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Time and body chemistry: questions and answers

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Summary. - This is an article based on questions and answers which are aimed at explaining of chronobiological principles from rhythms of chemical compounds of living matter. The chemical aspect of biological rhythms is important considering that any biological event is guided by chemical phenomena. The dialogue helps us to understand how biochemical rhythmicities are fundamental for clinical health, their deviations being demonstrable in pathophysiological conditions.

Key words: biological rhythms, chemical bioperiodicities, chronobiology, time structure.

Riassunto (*Aspetti cronobiologici della chimica della materia vivente: domande e risposte*). - Questo articolo è basato su domande e risposte finalizzate a spiegare i principi della cronobiologia. L'intervista riguarda gli aspetti cronobiologici della chimica della materia vivente, considerando che qualsiasi evento biologico è guidato da fenomeni biochimici.

Parole chiave: cronobiologia, ritmi biochimici, ritmi biologici, struttura temporale.

Introduction

Chronobiology is a discipline which is constituted by scientific principles and methodological procedures. Its teaching may be done in an axiomatic way as in the case of the reviews published in this issue. However, there is also a Socratic way to teach the maieutic method which consists in eliciting new ideas from another by means of questions and answers.

In the Socratic method, the teacher is the person who poses the question, and the disciples are the subjects who had to make a mental effort to give a response.

In the case of this article the Socratic method is hybrid. The question is posed by a hypothetical interlocutor who takes part in the dialogue, and the answer is given by the expert scientist. However, as the questions are taught by the responder, the technique is not an interview in which the questions have to be unknown to the interviewed person.

The present article may be regarded as personal interlocution in which the experts better explain the scientific matter by posing adequate questions.

The world of biological rhythm involves both biophysical and biochemical phenomena. But the physical events of biological functions are themselves provided by chemical events.

Therefore, to explain the periodicity of biological chemistry is a basic way to illustrate chronobiology.

Q. *How do we perceive body chemistry in time and space?*

A. Body chemistry embraces those neural (nerves), cellular (cells) and humoral (body fluids) processes of life that interact in four dimensions, comprising traditional three dimensional organic space and the dimension of time. Time is an integral component of human and other forms of organic life since it is concerned with functional synchronization. Time, in a biological sense, comprises organic time (neural, humoral, cellular, metabolic and so on) which straddles cosmic time (day, night, week, month and year). It is the synchronization of organic time with cosmic time which constitutes real time in which life moves through a sequence of events such as conception, birth, onset of puberty, reproduction, "ageing" and death. Body chemistry, together with organic time and cosmic time, has a crucial role to play in maintaining the functional integrity and well-being of the healthy individual at any "age". It is the influence of time-related changes in body chemistries (for example, biological rhythms of substances in blood) that controls health, determines work efficiency, moods, psychomotor skills and other activities. Quite apart from cosmic space and organic space there exists structural space, where nature and culture (societies) integrate, wherein body chemistry has an important role to play in relation to the expression of behaviour, emotion and so on.

Q. *What sorts of changes in body chemistry occur during our lifetime?*

A. A perusal of the family photograph album clearly indicates the results of *slow* "body chemistry" processes of *long* duration such as the growing child or the ageing adult since the photographs represent observed structural changes. Using a different piece of technology to that of the camera,

functional changes which are quick processes of short duration could also be observed. An example would be changes in blood pressure and heart rate as measured by the sphygmochron-sphygm (circulation) and chron (time). It is not surprising that human growth and development is accompanied by changes in hormone levels in biological tissues and fluids since they are intimately involved in the onset of puberty, reproduction and ageing. Endocrinology, which in the simplest sense is a study of hormones, has developed enormously in recent years to encompass molecular biology (genetic level), the immune system and neurobiochemistry, all of which involve time-related processes - hence the general term chronoendocrinology. White blood cells, red blood cells, neutrophils, blood platelets are also believed to change during one's lifetime as do the more classical "blood chemistries" such as albumin, urea nitrogen, chloride, cholesterol, glucose, iron, phosphorus and so on. The list is almost endless as one would expect intuitively since statistical form and function are predicted and enacted as a result of genetic programming in time and space.

What form do these time-related changes in "blood chemistry" take? Can these changes be quantified in health and disease? Will a knowledge of these time-related changes help in health promotion? We start by answering the first question in a slightly different way.

Q. *Do rhythms in body chemistry occur every second, every minute, hourly, daily, weekly, monthly or yearly?*

A. It was Professor Franz Halberg, considered by many to be the Father and Founder of modern Chronobiology, who in 1959 introduced the term circadian rhythm to mean one with a period of about 24h (circa, about; dies, day or 24h). Now many rhythms have been recognized which have other periods. Some are less than a day (ultradian: ultra, beyond; used here in the context of frequencies higher than circadian) such as processes concerned with metabolic events (generally seconds to minutes) and genetic expression (epigenetic) (generally minutes to hours). Indeed, events concerned with the internal mobility of proteins or membranes can now be measured in nanoseconds (10^{-9} s) or picoseconds (10^{-12} s).

One important rhythm concerned with pubertal development and reproduction is the 90 min rhythm (or more broadly - circacentumintan rhythm) of luteinizing hormone-releasing hormone (LH-RH). This hormone is secreted into the pituitary portal system where it acts on the pituitary to produce LH which is so crucial in signalling the onset of puberty and controlling the human ovarian cycle (circatrigintan, about 30 days) and hence reproduction. It is this LH-RH rhythm, the electrophysiological origin of which is in the area of the brain just above the pituitary (arcuate nucleus of the medio-basal hypothalamus) with additional hierarchical control in the higher centres of the brain, which may be of importance in determining one index of breast cancer risk leading to the development of the disease in premenopausal years. The effects of substances like noradrenaline, endorphin and vasoactive intestinal peptide on this LH-RH rhythm require further study. Indeed, the synthesis and administration of an analogue of LH-RH (Zoladex^{R}), which initially stimulates and then suppresses LH secretion (therefore suppressing testosterone in man and oestrogens in women) is used in the treatment of advanced prostate cancer and premenopausal breast cancer.

Circadian rhythms are ubiquitous in all living systems. In the human, circadian rhythms exist for: numerous haematological (blood) variables such as haemoglobin, blood cells of various types, blood platelets, etc; clinical chemistry variables found in serum or blood or urine such as albumin, enzymes, bilirubin, chloride, sodium, potassium, magnesium, iron and so on; hormones found in saliva, serum, or urine such as adrenal steroids (cortisol, aldosterone), testicular (testosterone) and pituitary hormones such as prolactin, LH and thyroid stimulating hormone. Readers requiring more information should consult the works of Halberg *et al.* [1] and Haus *et al.* [2]. Circadian rhythms exist for blood pressure, body temperature, breast temperature and many other physiological variables.

Monthly rhythms, described as circatrigintan (about 30 days), circamensan (or circalunar as appropriate) are also found in the human, particularly in the healthy premenopausal woman, an example of which is salivary or serum progesterone (an ovarian hormone) concentration. Monthly rhythms of breast skin temperatures, plasma prolactin and cortisol have been reported although urinary excretion of ketosteroids in urine was reported in 1965 for an adult man studied for 15 years.

Weekly rhythms exist and are referred to as circaseptan (about 7 days). Circaseptan rhythms of haemoglobin, haematocrit (the percentage of whole blood volume occupied by red cells following centrifugation), and red blood cells have been reported by Haus with the peak (acrophase) occurring somewhere between Sunday evening and Monday morning, similarly for serum cortisol, and glucose. Circaseptan rhythms of urine volume, creatinine, sodium and many other biological variables have been identified. The rejection of human kidney transplants has also been reported by Halberg to have a circaseptan bioperiodicity. Circaseptan rhythms of blood pressure in the newborn infant have been reported.

Circannual rhythms are, of course, well known to everyone since they are implicit in seasonal breeding and the flowering of plants in the springtime, but do circannual rhythms occur in the body chemistry of the human? The answer to this question is yes, but because of the difficulty in designing the

appropriate experiments they are more difficult to quantify. One such circannual rhythm, namely serum prolactin, was observed in a study of two populations of 20-year old women, one in Minnesota, USA and the other in Kyushu, Japan. Striking differences in this hormone, thought to be related to breast cancer risk, were observed with the Japanese having a very much larger circannual amplitude than their American counterparts. Peak prolactin occurred in winter and troughs in summer for groups of women from each country. The Minnesotan women also exhibited circannual rhythms for insulin, thyroid stimulating hormone although these were statistically not quite as pronounced. Circannual variations have certainly been found in many haematological, clinical chemistry and endocrinological variables but more work is needed in their precise quantification. Certain drugs have been shown to exhibit a circannual variation in their effect, one example being the circannual immunosuppressive effect of cyclosporine on rat heart and pancreatic islet allografts (a tissue graft taken from a genetically non-identical donor of the same species as the recipient). In animal studies, circaseptan and circannual rhythms have been well documented but are beyond the remit of this presentation. Nevertheless, man is only part of the ecological system and is very inter-dependent upon the chronobiological features of all life within the biosphere.

Q. *Are rhythms in body chemistry genetically or environmentally driven?*

A. To answer this question let us return to our original perception of body chemistry in time and space where we identified organic time, cosmic time and real time as being astride both. Rhythms are products of genes in time and space and experiments on flies have revealed a per gene (per-period) which codes proteins in the cell that regulates rhythms. The greater the protein output, the faster the cycles develop and hence the shorter is its day. The situation in the human is immensely complex but whatever the origins of rhythms in the genetic material, they are believed to be inherent. Plant leaf movements (such as in the heliotrope plant or mimosa) characteristic of night and day continue in constant light or constant darkness. It is the characteristic of rhythms, even in humans, that when they are isolated from the normal cyclical environment they persist or free-run; an example is given by salivary testosterone measured in samples from a man placed in an isolation unit, i.e. his normal cyclical environment was removed leading to internal desynchronization. Thus an environment cycle such as so many hours of darkness and light, or a meal schedule can force or entrain another cycle to itself within a limited range of frequencies. Such an entraining agent is termed a synchronizer or a zeitgeber (time giver).

Q. *Do these rhythms of body chemistry help us to define health?*

A. Health may be defined as a time-qualified state of dynamic equilibrium existing between the human body and its environment so as to maintain the body's structural and functional characteristics within harmonious limits. We must think of health and therefore body chemistry in chronobiological terms. A single time-unqualified measurement, such as plasma cortisol, can be clinically misleading. At the very least such a measurement should be time-qualified (time of day, time of year), better still characterized as a rhythmic quantity (e.g. circadian rhythm) or even more so viewed in the context of its rhythmic interaction with other rhythmic variables where the integrated system leading to an attenuated, amplified or zero effect can be explained. Such an integrated system in the human could be illustrated by the pituitary and adrenal hormone secretions, modulated by the pineal gland, and the whole integrated by the suprachiasmatic nucleus. These rhythms in body chemistry, as well as physiological variables such as blood pressure and heart rate, and those of neural and cellular networks change with chronological age. To define health more rigorously we need to identify, measure and map rhythms of time-related events in body chemistry (or physiological variables arising therefrom) from conception to death in both healthy male and females so as to provide a reference set of rhythms against which departures, leading to disease, can be assessed.

Q. *Can we recognize changes in body chemistry so as to identify departures from health leading to disease?*

A. This is one of the major goals in human chronobiology and the chronobiologist has made considerable progress in this direction. To illustrate what steps have taken place let us take an individual with a clinical condition, such as a patient with a stroke, which seriously impairs their quality of life. This condition may well have been detected sooner by blood pressure measurements, and therefore treated so as to circumvent this debilitating condition. The chronobiologist would indicate flaws in the current practice of taking a single blood pressure measurement and, since both diastolic and systolic blood pressure exhibit circadian rhythms, patients could be diagnosed as being hypo-"normo-" or hyper-tensive depending on what time of day their measurement was made. The chronobiologist would claim that the whole rhythm of blood pressure should be quantified, with the aid of, for example, the sphygmochron, and new quantities as mesor hypertension introduced into clinical medicine. Another example could be the case of a time-unspecified plasma cortisol value which could, in the absence of other clinical findings, belong to a

so-called "normal" patient or one with Cushing's or Addison's disease depending on what time of day the blood was taken. To detect such clinical situations, (hypertension, endocrine abnormalities) measurements of body chemistry or physiological variables must be time-qualified or rhythmically-qualified and compared to a reference group. This group requires careful selection so as to standardise for chronological age, sex, weight, height, activity/rest, whether hospitalised or domiciled, ambulant, supine and, of course, time of day, time of menstrual cycle, time of year and so on.

The reference group of individuals can provide data in the form of charts (chronodesms) or rhythmic bands (cosinordesms) that contain, within certain limits, the expected range of observations against which an individual being monitored can be assessed. The individual can, of course, produce similar charts based on their own longitudinal measurements for example, on values obtained early on in their history of self-measurement. Many other rhythmometric techniques exist to determine the number of component rhythms in a series of measurements (Power Spectrum obtained by Fourier Analysis) and for their comparisons so that it may be used to identify early changes in body chemistry that may lead to disease.

Q. *Can the chronobiology of body chemistry be exploited in the following areas of medicine: Dietary control of Diabetes Mellitus?*

A. In the healthy human, ultradian, circadian and circannual rhythms in the spontaneous intake of nutrients have been reported. It has also been noted that when the total daily intake of food occurs in the morning, there is a reduction in weight which is not related to calorific intake and which does not happen in the afternoon. When a healthy individual is given an oral glucose load at different times through the day, there is a marked circadian variation in blood glucose and plasma insulin response, with post-load blood glucose levels lower and plasma insulin levels higher in the morning. Although, in the diabetic patient, the same pattern of events may not be present, the possibility exists that to some extent the timing of dietary intake may lead to a situation in which the amount of medication can be minimised. The role of exercise in modulating rhythms of glucose also requires investigation.

Q. *Can chronobiology be used to determine endocrine cancer risk?*

A. Risk factors for breast cancer have certainly implicated adrenal and ovarian hormones in the history (aetiology) of the disease. Until 1980, most analytical work concerned the comparison of hormone levels in blood or urine obtained from groups of healthy women or those with breast cancer or benign breast disease without properly considering the importance of rhythms. Halberg in his 1981 study of Kyushu (low risk) and Minnesotan (high risk) women was able to collect round-the-clock blood samples in each season of the year from which he determined circadian, circatrigintan and circannual rhythms for a number of hormones. It was clear from this study that hormonal risk factors should be thought of in terms of their rhythmic components as illustrated by the circadian and circannual rhythms of prolactin. Scientists at the Tenovus Institute for Cancer Research, Cardiff who were collaborators in the Halberg study, developed sensitive specific steroid assays for saliva. Saliva sampling is non-invasive, stress-free and multiple samples are easily taken and is ideal for many clinical and population-based studies. Researchers at the Tenovus Institute have thus been able to study circatrigintan patterns of salivary progesterone (ovarian hormone) in adolescent girls resident in the United Kingdom or Thailand. The British girls exhibiting a greater activity of progesterone secretion than their Thai counterparts when matched for chronological age and age at menarche (puberty).

Salivary steroid assays have application to other areas of endocrine cancer where gonadal function are thought to be involved such as prostate cancer. The characterization of circadian or circannual rhythms of testosterone in the saliva of young and elderly clinically healthy men and young and elderly men with a familial endocrine cancer risk and those with untreated prostate cancer may reveal important findings concerning the role of this hormone in the aetiology of the disease. It is this sort of analytical technology together with automated equipment that will greatly assist our chronobiological understanding of endocrine cancers.

One area of body chemistry that is important is that which underlies the circadian rhythm of body temperature in the human and which, in the breast, can be modulated to give rhythms which differ in health and disease such as cancer. Work at the Tenovus Institute has focussed considerable attention on breast surface temperature rhythms at a number of frequencies as a possible means of detecting the high risk breast long before overt cancer is diagnosed. Pronounced changes in the properties of circadian rhythms were noticed in postmenopausal women with cancer in which the rhythms over the area of skin over the tumour were compared with a similar site on the contralateral breast. As tumours get smaller, however, these prominent differences fade and the complex breast temperature structures becomes more difficult to unravel for the purposes of early detection of primary breast cancer especially in the premenopausal women where screening benefits from mammography are not so pronounced. Nevertheless, the early development of the LH-RH oscillator in adolescent girls which, through either an unbalanced or a prolonged action of hormones

on the breast before the first pregnancy, may lead to increased breast cancer risk originating possibly in adolescent years. It is the ultimate aim of this particular breast temperature project to identify this breast at risk (as well as that in post-menopausal years) and by intervention therapy hopefully to treat this preneoplastic condition early before cancer develops.

Another area of chronobiology which holds considerable promise is in the use of cytotoxic drugs for the treatment of cancer. Cytotoxic drugs are agents which interfere with cell division, or the processes leading up to cell division. These cell-cycle processes may have a different timing to that of normal tissues and thus may be exploited by administering the drug at a particular time of day (for example). Professor Scheving, at the University of Arkansas, Little Rock, was able to demonstrate a circadian rhythm in the tolerance to an anticancer drug (Arabinosylcytosine(ara-c)) administered to leukaemic mice (chronotolerance) as well as one leading to increased survival (chronoefficacy). Dr. W. Hrushesky has investigated the circadian timing of two anticancer drugs used in the treatment of advanced ovarian cancer in women using adriamycin and cisplatin. Adriamycin was administered at 06.00 or 18.00 h followed 12 h later by cisplatin. The group of patients receiving adriamycin in the evening and cisplatin in the morning was more toxic leading to far more treatment delays, and the need to modify the drug dosage. Chronoefficacy leading to improved survival has also been reported by Dr. Hrushesky. Since circadian rhythms of toxicity in animal studies have been demonstrated in at least a dozen or more anticancer drugs, the possibility of exploiting the timing of administration of anticancer agents (or applying radiotherapy) for the improved treatment of a variety of cancers is closer to reality.

Q. *Can chronobiology be used to predict the risk of cardiovascular disease?*

A. The chronobiological approach to identifying individuals at risk of developing high blood pressure is of considerable importance and promises to bring major benefits. Halberg has shown that newborn infants with a family history of high blood pressure have a markedly higher circadian amplitude than those without a family history of blood pressure (for convenience we use the inaccurate term of hypertension). Clearly those infants will be in need of primary prevention. Circadian rhythms in diastolic and systolic blood pressure have been well-documented for clinically healthy individuals. These rhythms, suitably age-qualified and obtained for a group of such individuals, can be used as a reference standard against which the circadian rhythms of those suspected of being hypertensive can be compared. Subjects can be classified as being mesor-hypertensive or amplitude-hypertensive as be the situation. The individual can be treated with, for example, an anti-(mesor)tensive drug and the rhythm parameters (mesor, amplitude and acrophase) compared statistically before and after treatment to assess efficacy. As one moves initially from a healthy to a "hypertensive" state it is preferable to measure the excess pressure above a critical threshold (the hyperbaric index) using rhythmometric techniques and then when it is considered appropriate to apply preventative therapy. Although the body chemistry relating to increased blood pressure and time is complex, nevertheless the monitoring of blood pressure in the home and at school should be encouraged. It is an essential part of health promotion.

Q. *How can developments in modern technology be used to investigate body chemistry in health and disease in the future?*

A. Considerable advances have been made in developing "dry chemical" procedures for estimating, at low cost, many electrolytes, enzymes, drugs and a variety of blood chemistry metabolites commonly measured using other techniques. Such chemical procedures may, in some situations, allow the patient to carry out assays in their own home and so pave the way for improved chronobiological surveillance. The development of biosensor technology so as to provide information on body chemistry (electrolytes, glucose, hormones) in a variety of body fluids is an expanding area of research since measurements may be made inside or outside the body and, therefore used in intensive care, post-surgery or post-traumatic care, and in the care of the neonate. Many other technologies for measuring body chemistry are under development so that in the near future we can look forward to sensing devices that will provide data on a spectrum of rhythms in health and disease at low cost. Computer software, specially developed for rhythmometry, can now provide nearly all the basic information that is required using a relatively low-cost microcomputer. As with the use of the "dry" chemistry procedures and biosensors, the limitations of its use must be clearly stated.

Chronobiological engineering is now contributing significantly towards automatic data capture. The sphygmochron, is a device for automatically measuring blood pressure and heart rate; the Chronobra is able to measure breast surface temperature over long time spans; devices exist for monitoring human activity, body temperature, electrocardiographic signals, and many other physiological variables. Developments in the design of implantable programmable pumps mean that substances like insulin and some anti-cancer drugs can be delivered automatically at any time of the day or night, thus obviating difficulties of administering treatment of drugs at a particular time of the day or night. Modern technology is now providing the means by which the chronobiological monitoring of health and disease

can take place in the home, the school, the general practitioner's office and, of course, the clinical and research laboratory.

Q. *Are there other aspects of body chemistry and time that require investigation?*

A. Most definitely. This dialogue serves only to illustrate in a most infinitesimal way our understanding of body chemistry and time. Body chemistry, an all embracing term, and time are very much concerned with aspects of medicine involving growth and development; endocrinology; digestive and metabolic events; neural, cellular and organ processes; mental health; physical exercise (sports medicine); psychological and physiological function in international travel or shift work schedules; psychiatric disorders such as melancholia; epileptic seizures; pharmacology; cardiovascular disease and cancer to name just some of the more important areas.

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