

Chronobiology:

- removes the curtains of ignorance sanctioned by misconceptions of constancy (see above figure)
- increases diagnostic accuracy
- recognizes disorders earlier by changes in the dynamics of blood pressure variation, such as an enlarged circadian amplitude
- spots blood pressure elevation at odd hours when it is unlikely to be checked otherwise
- provides guidelines for telling when to treat
- leads to prevention by identifying indicators of a heightened risk before blood pressure disease becomes overt.

8. Applications toward primary and secondary prevention

Figure 17 illustrates the application of chronobiometry to blood pressure and the resolution of its variability within the physiologic range for primary and secondary prevention. Differences in mean arterial pressure of the order of 8 mm Hg are found within the range of currently acceptable values between women who are clinically healthy at the outset but will develop gestational hypertension or pre-eclampsia, and those whose pregnancy will remain uncomplicated (Figure 17/IA). This result also applies to systolic and diastolic blood pressure, with mean values well below 125/75 mm Hg. Such elevations in blood pressure, occurring well within the physiologic range, are observed on a group basis already during the first trimester of pregnancy when they cannot be picked up by casual measurements.

A similar finding was made for non-pregnant women, suggesting that pressures which are seemingly acceptable by current standards may be a cause for concern. Reference limits (chronodesms) that are specified as a function of the circadian scale are accumulating from newborns, children and adults of all ages, including pregnant women at different gestational ages (Figure 17/IB). Chronobiometry provides reference limits not only for the interpretation of single values, but also for that of rhythm parameters (Figure 17/IC). The systolic blood pressure profile of this pregnant woman in her 20th gestational week showed an excessive amplitude: whereas the 90% prediction limits for the double amplitude of the circadian systolic blood pressure rhythm of clinically healthy women extend from 1.7 to 30.9 mm Hg, hers was 38.6 mm Hg. It was hence flagged as being too large. The warning from this excessive circadian amplitude was disregarded, in view of a seemingly acceptable blood pressure mean. Action may have prevented convulsions 8 weeks later and the subsequent delivery of a very premature boy who was hospitalized on and off for the first 26 months of life and whose care during the first 13 (cost-accounted) months of life cost U.S. $615,000.

A form for the interpretation of blood pressure (and heart rate) profiles over 24 or 48 hours or preferably longer, called a sphygmochron (Figure 17/II), is illustrated for systolic blood pressure. It presents a comparison of a patient's profile with peer group limits, using a parametric and a non-parametric approach. Parametrically, estimates of the MESOR, double amplitude and acrophase of the circadian rhythm are listed along with 90% prediction limits derived from similar data obtained from healthy peers of the same gender and of a similar age. Nonparametrically, the patient's profile is compared by computer to the time-specified reference limits (chronodesms). When the patient's profile exceeds these limits upward and/or downward, blood pressure excess and/or deficit is recognized. The results are integrated over a full 24-hour cycle to assess a) the percentage time elevation, (PTE) b) the timing when most of the excess occurs within 24 hours and c) the extent of the excess, measured in mm Hg x h during 24 h and defined as the area delineated by the upper limit of the chronodesmic band and the patient's profile whenever it exceeds that limit, the hyperbaric index (HBI). On the basis of the parametric and non-parametric results, a recommendation is made regarding the need for any further follow-up or intervention.

The information listed in the sphygmochron can be utilized for timing treatment as secondary prevention (Figure 17/III). Profiles from two patients are shown (Figure 17/IIIA). Whereas both patients have a similar percentage time elevation (PTE = 84% and 75%), the severity of their blood pressure excess differs (HBI = 231 vs. 764 mm Hg x h during 24 h). By examining the distribution in time of the blood pressure excess, it is possible to determine when most of the excess occurs (Figure 17/IIIB and C) and to time the administration of treatment accordingly, when it is needed. The merit of this approach is shown for the case of propranolol (Figure 17/IIID). As compared to once-traditional treatment 3 times a day, chronotherapy was applied by giving the drug 1.5 to 2 hours before the daily blood pressure peak, determined by around the clock measurements for the preceding 3 days. While less drug is needed, blood pressure is lowered more and faster with chronotherapy as compared to traditional therapy, and is accompanied by fewer complications and less overdosage (Figure 17/IIID). Similar results are obtained for certain other anti-hypertensive drugs as well (Figure 18).

CHRONOBIOMETRY OF BLOOD PRESSURE (BP) WITHIN THE PHYSIOLOGIC RANGE FOR PRIMARY (LEFT) AND SECONDARY (RIGHT) PREVENTION

Parametric - Nonparametric BP Approach Assesses Percentage Time Elevation (PTE), Extent of Excess (Hyperbaric Index, HBI) and Timing of Overall Highest Excess During 24 Hours

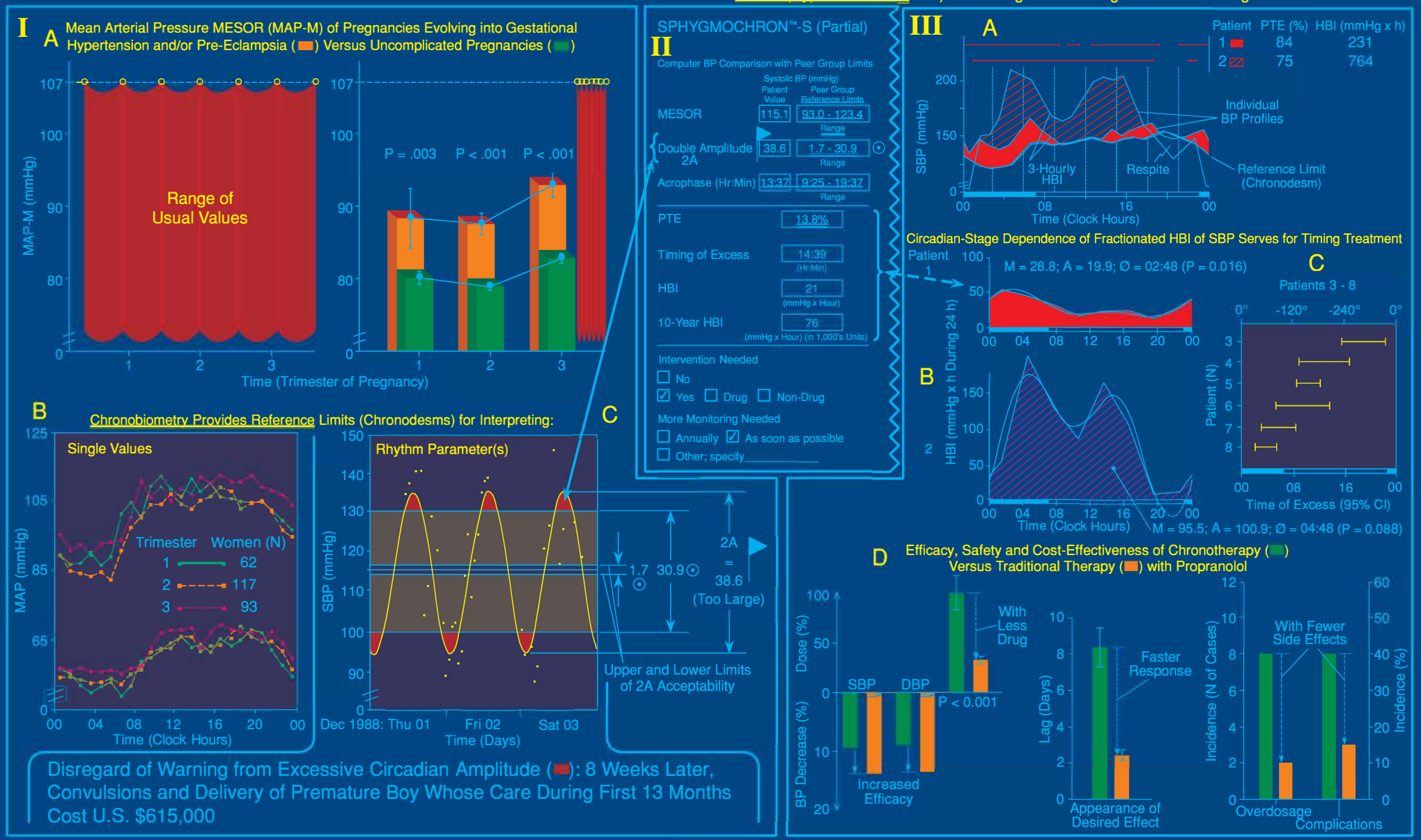


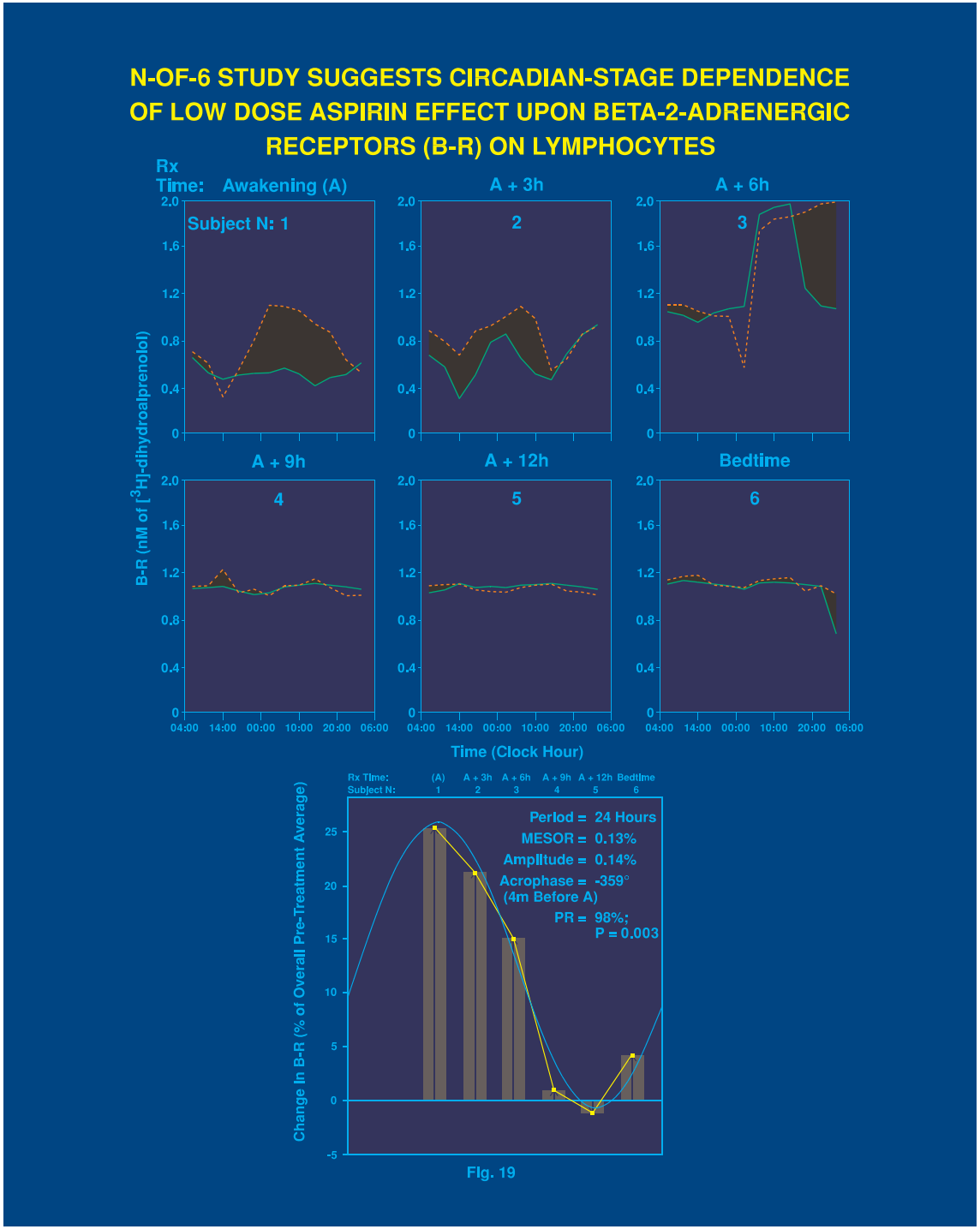
Fig. 17

Results noted earlier on a small sample of women suggest, with statistical significance, not only that low doses of aspirin affect prostaglandin and adrenergic pathways, but also that such effects vary as a function of the circadian stage at which the aspirin is taken. Six clinically healthy women, 20-30 years of age, volunteered to participate in a randomized pilot study consisting of a reference stage (lasting 2 days, starting after a 5-day adjustment to hospital conditions) followed by a 7-day span during which aspirin (100 mg/day) was administered at one of 6 different circadian stages: upon awakening, 3, 6, 9 or 12 hours after awakening or at bedtime. During the reference stage and during the last 2 days of the low-dose aspirin test span, venous blood samples were collected every 4 hours for the determination of, among others, lipoperoxide concentration in platelet-rich plasma (LP) and the affinity of lymphocyte beta-2-adrenergic receptors (B-R) for ^{3}H-dihydroalprenolol.

The circadian variation in lipoperoxide concentration in platelet-rich plasma before (upper curve) and on days 6 and 7 of prophylactic treatment with low doses of aspirin (100 mg/day for one week; lower curve) is shown in Figure 4/IVB. Each profile spans 2 days as shown on the horizontal scale. Whereas lipoperoxides are invariably depressed by aspirin, the extent of this effect varies as a function of the circadian stage of its administration. Aspirin use upon awakening (top row left in Figure 4/IVB) is associated with a clear (desired) inhibition.

Inhibition is also seen when aspirin is taken 3 hours after awakening (top row center in Figure 4/IVB). By comparison, the effect is very greatly reduced if aspirin is taken 12 hours after awakening (bottom row center in Figure 4/IVB).

The circadian variation in beta-2-adrenergic receptors on lymphocytes is shown in Figure 19. These receptors are said to counteract platelet aggregation. Again, the desired increase is seen when aspirin is taken each day on awakening or 3 hours after awakening, but not when it is used 12 hours later. The effect of daily low doses of aspirin for one week on B-R is reproduced as a vertical bar at the bottom of Figure 19 representing the extent of enhancement of B-R by low doses of aspirin. The extent of inhibition of LP by low doses of aspirin as a function of the circadian stage when aspirin is taken is shown in Figure 4/IVC. The differences in mean value between the low-dose aspirin test span and the reference stage computed for each subject were assigned to the circadian stage of treatment administration and fitted by least squares with a 24-hour cosine curve to assess the response (rhythm) to aspirin. When taken on awakening or 3 hours thereafter, aspirin depressed LP (perhaps by inhibiting thromboxane synthesis) and enhanced B-R: these effects were much smaller when the drug was taken 6 or 9 hours after awakening and were not demonstrated 12 hours after awakening. The circadian stage dependence of the effect is statistically significant (LP: P=0.012; B-R: P=0.003).



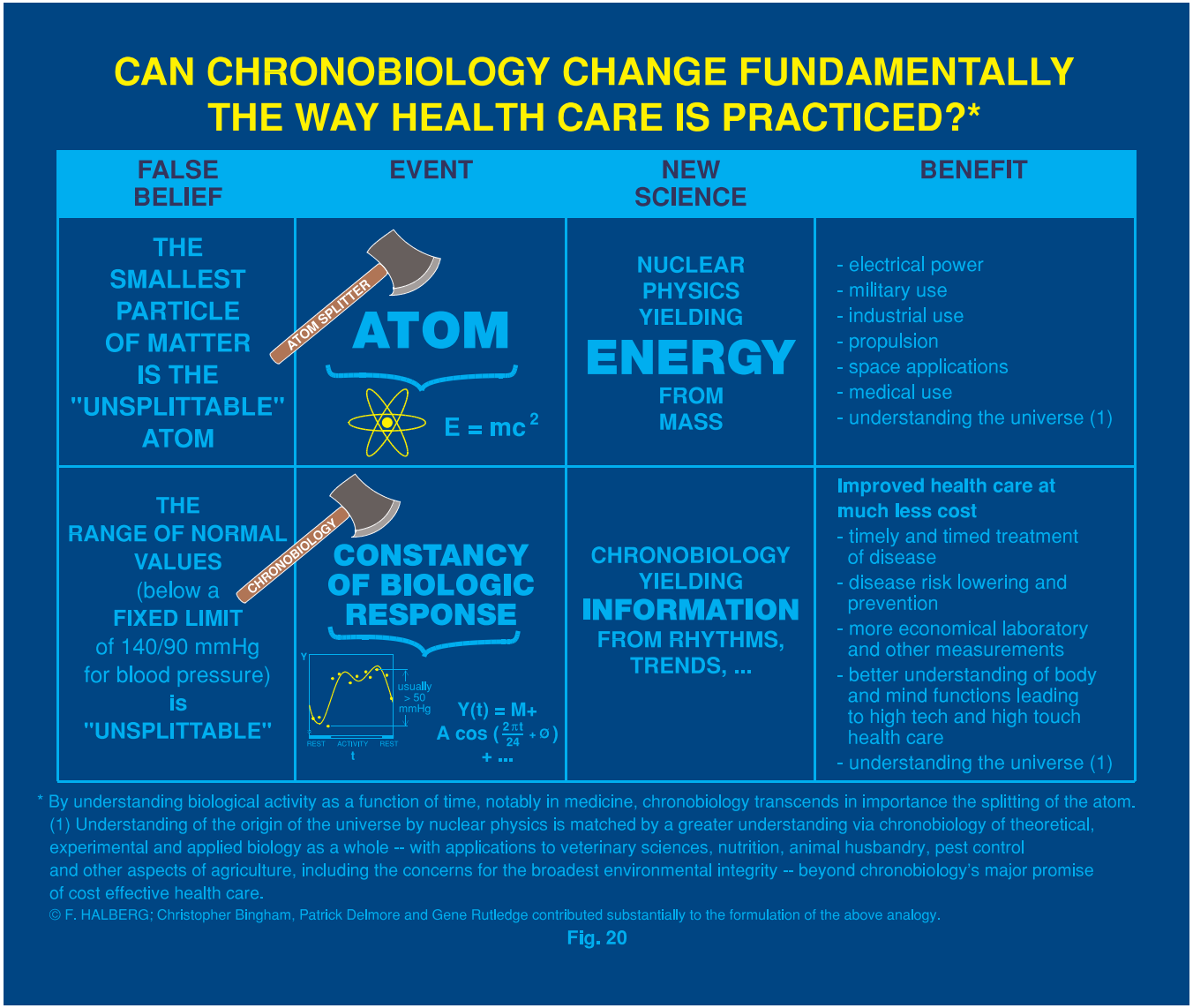


Fig. 20

A test of the clinical signification of timing (according to circadian and other chronome components) of drugs proposed or used for the case of vascular disease prophylaxis seems mandatory. Chronobiologic pilot studies are best initiated in all biologic tests or procedures, whether nutritional, hygienic, or involving exercise, drugs or prosthetic devices for prophylaxis and disease risk lowering or for treating overt illness. Such phase zero tests are best implemented to explore the role of all chronome components, not only the circadians but also those with periods of about a week, a month, and a year. At times specified by these phase zero studies, trials on larger groups can then be implemented to determine outcomes and thus the statistical signification of an intervention, in the best-tolerated dose (phase I trials), for the most effective dose (phase II trials), and as compared to the best current procedure (phase III trials). Pilot studies can suggest no more, yet may be cost-savers in providing the way to detect, sooner and with smaller sample sizes, desired or undesired effects that may otherwise be missed.

9. Conclusion

It was once believed that the 'atom' was the smallest particle that could not be further split. Breaking the atom opened the door to a new universe of particles governed by new forces and physical laws. The field of nuclear physics evolved and brought with new knowledge a new energy source and a wealth of practical applications (Figure 20).

Similarly, lifting the curtain of ignorance hiding the range of physiologic variation uncovered a new world of biologic functions that instead of striving for constancy in a homeostatic framework are coordinated by rhythms, the backbone of life itself. Entering the physiologic range revealed new biologic laws and yielded new information, the task of the budding science of chronobiology (Figure 20). Apart from providing a better understanding of the universe, common to both nuclear physics and chronobiology, perhaps one of the most challenging applica-

tions of chronobiology is that of improving the quality of health care while also reducing its cost (Figure 21).

Chronobiology offers the opportunity to quantitatively resolve the dynamics inside the normal range and hence to refine the definition of health, positively and individually. Early chronome alteration indicates earliest risk elevation. At variance with the black box of an airplane or current pacemaker-cardioverter-defibrillators, a 'chronome-box' could provide an adult lifelong record of the dynamic changes in systems of different physiologic variables. Single casual tests can hence be replaced with the synthesis, in a moment, of the whole past history, and on this basis, help can be sought long before a heightened risk has developed into overt disease. The hardware is available and is sufficiently unobtrusive to be carried by an animal as small as a rat for most of its lifespan. The software is available as modules for windowing, compacting, and recycling the accumulating information as-one-goes. Of two groups of physicians of similar competence, the one given only the history provided a better diagnosis than the one allowed only a physical examination. It would be much better yet if the salient features of the history would be dictated by the individual and stored along with continuously and automatically collected and analyzed vital signs. One could thus split and exploit the normal range, making it the custodian of our health in a telehygiene as well as telemedicine system for the home, whether it is near or far from a health care center.

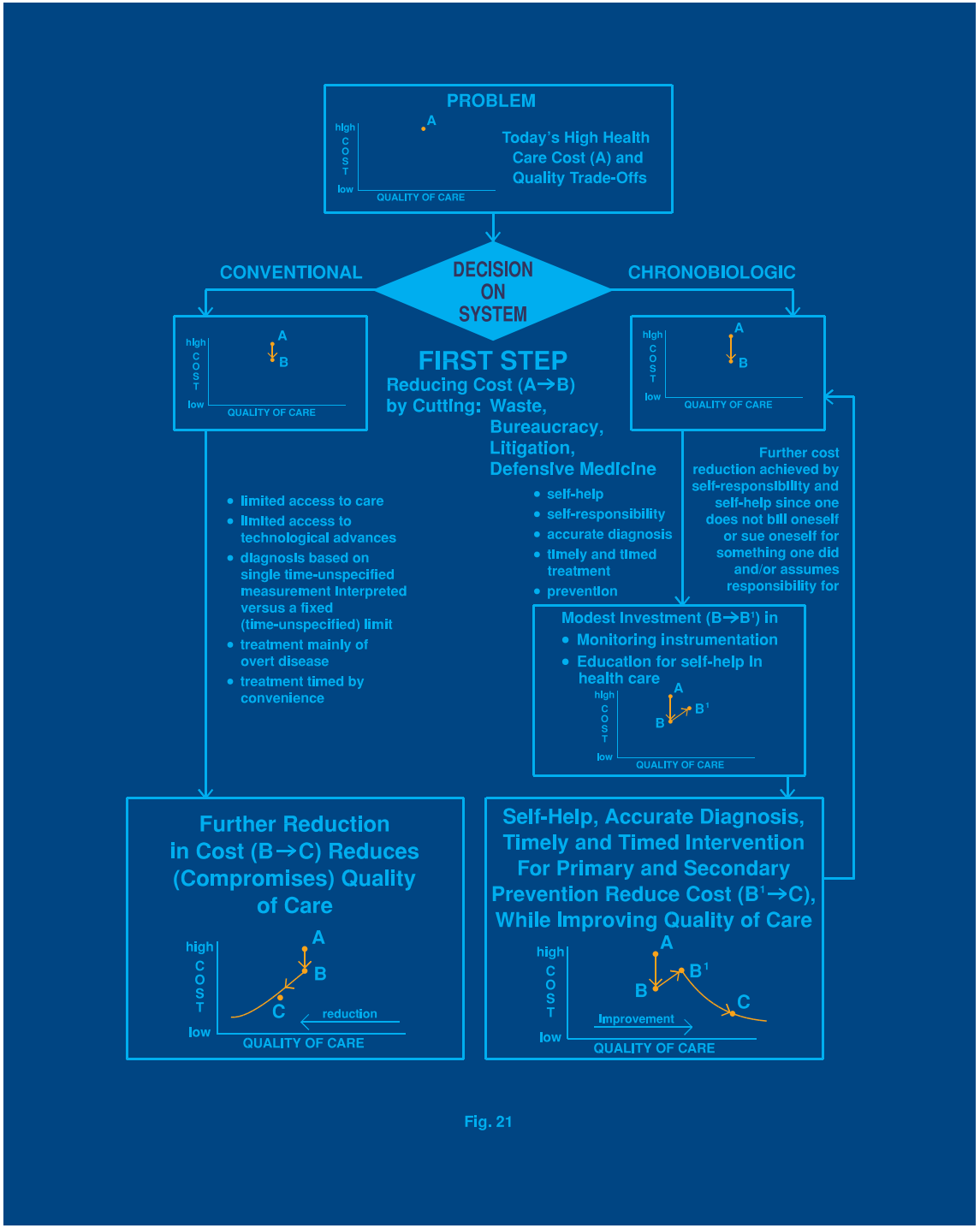


Fig. 21

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Acknowledgements and Updates

Who contributed? The results illustrated herein represent the fruits of both individual commitments (1-5), in particular to self-studies, and teamwork. These illustrations are snapshots of documentation in well over 2,000 publications. The latter provide experimental detail and/or numerical summaries of hypothesis testing and parameter estimations, the factual and conceptual bases of a new inferential bioscience. Thousands of individuals contributed from all ranks of academia and society, from university presidents in Montpellier, Nagoya, Paris and Szeged to very many patients at the then-Cambridge (Minnesota, USA) State School and Hospital for Epileptics. Thanks are due to friends, University of Minnesota and/or hospital professorial and technical staff members, students, senior and junior visitors, and volunteers in Minnesota and elsewhere. For decades, associations with the Clinical Center at the National Institutes of Health in Bethesda, Maryland, USA, and at the New Britain (Connecticut, USA) General Hospital, as well as at many universities around the world, including L'Aquila, Ferrara, Florence, Milan and Rome in Agostino's beloved Italy, proved fruitful. Many of those who contributed are co-authors listed in our bibliographies. Some of them have passed away. A few of these are explicitly honored elsewhere in this series (6).

Earl Bakken. During the early 1950s, the cooperation of Earl Bakken, DSc., hon., F.A.C.C., the founder of Medtronic, was second to none. Markers were needed to refer the endocrine and other biochemical changes to some readily measured variable. Earl Bakken built activity monitors for the study of what became the circadian system and has now become a set of yet broader chronomes. Genetically anchored yet socioecologically synchronized chronomes are found in each variable investigated thus far, once a data series is of sufficient density and length. A cosmic influence, long suggested, is becoming amenable to an experimental approach.

In the earliest 1950s, we had the problem of adjusting the sensitivity of the activity-measuring device, so that on the one hand, washing movements of a rodent could be assessed, but on the other hand, the energy-consuming horizontal displacement could be properly quantified. Only in the past few decades has this technology been transferred to humans. Activity meters have now become available to monitor not only the ultradian and circadian systems (7), but also to demonstrate about circasemiseptan and circaseptan components in human activity (8). In viewing periods that were close to 24 hours but statistically significantly different from the precise environmental 24-hour match, and also quite often different from one animal to the next, Franz postulated an endogenous component as the underlying mechanism. Earl Bakken suggested the analogy of a free-running oscillator and built a theoretical model for it (9). The circa-rhythms followed one another (10).

Earl Bakken then proceeded to build the battery-powered, transistorized cardiac pacemaker in 1957; the following year, he introduced it for long-term fully ambulatory use. Today, thanks to Bakken, much can be done to restore a most basic rhythm, by acting when the heartbeat stops, falters, flutters or fibrillates. Unknown as yet to many who produce and use it, the cardiac pacemaker is a milestone in both chronobioengineering broadly and in applied chronocardiology *par excellence.*

Patrick Delmore, Head of Communications at Medtronic, Inc. and co-author of a series of scientific publications, drew the abstract graphs in this volume. His continued help, intellectual and artistic, and that of his indefatigable assistant Marge Pekula, are gratefully acknowledged, as is the competent and patient artwork of David Lantto of ComputerChrome.

Hurdles. In Figure 1, the dispersion of the single individual glycogen values reveals their very large variability; this scatter may be one reason why Eric Forsgren, who discovered the rhythmicity of liver glycogen, was denied his medical doctorate in Sweden for very many years. Those who determined liver glycogen (or blood eosinophils; see their dispersion in Figure 1) on the basis of single determinations accepted data on rhythms in any and all variables except for the one they worked with. This attitude of ignoring the very large variation on hand for the sake of 'simplicitiy' was and is the major obstacle. The failure to

act on (and to accept as fact) a variation of over 60 mm Hg in *the* (human adult's) blood pressure each day may account for the fact that chronobiology as yet is still not in the mainstream of medicine. Cardiologists do not yet admit to themselves, much less to others, that taking *the* casual blood pressure, i.e., what they do in everyday practice, has over 40% uncertainty (11). It is easier to accept concepts of constancy and homeostasis, and turn to the next patient in the short time available for the patient's visit.

Complexities. To cite another example, studies in the 1950s resolved the circadian changes of rat liver metabolism and mitoses (9) and led to Figure 2. Such studies were complex, notably in the era before automation. Different techniques were often concomitantly used. Thus, for the Figure 2 problem (the rhythmicities of RNA and DNA), regeneration after partial hepatectomy was first investigated (9, 12, 13). Surgical procedures and techniques of wet chemistry were wedded to radioactive tracer studies, to differential centrifugation, and to more classical physiologic, hematologic and histologic approaches, with 180 partial hepatectomies done within 3.5 hours (12). All of this is background work to the main point in Figure 2, the circadian cell cycle in immature growing liver. Before the necessary teams of scores of investigators and their students working together could be assembled, much existing bias had to be overcome.

Positive attitudes. In 1950, it was customary to relate the results of chemical determinations to the most 'constant' material in a tissue: its DNA content. The possibility that DNA formation would undergo a circadian rhythmicity was then viewed as sheer heresy. The late Professor of Biochemistry, Cyrus P. Barnum was reluctant to let Franz have a technician paid on a Public Health Service grant to test for such a periodicity. Such a guess was to him wild and unlikely to yield any results. Barnum's straitlaced yet open mind could not justify the spending of taxpayers' money on testing for 'rhythms'. He did feel, however, that he could do what he wanted on his own time. He provided much enthusiastic and invaluable help himself, being at the helm of a homogenizer in many 24-hour sample collections that involved more than a sleepless night since the tissues collected had to be processed.

When data on mice given the tracer P^{32} at 1 hour before killing every few hours for 24 hours were plotted as a function of time (Figure 1 in reference 13), a second increase, 24 hours after a first peak in DNA formation, was barely seen. When the study was repeated on animals injected intraperitoneally 2 hours before killing, there was a second, small peak about 24 hours after the first large peak in the relative specific activity of DNA. This peak in DNA synthesis preceded, as would be anticipated, a much larger second peak in mitotic activity in regenerating liver. To 'isolate' this periodicity of RNA and DNA by new biologic data more clearly, separate groups of comparable inbred immature mice were studied. The animals were killed 2 hours after the injection of the tracer. Now the rhythmicity of both RNA and DNA came clearly to the fore.

Slow technology transfer. It was long mistakenly believed (14) that a 'basic' rhythm such as that in mitosis could not be shifted in its timing by the institution of changes in lighting and that the amenability to shifting of rhythms in motor activity as well as in blood eosinophils (15) was an exception, just as for a very long time rhythmicity, by those believing in homeostasis, was also dealt with as an exception rather than as the rule. By 1958, it was demonstrated that these basic cellular rhythms of mitoses of liver and skin and RNA and DNA formation, and those in circulating glucose and corticosterone and in liver glycogen, could all be changed in their timing along the 24-hour scale by manipulating the lighting regimen. It was concluded: 'The possibility to control timing of these rhythms by an easily manipulated environmental factor is of obvious practical interest…' (16). By the 1950s we also learned that the lighting-induced change applies to humans and that there was an early response of blood eosinophils to sunlight of high intensity. It is interesting today to see patents given for circadian light therapy (with mixed responses by colleagues, discussed at the time of this writing as a hotly debated topic of 'Technology transfer' [17]) when the true question revolves around an application of all treatments in the literal and figurative 'light' of the entire chronome.

New experiments or metachronanalyses: the economical approach. The studies leading to Figure 2 required teamwork for years and involved the change in model from a murine liver regenerating after partial hepatectomy to the immature growing liver. Rather than changing models, as done in order to map the cycle in growing intact rat liver (Figure 2), it is often much easier today to reanalyze data already in hand and to isolate a sought periodicity from any obscuring 'noise'. We faced a choice between preventing, at a cost of millions of U.S. dollars, a periodicity of 3 hours from entering data from a Biosatellite, versus allowing the periodicity to enter the data but then removing it mathematically at the cost of a few hundred dollars. The cheaper approach seemed acceptable as long as the 3-hour input did not gravely alter the animal's circadian behavior under study (18). A similar mathematical approach applied to available data was fruitful in the case of a meta-analysis of the chronome of *E. coli* and of cyanobacteria, as will be demonstrated below.

From Nathaniel Kleitman and sleep research to the chronome initiative. Chronobiology, like statistics, is first and foremost 'common sense applied'. We are all aware of our circadian system. To paraphrase Nathaniel Kleitman, the authority on sleep and wakefulness (19,20) and on a basic rest-activity cycle (21), eventually we all realize that we have to sleep. (As we have seen in Figures 1 and 2, our RNA and DNA formation, in this order [there is no *inverse* transcriptase], must also 'sleep'.)

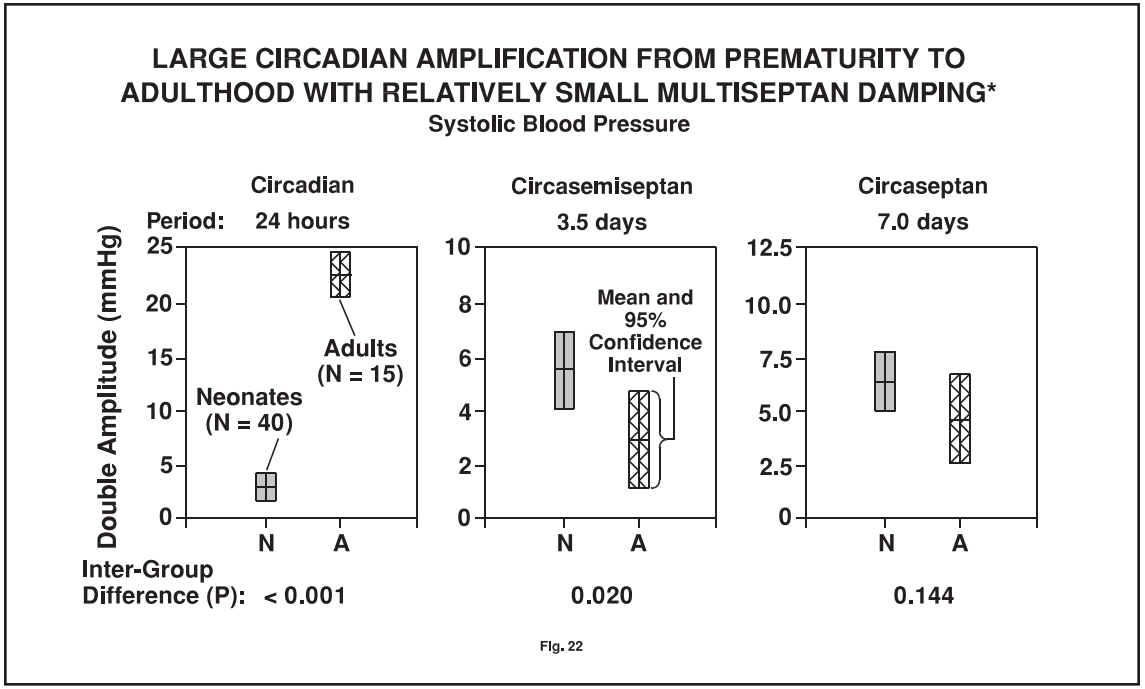


Fig. 22

Kleitman set an example in many ways. He was supportive rather than obstructive when the hypothesis of rhythms being mere conditioned reflexes, 'impressed from without and persisting from within' (20), was amended by the demonstration of genetically anchored, objectively quantified and inferentially statistically secured free-running components of a chronome (22-24). Nathaniel Kleitman is ready to set yet another example of self-assessment for science in everyday life, as he now, at 99 years of age, offers to monitor himself as a centenarian. In so doing, Kleitman, it is hoped for a long time and with many others, will be participating in an international endeavor, designed and coordinated by Germaine, involving over 100 investigators. This initiative maps systematically, from womb to tomb, the blood pressure and heart rate chronomes and opportunistically the time structures of other variables.

Basic results from womb to tomb. From this endeavor, we learned that multiseptans, such as the circasemiseptan and circaseptan components, are more prominent than circadians in many physiologic variables of human prematures, Figure 22. Before life adapted to the rotation of the earth around its axis, it may have resonated with effects related to the much slower rotation of the sun around *its* axis (25). (Religions have recognized our built-in circaseptans and have introduced a day of rest to mark this apparently earliest unit of our natural biochronology.) In adulthood, the multiseptans may

decrease only slightly while the circadians greatly increase. There are corresponding changes in the multiseptan/circadian amplitude ratios, shown in Figure 23 and Table 2.

Applied results. Figure 24 suggests an alteration of the adult circasemiseptan/circadian amplitude ratio in patients with occasional undue blood pressure excess. There is a need for long-term monitoring and analysis of infradians as well as of circadians and ultradians, if biologists are to resolve and fully exploit the dynamics within the physiologic range of variation, with applications in particular to health care.

In addition to circadian amplitude-hypertension (26), other new syndromes and yet broader biologic problems may be detected early for the sake of prevention in the broadest sense of the ills of our planet as well as of ourselves.

Primum redigere ad mensuram chronomata, periodicitates vel inclinationes, quæ non unas sed maximas proprietates vitæ sunt: quod est demonstrandum *(A hypothesis yet to be further tested is that chronomes, anchored in the genomes of life forms, are* ***the,*** *rather than* ***a*** *fundamental dimension of living matter).* The French neurophysiologist Alfred Fessard wrote that periodicity is a fundamental property of living matter (27), and we may add, much more than a clock or calendar. Toward this goal, this volume honors Agostino Carandente best by including, in keeping with his exhorta ion **'Gehen Sie weiter',** the most recent results in

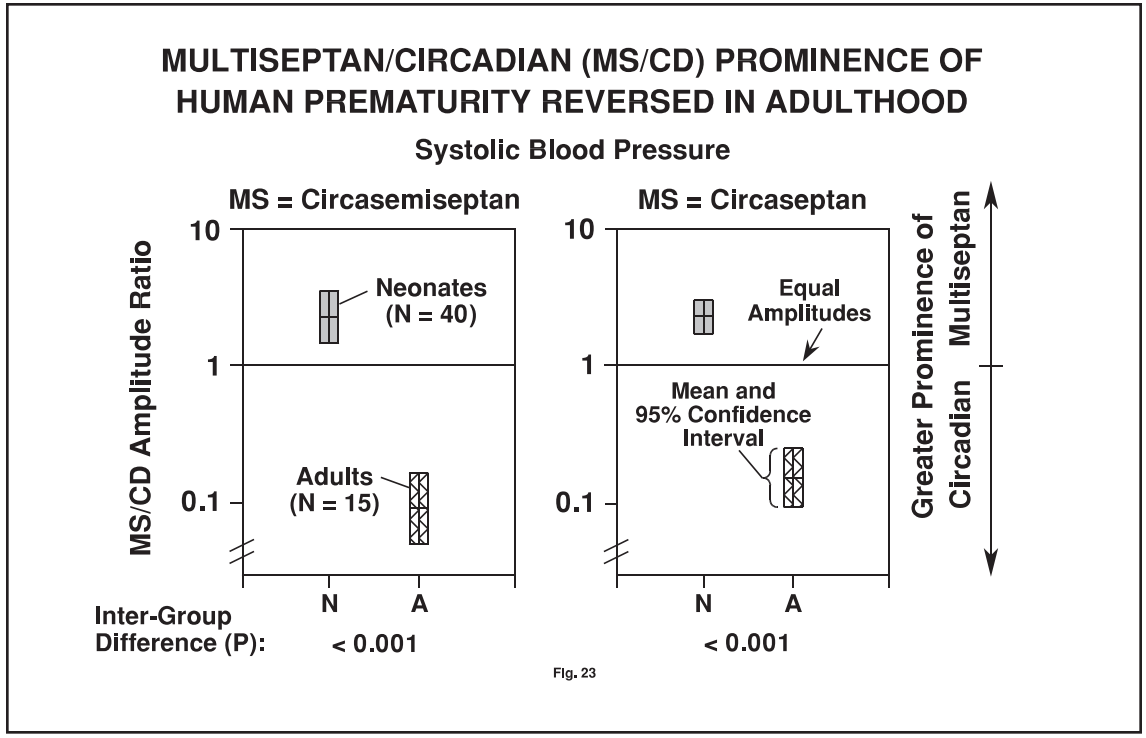
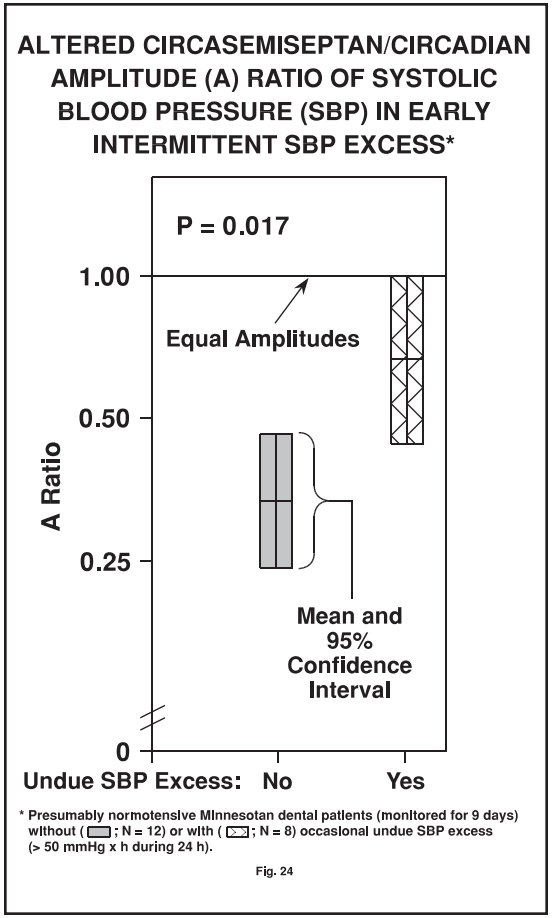
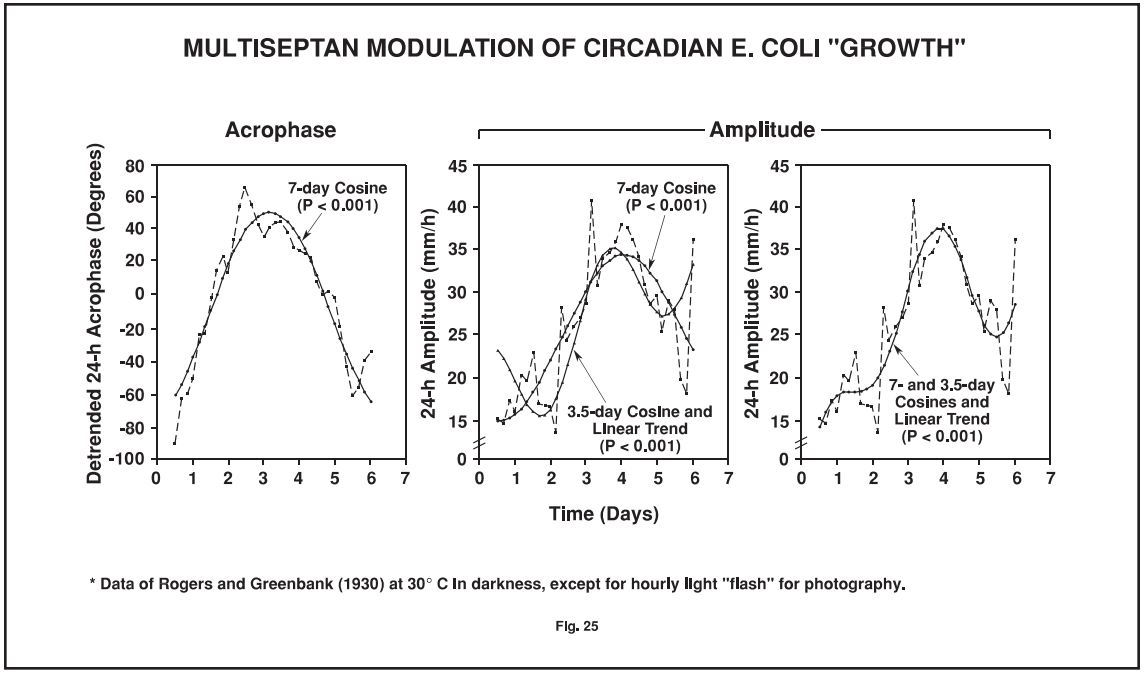


Table 2
LARGER CARDIOVASCULAR PROMINENCE OF CIRCASEPTANS IN NEONATES AND OF CIRCADIANS IN ADULTS, GAUGED BY AMPLITUDE RATIOS (AR)*

Variable	Mean AR and 95% confidence interval Neonates (40)			Adults (15)			Comparison Student *t*	(P)
Systolic BP	2.23	[1.66,	2.99]	0.15	[0.09,	0.24]	9.545	(<0.001)
Diastolic BP	1.57	[1.18,	2.09]	0.13	[0.08,	0.21]	9.024	(<0.001)
HR	2.32	[1.78,	3.01]	0.16	[0.11,	0.25]	11.848	(<0.001)

*Data series on blood pressure (BP) and heart rate (HR) analyzed by the least-squares (cosinor) fit of 24-hour and 7-day cosine curves to data series each covering at least 7 days around-the-clock. The prominence of circaseptans early in extrauterine life raises the question whether this chronome component may have evolved by resonance with solar wind-related (geomagnetic) disturbance and/or may (also) currently constitute a response to our cosmos. The endogenicity of BP and HR circaseptans is supported by their free-running under conditions of an isolette for prematures or in social isolation for adults or for systolic BP in an afebrile boy (with intermitent fever) on a daily routine in the presence of a 24-hour synchronized circadian of locomotor activity (59).





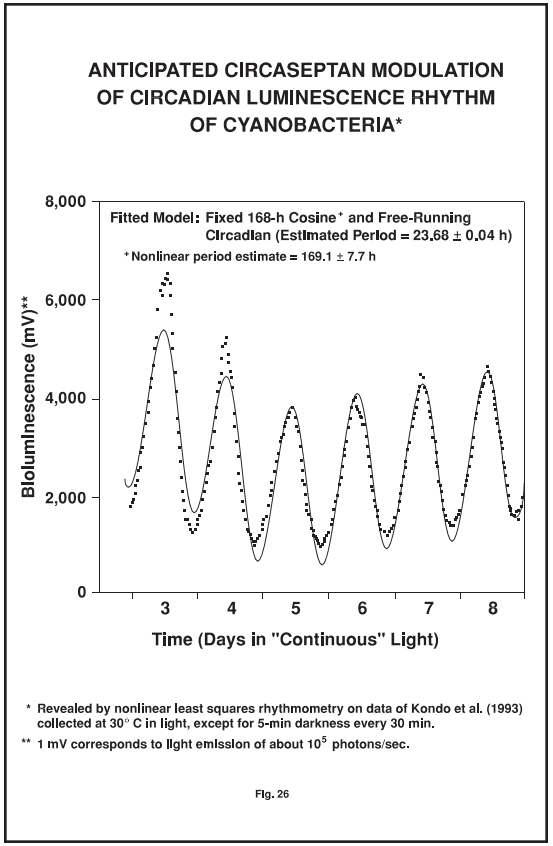
their historical context. Chronobiology is progressing by applying a spectral approach to smaller and smaller morphologic units. It did so to a time-macroscopic circadian record (28): the 1930 data of Lore A. Rogers (a noted bacteriologist described by a Cosmos Club Vignette of December 1967 as 'the bright star in the [U.S. Department of Agriculture's] scientific horizon before World War II'). By 1961, we had demonstrated the circadian free-run of about 21 hours and subsequently the indication of an infradian, in the growth and/or colony advance of *E. coli,* Figure 25 (29,30). In his nineties, Rogers (personal communication) had also ascertained, from his technician and co-author G.R. Greenbank, the lack of any known external 21-hour cycle.

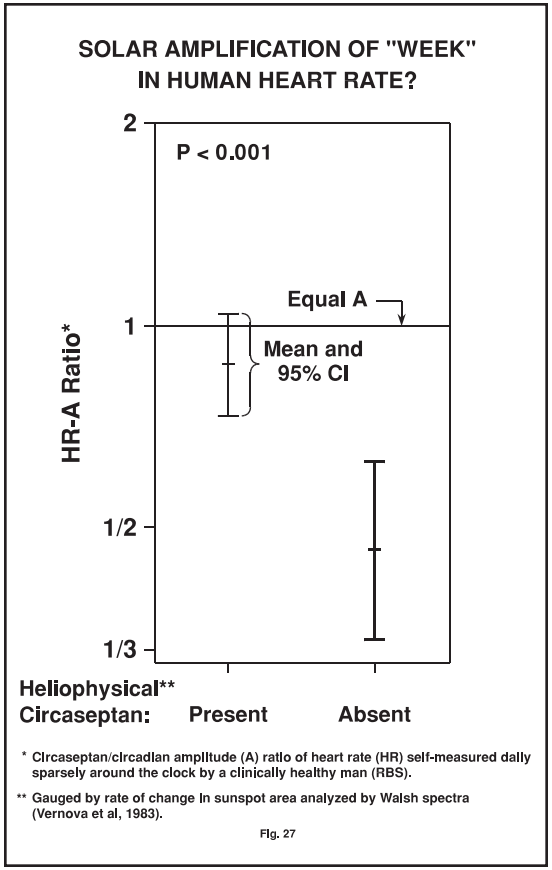
Nonetheless, in 1976, a committee reported that the minimal biologic unit capable of exhibiting circadian rhythmicity was the single cell (31): experiments reporting circadian rhythms in *Klebsellia* (32) and *E. coli* (33) were criticized on methodological grounds and unpublished negative experiments reported to the committee by several authors (Sweeney, Edmunds, Schweiger, Brinkman, Bünning and Bruce) believed instead (31). It is never easy to rule out periodicity. By 1994, with the early follow-up work (32,33), a series of more recent added publications explicitly rejects the dogma 'eukaryotes only' (34-38), thus fully vindicating our 1961 analyses (29).

Turning to the question of the basic nature of multiseptans, circaseptans and/or circasemiseptans are quantified in the firing of an isolated retina (39,40), in the beating of an isolated myocardial cell (41), in unicellulars (42-45), in isolated platelets (46) and even in *E. coli* and cyanobacteria, Figures 25 and 26. The brevity of the time series in many of these models is a grave limitation. The vitality of the isolated platelet gauged by its glutathione content and that of some other models showing a decreasing trend of slow death is another limitation. These circumstances help us the more to emphasize that human prematures are perhaps the most challenging model, since relatively long data series are readily obtained on neonatal critical care units. The promise of chronomes to bear prognostic, diagnostic and therapeutic fruit in health care broadly is thus testable at a critical time when preventive measures may be most effective, an approach currently followed by Elena V. Syutkina of the Institute of Pediatrics in Moscow. Infradians are now documented in one boy born in the 28th gestational week by around-the-clock measurements, with interruptions, for 26 months (25). The prominence of circaseptans in the blood pressure and heart rate of prematures is supported by 40 series, Table 2 (47) and confirmed on 30 more babies as this seminar goes to press (60).

There are also limitations to studying prematures. At an age of very active growth, the data have to be detrended for this reason and also because many babies investigated are sick and their disease and treatment can generate added trends. In the light of these complexities, the claim to the relative simplicity of bacterial and unicellular models must seriously be considered. In any event, nobody should want to be an investigator only of one model, if not book *(homo unius libri).* The proper study of humankind, with Charron (48) and Pope (49) paraphrased, is humans, from womb to tomb, yet indeed our perspective is broadened by unicells and prokaryotes. While a chronome initiative focuses, first and foremost, on humans, other models, under certain conditions, offer relatively inexpensive and more readily manipulable approaches to the time structure of life, leading to testable hypotheses for optimizing both health and the environment relevant to humankind, notably in the neonatal stage and for shift-workers.

Cosmos. On humankind, we have explored effects (upon chronomes) of the moon and the sun. These effects are difficult to separate. We have used the term 'circatrigintan' to refer to cycles with a period of about 30 days, rather than terms implying a direct relation to any *lunar* cycles (50), some of which may turn out to be *solar* (25). The role of the sun has been debated strongly since early in this century, when Chizhevsky described life on earth as 'an echo of the sun' (51). We may have gone a few steps (see chapter 6, Figure 12) beyond previous endeavors reviewed in a scholarly book by Dubrov (52). Furthermore, in Figure 27, Germaine's analysis suggests that cir-





caseptans are not necessarily only a reflection of past adaptation, as a genetic multiseptan anchor, but may still be influenced by the sun's activity: a decrease in the circaseptan prominence of heart rate of a clinically healthy man is observed in association with a drastic reduction in circaseptan features in solar activity, determined by Walsh spectra (53), Figure 27. The approach, involving 'remove and replace' experiments broadly, rather than the result on this particular subject itself, deserves further consideration in future investigations on the effects of subtle geophysical factors.

We need not conclude, as did Hobart A. Reimann, a former professor of medicine at the University of Minnesota, in his book on periodic disease (54): '…according [presumably] to Montaigne, whenever a new idea is offered, many say, "It is probably not true." When it is confirmed they say, "Yes, it may be true, but it is not important." When its importance is validated, they say, "Yes, surely it is important, but it is no longer new."' The chronome was then missing, just as the importance of the genome then went somewhat unrecognized.

The circaseptans are old (55-57) yet new, as were the circadians in the 1950s. So is the chronome, obvious as the sunrise yet usually in need of data collection and quantification by the computer, the equivalent of both the microscope and the telescope of life in time. This introduction to chronobiology may prevent the next generation from sharing the fate of Molière's M. Jourdain (58), who only late in life learned that he had all along spoken prose (read: chronobiology).

After reading this introduction, we trust that today's bioengineering, biomedical and political communities at large will no longer have to be told that all their lives they have been and are speaking chronobiology, which for Agostino and us is beautiful poetry. All of life, starting in earliest public instruction, in town and gown, awaits quantification by chronobiometry in all disciplines, from sociologists writing about the week to ecologists with blueprints about the environment. With the philosophical and bioethical recognition that all manifestations of life can be meaningfully measured in time, chronobiology becomes the *'commune vinculum omnibus artibus, omnibus scientiis et omnibus disciplinis'.*

Omnia metire tempestive et ergo significative
quæcumque licet
et immensa ad mensuram eandem redige
Measure in time (hence meaningfully)
what is measurable
and render thus measurable
what as yet is not

Germaine Cornélissen
Franz Halberg

Prof. ROBERT P. SONKOWSKY, former Head of the Classics Department at the University of Minnesota, invaluably and promptly helped in translating into the tradition of the classics the results of chronobiology, obtained by mathematics, disciplines dear to Agostino Carandente.

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