CHRONOPHARMACEUTICS
CHRONOPHARMACEUTICS
Science and Technology for Biological Rhythm-Guided Therapy and Prevention of Diseases

Edited by

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“We must use time creatively.”
— Martin Luther King, Jr. (1929–1968)

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PREFACE

“Education is the most powerful weapon which you can use to change the world.”
— Nelson Mandela (1918– )

Chronopharmaceutics is an emerging discipline combining the traditional goal of pharmaceutics (sciences of drug delivery systems) with recent knowledge in different disciplines derived from advances in chronobiology. Basically, the advances in chronobiology and related disciplines have led to a plethora of data demonstrating the extent of generality and precision of biological rhythms that may be used intelligently for the development of novel drug delivery systems, as well as drugs, to optimize their efficacy and safety. Furthermore, advances in genomics and nanobiotechnology provide new horizons in disease therapy and prevention. Hence a convergence of pharmaceutics and modern chronobiology is taking place along with the alignment of concomitantly locally and globally monitored environmental and organismic time structures—chronomes. Chronomics is the most ambitious way of optimizing medicines, and thus health care practices so that maximal therapeutic efficacy is achieved with minimal side effects, and is critical in the treatment of cancer and other diseases.

As a new and evolving discipline, chronopharmaceutics has attracted considerable attention from academia and industry. Over the past several years, various courses and workshops in this area have been offered on many campuses worldwide. At the international level, the American Association of Pharmaceutical Scientists, on my initiative, is one of the first to offer a 2004 sunrise school on the topic of “Chronopharmaceutical drug formulation.” Frequent requests for a standard textbook from several scholars and publishers on chronopharmaceutics prompted me to propose the current edited book on chronopharmaceutics. At present, there is no comprehensive textbook available on chronopharmaceutics. The various graduate level courses on this interesting topic are being taught by professors who must gather materials from diverse sources. In the search for an ideal controlled release system for a drug, there is no comprehensive book to bridge the gap between current knowledge in pharmaceutics, usually based on physical chemistry, and chronotherapy for effective and safe drug delivery system design, notably for disease prevention.
using current advances in chronobiology, chronomics, and chronogenetics. This book is intended to increase awareness and initiate discussion in the pharmaceutical and biomedical communities about the importance of rhythms in us and around us in the early design of a delivery system for treatment and more so for the prevention of diseases. Real challenges remain to be solved for ideally controlled drug release and health, for risk elevation, as well as disease monitoring, based on what is known today. To satisfy this urgent need, we have assembled a group of experienced investigator-educators around the world who are on the frontline of chronogenetics, chronopharmacokinetics, chronopharmacodynamics, and chronopharmaceutical drug delivery research to develop a textbook on chronopharmaceutics. This book and specifically the “chronopharmaceutics” topics are intended to be a “hybrid” of both fields (“controlled drug delivery” and “chronotherapy”).

This book is intended for undergraduates in their third and fourth year in college and for first and second year graduate students in biomedical and pharmaceutical sciences, first year medical, veterinary, nursing, and dental students and professionals, and researchers in these fields seeking to update their knowledge in a lifelong learning process.

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Bi-Botti C. Youan
ACKNOWLEDGMENTS

The idea to start this book on chronopharmaceutics came from Jonathan T. Rose, Editor, Scientific, Technical, and Medical Division, John Wiley & Sons, and caught me by surprise, when we first met at the 2004 meeting of the American Association of Pharmaceutical Scientists (AAPS) in Baltimore. Probably Jonathan had read our first manuscript on the topic, which shortly after publication became one of the 25 hottest papers in the *Journal of Controlled Release* at that time. Shortly after meeting with Jonathan, I developed a book proposal that was kindly reviewed by international experts in the field: Ronald Siegel (Professor and Head, Department of Pharmaceutics, College of Pharmacy, University of Minnesota), Michael M. Smolensky (Professor of Environmental Physiology, The University of Texas, Health Science Center at Houston, School of Public Health), William J. M. Hrushesky (Norman J. Arnold School of Public Health, W. J. B. Dorn Veterans Affairs Medical Center, Columbia, South Carolina), Andrea Gazzaniga (Professor, Istituto di Chimica Farmaceutica e Tossicologica, Faculty of Pharmacy, University of Milan, Italy), and Bjoern Lemmer (Professor, Institute of Pharmacology and Toxicology, University of Heidelberg, Germany). Their expert insights encouraged me to pursue this project at the early stage. I am also extremely thankful to the editorial board for the time and effort they contributed to this endeavor: Robert Langer (Institute Professor, Department of Chemical Engineering, Massachusetts Institute of Technology, Boston, Massachusetts) and Franz Halberg (Professor and Director, Halberg Chronobiology Center, University of Minnesota)—two pioneers in the area of drug delivery and chronotherapy, respectively. I am also indebted to my UMKC colleague and basic circadian rhythm investigator, Jeffrey Price (Associate Professor, School of Biological Sciences, University of Missouri–Kansas City), for the time taken to review and provide constructive comments on my first draft chapter related to chronobiology.

I am grateful to Jonathan T. Rose (John Wiley & Sons), who has always been there to help and support, and perhaps most importantly, to the team of authors from around the world who contributed to this book and assisted in the peer-review process of each chapter. Among reviewers, special thanks are due to Dr. Elena Losi (Dipartimento Farmaceutico, Università di Parma) and Dr. John Santini Jr. (President, Microchips, Inc., Bedford, Massachusetts),
who unfortunately could not contribute to this book due to other commitments.

I would like to sincerely apologize for the delay in the publication of this book due to several life changing events. My first son (Traby) was born on November 12, 2004 and my second son Bobby on August 15, 2006. These changes were also followed by our relocation from Texas (Texas Tech University) to Missouri (University of Missouri–Kansas City), where I began my new position in October 2006 with several new challenges and adaptation issues, as one could imagine. To my wife (Lou Antoinette) and family, I am eternally grateful for always being patient, even on the days when they hardly saw me during the development of this exciting and provocative project.

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<td>Ambulatory blood pressure</td>
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<tr>
<td>ACTH</td>
<td>Adenocorticotropic hormone</td>
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<td>AD</td>
<td>Alzheimer disease</td>
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<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
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<td>AMS</td>
<td>Accelerator mass spectroscopy</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARNT</td>
<td>Aryl hydrocarbon receptor nuclear translocator</td>
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<tr>
<td>ASA</td>
<td>Acetylsalicylic acid (Aspirin)</td>
</tr>
<tr>
<td>5-ASA</td>
<td>5-Aminosalicylic acid</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>AVP</td>
<td>Arginine vasopressin</td>
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<tr>
<td>bHLH</td>
<td>Basic helix-loop-helix, a that characterizes a family of transcription factors</td>
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<td>BCNU</td>
<td>bis-Chloronitrosourea (Carmustine)</td>
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<tr>
<td>BHAT</td>
<td>Beta-Blocker Heart Attack Trial</td>
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<td>BIOSPHERE</td>
<td>BIOSphere in the COSmos</td>
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<tr>
<td>Bmal</td>
<td>Brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like</td>
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<td>BZ</td>
<td>Belousov–Zhabotinsky</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<tr>
<td>CCGs</td>
<td>Clock-controlled genes</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<tr>
<td>CGMS</td>
<td>Continuous glucose monitoring system</td>
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<tr>
<td>CHAT</td>
<td>Circadian hyper-amplitude-tension</td>
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<tr>
<td>ChrDDS</td>
<td>Chronopharmaceutical drug delivery systems</td>
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<tr>
<td>CKI(e)</td>
<td>Casein kinase I(e)</td>
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<tr>
<td>CKI(\delta)</td>
<td>Casein kinase I(\delta)</td>
</tr>
<tr>
<td>CLK</td>
<td>Clock</td>
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<tr>
<td>CODAS</td>
<td>Chronotherapeutic oral drug absorption system</td>
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<tr>
<td>COER</td>
<td>Controlled-onset extended-release</td>
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<tr>
<td>CoQ10</td>
<td>Ubiquinone</td>
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<tr>
<td>CREB</td>
<td>cAMP response element-binding</td>
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<tr>
<td>CRM1</td>
<td>see Exportin 1</td>
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<tr>
<td>CRR</td>
<td>Circadian rhythm release</td>
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<tr>
<td>Cry1</td>
<td>Cryptochrome 1</td>
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<tr>
<td>Cry2</td>
<td>Cryptochrome 2</td>
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<tr>
<td>CUSUMs</td>
<td>Cumulative sums</td>
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<tr>
<td>CYC</td>
<td>Cycle</td>
</tr>
<tr>
<td>DBP</td>
<td>D-Element-binding protein</td>
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<tr>
<td>D-CHAT</td>
<td>Diastolic circadian hyper-amplitude tension</td>
</tr>
<tr>
<td>DHRV</td>
<td>Decreased heart rate variability</td>
</tr>
<tr>
<td>DM1</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>3DP</td>
<td>Three-dimensional printing</td>
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<tr>
<td>EBM</td>
<td>Evidence-based medicine</td>
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<tr>
<td>E-box</td>
<td>sequence which usually lies upstream of a in a region</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EEGs</td>
<td>Electroencephalograms</td>
</tr>
<tr>
<td>EPP</td>
<td>Elevated pulse pressure or excessive pulse pressure</td>
</tr>
<tr>
<td>ESS</td>
<td>Error sum of squares</td>
</tr>
<tr>
<td>EVA</td>
<td>Ethylene-covinyl acetate polymer</td>
</tr>
<tr>
<td>EVAc</td>
<td>Ethylene vinyl acetate copolymer</td>
</tr>
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<td>Exportin 1</td>
<td>CRM1 homolog, also known as XPO1, is a human. The protein encoded by this gene mediates leucine-rich nuclear export signal (NES)-dependent protein transport</td>
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<td>FAPS</td>
<td>Familial advance sleep phase syndrome</td>
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<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
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<tr>
<td>5-FU</td>
<td>5-Fluouracil</td>
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<tr>
<td>GERD</td>
<td>Gastroesophageal reflux disorder</td>
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<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
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<tr>
<td>GH</td>
<td>Gestational hypertension</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
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GPC: Gel permeation chromatography
GPCR: G-Protein coupled receptor
GSK3β: Glycogen synthase kinase 3β
HALO: Hours after light onset
5-HT: 5-Hydroxytryptamine or serotonin
hGH: Human growth hormone
HIV: Human immunodeficiency virus
HLF: Hepatocyte leukemia factor
HPLC: High-performance liquid chromatography
HPMC: Hydroxypropyl methylcellulose
HPMCP: Hypromellose phthalate
IBD: Inflammatory bowel disease
ICD10: International Classification of Diseases, 10th Revision
ICU: Intensive care unit
IL-2: Interleukin-2
IR: Immediate release
IVIVC: In vitro–in vivo correlations
LC: Lipid clinic
LDL: Low-density lipoprotein
LPs: Lipoperoxides
LSC: Liquid scintillation counter
LVMI: Left ventricular mass index
MAP: Mitogen-activated protein
MAP: Mean arterial pressure
M-CSF: Macrophage colony-stimulating factor
MEMS: Microelectromechanical systems
MESOR: Midline estimating statistic of rhythm
MMC: Migrating myoelectric complex
mRNA: Messenger RNA
NA: Noradrenaline
NCEP ATP III: National Cholesterol Education Panel-Adult Treatment Panel III
NES: Nuclear export sequence
NLSs: Nuclear localization signals
NPAS2: Neuronal PAS domain protein 2
ODT: Orally disintegrating tablet
OROS: Osmotic release oral systems
PAI-1: Plasminogen activator inhibitor-1
PAMPS: Poly(2-acrylamido-2-methyl-1-propanesulfonic acid)
PAR bZIP: A family of proteins that are transcription factors; that is, in response to various signals, they combine to other transcription factors to express a gene.

The three members of the PAR bZIP family – DBP, HLF and TEF – are involved in the complex world of circadian rhythms: those physiological rhythms which are regulated according to our 24-hour day

PAS: contained in many signalling proteins where they are used as a signal sensor. It was named after three proteins that it occurs in: Per (period circadian protein), Arnt (aryl hydrocarbon receptor nuclear translator protein) and Sim (single-mined protein).
Pdpl: PAR domain protein 1;
Pdpx: Pyridoxal kinase
PE: Pre-eclampsia
PEG: Polyethylene glycol
PEO: Polyethylene oxide
Per: Period
PGA: Polyglycolic acid
PK2: Prokineticin 2
PK/PD: Pharmacokinetic/pharmacodynamic
PLGA: Poly(D,L-lactic-co-glycolic acid)
PLL: Poly(L-lactic acid)
PORT: Programmable oral release technologies
PP2A: Protein phosphatase
PTh: Parathyroid hormone
QTL: Quantitative trait loci

Rev-erbα: Also known as NR1D1 (nuclear receptor subfamily 1, group D, member 1), is a member of the Rev-ErbA family of nuclear receptors and is a transcriptional repressor. Rev-erbα is highly expressed in the liver, skeletal muscle, adipose tissue and brain, participating in the development and circadian regulation of these tissues

RNA: Ribonucleic acid
RORz: RAR-related orphan receptor alpha (ROR-alpha), also known as NR1F1 (nuclear receptor subfamily 1, group F, member 1), is a nuclear receptor encoded by the RORA (RAR-related orphan receptor A)

DNA: Suprachiasmatic nuclei
SEM: Scanning electron microscopy
SiO2: Silicon dioxide
SIF: Simulated intestinal fluid
SIRT1: Silent mating type information regulation 2 homolog, S. cerevisiae stands for sirtuin (silent mating type information regulation 2 homolog) 1 (S. cerevisiae).
SIRT1 is an enzyme which deacetylates
proteins that contribute to cellular regulation (reaction to stressors, longevity)
STN: Subthalamic nucleus
SITT: Small intestinal transit time
SU4885: Metopirone (metyrapone) is a drug used in the diagnosis of adrenal insufficiency and occasionally in the treatment of Cushing’s syndrome (hypercortisolism)
SUMO: Small ubiquitin-related modifier protein

TEF: Thyrotroph embryonic factor
TEPP: Time equivalent process parameter

TETP: Time equivalent thickness parameter
Tim: Timeless, timeout
t-PA: Tissue plasminogen activator
TPR: Timed, pulsatile release
TSR: Timed, sustained release
TSS: Total residual sum of squares

UC: Usual care
UGP: Urinary gonadotropin peptide
USP: United States Pharmacopeia

Vri: Vrille
“Surely... [in all of transdisciplinary science, including therapy] the thing to hunt down is a cycle ... and if found, then above all things, and in whatever manner, lay hold of, study it, record it and see what it means.”

— *Sir J. Norman Lockyer (1836–1920)*

The 2-year survival rate of patients with perioral cancers has been doubled by radiation treatment at the peak of the previously monitored tumor temperature. A risk of stroke greater than that associated with hypertension has been detected by taking time structure into account. Aspirin has been found to have drastically different effects with different timing, and agents acting on the central nervous system show different times of maximal toxicity, (Figure 1). While a treatment guided by the circadian tumor temperature rhythm was documented in 1977 (Refs. 532 and 551 in Ref. 1), periodicity in health was experienced and then known as the change from sleeping to waking to the first organisms that sleep or rest, more or less regularly. Periodicity was known in antiquity, probably as fevers of infectious diseases for millennia. For centuries, epilepsy was described to occur in some patients mostly during waking, in others mostly during sleep, or in still others on awakening (if not diffusely during 24 hours—reviewed in Ref. 26 in Ref. 1). It was hence hardly surprising to find circadian rhythms in the human electroencephalogram in disease by 1952 (Ref. 15 in Ref. 1) and in health in 1966 (Ref. 211 in Ref. 1). A history of Minnesota chronobiology is given in Ref. 2542 of Ref. 1. That noise of a fixed intensity to which mice of susceptible strains were exposed can induce convulsions and death at one circadian stage but not at another was reported.
Figure 1. Timing can be as important as dosing, or more so if circadian stage determines the chances of life versus death in response to the same stimuli, as demonstrated for many drugs under the environmental conditions available in a modern laboratory with standardized lighting, environmental temperature and humidity, noise, and odor (while, any associations with magnetic storms that also affect rhythms remain uncontrolled). Part (1A) shows the difference as a function of timing in the response of susceptible mice to noise. On a given lighting scheme (light by day, but not by night), exposure of mice to the ringing of bells at 0800h elicited dashing in very few animals, as seen in the first gray column on the left. Even fewer mice showed clonic or tonic convulsions (the next two columns) and none died (the last column with no entries). By contrast, over 40% of comparable mice of the same susceptible inbred strain exposed to noise in a different stage (at 2100h) dashed, over 20% convulsed, and all of these died from the same stimulus on the same day. The data demonstrate that exposure timing accounted for the difference between life and death in response to the same stimulus. Four days after reversal of the lighting regimen, in addition to an overall elevated susceptibility, there were more rather than fewer deaths at 0800h as compared to exposure at 2100h (Ref. 53 and Ref. 106 in Ref. 1). Part (1B) shows a free-running circadian rhythm in survival after injection every 4 hours of a fixed dose of ethanol to separate groups of comparable mice (Ref. 126 in Ref. 1). Part (1C) (Ref. 137 and 188 in Ref. 1) to (1F) show other circadian toxicity rhythms (Ref. 308 in Ref. 1). A (for us indispensable) point and interval parameter estimation (e.g., by cosinor) is seen in Part (1E) (right) and (1G). For details of the latter, see (Ref. 246 in Ref. 1) [6, 10, 41].
by 1955 (Ref. 53 in Ref. 1) and again in 1958 (Ref. 106 in Ref. 1). The time-varying circadian susceptibility of the nervous system also reveals different phases for the effects of different drugs such as Librium (Ref. 137 in Ref. 1), pentobarbital (Ref. 308 in Ref. 1), nomifensine (Ref. 659 in Ref. 1), ethanol (Ref. 110, Ref. 116 and Ref. 126 in Ref. 1), and anesthetics such as halothane (Ref. 188 and Ref. 238 in Ref. 1) and methohexital (Ref. 915 in Ref. 1).

That we are not dealing with a relation to clock-hour [1] was shown not only by the ability to phase-shift rhythms by manipulating the lighting regimen for mice, (Figure 1A) and by changing the sleep–wake schedule for humans (Ref. 107 in Ref. 1), but also by an effect of ethanol that was circadian periodic but free-running with a period close to, but different from, exactly 24 hours in mice kept in continuous darkness, (Figure 1B) (Ref. 126 in Ref. 1). Also in 1958, the effect of ouabain was found to be circadian stage dependent (Ref. 110 and Ref. 119 in Ref. 1). In 1964 (Ref. 188 & Ref. 238 in Ref. 1), the susceptibility resistance cycle to halothane had been extensively documented, to be followed by studies reviewed by Chassard et al. [2]. A circadian rhythm in an experimental oncological therapeutic index appeared in 1973 (Ref. 316 in Ref. 1).

Notwithstanding a mountain of evidence provided from many quarters in this book, indications of timing the use of physical agents [1], drugs [1–7], nutriceuticals [6, 8], or food [4, 9] as yet are missing in their scheduling (e.g., on package inserts or labeling). This undesirable status quo [10] may be due to a central idea in physiology, as also recently noted by Chassard et al. [2] under the title “Timing Is Everything.” For too many, nothing in biology makes sense except in the light of homeostasis [11; cf. 12], a concept often used as an excuse to ignore time structures mistakenly assumed to be negligible. A deus ex machina keeps us in a theoretical steady state and, for treatment, leads to what Arthur Jores [13] called “the idiocy of ‘three times a day.’” On the left of Figure 2, we resign ourselves, as the aging Claude Bernard [12] did, to assume a relative constancy of the internal environment: homeostasis, which, whether explicit or implied, leads to the treatment of a presumably “true” yet really imaginary steady state, such as a “true” blood pressure or a “true” blood cell count or a “true” hormone level. Alejandro Zaffaroni [14], the original developer of novel drug delivery systems intended for chronotherapy, received his inspiration from his work on steroids, and abandoned “4 tablets a day.” Homeostasis can lead to blunders in interpretations of “stress” or “allergy”, (Figure 3) and thus to erroneous or incomplete diagnoses and unwarranted treatments. When two groups differ only in the phase of a rhythm (Figure 3) or in its frequency, depending on the time when the groups happen to be compared, one can then encounter large differences in opposite directions, to falsely advocate either (unwarranted) substitution treatment or the removal of (a nonexistent) excess by an inhibitory drug or surgery [15].

Before the era of institutional review boards, endocrine glands were, in good faith but indiscriminately, removed from various kinds of patients simply based on homeostatic ideas underlying research. Alternatively, in this age of computerized data collection and analysis and of programmable pumps, we can do
Figure 2. Let us turn from an imaginary master clock serving an equally imaginary constancy, to an integrative internal–external collateral hierarchy for physiological–environmental coordination. Homeostasis postulates (and laboratory medicine today implements the idea) that physiological processes remain largely within a certain range in health and seek departures from such “normal values” to diagnose overt disease. Thus, variability within the normal range is often dealt with as if it were narrow, random, and trivial—the body striving for at least a relative “constancy.” This status quo (on the left of this figure) should be (but without action by chronomics, as yet is not) improved by learning about a “biological clock” that somehow enables the body to keep track of time. In fact, by removal and replacement experiments, a mechanism was located first in the adrenal and thereafter in the broader pineal–hypothalamic–pituitary–adrenal network [1] and was shown to be responsible for some but not for other circadians that persisted after brain ablation, but all were altered after these procedures. Single cells and even bacteria are genetically coded for a spectrum of rhythmic variation, as are mammalian liver cells. These facts indicate that the concept of a “clock” needs extension, as do biological calendars, when we find biological years longer than a calendar year and recognize, among others, a biological week, an about 0.42 year and a biological decade or two or those in us, and find other new rules in biological variability, such as deterministic chaos and long-known trends, some of which may turn out to be cycles with longer and longer periods. When the giant alga Acetabularia acetabulum, a prominent model of a circadian “clock”, is placed in continuous light, its spectrum of electrical activity reveals, after signal averaging, the largest amplitude for a component of about 1 week rather than 1 day. When 14 years of studies on this alga are pooled, a cycle slightly but statistically significantly longer than a calendar year, a transyear and an about 10-year cycle, among others, emerge in the data set as a whole and in other data covering decades. The alignment of spectral components and chaos and trends in and around us has also begun (right). Long-term longitudinal, but not yet entire lifetime, monitoring of critical variables complements current linked cross-sectional (hybrid) reference values required for preventive health and environmental care. Changes occurring within the usual value range, as increasingly longer cycles, resolvable as chronomes with a (predictable multifrequency) rhythmic element, allow us to measure the dynamics of everyday life, in order to obtain warnings before the onset of disease, so that prophylactic measures can be introduced in a timely manner. Thus, we find heretofore undetected or largely unquantified, sometimes harmful, environmental effects, all information for timely and timed treatment. The abstract idealized presentation of the sector structure in the interplanetary magnetic field, shown on the top right, consists of three visible arrows, the fourth being covered by a sketch of irregular solar flares, with parameters that are much more variable than originally visualized. Associations between helio- and geomagnetic variability on the one hand and, on the other, of vital signs in health or cardiac arrhythmia, myocardial infarction, stroke and sudden cardiac death are accumulating: they are just the tip of the iceberg, with a highly significant effect of magnetism (recognized by Gilbert in 1600), apparent in the human electrocardiogram in health and disease. In external–internal interactions, a broad spectrum of rhythms (both in the environment and in living matter) organizes deterministic and other chaos and trends. Trends pursued long enough may become low-frequency cycles (e.g., for the detection of any increased risk) so that timely preventive action may be taken (see Figure 6).
something about recording and analyzing changes in the usual range and about treating accordingly. When asked, the younger Bernard identified as one of his two major discoveries “the extreme variability of the internal environment” [16; cf. 17]. Rhythms and broader time structures, or chronomes [18–22], also consisting of trends when the time series are long enough, and of probabilistic and other chaos when the data are dense enough, are a fact of life, and there are many of them, not only circadians [18–22]. Mapping them all, and eventually correcting their alterations, requires a concerted international effort, a mapping of the chronome project, an endeavor exceeding the scope of mapping genomes. This task can now be implemented to pursue, with prevention in mind, the diagnosis and treatment of conditions such as disorders of variabilities (not only putative “levels”) of blood pressure, heart rate, and glycemia [23, 24], among many others, a task not achieved by computing day–night ratios, which latter do not separately assess changes in amplitude and phase and limit one’s perspective to only one confounded aspect of circadian systems [25, 26].

Beyond biological clocks and calendars, shown in the middle of Figure 2, chronome elements are shown on the right side of this figure and new and old spectral components are on the bottom right of Figure 4. There, we introduce reciprocal periods in and around us, pertinent to those interested in diseases and threats not only of individuals but also of societies, nations, and ecosystems, and more broadly the environment near and far. We need to recognize and assess these many rhythms in their own right, both in individuals and in populations. For instance, the mapping of circadecadals (Figure 5), has to be considered in seeking indications for hormone replacement therapy [18]. Blunders at many other periods, τ, as in the case of circadians, could be avoided
as soon as we knew what to spotcheck with a few strategically placed samples in already mapped rhythms. Unless we sample for a lifetime, and such monitoring is already done for very many thousands of patients with diabetes and should be done in blood pressure disorders, we may as a rule deal with a situation wherein the observation span, $T$, is much shorter than a decadal and/or many other infradian $r$'s. We had then better consult maps or prepare them when as yet they are unavailable. There is also a much more important perspective. Some of these periodicities characterize the presumed good and bad: religious motivation, crime and war bear signatures of our cosmos that we can also find in individuals [19–22]. A focus on global diseases is the greatest challenge for industry: by some means we have to shield from or compensate for magnetic effects, as we do already with heating and air conditioning once the still hidden environmental factors underlying mass psychoses are clarified.

Chronome mapping may seem complex and utopian at first, like the building of roads in an unknown terrain or the suggestion to take to the air. But in

Figure 3. Neglect of timing can lead to inexcusable blunders that were avoided in Minnesota, where they led to chronobiology, and can be avoided everywhere. (A) Eosinophil counts seemingly (but only apparently; see below) lowered by “fasting” and/or “stress.” Effect of a 50% reduction in dietary carbohydrates and fats (with proteins, vitamins, and minerals as in control group) in C3H mice with a high breast cancer incidence, which is greatly lowered by a diet reduced in calories (not here shown). Is an adrenocortical activation, then assessed by eosinophil depression (as an internal bioassay, in the absence at that time of a chemical assay) the answer for treating breast cancer and for prolonging life? It seemed to be an exciting finding at first. Steroids that depress eosinophil cell counts and depress mitoses could be a mechanism through which caloric restriction (and ovariectomy, also done on the calorie-restricted mice) acts in greatly reducing cancer incidence. This seemed to be the mechanism to prevent breast cancer, or was this very reasonable and plausible hypothesis a premature extrapolation? (The senior author’s chief had taken these results as a statistically significant (and to that extent validated), most promising result to a conference in Paris.) But once the difference in eosinophil count between two groups of mice was found, we attempted to replicate it, because of the importance of its findings to the etiology of cancer, using a larger group of animals, and started earlier in the day. (B) One week later, a follow-up with more animals starting at an earlier clock-hour shows “no difference.” By then, a phase difference between the two groups was one explanation for the fact that the large intergroup difference in eosinophil count seen in (A) was not replicated in (B) when more animals were used with an earlier start, as shown in (C). (C) An opposite outcome was observed 1 week later, with a still earlier start: the (without periodicity meaningless) “stress” in (A) had become an equally spurious “allergy” in (C). Erroneous conclusions concerning therapy result from ignoring in diagnosis a circadian (or other) difference between the timing of rhythms due to differences in their synchronization. This is shown by results from added sampling on the same day, with a validation of a difference in the opposite direction as compared to the difference observed first in (A), as seen in (D) with data and theoretically in (E).
building highways or airplanes to bridge distances, it must be kept in mind that once the roads or the planes are available, they must not be built over and over again for each trip. Users may pay taxes, pay a toll, or buy a ticket. This is what the BIOCOS project is all about, aiming at collecting reference standards that are complex and costly [22]. The new available reference database, used in the interim, will have to be augmented and improved by further work. In science and health care, the introduction of maps about timing means changing a status quo, described by a journal editor in a spontaneous note as “flying blind” [27], and is a raison d’être of any book considering the use of time structures in diagnosis and treatment. To illustrate this need, we refer to the opportunity of dealing with blood pressure and heart rate variabilities [23, 28].

Soon, diagnosis and treatment on repeated yet single blood pressure measurements will be recognized as tunnel vision (which of course is immensely superior to “flying blind” most of the time in the absence of systematically repeated measurements and their chronobiologic interpretation). An about 24-hour profile-based diagnosis and treatment will be identified as bitemporal hemianopsia; albeit much superior to tunnel vision, it is still unsafe in cases of variability disorders (Figure 6), in view of day-to-day variability and rhythms with longer than 24-hour periods, some longer than a year. The circadian and circannual rhythm-based approach (Figure 2 middle), will in time be replaced by continuous chronically (time structurally) interpreted as-one-goes surveillance with

Confusing results, that could wrongly be interpreted as “stress” or “allergy”, are accounted for by the action of food (offered mornings) and light as competing synchronizers of circulating eosinophils in C3H mice with high breast cancer incidence that can be drastically reduced by calorie restriction.

Denser sampling (in 3, 4, 5) suggests antiphase.

www.T CircadianRhythms.com/content/pdf//1740-3391/1/2.pdf (Halberg F. et al., 2003)
Figure 4. Chronoastrobiology strives to elucidate the past and present integration of organisms into their largely wavy environment. Reciprocal periods in the chronomes of variables in us and around us came about by the eventual genetic coding of the latter in the former. Internal–external interactions await further exploration in health care, notably for stroke prevention in individuals and for the prevention of war, crime, and other diseases of societies and nations. (See color insert.)
Contradictory Correlations of Hormonal Metabolites with Age (I, II, IV - 5 of them) or with Solar Activity (V - 5 of them) Stem in part from a Common Years-Long Cycle (III, IV, VI)

Need for Hormone Replacement therapy (HR-Rx)? No Indication for HR-Rx? Mostly a Natural Cycle

Figure 5. Variabilities along the scale of decades, a confusing source of variation if ignored, can become a new, quantified reference value [18].

sequential testing [29] and parameter tests [30], interpreted in the light of maps, many of them yet to be built and those available yet to be perfected.

Once continuous monitoring is implemented, we can immediately introduce into everyday practice procedures that have become sine qua non in research, including $P$-values and uncertainty estimates in the form of 95% confidence and tolerance or prediction intervals [31, 32]. Today, we can diagnose prehypertension and other increased risks as variability disorders (Figure 6), and can treat them at times of pertinence rather than convenience. We can check the effects of treatment prescribed systematically by sequential tests, in dealing already with prehypertension, with prediabetes and, after more homework is done down the line, with preobesity, eventually to nip a premetabolic syndrome in the bud.

Those who take into account a broader-than-clock-hour and calendar-date-based timing according to time structures can make a big contribution. A bit of attention to the role played by the cosmos (Figure 4) cannot hurt and may help. We can manipulate (treat the amplitude of) a transyear component of blood pressure statistically significantly different from a calendar year, thanks to a contribution by Yoshihiko Watanabe and co-workers [33]. It appears that transyears, at least in sudden cardiac death, are geographic (magnetic) site specific and we have much more to learn [34, 35]. We certainly do not wish to rely only on spotchecks at long intervals and in so doing ignore any variability disorders and their contributions as vascular variability syndromes, VVS...
Figure 6. Abnormalities in the variability of blood pressure and heart rate, impossible to find in a conventional office visit (the latter aiming at the fiction of a “true” blood pressure), can raise cardiovascular disease risk in the next 6 years from <4% to 100%. As compared to an acceptable variability, the relative vascular disease risk associated with a circadian hyperamplitude tension (CHAT), a decreased circadian heart rate variability (DHRV), and/or an around-the-clock elevated pulse pressure (EPP) is greatly and statistically significantly increased. These silent risks are very great, even in the absence of hypertension. They can often be reversed, notably the risk of CHAT, by a nondrug (relaxation) or drug (specified in timing as well as in kind and dose) approach; and the need for intervention can be found when it occurs by the combination of a closed-loop diagnostic and therapeutic system for chronotheranostics [28]. (See Chapter 11 in this volume.) (See color insert.)

(Figure 6). Thirty years ago, hoping that a time structure assessing (chronome-assessing) medicine would eventually come into its own, one of us wrote [5; cf. 4]:

Once the feasibility of manipulating a rhythm’s timing and preferably also its amplitude has been demonstrated for a majority of individuals in a population, the procedure may be carried out without individual validation of the specific timing. However, whenever such procedures [for deciding on a timing] are unavailable and individuals differ from each other in terms of the timing of their susceptibility rhythms, sensitivity tests will have to be developed for the given
individual by the use of his own markers—say, for bone-marrow depression … in the case of certain chemotherapeutic [and for blood pressure lowering in the case of antihypertensive agents]. These markers will then have to be monitored in close cooperation with the patient and the data thus obtained will have to be analyzed by special techniques. These should allow the identification, inter alia, of individualized acrophases, when the latter correspond to orthophases, while other methods are needed to define the optimal treatment time(s) whenever the acrophase differs from the orthophase.

By 1973, the gains from chronochemotherapy with a sinusoidal pattern, including an improvement of therapeutic index by timing, could be shown in the laboratory. Recently, the optimization of timing nutriceuticals was started with ubiquinone (Q-Gel) [8]. In 1991, Bakken and Heruth [36] cited studies carried out with their then-modern instrumentation that objectively revealed an improved outcome from cyclosporine infused in a circadian sinusoidal pattern versus a constant rate [37; cf. Chapter 11 in this Volume]. A pump (and any treatment more broadly) has to be scheduled by clock-hour. Such scheduling has been a boost to chronotherapy and has worked [38], albeit not invariably [39]. Half a century ago, the manipulability of optimal timing was reviewed [4; cf. 40]. Using circadian and circaseptan rhythms in cancer markers [41, 42], such as CA125 or preferably CA130, to guide treatment, e.g. of ovarian cancer, is a desired individualized alternative to a specification only of clock-hour for all comers [6] and has added years to a cancer patient’s survival [43], but as yet is costly and will remain so until its merit further is documented, another challenge for the cooperation of clinicians with industry.

While suggestions made in 1975 and 1991 remain pertinent in 2009, there are now also new diagnostics of disorders of the young Bernard’s “extreme variability” for the care, among others, of an above-peer-threshold circadian amplitude of blood pressure and a below-peer-threshold circadian standard deviation of heart rate (Figure 6).

Our editor’s thorough review of the background to what he dubs “chronopharmaceutics” rightly raises the question of clinical relevance [44]. Individualized timeliness and timing are obviously needed in support of the promise of chronopharmaceuticals and of corresponding drug delivery systems. In an era of major concerns about the epidemic of obesity and a metabolic syndrome, we learned already that prehypertension [45, 46] and prediabetes [47, 48] may be detected as CHAT. There are also technologies for closing the loop between time structure-based monitoring and analyses for both early diagnosis of elevated risk before actual disease and then timely and timed treatment, with procedures to assess the uncertainties of what is done in individualized practice rather than only in research on groups (See Chapter 11 in this volume). We need not respond only to the values at a given moment, like blood sugar or blood pressure values. When risks higher than those of hypertension are found in variability and when such conditions are
documented worldwide [23], as in the case of blood pressure overswinging, there is no safe alternative but to take the broader time structure into account in a budding chronotheranostics (See Chapter 11 in this volume). Figure 7 should convince any reader with high blood pressure that the treatment he or she receives needs to be individualized. We must try not to fly blind [27].