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B. B. LLOYD

The Concept of Regulation in Physiology

M. LINDAUER AND H. MARTIN

Special Sensory Performances in the Orientation of the Honey Bee

Discussions

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THE CONCEPT OF REGULATION IN PHYSIOLOGY

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The rapporteurs of this conference have been asked to set a broad framework for subsequent discussion and not to cover topics in detail and entirety. After a brief discussion of homeostasis I shall therefore concentrate on some quantitative and speculative aspects of respiratory regulation, describing controller and material equations, and briefly discuss frequency of breathing and the effect of high altitude. The problem of physiological set point is then related to the respiratory regulation of pH. Regulation in exercise and voluntary interference with ventilation are then briefly discussed.

Energy, material and information pass through the individual animal at various and varying rates during its existence. Body temperature and posture are typical resultants of a balance between energy input and output, and body weight a typical resultant of a material balance, and are examples of what Cannon [1] called homeostasis, which may be defined as the maintenance of a variable that is subject to throughput within a tolerable and usually fairly narrow range of an average value. Regulation may be defined as the sum total of devices and processes by which homeostasis is achieved.

The information throughput is different from those of energy and matter. Wiener [2, p. 121] has likened the activity and development of a human brain during its life to a single run on a computer: memory and learning, whether neural or immunological, are scarcely examples of homeostasis. And the transmission and multiplication of genetic material by reproduction is largely restrained by the environment, and not subject, except perhaps in civilized man, to homeostatic regulation [see 3 for a different view], which is based on negative feedback, whereas much sexual behaviour, such as mating and parturition, provides examples of positive feedback, which is otherwise rather rare in normal biology, though perhaps not in pathology.

The variables which show homeostasis are legion and the ones most easily and frequently studied are chemical concentrations in the blood. These may be classified into respiratory, ionic, acid-base, nutritional, excretory and hormonal, each of these classes showing more or less overlap with the others; and the list of hundreds of normal values in any reference book of medical chemistry [4] is a list of homeostases, which are also seen in growth and organ size, in the hydrostatic problems of the circulation of the blood, and in the regulation of the frequency, amplitude and wave form of cyclic processes, such as sleeping and

waking and the sexual rhythms, which often profoundly influence supposedly steady levels.

Claude Bernard "Le plus illustre physiologiste de notre époque" (the most illustrious physiologist of his epoch) according to Vulpian [5, **1**, vii], once wrote [5, **1**, 340] that "Tout acte d'un organisme vivant a sa fin dans l'enceinte de cet organisme" (every act of a living organism has its aim within the ramparts of that organism), which may have prompted J. S. Haldane [cited in 1 & 3] to state that "No more pregnant sentence was ever framed by a physiologist" about Bernard's [5, **1**, p. 113] proposition that "La fixité du milieu intérieur est la condition de la vie libre, indépendante". This most famous of physiological generalizations comprises three notions of great importance, the free life, the internal milieu, and the latter's constancy. Physiologists have spent little time debating whether Bernard's 'La vie libre' referred to the mobility of a mammal or to the political freedom of man, but in the last century there has been immense and intense physiological investigation of the identity, composition and regulation of the internal environment, of which Bernard [5, **2**, p. 5] stated 'C'est le sang; non pas à la vérité le sang tout entier, mais la partie fluide du sang, le plasma sanguin, ensemble de tous les liquides interstitiels, source et confluant de tous les échanges élémentaires.' (It is the blood; not indeed the whole blood, but the fluid part of the blood, the plasma, including all the interstitial liquids, the source and confluence of the elementary exchanges.)

This concept of the internal environment is quite complex. Our skin exists in atmospheric air, but the alveolar air in chemical contact with blood in the lungs contains much less oxygen (14 as opposed to 21 %) and far more CO_2 (6 as opposed to 0.03 %) than atmospheric air. We can thus be said to live in an atmosphere containing a partial pressure of oxygen, P_{O_2}, of 100 torr, and a P_{CO_2} of 40 torr, which at once differentiates us from the creatures in the sea, where both these pressures are usually lower.

The arterial blood exchanges gases with the alveolar air, so that for most purposes in the normal man we can accept the equality of the alveolar and arterial partial pressures (P_A and P_a) of oxygen, nitrogen, CO_2 and water, but, just as we really live in alveolar, not atmospheric, air, our tissues live in venous rather than arterial blood, with a P_{O_2} less than 50 and a P_{CO_2} above 40 torr. The immediate environment of cells is the tissue fluid, which tends to be slightly more venous than venous blood, with lower P_{O_2} and higher P_{CO_2}, and the intracellular fluids, in which the cellular components such as mitochondria function, may have P_{O_2}'s of only a few torr, and at the actual points in the cell where oxygen is converted to water its concentration tends to zero. The chemical reduction of oxygen supplies energy to the cell, and the continuous but fluctuating energy demands of the cell depend on an appropriate transport of O_2 from air to the mitochondrion, and of CO_2 from the enzymes of the Krebs cycle to the air. The maintenance of appropriate flows of CO_2 and O_2 is the primary task of ventilation and cir-

culation, and in discussing the concept of regulation in physiology I shall speak mainly about this task. This needs no apology, for not only did Bernard [5, **2**, p. 141] state that 'La respiration est le phénomène le plus caracteristique de la vitalité, c'est-à-dire de l'être en activité vitale', (Respiration is the most characteristic phenomenon of vitality, that is to say of the being in vital activity) but the physiology students being examined in Oxford a fortnight ago were asked to discuss the proposition that 'Respiratory physiologists are the most quantitative neurophysiologists'. Respiration has indeed been much investigated, as its inputs and outputs are relatively easy to measure; it operates over a 30-fold range of activity, and it provides the most characteristically quantitative examples of chemoneural interactions.

We are still in doubt, owing to inadequate experimentation, as to what chemical variables directly affect the ventilation, but it is generally agreed that the H^+ concentration of the blood is effective at the chemoreceptors in the aorta and the fork of the carotid artery, and that there is an effective H^+ concentration inside the cranium. It is also accepted that the partial pressure of oxygen in the arterial blood, $P_{a}o_2$, has a potent effect on the arterial chemoreceptors. The experimentally observed relations between chemical factors and ventilation $\dot{V}$ have been summarized in various equations, of which the following is a recent, though still controversial, example [6].

$$\dot{V} = h\{\psi(\lambda + \log H_a^+/H_{a\theta}^+)/(P_{a}o_2 - \gamma) + \mu + \log H_c^+/H_{c\theta}^+\}. \tag{1}$$

$\dot{V}$ represents the total air breathed out by a subject in unit time (l./min.) and is easily measured by means of a gas meter, while h is partly a size parameter (an elephant will breathe more than a mouse having the same blood chemistry), and partly a measure of overall sensitivity.

The first term inside the curly bracket is meant to represent the activity of the chemoreceptors. These small bodies, situated on the aorta and the carotid artery, fire more nerve impulses to the brain when the pH or Po_2 falls in the arterial blood reaching them. The term shows a linear function of the logarithm of blood hydrogen ion, $(\lambda + \log H_a^+/H_{a\theta}^+)$, multiplying a reciprocal function of oxygen $\psi/(P_{a}o_2 - \gamma)$, and represents the notion, which is still being investigated, that the product of these terms generates the impulse traffic in the chemoreceptor nerves. This traffic adds to two central terms, μ representing a residual nervous effect independent of chemical stimuli, and a final term containing H_c^+, a central hydrogen ion concentration, perhaps in cerebrospinal fluid or brain interstitial fluid. When H^+ falls below the threshold value H_θ^+ in either log term the effect is taken to be zero rather than negative.

We now turn from these informational relationships to the metabolic or material relationships that normally exist between ventilation and the respiratorily important variables. A simple example is the equation relating Pco_2 with ventilation:

$$\dot{V}(P_{A}\,co_2 - P_{I}\,co_2) \simeq j\dot{V}co_2. \tag{2}$$

This states that the product of ventilation and the difference between alveolar and inspired P_{CO_2} is approximately proportional to the metabolic production of CO_2, so that if $\dot{V}$ is doubled at constant $\dot{V}_{CO_2}$, $P_{ACO_2} - P_{ICO_2}$ is halved, and so on, a relationship we term the metabolic hyperbola. This means that if $\dot{V}$ changes for any reason, such as a change in one of the terms in the right-hand side of equation (1) there will usually be a change in P_{ACO_2}, which is itself a main determinant of H_a^+ and H_c^+ and hence of $\dot{V}$. The form of the equations shows that when $\dot{V}$ rises, P_{ACO_2} approaches P_{ICO_2} and hence H_a^+ and H_c^+ tend to fall, causing a depression of ventilation. This negative feedback is an example of the myriad of negative feedback arrangements in physiology. A similar negative feedback applies to oxygen, for which we may write

$$\dot{V}(P_{I\,O_2} - P_{A\,O_2}) \simeq j\dot{V}_{O_2}. \tag{3}$$

When $\dot{V}$ rises, $P_{IO_2} - P_{AO_2}$ falls, so that, at constant P_{IO_2}, P_{AO_2} rises. This reduces $1/(P_{aO_2} - \gamma)$ in equation (1), so that the stimulus is reduced and $\dot{V}$ tends to fall. In short, knowing P_{IO_2}, P_{ICO_2} and $\dot{V}_{O_2}$ ($\dot{V}_{CO_2}$ is usually close to it in value) and the basal characteristics of the blood and c.s.f. of the subject, we should be able from equations (1), (2) and (3) to predict the steady-state values of $\dot{V}$, H_a^+, H_c^+ and P_{aO_2}. The non-steady state is more complicated, and a masterly treatment of the relationships corresponding with equations (2) and (3) in the non-steady state for lungs, blood, tissues, brain and c.s.f. is to be found in the paper of Grodins, Buell and Bart [7].

Their paper, like our discussion so far, treats ventilation as a steady process, represented by a steady current of air passing over the lung surface, but breathing, as Yamamoto and Raub [8] amusingly point out in an intriguing theoretical paper, is of course rhythmic, involving V_T, the tidal volume or amplitude, and a frequency f, such that

$$\dot{V} = fV_T. \tag{4}$$

Hey et al. [9] have shown that over a wide range of ventilations with a variety of respiratory stimuli (increased body temperature is an exception, causing m to increase) there is a linear relation between $\dot{V}$ and V_T of the form

$$\dot{V} = m(V_T - k). \tag{5}$$

The parameter m has the dimensions of a frequency, min^{-1}, and it is highly correlated with the sensitivity-size parameter h of equation (1), which has the dimensions $l.min^{-1}$. This high correlation implies some important overlap between chemical and neural phenomena, and raises the question of the origin of the rhythm of ventilation and of its optimal frequency. The latter is related to work of breathing, to the volume and resistance of the respiratory tubes that do not exchange gases with blood, and with the notion of length-tension appropriateness

[cf. 10, 11]. Mathematical ideas of optimality are of growing importance in biology [12].

Although equations such as (1), (2) and (3) provide a basis for respiratory regulation, they are far from adequate as explanations of every important respiratory phenomenon. Altitude and exercise provide the most striking examples of physiological respiratory alteration, and both provide interesting examples of regulatory peculiarities.

At altitude P_{IO_2} and hence P_{AO_2} are reduced, and equations (1), (2) and (3) permit a prediction of $\dot{V}$ in the steady-state which is reasonably borne out by experience. The increase in $\dot{V}$ without an increase in P_{ICO_2} will by equation (2) lower P_{ACO_2}, and this will cause $H_a{}^+$ and $H_c{}^+$ to fall below their threshold values $H_{a\theta}^+$ and $H_{c\theta}^+$, so that the log terms are effectively zero. Whereas an abnormal alkalinity of the blood is only slowly rectified by renal excretion of HCO_3^-, an abnormally alkaline c.s.f. is rapidly rectified by a secretory lowering of HCO_3^- so that the pH of c.s.f. is soon restored to its usual figure of about 7.32, at which $H_c{}^+$ is sufficiently high to promote ventilation and hence a raised P_{AO_2}. This extremely interesting phenomenon, by which c.s.f. pH is maintained and P_{AO_2} considerably augmented at altitude, was hinted at by Kellogg [13] and confirmed by Mitchell, Severinghaus and their collaborators [14], when they heroically analysed their own c.s.f.'s at an altitude of 3800 m.

A well-known effect of altitude is the increase in red cells per unit volume of blood. This is yet another example of negative feedback, the hypoxia calling up a response in the blood-forming system which tends to negative the effect of hypoxia. There are, however, two disadvantages of polycythaemia, the obvious one being the rise in blood viscosity, which can overload the heart and circulation. Secondly, and this is less obvious, because haemoglobin is the main blood buffer polycythaemia depresses the slope of the blood log H^+, log P_{CO_2} line and thus tends to cause a reduction in ventilation which could lead to further hypoxia and hence more haemoglobin, a positive feedback to which there is no defence except blood-letting or a return to lower altitudes. This is a speculative contribution to the aetiology of the undesirably high polycythaemia of chronic mountain sickness, but brings out the point that where an element in a normally effective homeostatic mechanism develops positive feedback with an open-loop gain greater than 1, disease and perhaps death ensue. This question is discussed in Milhorn's [15, pp. 102, 358] comprehensive text, in which control theory is systematically expounded in a physiological context, at much greater length than in the useful shorter book by Bayliss [16].

The phenomenon by which c.s.f. pH tends to be jealously regulated near 7.32 without a steady-state error, by a combination of ventilation, secretion, brain bloodflow and metabolism, raises the whole question of set-points in physiological regulation. Why is the blood pH 7.4, the c.s.f. pH 7.318? On the former, Priban and Fincham [17] have made the ingenious and stimulating suggestion that the

normal pH of blood is set at 7.4 because this is the pH at which haemoglobin is most effective as a physiological acid-base regulator. Haemoglobin is a buffer system which accepts protons and lessens pH change as CO_2 diffuses into blood from the tissues, but this simple physicochemical buffering is further supplemented by the Bohr-Haldane effect, by which oxyhaemoglobin is a stronger acid than reduced haemoglobin, and oxygen is driven off haemoglobin by an increase of CO_2 or H^+ concentration. Priban regards this effect as being most marked at a pH of about 7.4, and suggests that this property of haemoglobin acts on the ventilation to bring blood pH back to the figure at which haemoglobin is most effective, using the device known to control engineers as hill-climbing to provide a mechanism in detail.

This suggestion is open to serious criticism as a full explanation of respiratory regulation, but it contains the important idea that the set-point to which a regulatory system returns may be embodied in the properties, physicochemical or otherwise, of a large molecule evolved in more primitive evolutionary conditions. Once evolved the large molecules can tend to improve their biological effectiveness by promoting the selection of an internal environment which enhances the biological value of their physicochemical peculiarities. Priban's idea is also close to the notion that an enzyme could during the course of evolution bring its intra- or extracellular environmental pH to the value at which it is most effective as a catalyst.

A further suggestion as to the respiratory regulation of blood pH comes from the work of Rahn [18], who has shown in cold- and warmblooded animals that over a range of body temperatures the pH to which the blood is normally brought by regulation varies with temperature in parallel with the pH of neutral water, so that the pH of blood is about 0.6 above that of physicochemical neutrality. Albery [19] has argued that this may be related to the change with temperature of the pK of some biologically important buffer system.

Yet another aspect of blood pH regulation is the possibility that it is set to give a minimum effective total of $[H^+]+[OH^-]$, both H^+ and OH^- being powerful hydrolytic catalysts for biologically important polymers such as proteins, esters and polysaccharides. If we can argue that because H^+ is about twice as mobile as OH^-, and that the ideal compromise pH is reached when $[H^+]$ is 0.5 $[OH^-]$, we should expect at 37° a biological pH of 6.95, 6.8 being the pH of neutral water. This seems rather far from that of blood, but is probably close to intracellular pH's [20].

The point emerging at this stage of our discussion is that the pH's of blood, interstitial fluid and cells undoubtedly show homeostasis and are subject to regulation, but that the set point to which they regulate is not some standard pH tucked away in a little compartment to which reference is continually made, or a pointer reading as in a chemical engineering plant, but may be embodied in the physicochemical or biochemical properties of key macromolecules. The

links between these properties and the regulatory devices have yet to be worked out, and until this is done in detail this notion remains speculative.

In discussing the pH regulation of the blood it may be convenient to think of factors tending to disturb it and of those tending to correct it. A low-protein vegetarian diet tends to cause alkalinity, and a high-protein diet acidity by the oxidative production of sulphuric acid: these are relatively slight effects dealt with in the long run by the kidney. The ingestion of any sort of food leads to the secretion of acid in the stomach, and Dodds [21] showed that the resulting alkalinity of the blood is dealt with by a depression of ventilation, a consequential retention of metabolic CO_2, and a partial restoration of pH to the norm. Haldane [22] showed that 10 or 15 g of ammonium chloride could be ingested daily without apparent ill effect and that it gave an effect very similar to that of the administration of hydrochloric acid. This causes acidity of the blood, an increase in ventilation, a consequential lowering of the CO_2 concentration of the blood and hence a partial restoration of the pH to normal.

As long as the daily ingestion of 10 g of ammonium chloride continues, the blood pH remains at about 7.3, this giving the steady-state error of 0.1 pH needed to drive the kidney to dispose of the 10 g of ammonium chloride into an acid urine: similarly the oral ingestion of sodium bicarbonate can lead to a steady-state blood pH of 7.45, this being the pH at which the kidney will dispose of the bicarbonate into an alkaline urine. This dietarily imposed range of 0.15 pH is quite large, and implies that the kidney operates as a proportional controller with a steady-state error, that is as if the rate of excretion of acid is a direct function of the acidity of the blood, and there is no evidence, from the persistence of abnormal pH's when the diet is abnormal, that integral control operates to bring the final steady-state error to zero. The kidney seems also to lack derivative control responding to the rate of change of blood $[H^+]$, but this is not a defect, for the most precipitate change in blood pH comes during and after the most violent exercise, such as the running of 400 m. in 45 seconds. Energy for this comes partly from oxidation and partly from the conversion of some 40 g of glycogen to lactic acid in the muscles. The concentration of lactic acid in the blood may rise from 10 to 150 mg per 100 ml, and, if there were no ventilatory adjustments, the pH would fall to 6 or less. As we all know from personal experience there is during or immediately after violent exercise a rise in ventilation (partly predictable from equation (1)) which drives off CO_2 and moderates the fall in pH so that the lowest value reached is about 7, and then during recovery there is a steady and rapid return to normal as lactic acid is removed from the blood, mainly by the liver. If the kidney responded immediately to the low pH of exercise, we should be in grave difficulties. Lactic acid, a valuable source of calories under aerobic conditions, would be lost in the urine, and the metabolic cost of violent exercise would rise ten-fold, a situation of no advantage to primitive man and of benefit only to the obese of the twentieth century (if they could be persuaded to make use of it).

This short-term and very effective ventilatory regulation of blood pH depends on the volatility of the main acid product of metabolism, CO_2. By hyperventilation we can lose roughly one molecule of CO_2 for each molecule of lactic acid added, though once again, as with the longer-term kidney, there will be a steady-state error, showing the the ventilation-blood-H^+ system seems to work as a proportional controller without integral control. The importance of the blood H^+-CO_2-ventilation system is its rapidity of action, which is second only to that of the first line of defence, the physicochemical buffers of the body fluids, of which haemoglobin and bicarbonate are the most important.

When $P_{A}co_2$ is raised by inhalation of CO_2, the blood supply to the brain generally rises through the agency of a roughly proportional control mechanism by which the diameter of the brain arterioles is a direct function of $P_{A}co_2$, so that $P_{c.s.f.}co_2$ rises less than $P_{A}co_2$. This means that the stimulus to raise c.s.f. [HCO_3^-] by secretion is smaller than it would otherwise be. If this regulation of brain bloodflow did not exist, raising blood CO_2 would entail a secretory rise in $HCO_{c.s.f.}$, a change which could lead to serious underventilation if the extra CO_2 were suddenly removed. The raising of brain bloodflow in response to high $P_{A}co_2$ is thus a valuable short-term regulation by a rapidly reversible process, similar in function to the respiratory as opposed to renal adjustment of blood pH during the lactacidaemia caused by violent exercise. It should be noted, however, that the final regulation of c.s.f. pH to 7.32 in a wide variety of conditions [14: but see 23] may imply integral control without a steady-state error.

Exercise is the most normal and least understood stimulus to ventilation. It can raise $\dot{V}co_2$ and $\dot{V}o_2$ of equations (2) and (3) 25-fold above their resting values, and this must cause an increase in either or both of $\dot{V}$ and $(P_{A}co_2 - P_{I}co_2)$. It is obviously possible in principle to calculate $\dot{V}$ in exercise from equations (1), (2) and (3) and the appropriate subsidiary equations relating H^+ to Po_2 and Pco_2, but it is found in practice that $\dot{V}$ is nearly always greater than the prediction, and indeed greater than the value predicted when values actually measured in exercise are substituted in the controller equation. It is therefore suggested that exercise increases μ, possibly by impulses coming from active limbs, and we [24] have found in man, following in the wake of M. Dejours [see e.g. 25] that the increase in $\dot{V}$ at the beginning of exercise is independent of the pre-existing Po_2 and Pco_2 in the chemical background, which argues strongly that this initial exercise effect is an increase in μ. We [26] are currently investigating short-term and steady-state aspects of the hypoxia parameters (ψ and γ) in exercise, which now appears to evoke a change in the form of equation (1). The hyperventilation seen in exercise over and above the prediction of equation (1) is obviously of great interest, and has been attributed to feed forward, showing up as an increase in μ. The absence of changes (steady-state error) in $P_{a}co_2$ and $P_{a}o_2$ in moderate exercise points also to integral control.

Professor Dejours has always grasped the most interesting nettles, seldom if

ever being stung, of respiratory physiology, and I see from the programme of the June 1967 meeting of French-speaking physiologists that he is now working on respiratory aspects of speech. Speech is the most human of motor activities, using one of the most primitive of quasi-autonomic activities, respiration, for its purposes: and it has for some time been known [27] that during speech the respiratory muscles are used primarily not for maintaining the homeostasis of blood pH or brain P_{O_2}, but for keeping a constant pressure of gas against the vocal cords. This capacity for over-ruling the usual chemoneural respiratory rhythms is of immense biological importance for drinking, swimming, talking, spitting, yawning, grimacing, laughing, sobbing, cleaning one's teeth, snorting, singing, diving, sneezing, sniffing, coughing, blowing, sucking, eating, drinking and for rejecting some hostile atmospheres and inhaling others, and it is interesting to speculate as to where the will to hold one's breath, presumably cortical in origin, conflicts with the chemical drive which ultimately forces one to breathe. Measurements during and after breath-holding provide, albeit at a low level, objective and reproducible data on the will and its pharmacology [28, 29], and it is of interest that Dr Bhattacharyya has found in our laboratory that at the breath-holding breaking point the movement of the chest is affected by the blood gases, but the effects, though fairly consistent for an individual, differ widely between subjects.

By definition, but by nothing else, the gaps in this presentation cannot be its most salient features. By largely confining it to respiratory regulation we have been able to go into some detail, though the treatment of the only controller equation we have discussed has been brief and dogmatic. The unsolved problems in regulatory physiology are the details, largely requiring physiological experimentation, of these control equations, the conceptual problems of the non-steady-state, in which Grodins has given an admirable lead, and the search for the physical counterpart of the set point to which many homeostatic systems return, with or without steady-state error.

The problem of ventilation in exercise remains partially unsolved, and the central nervous links between the chemical input and the rhythmic neuromuscular output remain largely unexplored. There is no doubt that the physiology of regulation has already benefited from the limited application of the techniques and ideas of mathematics, physics, engineering and physical chemistry that has so far been made. During the next twenty years a systematic extension of these applications to the scores of regulations which are now merely verbally described will transform this branch of physiology into a rigorous quantitative discipline. Regulation was made for man and his free life, not man for regulation. May this neo-Bernardism bring many more physically trained theorists into biology!

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SPECIAL SENSORY PERFORMANCES IN THE ORIENTATION OF THE HONEY BEE

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Adaptation, regulation and homeostasis, these fundamental performances of the living organism, are only possible when the latter can gain information about conditions and changes in its environment. Every animal, including human beings, can however register only a diminutive fraction from the *objective* environment through its "interpreters"—the sense organs. In order to understand the behaviour of a living organism it is important to recognize the *subjective* part of its environment.

Three examples, drawn from the orientation behaviour of the bee, may us reveal a section of a world, inaccessible to our sensory system.

1. The suncompass-orientation

Bees use the sun as a compass. It is important to realize, that they use the sun not only for their private orientation but mainly as a point of reference in their mutual communication: When a forager bee in the darkness of the hive indicates the location of a food source by the tail-wagging dance [1] the angle between sun and goal is transposed into the field of gravity. Astonishingly enough—our bees communicate by dances even if the sun is hidden behind clouds and if they can see only a patch of blue sky. That is possible because the light that comes from the blue sky is polarized showing definite relations to the sun's position with both the direction of vibration and the intensity of polarization. V. Frisch discovered this twenty years ago and today we know that there exist true analyzers for polarized light in the bee's eye. The fine structure of the rhabdomeres gives the prerequisites for the dichroic absorption of polarized light [2, 3, 4, 5].

Another question is whether the bees are familiar with the whole pattern of polarization on the blue sky and its regular arrangement around the sun. If you keep young unexperienced bees in a closed room under artificial light and let them fly out for the first time after three weeks than they are unable to use the sun as a compass. It takes at least 5 days, or 500 foraging flights until they learn to use the moving sun and the pattern of polarization on the blue sky as a compass. Another experiment demonstrates that this suncompass is not innate but must be learnt by the bees: I brought a bee colony from Ceylon to Poona (India) and from Ceylon to Munich. The bees behaved in the northern

hemisphere as if they would be still under the Ceylonese sky e.g. they calculated the sun movement anticlockwise instead of clockwise; it took 40 days until they got familiar with the orientational cues on the new sky [6, 7].

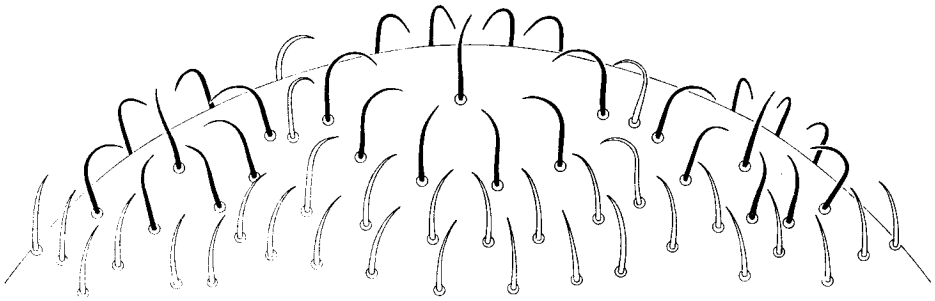
A prerequisite for suncompass-orientation is an exact time memory. The bees have to know the azimuth of the sun every minute of the day for calculating the changing angles between the moving sun and the fixed position of the food source. Beside the findings of Beling [8], Wahl [9] ,and Renner [10, 11] the following experiment may surprise every one how precisely the time sense works in suncompass-orientation: In specific situations [6, 12] you can induce "marathon-dances"; in this case the bees continue dancing for many hours without leaving the hive. Therefore they cannot control in the meantime the changing angle between sun and goal; time memory and knowledge of the sun movement exclusively have to save this critical dilemma. Even at night with artificially induced dances the bees show where on the sky the sun could be found; and —the human observer hardly can believe it—they don't calculate the sun's movement at an average speed: they take into account the seasonal variations of the change of azimuth of the sun [13].

2. Problems of orientation during building activity

A masterpiece of the regulated communal work in the beehive is the building of the honeycomb, a performance of orientation marvelled at since long time not only by biologists but mainly by mathematicians, and physicists, in front of all by REAUMUR. In this situation the mechanical senses are alone responsible. The honeycomb must be built in the darkness of the hive; without the guidance of optical clues the builders have to measure the overall dimensions of the space within the cells and have to control the angles between the comb-cell walls as well as their inclination towards the foundation. Of the different control mechanisms in the building activity we know only two:

1.) The bristle fields in the neck of the bees are used as gravity receptors to orient the cells in the gravity field. If you glue these sensory bristles with paraffin in all bees of a colony not a single comb cell can be built (even during 4 weeks); but if you remove the paraffin, the bees start with construction of combs immediately.

2.) A group of highly specialized pressure receptors on the antennal tip controls the thickness of the cell wall, which is strictly 72 μ in worker cells and 95 μ in drone cells (fig. 1a/b). These receptors work on the following principle: after the foundations of the wall are laid down the builders move along one side of the wall, continually making pressing movements with the rounded edges of their mandibles. The cell walls get indented about 5 μ deep under controlled pressure of about 4 dyn—just as when one draws two fingers over a tightly stretched curtain. The tips of the antennae—by bipolar scanning movements—register the change dynamically during the indenting and return movements. At the same time they perceive through the sensory hairs on their tips any counter



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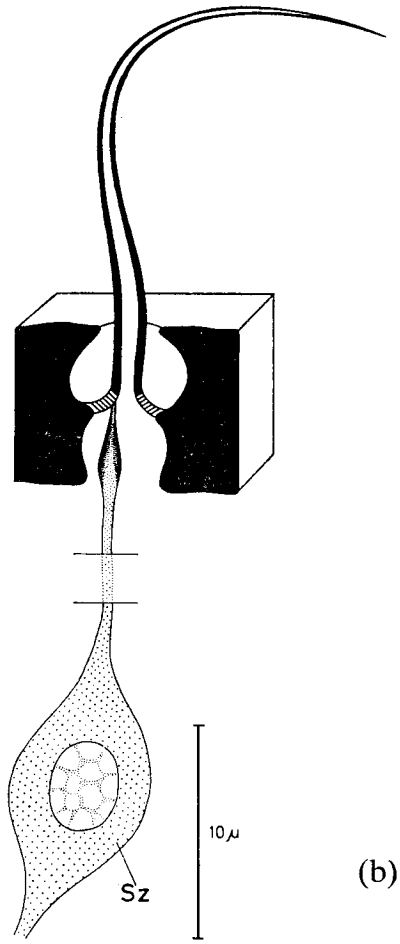


(b)

Fig. 1. Three groups of specialized sensory hairs on the tip of a bees' antenna; in the center of each is a straight bristle surrounded by 7–10 hooked bristles in a circle which measure the counterpressure of the wax wall, when it is dented by the mandibles. Note in fig. 1b that the chitinous membrane on the basis of the bristle allows a downward movement of the hair for less than 5 μ. This is exactly the range in which the builders indent the cell walls.

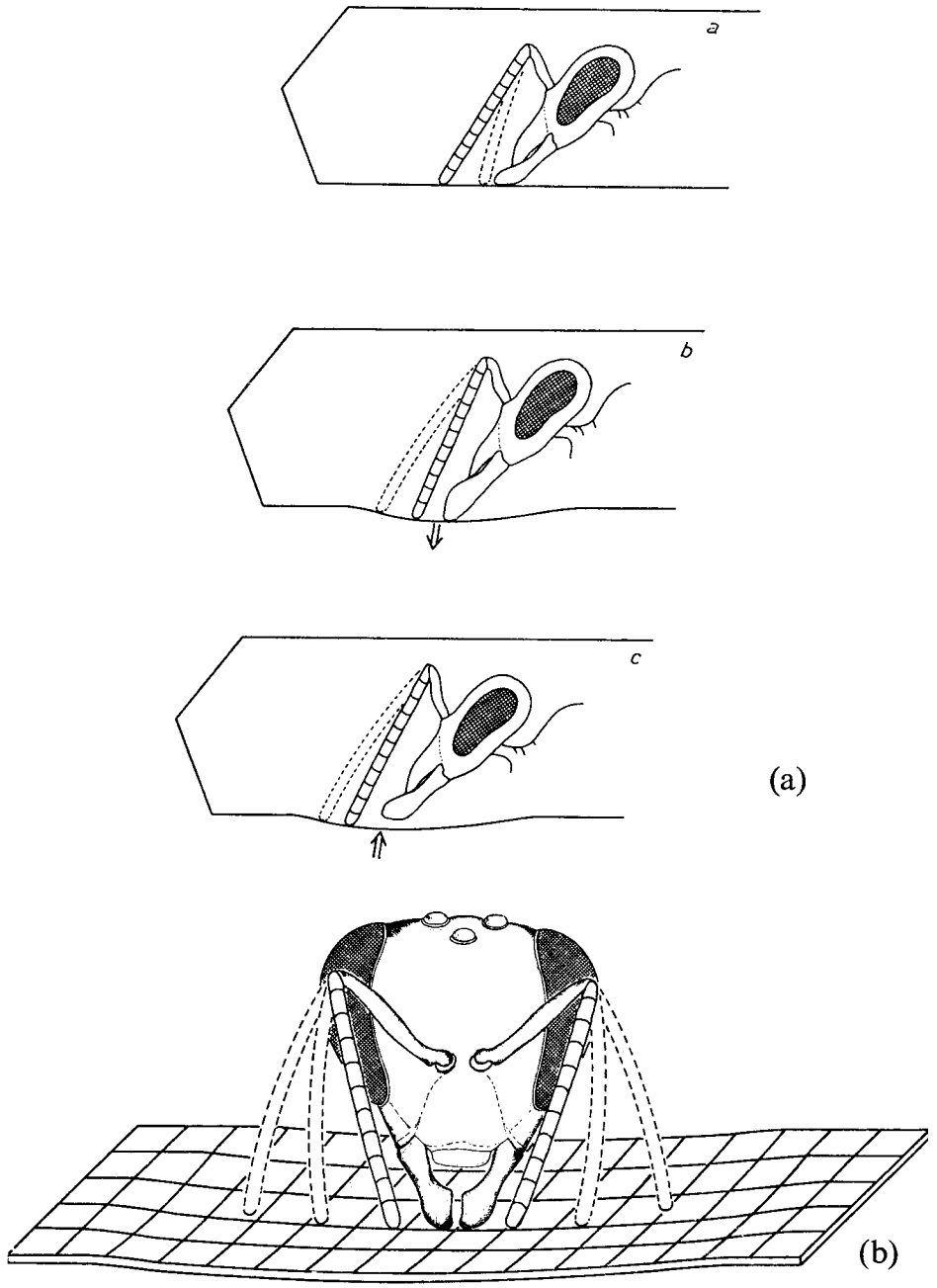
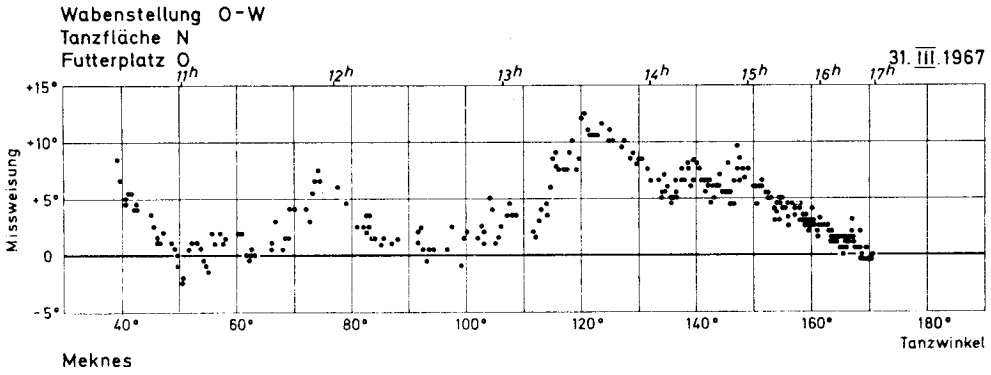


Fig. 2. (a): Pressing movements of the mandibles (a, b), the elastic aperiodic returning movement of the wall (c) and the simultaneous control of the counterpressure by the tips of the antennae provide the information to measure the thickness of the wall.
(b): The tips of the antennae measure three parameters relevant to the thickness of the comb-wall: counterpressure, change in shape and its speed.

pressure (fig. 2a/b). This control mechanism—in order to be effective—has two prerequisites:

1.) One has to use always the same raw material; in fact, this is the case: the building material is intrinsic wax (palmitic acid ester and myricil-alcohole) which is transformed into an amorphic stage by kneading them together with the secretion of the mandibular glands.

2.) The temperature must be kept exactly at 35° C because in this temperature range only the wax wall—after mandibular pressure—shows "aperiodic displace-



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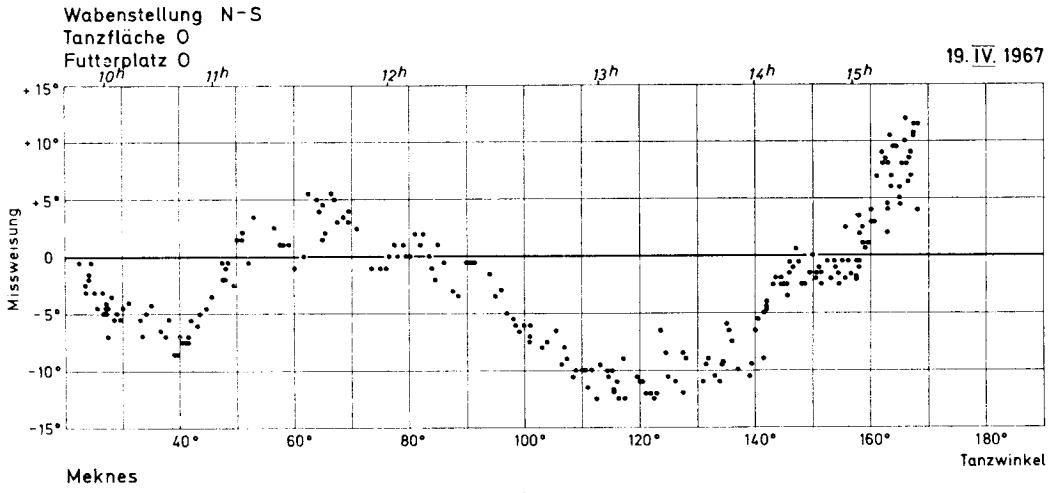


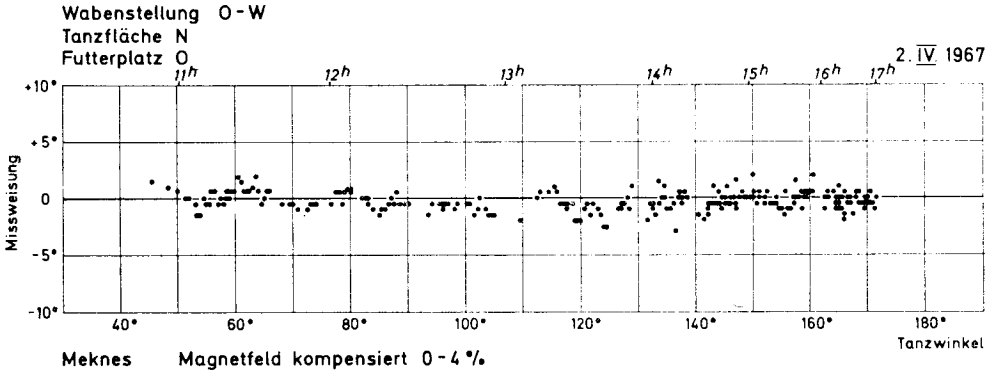
(b)

Fig. 3. Bees visiting a feeding table 400 m eastwards of the hive announce by their dances on the vertical comb the angle between sun and goal (transposed into the gravity field). The abscissa gives the changing angle between goal and the advancing sun throughout the day. All spots on the 0-line correspond to this angles performed by the dancing bees indicating the direction to the goal without mistake. All spots above or below the 0-line are deviations. The diurnal curve of these deviations depends on the position of the dance floor in the earth magnetic field. (In fig. 3a the dance floor faced northwards, in fig. 3b eastwards.)

ment". In fact since long time it is known that the temperature in the building cluster is kept day and night exactly at 35° C [14].

3. Orientation in the gravity field influenced by the earth's magnetic field

In the last ten years studying of orientation of dancing bees we encountered much trouble when the bees apparently made small genuine "mistakes". But there was a system in these errors: As I have mentioned already the optical angle between sun and goal is transposed into the gravity field; the wagging line of the dancer keeping the same angle with the perpendicular. When one measures, for a whole day long, this dance angle on the vertical comb, than—as the angle between the advancing sun and the goal changes the dance angle deviates in a characteristic course up to 25° from the expected normal (fig. 3a). The recruits in spite of this



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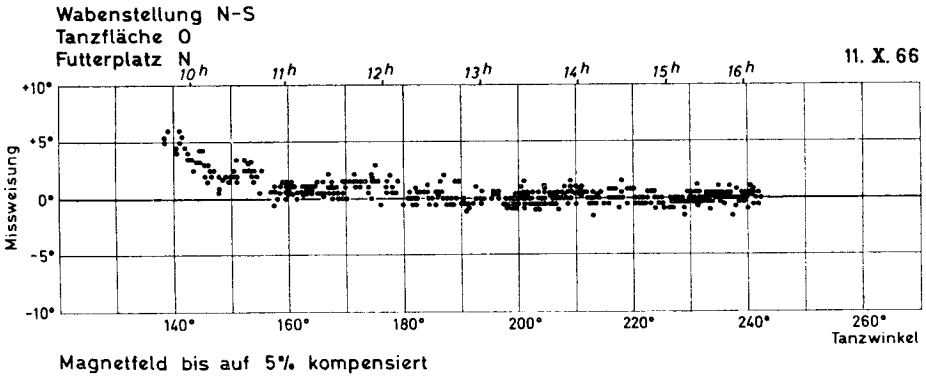

(b)

Fig. 4. The experimental situations in fig. 4a and 4b are identical to those of fig. 3a and 3b respectively. In both cases the dance floor was put in the center of a Helmholtz-drum and the earth's magnetic field was compensated up to 4 percent. The bees dance almost without deviation —they transpose the angle between goal and sun exactly into the gravity field throughout the day.

indicated deviation search for the right direction without misorientation. The communication system therefore in the "bee language" is correct, it demands however the right interpretation. To our astonishment we found the last year, that this diurnal „deviation" curve ran a quite different course when the vertical "dance floor" was put at different position in the earth magnetic field (fig. 3a/b). In the same way this deviation curve alters when the bees from Frankfurt are taken to Marocco where the earth's magnetic field has a different intensity and inclination. (Frankfurt: total intensity 0,43 Oersted; inclination 64°. Marocco: total intensity 0,41 Oersted; inclination 49°.) The deviations disappear almost completely when the hive, with its dancing bees, is put under a Helmholtz drum and the magnetic field is compensated (fig. 4a/b). The results will be discussed in detail elsewhere [15].

From this it seems that the bees are receptive to the fields of the earth magnetic force. We do not yet know the receptive mechanism for this nor its exact biological meaning. We hope the bees will give us some chance to enter in this unknown field step by step.

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DISCUSSIONS

H. C. LONGUET-HIGGINS: Perhaps one could be told how big the measurements of the vertical squares are. The deviations shown are deviations between the angle of the dance and the correct angle for locating the flowers?

M. LINDAUER: It is just at the 0 line.

H. C. LONGUET-HIGGINS: Your interpretation, then, is that the bees make a composite function of the earth's magnetic field and the polarization of the light from the sky?

Dr. MENDELSSOHN: Just one short question. It looks immediately of course, since the acting mechanism is a polarization of the light and is affected by magnetic field, that there should exist somewhere in the mechanism of the bee a rotation of polarization by a magnetic field. Has it been tried to see what happens if you do not just compensate the magnetic field but put a rather strong magnetic field in another direction, I mean, can you force the bee that way.

M. LINDAUER: It has been done. But I mentioned that when they go from Francfort to Meknes, the total intensity is different. Then the curve is different.

F. HALBERG: La figure 1 est intéressante d'un point de vue historique. Elle a été publiée à Leipzig, en 1840, par Gauss et Weber dans "Resultate aus den Beobachtungen des Magnetischen Vereins im Jahre 1839". Cette figure représente des courbes de déclinaison magnétique. Gauss et Weber ont utilisé des informations recueillies en différents points du globe, et, en particulier, à Milan, à Berlin et à Uppsala.

Malgré le déplacement géographique Milan–Berlin–Uppsala, les courbes sont presque identiques entre elles. Vous voudrez bien remarquer que ces graphiques sont publiés sans analyses complémentaires, par exemple, sans faire appel à la méthode des moindres carrés. Ce qui est intéressant est que cette publication a été sanctionnée par Karl F. Gauss, le "roi des mathématiques" et père de la méthode des moindres carrés, bien que les résultats n'aient pas été soumis à une critique statistique. En effet, la figure ne comporte pas d'étude de la variation statistique des valeurs représentées. Une telle étude était réellement superflue même pour ne discuter que de "l'harmonie des courbes", c'est à dire, sans vouloir essayer de quantifier les composantes du phénomène.

Les aspects statistiques du géomagnétisme ont été discutés d'une façon élégante

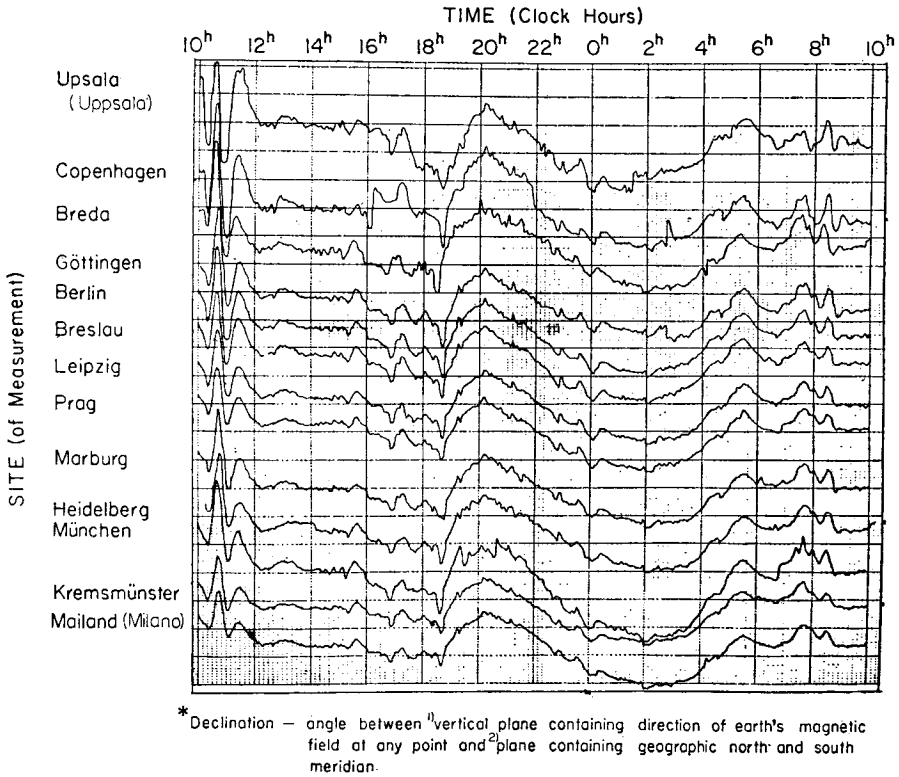

Fig. 1. The "harmony of curves" in itself can definitely be documented in instances such as these without any inferential statistics. [cf also Gauss, Carl Friedrich u. Wilhelm Weber. (Resultate aus den Beobachtungen des magnetischen Vereins im Jahre 1839. Herausgegeben von Carl Friedrich Gauss und Wilhelm Weber. Mit 4 Steindrucktafeln. Leipzig, im Verlage der Weidmannschen Buchhandlung, 1840)].

par MM. S. Chapman et J. Bartels dans "Geomagnetism, Volume II, Analysis of the Data and Physical Theories, Oxford Univ. Press, 1940".

M. Lindauer – comme MM. F. Brown et D. Beischer aux U.S.A. – a eu le grand mérite d'avoir pris en considération des équivalents biologiques des phénomènes représentées à la figure 1 et aussi les effets biologiques d'autres variations géomagnétiques. Ces effets biologiques ne sont peut être pas tout à fait en relation de causalité classique.

Dans ces conditions, je pose à M. Lindauer la question suivante: quelle est l'erreur qui peut dépendre de l'échantillonnage? Ce qui m'amène à poser deux questions complémentaires: 1) y a t-il une ressemblance entre les effets du géomagnétisme mesuré par vous en des emplacements différents et les phénomènes rapportés par Gauss et Weber? 2) Avez-vous une notion des récepteurs physiologiques sensibles aux phénomènes magnétiques dont vous faites état?

M. LINDAUER: Les différences quotidiennes sont peut-être les mêmes. Mais la déclinaison entre, par exemple, Francfort et un autre point, serait de 12 degrés. Il y a des différences de 60 degrés et d'autres de 49 degrés par rapport à Francfort.

F. HALBERG: Quel est l'ordre des déviations?

M. LINDAUER: Les déviations sont de l'ordre de 100 gammas; les différences de l'inclinaison sont supérieures à 1000 gammas.

W. ELSASSER: I would like to comment only on a quantitative relationship. I think there is a misunderstanding here. These daily variations of the magnetic field are very small. If that was not so no sailor could use a compass for navigation.

The deviations that you showed were of the order of 10–15°. So I don't think there is any kind of contradiction. Evidently, what the bees see is not the magnetic daily variation, it's not anything produced by the external atmospheric field which is so small that it can be measured only with very delicate instruments.

W. REICHARDT: In relation to Dr. Lindauer's paper I would like to add a remark concerning dichroitic absorption in the rhabdomeric structures of the insect compound eye. We have recently studied dichroitic absorption in rhabdomers of the housefly (Diptera) using polarized light. If polarized light is thrown onto the distal end of a single rhabdomer and the vector of polarization rotated, a degree of 50 % modulation is found in the light beam leaving the rhabdomeric structure. This finding is consistent with the hypothesis that the pigment molecules are located and randomly distributed in the membranes of the tubuli of which the rhabdomers are made up. Therefore dichroitic absorption in the insect eye seems to be entirely due to the geometry of tubuli oriented perpendicular with their long axis to the Poynting vector of the stimulating light.

E. D. BERGMANN: If Dr. Lindauer permits me, I would like to add to his beautiful statement some data which show that insects seem to be as well versed in chemistry as they are in physics. These data refer to the well-known phenomenon of sex attraction in insects.

It is known that male insects are attracted to the females over distances of many kilometers, a fact which has puzzled the scientists for a long time. One has recently isolated several such sex attractants and has determined their structure. I would like to take the data of Butenandt for the virgin silk moth. The quantity required to attract the males is only 10^{-10} micrograms. If one wants to translate this figure into something more meaningful, one can calculate from the vapor pressure of the compound that 1 cm^3 of air over a thin layer of this compound contains 192 individual molecules. The total quantity of sex attractant which is produced by one female silk moth is 10^{14} individual molecules. Thus one can see

that, given a normal current of air, one can supply a considerable volume of gas with the concentration of the sex attractant which I mentioned.

This is, indeed, a very small quantity; let us compare it with other data which we possess, pertaining to the sensitivity to smell, the sensitivity of the olfactory receptors. There are two data which I remember. One is that Alsatian dogs, which are very sensitive to butyric acid, require 190 individual molecules/cm^3 of air in order to feel uncomfortable. The other figure is that 42 individual molecules only of secondary butyl mercaptan/cm^3 of air are sufficient to make it impossible for a human being to remain in this atmosphere.

So we are coming down now to figures which are in the neighbourhood of one individual molecule per chemoreceptor. This, I think, is a very remarkable feat of nature. It also shows that the chemoreceptor and the active chemical compound, e.g., the sex attractant, must be structurally very well fitted to each other in order to give this extraordinary sensitivity.

P. DEJOURS: Mon intervention a trait à la présentation de M. Lloyd. M. Fessard nous a dit: "Vous, les physiologistes de la respiration, vous avez bien de la chance, car, au moins, vous pouvez parler en termes quantitatifs". M. Lloyd, aujourd'hui, nous a donné un exemple de la façon dont les physiologistes respiratoires utilisent ces termes quantitatifs: vous avez vu un certain nombre d'équations. En réalité, nous rencontrons d'énormes difficultés pour nous exprimer quantitativement. Lorsque nous utilisons le symbole $\dot{V}$, on veut dire volume ventilé par unité de temps ou débit ventilatoire. Mais la ventilation est un phénomène rythmique, périodique, produit d'un volume et d'une fréquence. Il est bien évident que l'utilisation du seul terme $\dot{V}$ dans nos équations est tout à fait insuffisante, car ce terme n'est pas représentatif du phénomène ventilatoire et ne peut être considéré que comme un index imparfait. Il arrive souvent que dans deux circonstances on mesure un même débit ventilatoire, mais que dans un cas le volume est faible et la fréquence élevée, et que dans l'autre cas ce soit le contraire. Il est bien évident qu'il n'est pas permis de dire que les régimes ventilatoires sont identiques dans ces deux circonstances. Il est évidemment impossible d'utiliser une seule quantité pour rendre compte d'un phénomène périodique; mais tous les jours nous nous heurtons à ce problème car, en physiologie respiratoire, l'expression mathématique complète de la fonction ventilatoire serait trop complexe pour représenter une expression, facilement maniable, du phénomène. J'aimerais connaître votre opinion, M. Lloyd, sur ce point.

Vous avez beaucoup parlé des changements de respiration en différentes circonstances, par exemple au cours de la parole, du cri, du chant, etc. Cela est très important, car la plupart des physiologistes de la respiration, à la différence des neurophysiologistes, les physiologistes de la respiration ont la très mauvaise habitude de toujours rechercher un régime stationnaire qui leur parait plus facile à analyser. C'est une déplorable habitude. Car, dans la nature nous sommes

soumis à des sollicitations multiples qui modifient notre ventilation. On peut dire, si l'on veut, que le système opérationnel qu'est notre organisme est soumis à différentes stimulations qui peuvent être des fonctions échelon, rectangulaires, sinusoïdales ou de n'importe quelle forme, et que ce système réagit. Sans cesse, de nouvelles sollicitations s'opposent à un régime stationnaire de la ventilation. A cet égard, le régime stationnaire de repos est artificiel: il n'existe pas dans la nature, on devrait parler de repos forcé ou imposé.

B. B. LLOYD: Dr. Dejours' two questions are of course connected. He asked why we don't pay more attention to the fact that the ventilation is a function in time of the amplitude. Luckily ventilation is almost exactly sinusoidal, so one can fit a simple sine curve, which represents ventilation really rather well:

$$V = a + \tfrac{1}{2} V_T \sin 2\pi ft.$$

There are three things you can alter in this pattern. First you can alter the setting of the point a, the average volume of the chest: this is an important notion, in terms of buoyancy or of oscillations in arterial P_{CO_2}. Secondly you can alter amplitude, or tidal volume V_T, and thirdly the frequency f. We know that these three things alter. The linear plot of ventilation $fV(=\dot{V})$ against V_T shows that up to a certain limit if you know $\dot{V}$ or V_T, you know the other. You could rewrite all the equations that I gave you in terms of V_T as a function of H^+ and P_{O_2} and you could also write frequency as a similar function. So I am not absolutely convinced that when we are doing steady-state experiments we need to bother much about the frequency and the amplitude, at any rate in man. In some animals it's quite different because ventilation regulates temperature. As soon as you start transient analysis, you have to be careful about the phase of the ventilation when you impose the transient. This is a situation where we undoubtedly need the help of people who know how to analyse these rhythmic phenomena.

It is, however, interesting that the chemical variables and the amplitude-frequency parameters are related. Dr. Bhattacharyya working in our laboratory has been looking at the movement of the chest after breathholding. The subject is suddenly told to hold his breath at a given point and then after a certain time he has to breathe again. Obviously the direction in which he starts again will depend on what part of the cycle he started holding his breath in. If he is holding his breath on low oxygen he tends to breathe in even when his chest is on the full side. On the other hand, if you make him hold his breath on high CO_2 then in general there is a tendency for him to expire.

L. ROSENFELD: Simply as a matter of curiosity, I should like to point out that there is a case in which men did nearly as well as bees in the way of orientation. The Vikings are known as hardy navigators and they were as ignorant of physics as the bees. They were able to find their bearings in circumstances in which there

was no sun to guide them. In a saga there is an allusion to a "sun stone" by which they could achieve this. Now, it is well known that in Iceland there are several strongly anisotropic minerals, in particular the famous Iceland-spar which led Huygens to the discovery of the polarization of light. So it is an easy induction that they used such anisotropic stones to analyse the polarization of the light from the sky, and that they had found empirically the correlation between the appearances they observed and the position of the sun.

B. B. LLOYD: We have mechanisms such as those in the foetus which enable us to grow up and become adults. Then if we go to altitude we may find that these mechanisms let us down. This I suppose is the origin of some disease which cannot be attributed to the invasion of an outside organism such as a virus or a bacterium. Eventually our homeostatic negative feedbacks start letting us down and in various regions of physiology go over into positive feedback and ultimately disaster. There are other parallels with this hypertension effect at high altitude; for example there is an increase of red cells in the blood at high altitude. This can be advantageous, but beyond a certain point it becomes disadvantageous and some subjects have either to be bled or come down to a lower altitude if they wish to survive. This takes us away from the physiology of regulation into its pathology, which is enough for at least three other conferences.

Journée du 30 juin 1967

MECANISMES PHYSIOLOGIQUES

2ème séance

PRÉSIDENT G. CARERI

F. HALBERG

Chronobiologie, Rythmes et Physiologie Statistique

Discussions

Theoretical Physics and Biology, © 1969, *North-Holland Publ. Co., Amsterdam*

CHRONOBIOLOGIE; RYTHMES ET PHYSIOLOGIE STATISTIQUE *)

FRANZ HALBERG

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1. Introduction

Au cours de cette réunion, nous avons discuté surtout jusqu'à maintenant de la Biologie moléculaire que l'on essaie de transformer en Biologie "*sub-moléculaire*". Nous avons examiné non seulement ses molécules, mais aussi l'organisme global, plus ou moins considéré comme une structure uniquement ou exclusivement spatiale.

Une autre dimension des organismes doit être étudiée: celle du temps, de manière à connaître leur structure temporelle. Certains aspects de haute fréquence, d'une structure temporelle ont été examinés par des chercheurs, tels que M. A. Fessard, il y a une vingtaine d'années [1, 2]. Fessard traitait, dans une monographie publiée en 1936, de l'activité rythmique spontanée du système nerveux et des systèmes excitables. Ce sera mon privilège de vous soumettre ici des documents sur le degré d'activité rythmique spontanée de processus biologiques dont les périodes ont une durée d'un jour, d'une semaine et même d'un an (tableau 1).

Les recherches relatives à ces périodes introduisent certaines "propriétés" du *temps* ou de la *durée* dans la représentation des phénomènes biologiques: (fig. 1). Celle-ci est habituellement donnée en terme de *structure spatiale* (siège anatomique, histologique et même moléculaire d'un processus) et en terme d'action et de réaction biophysique et (ou) biochimique. Ce faisant, on cherche à préciser la nature (le "quoi") d'un ensemble de processus et *où* il se situe dans l'espace de l'organisme. L'analyse des variations rythmées de cet ensemble de processus doit nous permettre de le situer dans le temps. On cherche alors *quand*, dans l'échelle du temps, peut être située telle ou telle variation intéressante du processus considéré. Il est bien évident que les réponses aux trois questions: *où*?, *quoi*? et *quand*? sont liées et que la réponse à la question *quand*? doit apporter aux deux autres un complément indispensable [2].

*) Les traveaux ici discutés ont été subventionés par l'U.S. Public Health Service (5-K6-GM-13981) et la N.A.S.A. (NAS 2-2738 and NGR-24-005-006). C'est un plaisir de remercier Alain Reinberg, Maître de Recherches, C.N.R.S., pour une coopération inestimable dans plusieurs lignes de recherches presentés dans cet essai. De plus, lui et Hélène Astier, Docteur ès Sciences à l'Université de Montpellier, ont eu l'amabilité d'examiner le style d'une partie de ce texte.

TABLEAU 1

Domaines du Spectre des Rythmes Biologiques

DOMAINE *	Haute fréquence $\tau < 0.5$h	Moyenne fréquence 0.5h $\leqslant \tau \leqslant$ 6j	Basse fréquence $\tau >$ 6j
REGION	$\tau \sim 0.1$ s $\tau \sim 1$ s et cetera	ULTRADIENNE ($0.5 \leqslant \tau < 20$) CIRCADIENNE ($20 \leqslant \tau \leqslant 28$h) INFRADIENNE ($28 < \tau \leqslant 6$j)	CIRCASEPTIDIENNE ($\tau \sim 7$j) CIRCAVIGINTIDIENNE ($\tau \sim 20$j) CIRCATRIGINTIDIENNE ($\tau \sim 30$j) CIRCANNUELLE ($\tau \sim 1$ année)
RYTHME	Electroencéphalographique Cardiaque Respiratoire	Sommeil rapide (REM) Veille – sommeil Repos-activité Réaction aux médicaments Constituants sanguins Variables urinaires Processus métaboliques en général	Menstruation Excrétion des 17-céto-stéroides avec composantes spectrales dans toutes les régions montrées ci-dessus et dans d'autres domaines

* Domaines et régions [(nommées selon des critères de fréquence (f)] sont partagées selon la fréquence réciproque c'est à dire la période, τ, de la fonction utilisée pour l'approximation d'un rythme. s = seconde, h = heure, j = journée.

Pour les variables examinées jusqu'à maintenant on trouve des composantes statistiquement significatives dans plusieurs domaines spectrales.

C'est à l'étude des réponses à la question "quand" que s'adresse la chronobiologie—une discipline *in statu nascendi* s'occupant *inter alia*, de l'activité rythmique.

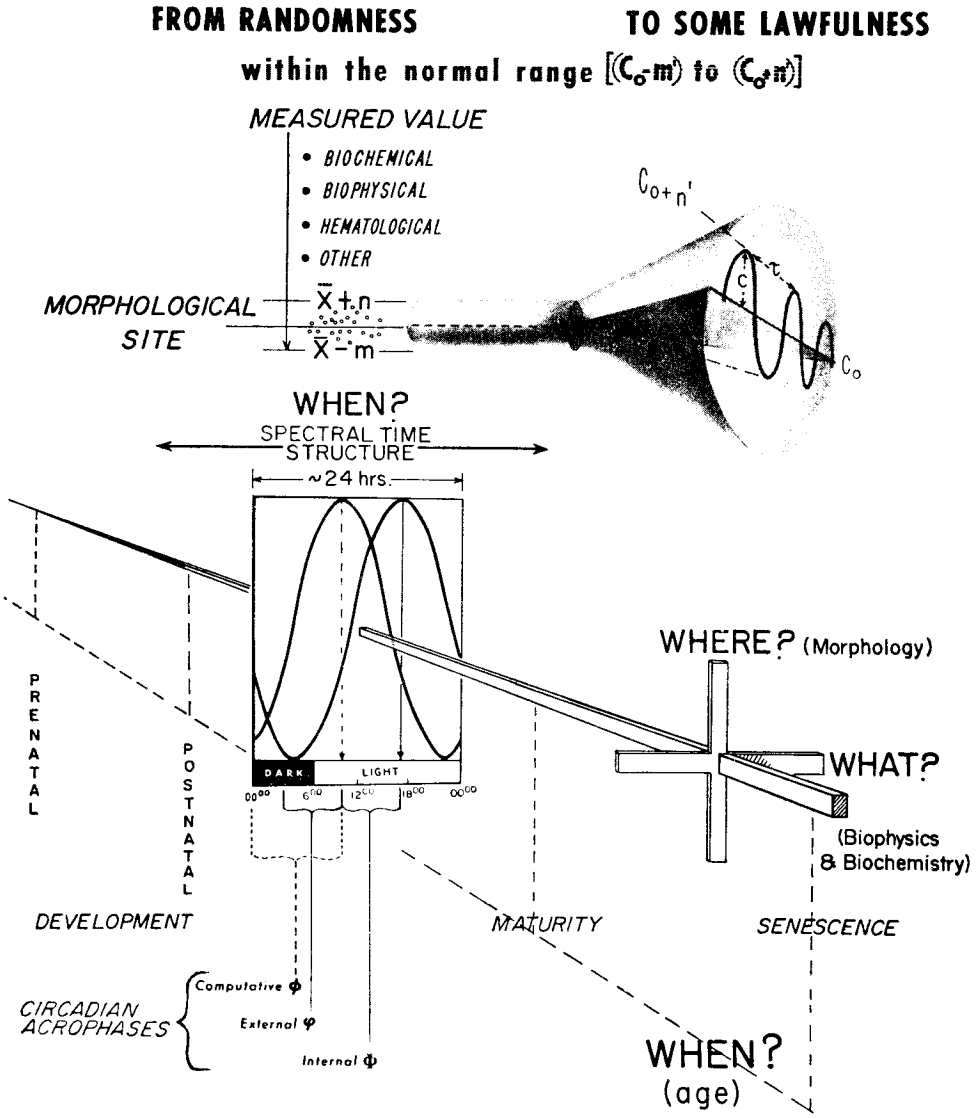
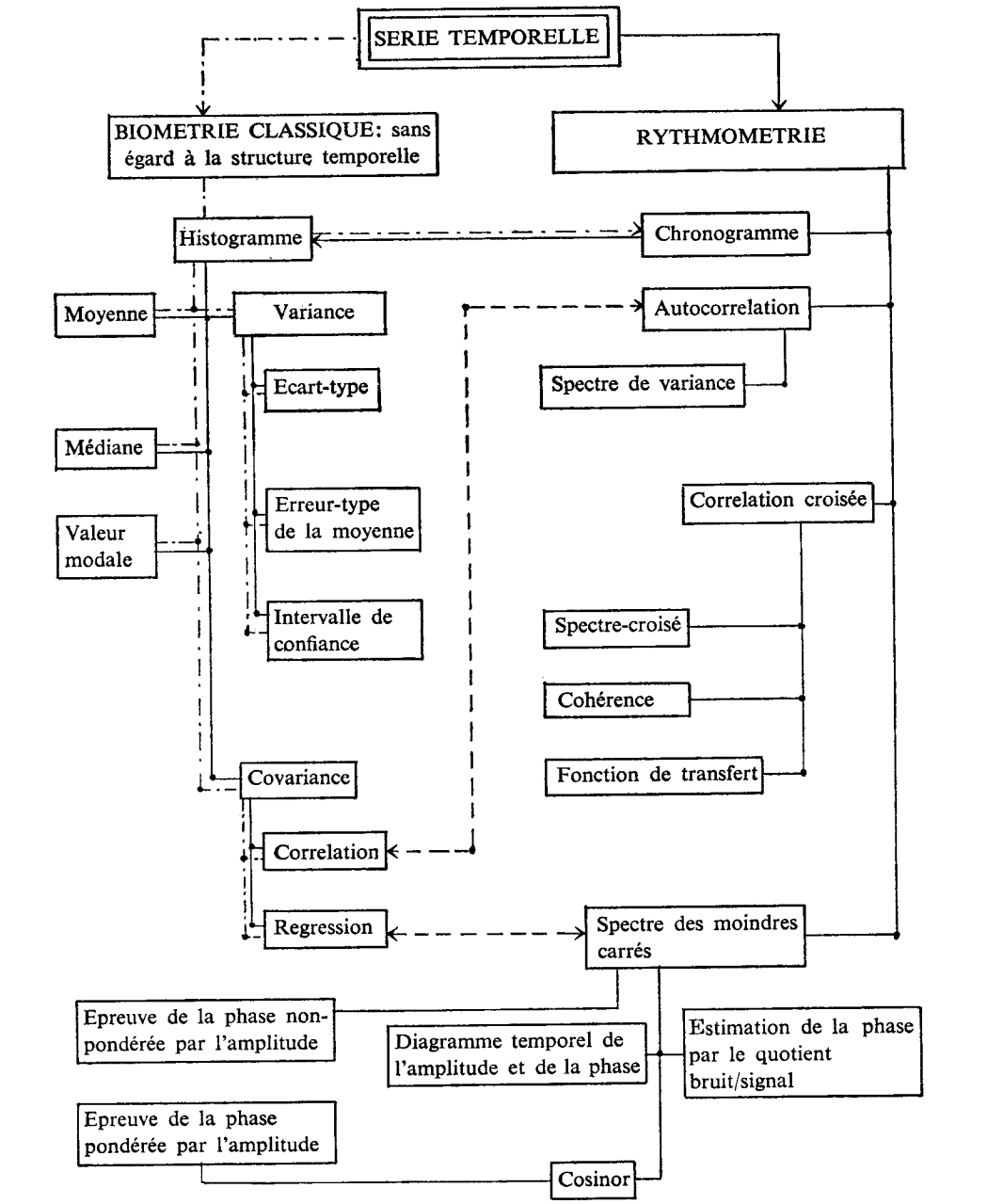


Fig. 1. Rhythmometry for the study of circadian and other rhythms adds a new domain to organisms and yields new endpoints such as the circadian acrophases shown at the bottom of the figure.

TABLEAU 2

La rythmométrie, complément de la biométrie classique



2. La chronobiologie

La chronobiologie a pour but d'inventorier, de définir, de caractériser et de mesurer par des moyens appropriés (e.g., tableau 2 et fig. 2) les caractéristiques temporelles des phénomènes biologiques, tels que l'activité rythmique qui apparaît comme une des propriétés fondamentales de la matière vivante. La chronobiologie, illustrée par des données relatives aux rythmes circadiens [2–11] comprend déjà les branches ci-après:

a) *La chronophysiologie* étudie, chez le sujet sain, les caractéristiques temporelles de tout un ensemble de processus physiologiques—métaboliques, endocriniens, nerveux, etc.... Le chronophysiologiste étudie certains rythmes après une manipulation (définie le mieux possible) de l'organisme—par exemple après une ablation glandulaire (fig. 3a), et une substitution hormonale ou après des modifications de l'ambiance (fig. 4) suivant des horaires modifiés avec ou sans vols transmeridiens ou dans des situations expérimentales aussi différentes et particulières que le séjour dans une grotte (figs. 5 et 6) ou dans l'espace extra-terrestre.

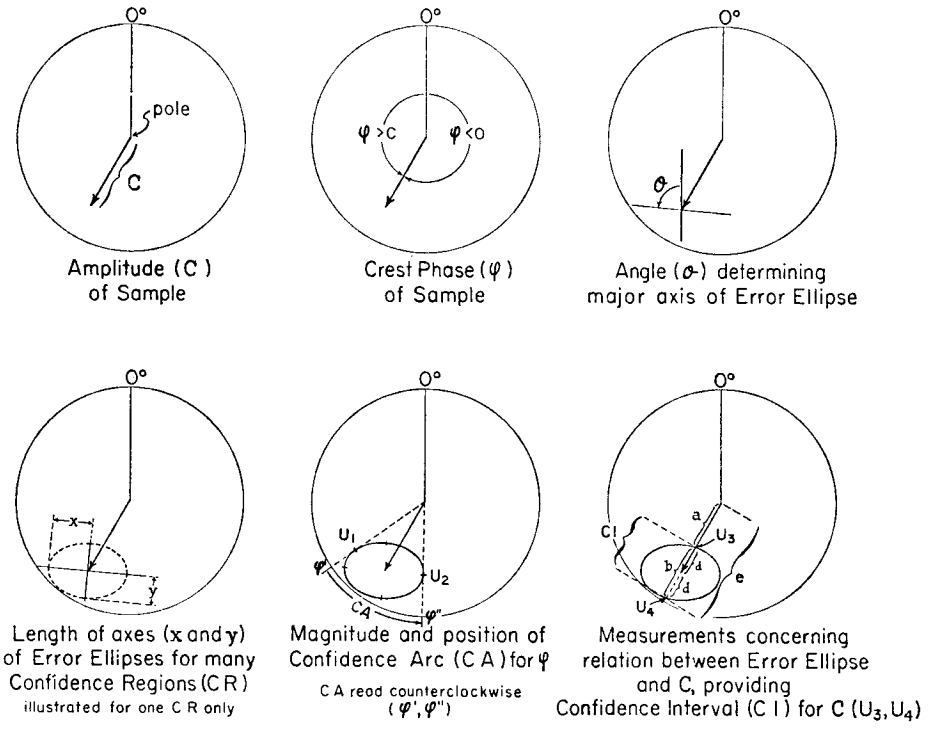


Fig. 2a. Numerical estimates provided by routines for the drawing of a cosinor.

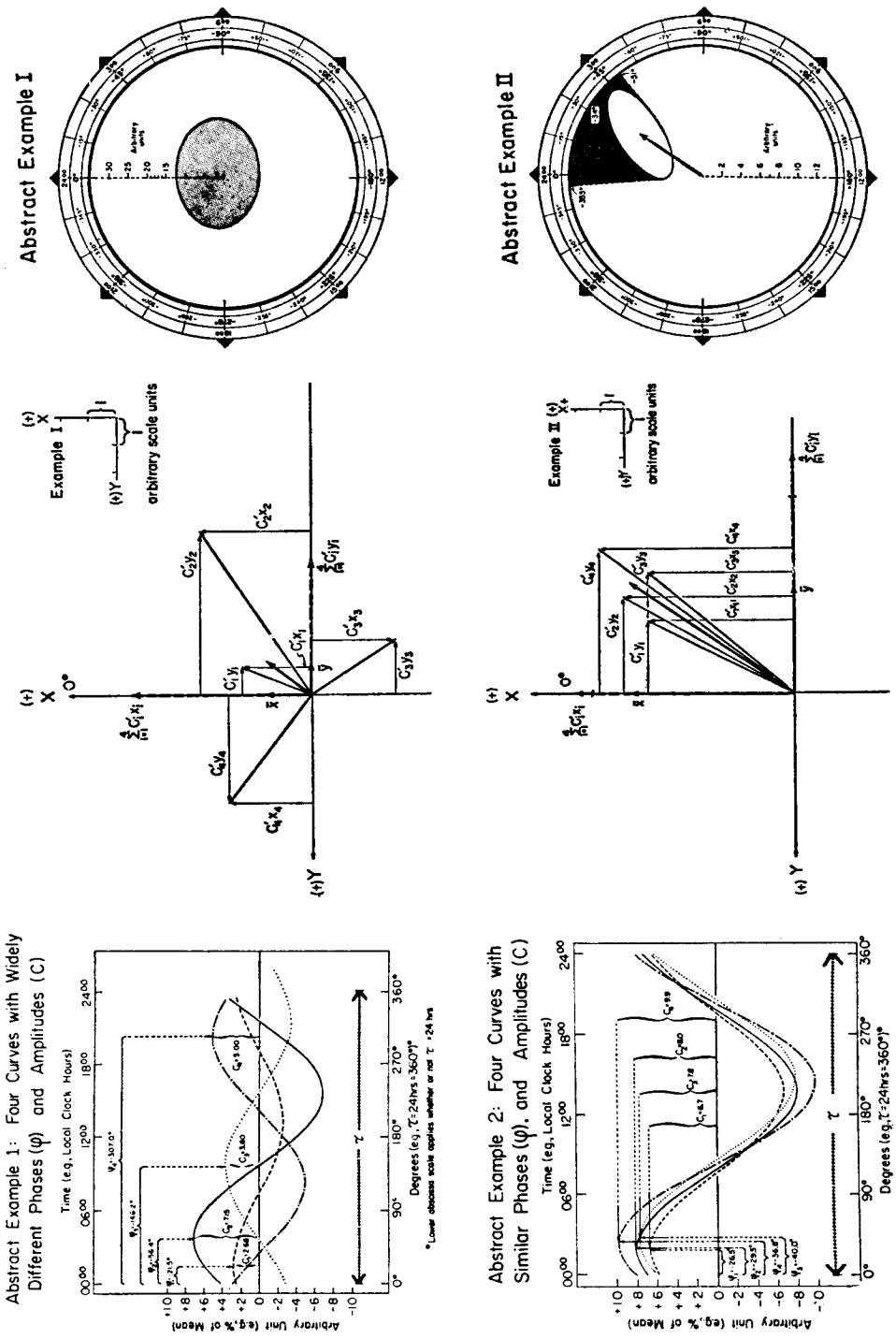
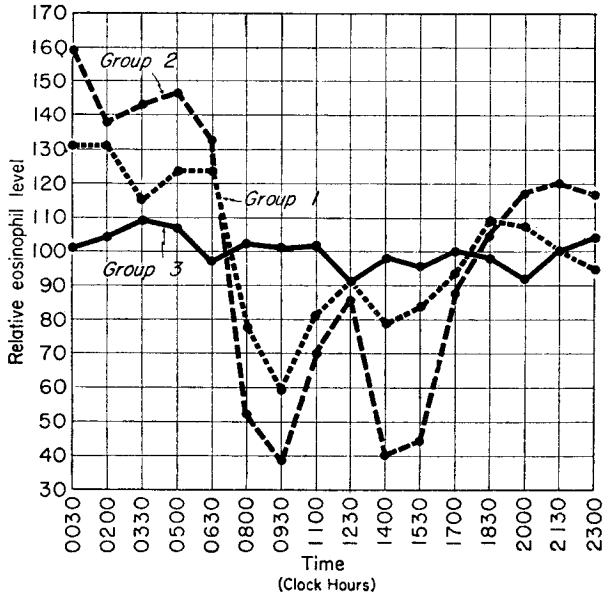


Fig. 2b. Abstract illustrations of cosinor procedure. From the curves displayed in the time domain on the left, one arrives via a vectorial transformation in the middle to the cosinor plots shown on the right [cf. 5]. Display of cosinor results by electromechanical plotter [discussed in ref. 5].



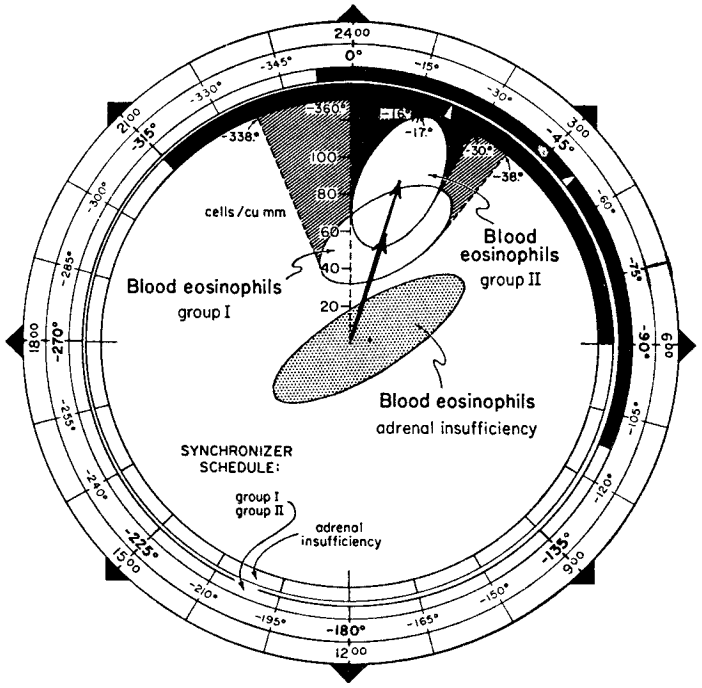
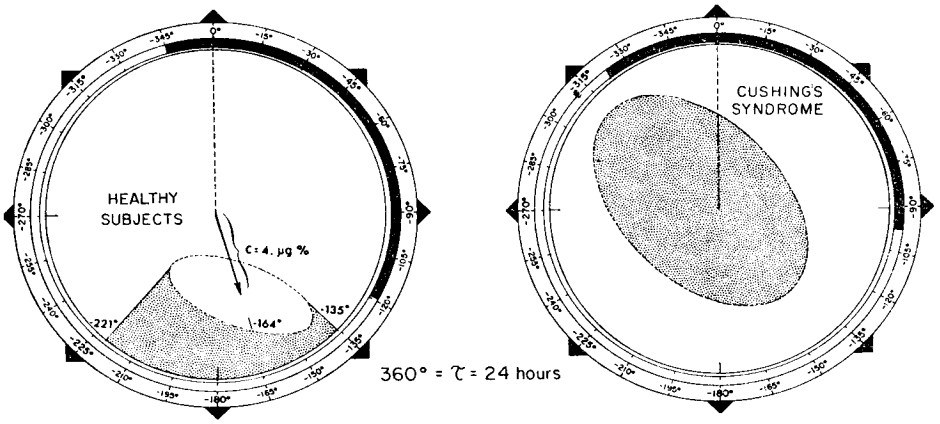
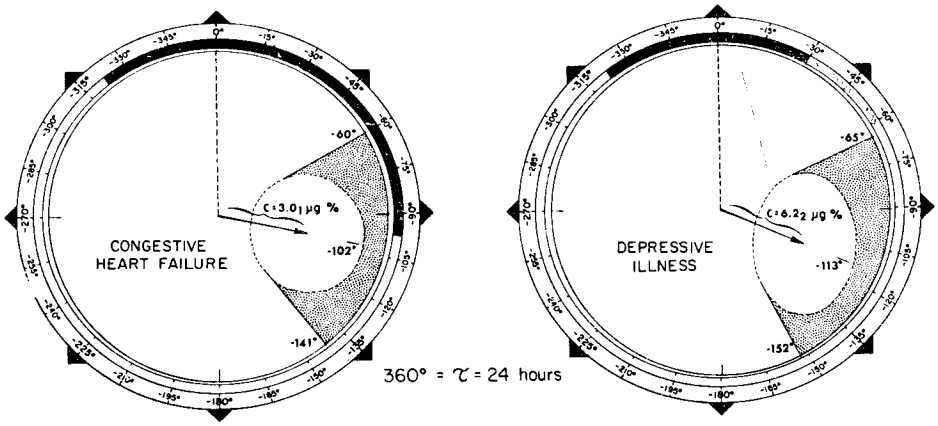


Fig. 3a. Chronogram of 3 sets of transverse data, on the top, summarized by cosinor at the bottom. Note failure to detect a rhythm in Addison's disease (adrenocortical insufficiency).

Group I — healthy, limited activity;
Group II — healthy, unrestricted activity;
Group III — adrenal insufficiency, limited activity.



No pole overlap if confidence coefficient $\leq .998$
.95 confidence interval of C: 2.7_2 to 7.0_8 µg %



No pole overlap if confidence coefficient $\leq .995$
.95 confidence interval of C: 1.1_1 to 4.9_1 µg %

No pole overlap if confidence coefficient $\leq .992$
.95 confidence interval of C: 2.5_9 to 9.8_5 µg %

Fig. 3b. Cosinor evaluation of circadian rhythm of plasma 11-hydroxycorticosteroids in congestive heart failure and depressive illness—rhythm not detected in Cushing's syndrome. Data of M. S. Knapp, P. M. Keane and J. G. Wright (British Medical Journal **2**: 27–30, 1967). Failure to detect a rhythm in plasma 11-hydroxycorticosteroids in patients with Cushing's syndrome.

b) *La chronopathologie* étudie les altérations temporelles des biorythmes (fig. 3) de sujets malades: 1) altérations qui résultent de la maladie et, ce qui semble plus important encore, 2) altérations qui peuvent jouer un rôle déterminant dans l'état pathologique [2].

c) *La chronopharmacologie* étudie les effets de substances actives (médicamenteuses ou autres): 1) sur les caractéristiques temporelles biologiques (figs. 7–9);

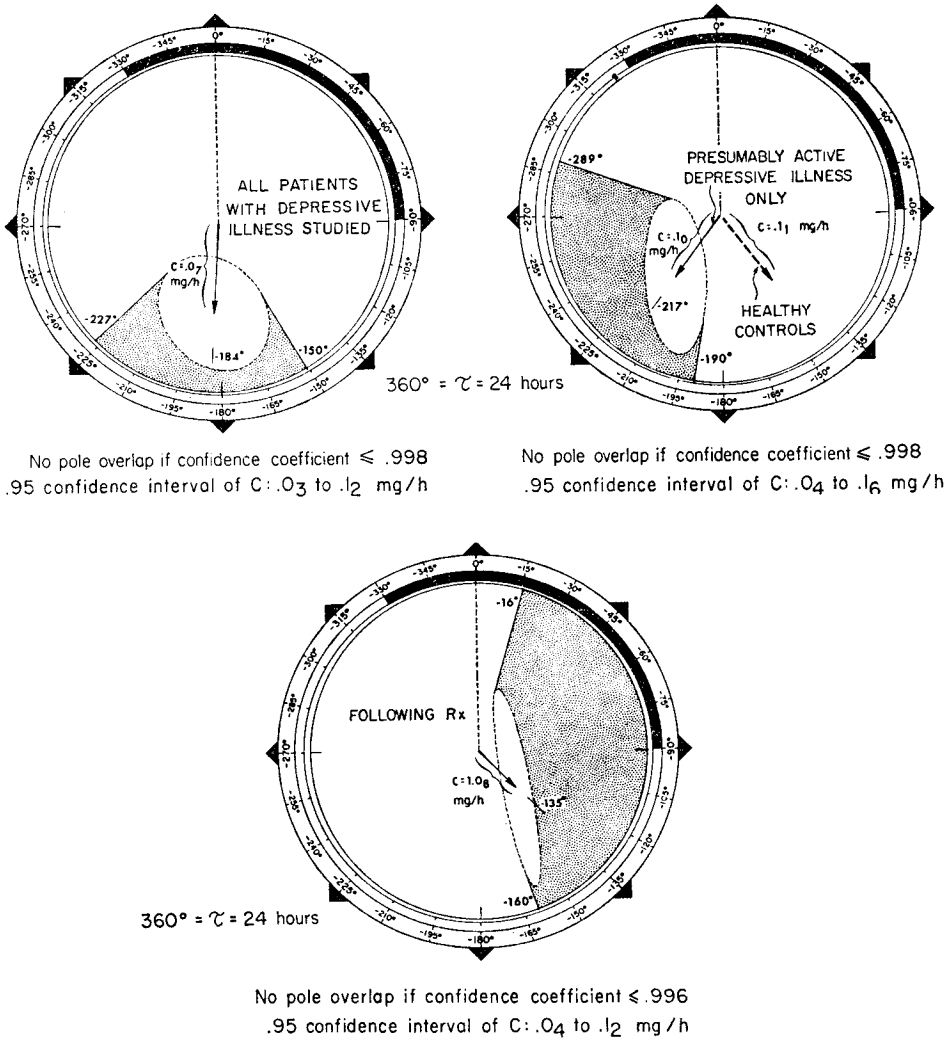


Fig. 3c. Possible circadian dyschronism of 17-ketosteroid excretion in untreated patients with depressive illness. Original data of M. Sakai (Yokohama Medical Bulletin II: 352–367, 1960). Synchronizer schedule extrapolated from timing of urine collection spans [cf. also 14 and 15].

et 2) en fonction de leur distribution dans le "temps biologique" déterminée par les mesures d'acrophase (sommet de la fonction périodique) (fig. 10). Dès lors, en augmentant le rapport: "activité thérapeutique/activité toxique" d'un médicament, la chronopharmacologie peut apporter des informations complémentaires dans le domaine de la pharmacologie classique (réduction des effets secondaires et meilleure tolérance, par exemple). En outre, dans la mesure ou une altération rythmique peut jouer un rôle déterminant dans une maladie (désyn-

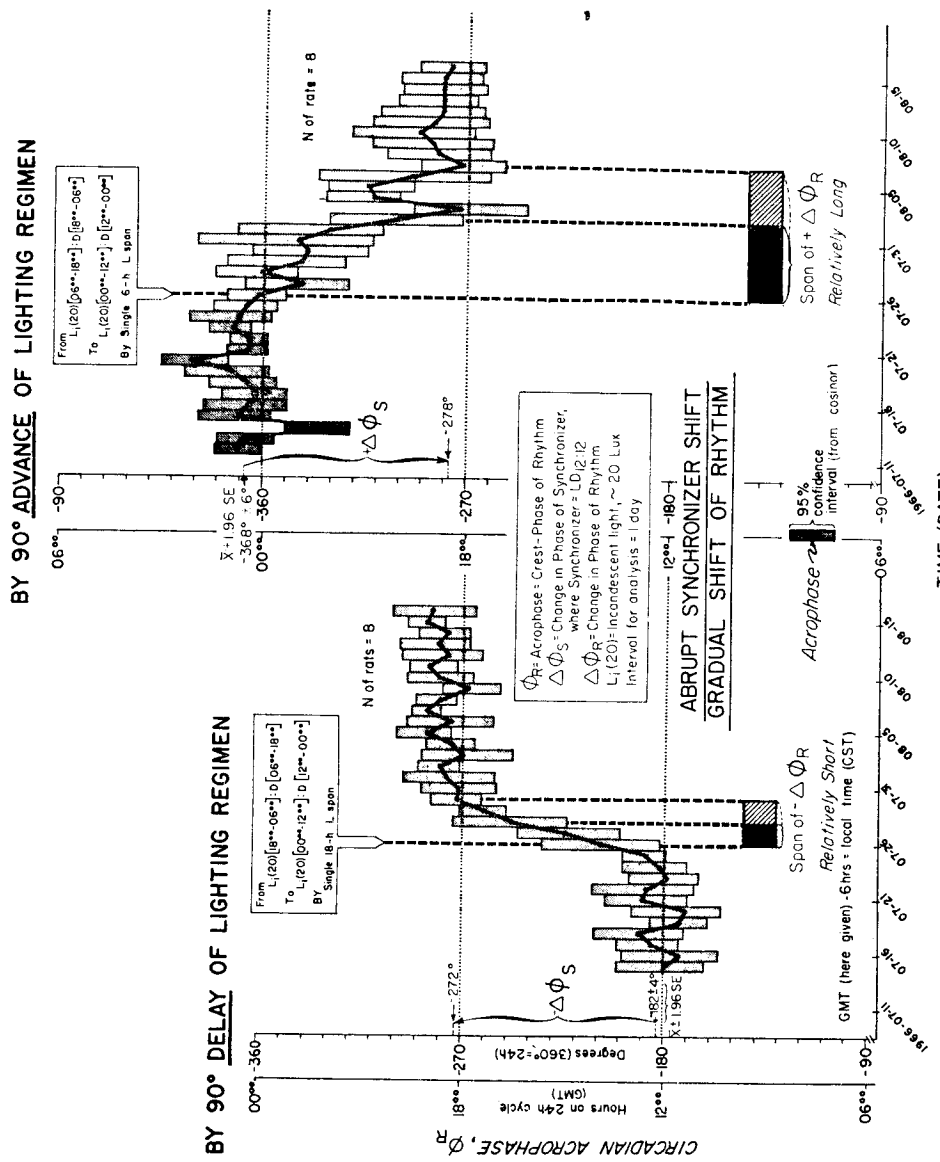


Fig. 4. "Polarity" in the circadian system of the rat—more rapidly delayed than advanced [cf 59–62]. Phase shifting of 24-hour-synchronized circadian rhythm in intraperitoneal temperature of female MSD rats [35, 36].

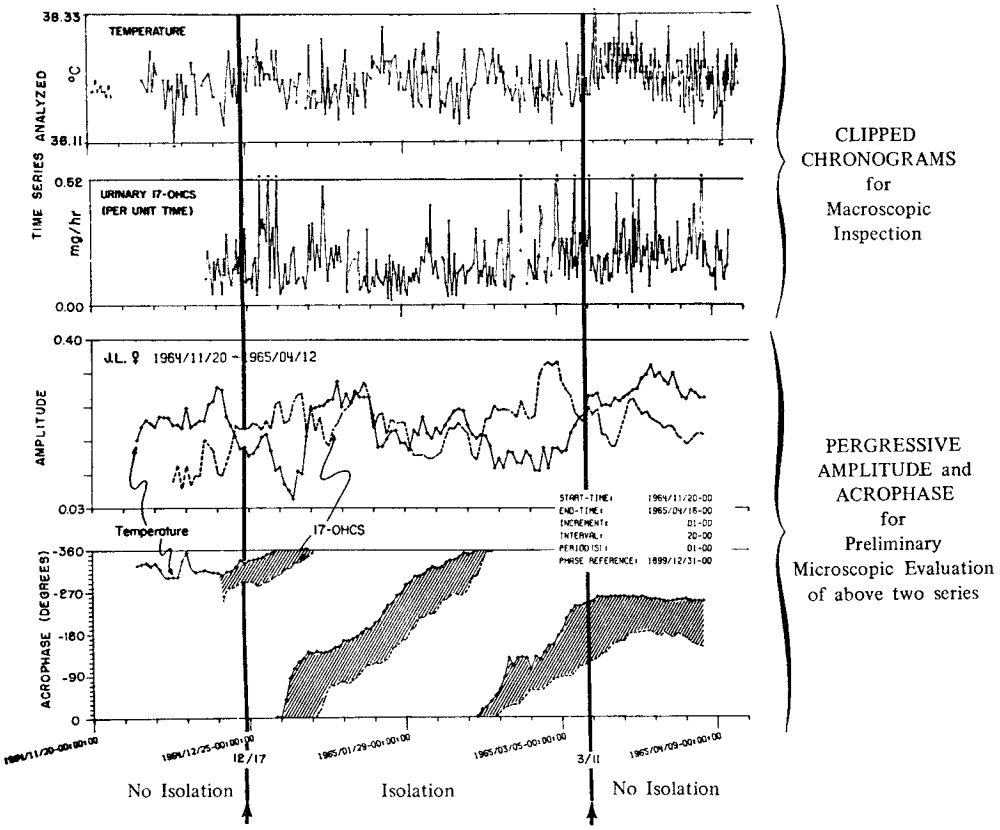


Fig. 5. Phase relations of circadian rhythms in the urinary excretion of 17-hydroxycorticosteroids and in rectal temperature of a woman during isolation in a cave and following resynchronization with a 24-hour-cyclic societal routine. Clipped chronogram of the time series shown on top. (For clipping, the mean and standard deviation were repeatedly computed and all values above and below the mean $\pm$ 3 standard deviations were repeatedly equated to the nearer of these limits until the result of this iterative procedure was no longer associated with a change in mean and standard deviation to the nearest four decimal places. Extreme values thus "clipped" are indicated by a dot on top of the corresponding chronogram).

A macroscopic inspection of these time plots is barely contributory. Long-period changes of some regularity—corresponding presumably to the menstrual cycle [69]—are apparent for rectal temperature in particular; changes with shorter period also are suggested by the record, yet it seems unjustified on the basis of inspection alone even to attempt to ascribe a precise period to a circadian rhythm and certainly it would be cumbersome, to say the least, to discuss the phase relations to each other of circadian components in the two time series.

By contrast, the display of acrophase in the bottom row—part of the microscopic approach—indicates first that the rhythms of both functions changed their period during isolation—only to be resynchronized with a 24-hour-cyclic routine thereafter; second, that the rectal temperature acrophase lagged behind that for 17-hydroxycorticosteroid excretion during isolation, as well as following resynchronization; and third, that resynchronization of body temperature occurred considerably faster than that of 17-hydroxycorticosteroid excretion. The latter finding may be related at least in part to the circumstance that the ϕ of rectal temperature was nearer its usual temporal placement in relation to the synchronizer than the ϕ of 17-OHCS, on the day of emergence from the cave.

From the pergressive amplitude diagram—third row from top—it can be seen that the amplitude, notably that of the rhythm in 17-OHCS excretion during isolation, showed no indication of damping as a conditioned reflex phenomenon might be anticipated to do. If there was a difference between the amplitude at the end of isolation and that upon resynchronization, this measure of the extent of circadian periodic change in 17-OHCS excretion indicated a more marked rhythm at the end of isolation than following resynchronization.

chronisation circadienne, par exemple), de nouvelles applications thérapeutiques peuvent être mises en évidence pour des drogues déjà connues (par exemple l'ACTH pour une resynchronisation) ou de nouvelles drogues peuvent faire l'objet d'applications intéressantes et, nous l'espérons, importantes.

d) *La chronotoxicologie* étudie les effets non désirés et/ou dangereux d'agents chimiques, physiques ou autres, y compris ceux de poisons, de substances polluantes ou contaminantes, et l'éventualité de surdosages de médicaments utiles. Ces effets sont étudiés: 1) en relation avec les altérations caractéristiques temporelles (fig. 11) et, 2) en fonction de leur distribution dans le temps biologique (figs. 12 et 13) [26–33].

Toute recherche dans l'une de ces branches entre dans le domaine de la chronobiologie, puisque la validité du travail ainsi réalisé dépend étroitement du degré de quantification des rythmes et des changements de phase lorsqu'on étudie les interactions et les interrelations de ces rythmes entre eux.

3. Analogie physique

Un spectre de rythmes physiologiques comportant des fréquences caractéristiques, différentes entre elles, peut être comparé au spectre des radiations électromagnétiques. Ce dernier fait apparaître la distribution de l'intensité spectrale des oscillations composantes pour différentes fréquences, comprenant, entre autres domaines, celui de la lumière visible. Les spectres physiologiques révèlent dans quelle mesure les "intensités" ou les amplitudes d'oscillations biologiques, pour différentes fréquences, contribuent à la variabilité globale rencontrée dans les mesures biologiques, effectuées en séries temporelles. Comme les spectres électromagnétiques, les spectres physiologiques couvrent de larges domaines de fréquences.

On peut pousser plus loin cette analogie: de même que la lumière blanche peut être perçue directement à l'œil nu lorsque l'intensité des fréquences composantes se situe au-dessus du seuil de la vision, de même par le report des données expérimentales dans l'échelle des temps, certains des rythmes biologiques, tels que certains rythmes circadiens, peuvent également être clairement discernés sans l'emploi de procédés spectraux—si et seulement si, ces rythmes sont suffisamment accentués.

Certains autres rythmes biologiques peuvent ne présenter qu'une faible amplitude, et—ce qui est souvent le cas—la contribution d'un rythme aux changements observés sur un diagramme, où les résultats expérimentaux sont simplement représentés en fonction du temps, peut être estompée par des interférences appelées "bruit"; de tels rythmes sont alors brouillés, ou même situés au-dessous du "seuil de visibilité", dans ce type de diagrammes—le chronogramme. Leur détection relève alors des techniques spéciales. Entre autres procédés, les spectres de variance, les spectres des moindres carrés et la méthode dénommée "cosinor" nous permet-

tent de discerner directement ces mêmes rythmes en dépit d'un rapport: "signal (rythme)/bruit" peu favorable, tout comme on peut discerner les diverses couleurs si l'on interpose un prisme dans un faisceau de lumière blanche. De telles comparaisons n'ont pas le dessein de signaler de quelconques interactions [65] entre les rythmes biologiques et les oscillations électromagnétiques, mais elles permettent de souligner la nécessité de méthodes spéciales si l'on désire identifier toutes ces oscillations [5, 10]; des objectifs chronobiologiques spécifiques [2] peuvent être atteints par ces méthodes.

4. L'Analyse de données chronobiologiques

Pour commencer par un exemple relativement simple, un certain nombre d'étapes ont conduit au développement de ce que l'on nomme maintenant la méthode cosinor (figs. 2–4, 7–9, 13, 14, 20) [5]. Par ce procédé, on arrive éventuellement à réaliser l'étalement, sous forme de représentation en coordonnées polaires, de l'amplitude (C) d'un rythme, de sa phase (Ø) et de l'ellipse d'erreur correspondante—puis à procéder ultérieurement au développement d'un "spectre cosinor". Du cosinor on peut tirer, par exemple, des renseignements relatifs à l'amplitude C et la phase Ø de certains rythmes de sujets en bonne santé (fig. 14) [13]. Par la même méthode, on peut voir apparaître également des altérations de C et/ou de Ø dépendant d'un état pathologique tel que par exemple un cancer [11] une psychose dépressive [14, 15], la maladie d'Addison (fig. 3a) et le syndrome de Cushing (fig. 3b) [16–18]. En effet, même un résultat négatif, par le cosinor, peut faire l'objet d'une constatation positive pour un rythme donné; autrement dit, il peut révéler un état pathologique (fig. 3). C'est ce fait qui a conduit au choix de l'analogie entre la "microscopie" de rythmes au moyen d'analyses faites par un ordinateur électronique et l'examen des tissus à l'aide d'un microscope qui peut révéler les altérations d'un tissu—modifications qui sont souvent plus fines et qui peuvent éventuellement se révéler plus importantes que ne l'est un effet terminal global, tel qu'un manque de cellules dans un tissu nécrotique.

En outre, de même que l'anatomopathologiste emploie plus d'une coloration et plus d'un outil (il peut même recourir, à l'occasion, à la microscopie électronique), le chronophysiologiste et chronopathologiste doivent employer plus d'une série de méthodes d'analyses par un ordinateur et plus d'un diagramme (tableau 2). A part la méthode du Cosinor, il doit appliquer des méthodes qui servent à détecter un changement de fréquence—tel que la désynchronisation d'un rythme circadien provoqué par la cécité—lorsque la période de ce rythme devient égale p. ex. à 23.5 heures au lieu de 24 heures (fig. 15) [21]. Il doit pouvoir disposer des instruments nécessaires à mesurer une transposition de variance, telle que celle qui intervient dans le développement normal de l'enfant (fig. 16) ou dans une réponse caractéristique de sujets adultes à des drogues telles que la réserpine [22].

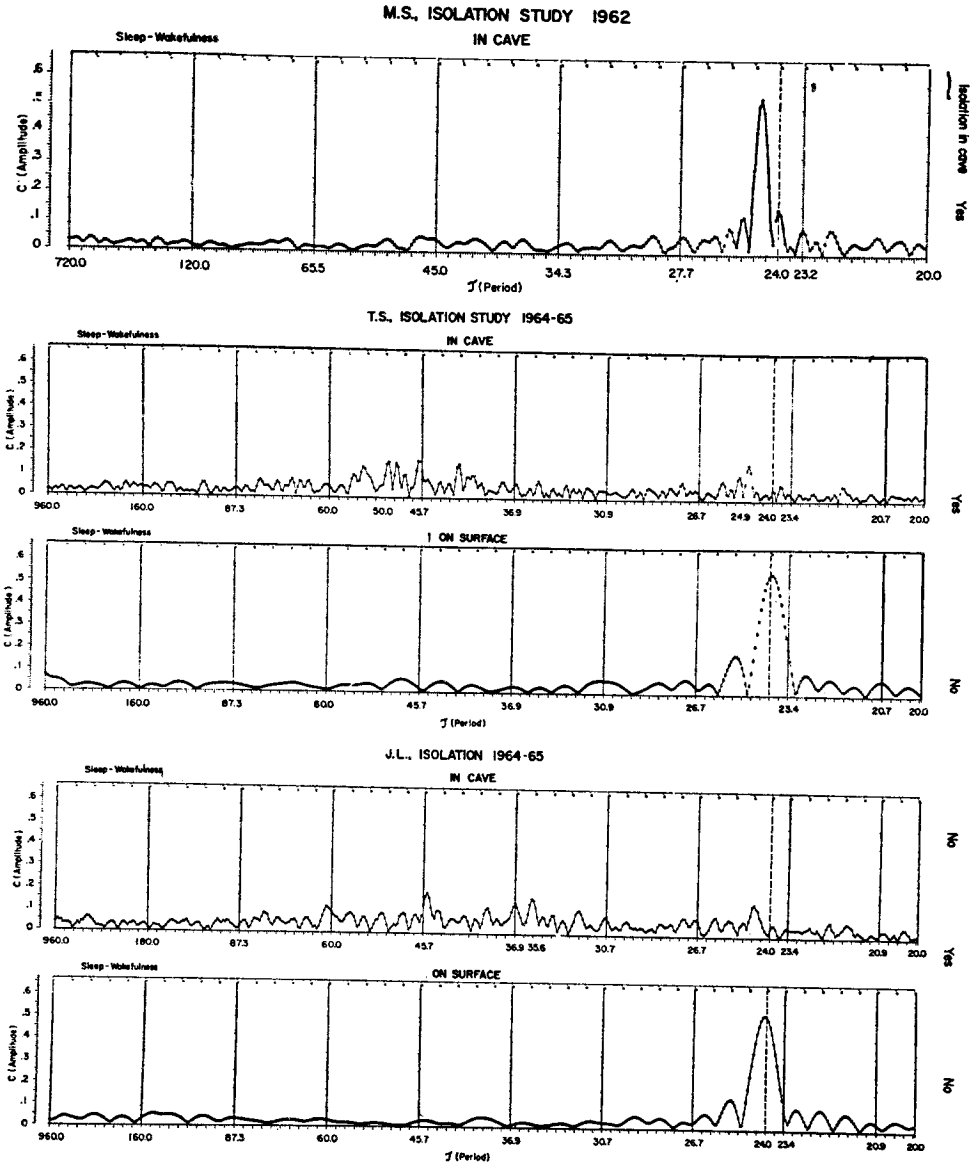


Fig. 6a. Quantification of circadian spectral components in sleep-wakefulness during isolation and upon return to a 24-hour-synchronized societal routine. Note decrease in amplitude of circadian component and appearance of ill-defined infradian components, described by Siffre *et al.* [24].

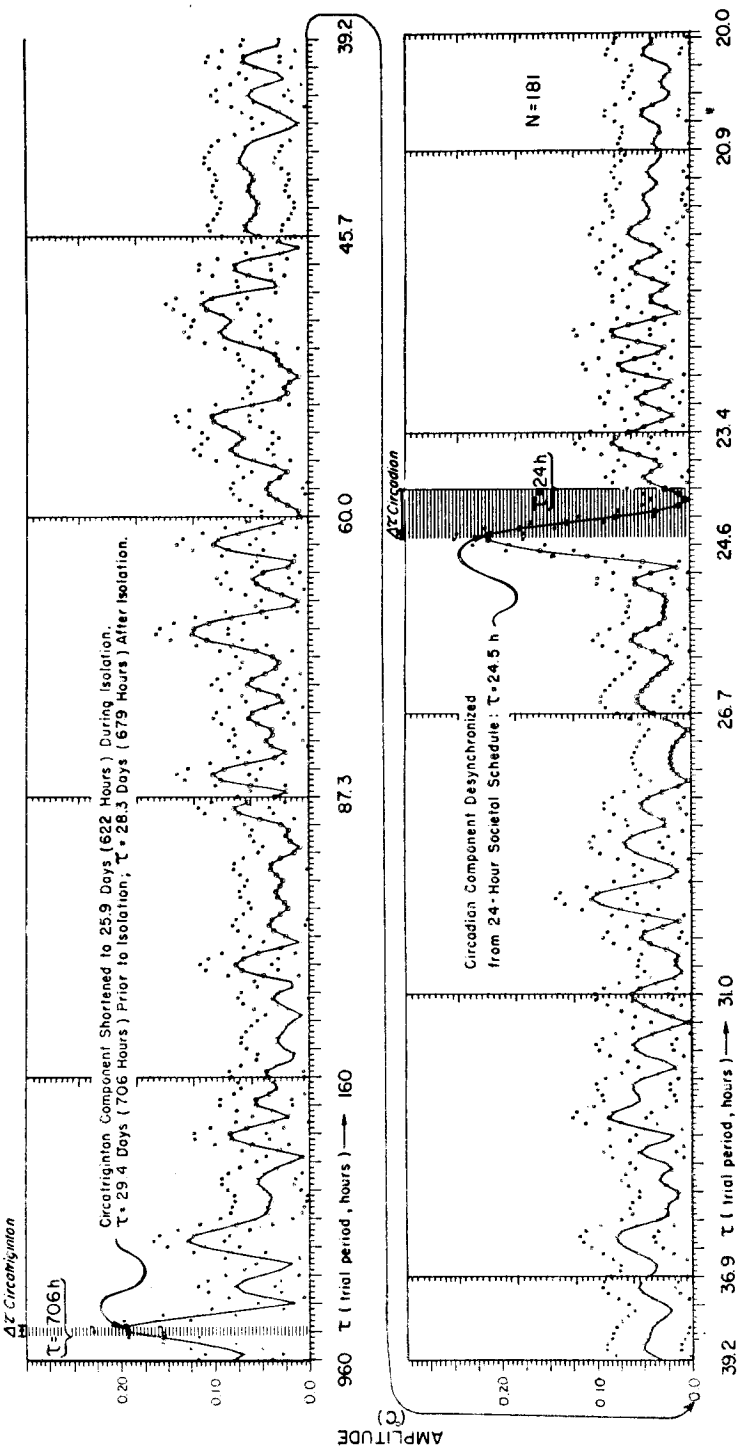


Fig. 6b. Shortening of circatrigintan component (corresponding to menstrual cycle) in rectal temperature series of a healthy woman—concomitantly with a lenthening of circadian component, during isolation in a cave [69]. (See ref. 74–78 for work on gross time relations of the circadian body core temperature rhythm to other variables.)

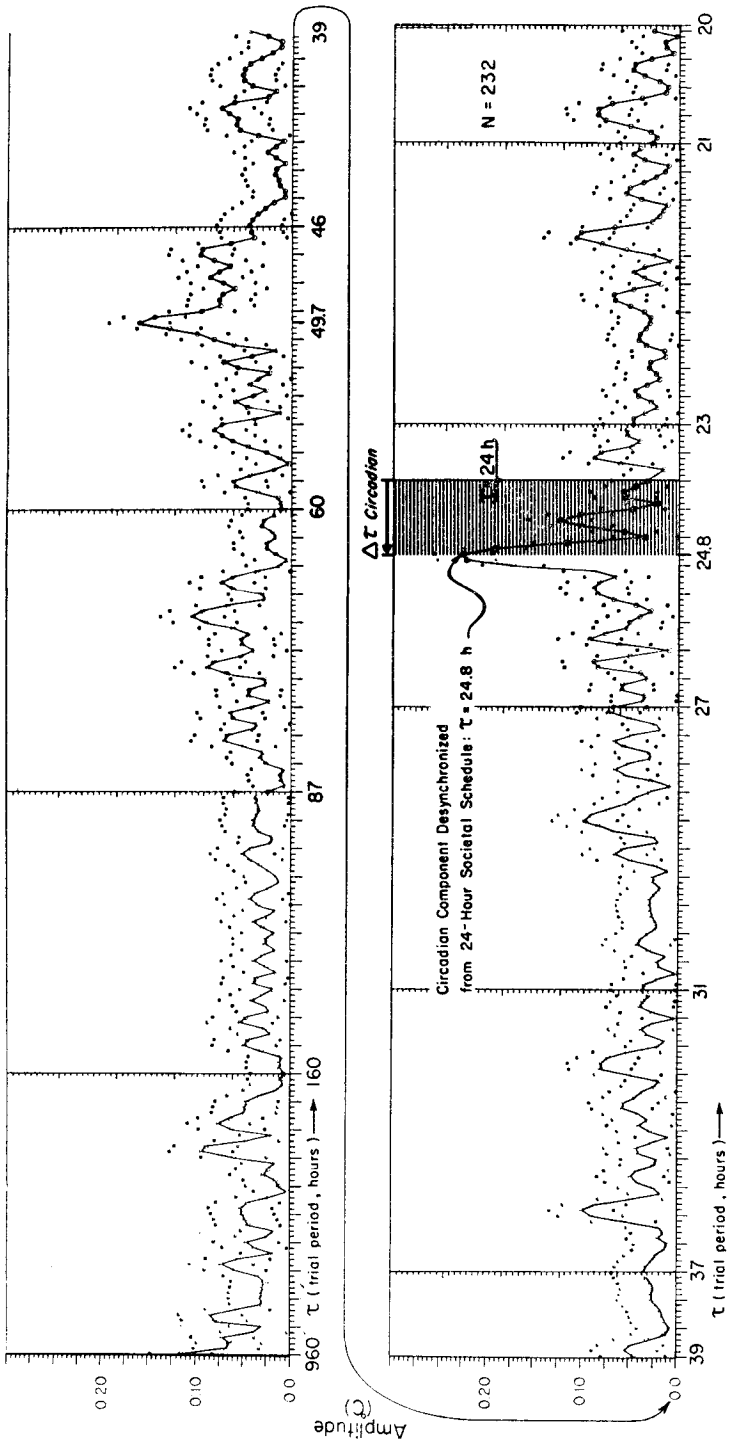


Fig. 6c. Circadian desynchronization with the appearance of an infradian component in the temperature series of a healthy man during isolation from known time cues.

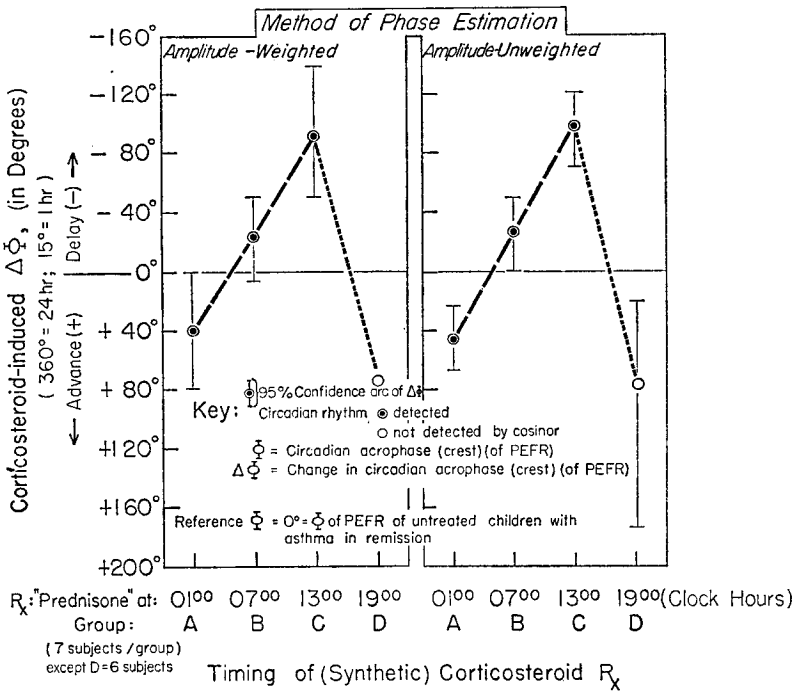


Fig. 7. Drug-induced phase shift of a circadian respiratory rhythm [cf. 25, 26]. Phase shift ($\Delta\Phi$) of peak expiratory flow rhythm (PEFR) as a function of timing of prolonged corticosteroid therapy in children with severe asthma.

En développant des telles méthodes "microscopiques", on dispose déjà des premiers moyens nécessaires à l'étude des informations relatives aux altérations de rythmes en tant qu'aspect de la chronopathologie. Un tel ensemble de techniques nous permet de donner des résultats plus complets que: "présence ou absence de rythme(s)", tout comme l'histologiste ne se contente pas exclusivement, ou même primordialement, de répondre à la question "présence ou absence de cellules". La signification possible, pour la chronopathologie, d'une constatation telle qu'une transposition de variance de la bande des fréquences circadiennes vers la bande des fréquences ultradiennes, dans le spectre d'une variable physiologique relative à une tumeur pourrait vraisemblablement être similaire à celle de la constatation d'une dédifférenciation dans le tissu cancéreux [11] dans le contexte de l'anatomopathologie microscopique classique.

Pour certaines variations rythmiques de l'Homme, telles que celles du rythme veille-sommeil, la composante ultradienne du spectre pourrait représenter un rythme plus "primitif" par rapport à sa composante circadienne, puisque la première apparaît plus tôt dans le développement néonatal et puisqu'on peut mettre en évidence, au cours du développement, un glissement de variance du

domaine ultradien vers le domaine circadien du rythme veille-sommeil dans les premiers mois de la vie. Cependant, il reste à savoir si de telles considérations sont susceptibles d'être généralisées à des fonctions cellulaires tels que les rythmes des mitoses [4] ou, comme l'a rapporté Pierre Passouant de Montpellier, à propos du sommeil "rapide" (REM) des narcoleptiques [12].

5. Le degré de généralité de la structure biologique temporelle

En désignant la totalité des changements réguliers et prévisibles dans le comportement des organismes, ou de leurs subdivisions anatomiques, physiologiques etc. . . . et, en complétant ainsi la structure spatiale classique, la structure temporelle peut-être caractérisée à tous les niveaux d'organisation—l'organisme *in toto*, les systèmes d'organes, les organes, les tissus et les cellules. L'organisation circadienne, discutée jusqu'à maintenant, en est un exemple démonstratif (fig. 17) [2]; de tels rythmes ont même déjà été mis en évidence dans les variations de processus intracellulaires. Il semble donc justifié de considérer la possibilité d'une structure biologique temporelle au niveau des interactions moléculaires et même au niveau des phénomènes atomiques, y compris les phénomènes "microscopiques", dans

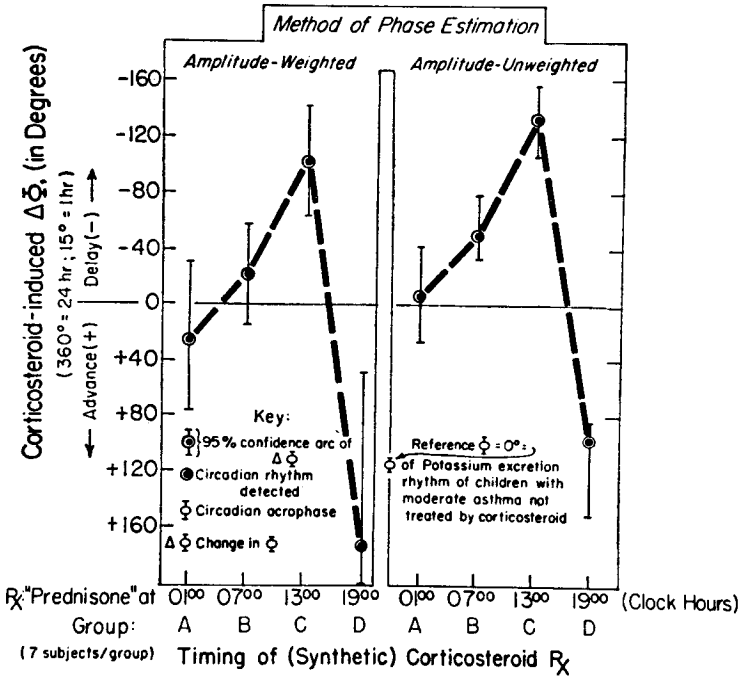


Fig. 8. Drug-induced phase shift of the circadian rhythm in urinary potassium excretion [cf. 25, 26].

le sens que la physique théorique donne à ce terme. C'est là un des buts des recherches à venir qu'il conviendrait de discuter, lors de cette conférence entre physiciens et biologistes. Mais pour le moment, le biologiste a le devoir de préciser les conditions d'application de la méthode chronobiologique.

Pour qu'un certain aspect temporel du comportement biologique, à tous les niveaux d'organisation, soit accepté comme élément de "structure temporelle", selon la description ci-dessus, il faut que l'on puisse montrer, objectivement et avec une chance d'erreur pouvant être estimée, que ce phénomène n'est pas dû au hasard.

En ne considérant qu'un seul individu, certains aspects de la structure biologique temporelle sont unidirectionnels et non-répétitifs, par exemple le développement, la croissance ou la sénescence de cet individu. D'autres aspects temporels, tels que la naissance et la mort, correspondent à des événements uniques—si de nouveau on se borne à ne considérer que la vie d'un seul organisme.

Si l'on adopte un point de vue plus général, c'est à dire si l'on passe du comportement de l'individu à celui d'une population, les événements auparavant uniques, comme la naissance ou la mort, deviennent, pour la population, des phénomènes rythmés [3]. On peut donc, plus généralement, parler de rythmes comme éléments principaux de la structure temporelle.

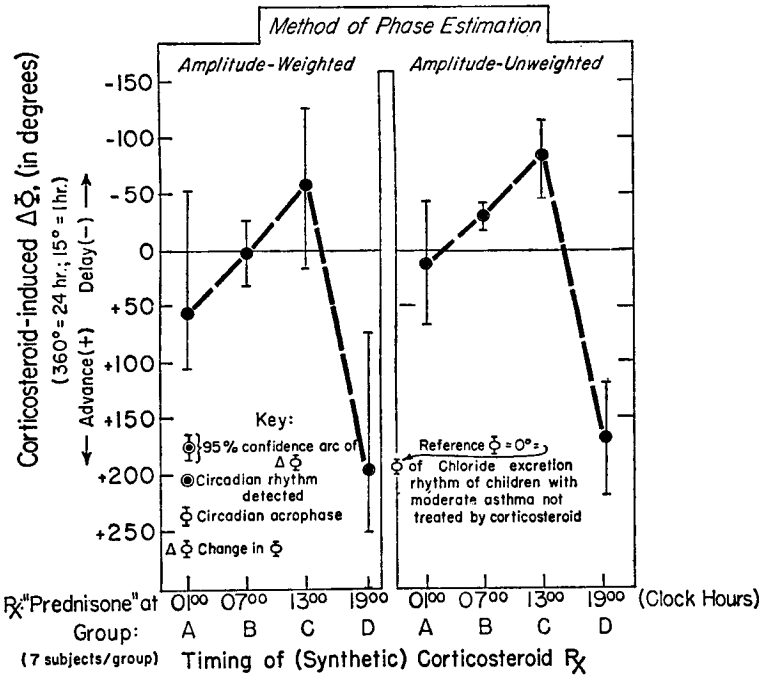


Fig. 9. Drug-induced phase shift of the circadian rhythm in urinary chloride excretion [cf. 25, 26].

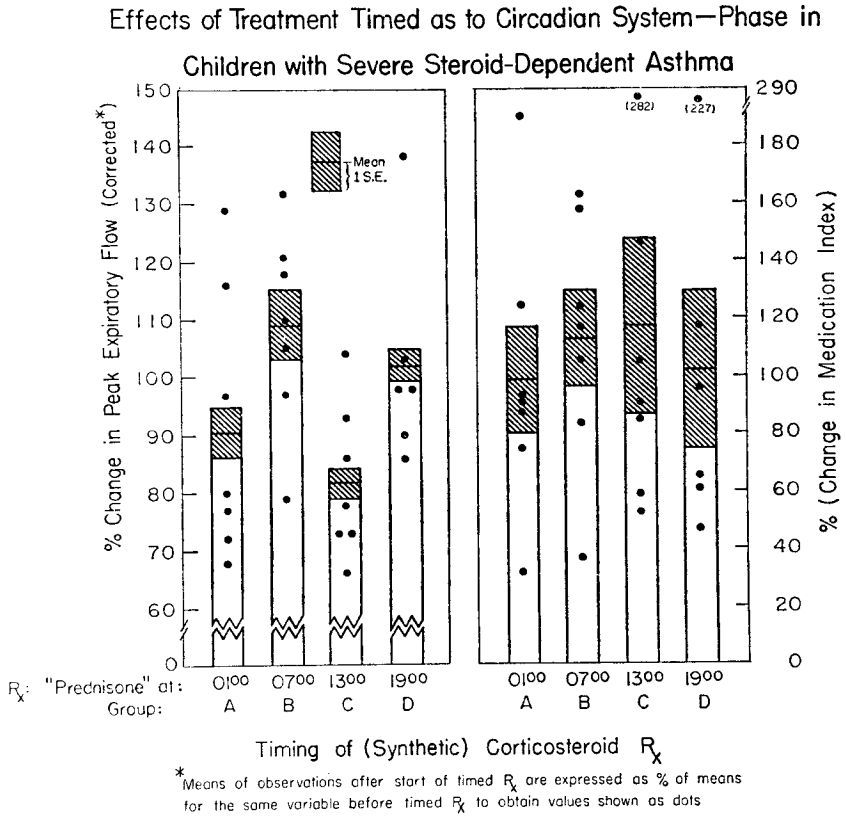


Fig. 10. Some preliminary chronopharmacologic findings suggesting but not proving the merits of corticosteroid therapy as a function of circadian system phase [cf. 25, 26].

Des orientations spéciales dans des disciplines telles que l'embryologie, la biologie du développement (au sens le plus large), aussi bien que la gériatrie, sont consacrées à l'étude de certains aspects de la structure biologique temporelle. Une grande variété de rythmes de haute fréquence, étudiés en physiologie classique,—*inter alia*, nerveuse, circulatoire, respiratoire—constitue d'autres aspects de cette structure biologique temporelle.

6. Aspects de basses et de moyennes fréquences dans l'étude des rythmes de haute fréquence

Les informations, dans le domaine des hautes fréquences biologiques, peuvent être obtenues à l'aide d'une instrumentation devenue d'usage courant en pratique médicale. Les applications cliniques quotidiennes de l'électrocardiographie et de l'électroencéphalographie sont devenues indispensables au diagnostic et au traite-

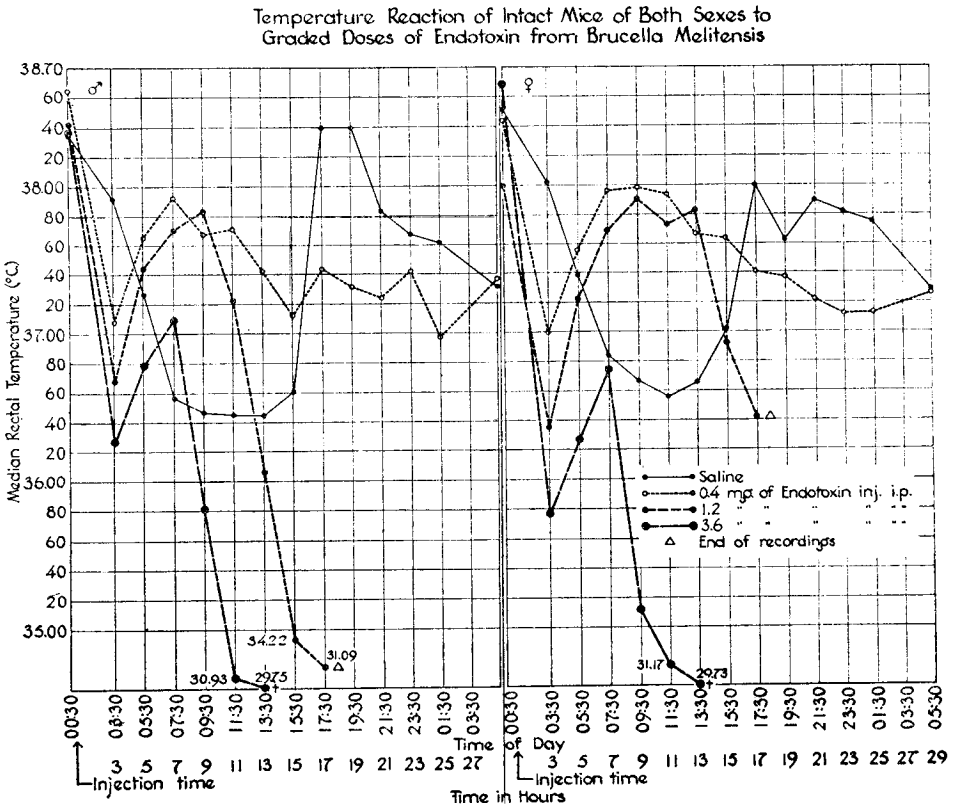


Fig. 11. Alteration of circadian temperature rhythm of intact ABC mice of two sexes following the administration of graded doses of *Brucella* endotoxin.

ment des malades souffrant d'affections cardiaques ou neurologiques. C'est donc le clinicien qui peut répondre d'une manière relativement satisfaisante à la question "*cui bono*", qui se rapporte à cet aspect de la structure biologique temporelle. Naturellement, il cherche à améliorer l'interprétation de ces rythmes "macroscopiquement" visibles dans un ECG ou EEG et, pour ce faire, il examine actuellement l'utilité potentielle d'analyses additionnelles réalisées à l'aide de programmes spéciaux établis pour les calculatrices électroniques [4, 5]. Si, dans la plupart des cas, de telles analyses sont limitées à des régions spectrales étroites de l'EEG ou de l'ECG usuel, cette limitation peut être levée par l'exploration concomitante de diverses composantes périodiques; c'est le cas des composantes de fréquence circadienne et de fréquence plus basse, qui se trouvent par exemple dans les diverses variations périodiques du pouls, de l'ECG ou de l'EEG superposées aux régions de fréquences ("spécifiques") habituellement examinées. De cette façon, on trouve dans l'EEG, en plus de ces fréquences "classiques" situées

dans les régions de 30 à 1,5 cycles par seconde, des composantes additionnelles ultradiennes, circadiennes et autres; (fig. 19) [6, 7].

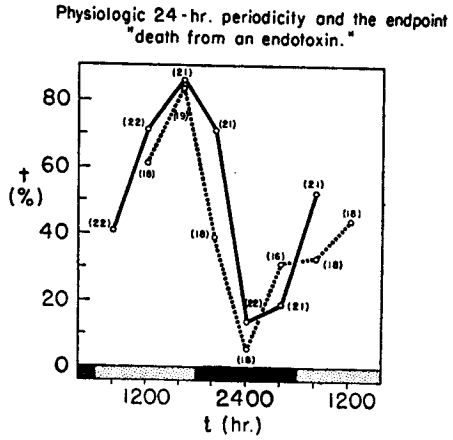


Fig. 12a. Circadian rhythm in susceptibility to *E. coli* endotoxin and its reproducibility in separate experiments.

Ordinate † (%) { % death of group of *standardized* mature C mice from *E. coli* lipopolysaccharide (Difco, 100 μg./20 gm., i.p.).

Abscissa t (hr.) { times of injection, in 2 experiments (injections begun at 2 different time points, during daily light period).

N of mice / group in parentheses.

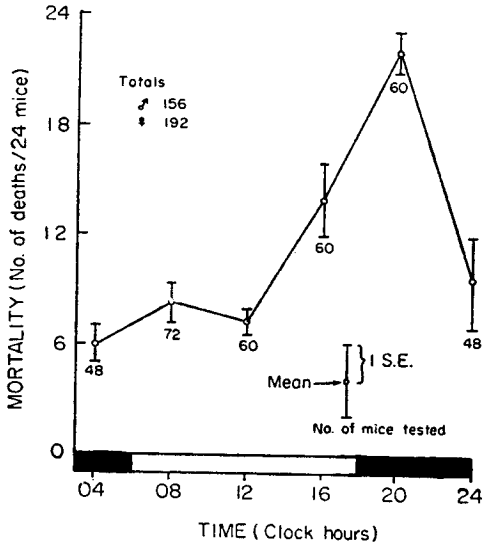


Fig. 12b. Circadian rhythm in susceptibility to ethanol [cf. also 32].

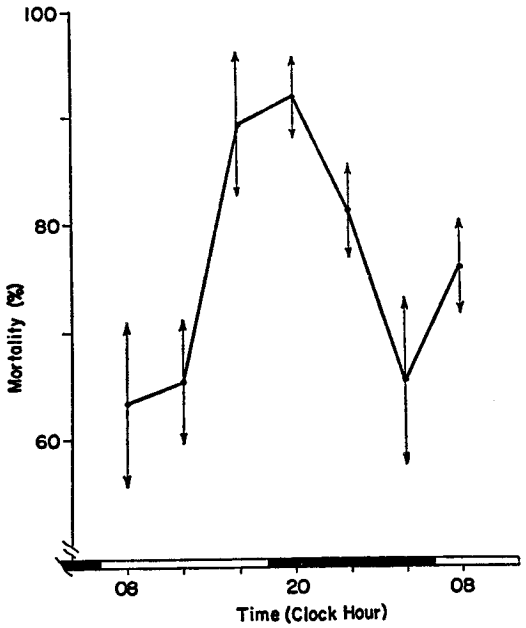


Fig. 12c. Mortality from acetylcholine also is circadian system phase dependent. Circadian susceptibility – resistance cycle to acetylcholine. B_6 male mice, about 1 yr. of age. (Pool of results with 3 dose levels (4.0, 4.2 and 4.4 mg/20 gm body wt.) at 4 hr. intervals for 48 hrs. $N > 45$/time point).

7. Détecteurs physiologiques pour l'étude des rythmes de fréquences moyennes et basses

En plus de ces équipements désormais courants, une variété d'instruments complémentaires modernes nous a été fournie par la nécessité d'une recherche biologique scientifique adaptée aux conditions particulières de l'espace extra-terrestre. Ce sont d'abord les "détecteurs physiologiques" que l'on appelle "transensors" aux U.S.A. Ces sondes "sensibles" sont liées à des radio-émetteurs, l'ensemble étant miniaturisé, ce qui facilite beaucoup l'étude de toutes les fonctions rythmées, quelle que soit leur fréquence. Mais pour analyser des rythmes à fréquences moyennes et basses, ces mêmes "détecteurs physiologiques" deviennent presque indispensables. Il en est ainsi pour l'étude dite longitudinale des divers aspects d'une structure biologique temporelle, c'est à dire, pour les collectes des séries de mesures continues ou à intervalles fréquents, pour un individu donné et pour des espaces de temps beaucoup plus longs que les périodes des rythmes évalués. Il convient d'ajouter qu'actuellement la collecte des données expérimentales, par des moyens classiques, demeure encore souvent le procédé de beaucoup le plus sûr et pour cela il est plus raisonnable de l'utiliser. Certaines méthodes

classiques de collecte de données physiologiques en série sont aussi "meilleures" que les méthodes de collecte par télémétrie et, souvent, elles sont aussi sensibles et même plus sensibles que ces dernières.

Néanmoins, un avantage très important de ces microsondes sensibles, télémétrant des mesures physiologiques, est que leur utilisation permet souvent de réduire, sinon d'éliminer, l'interférence du procédé de mesure avec les comportements du sujet étudié, notamment les changements de son mode de vie, et les interruptions de son sommeil en particulier. Par exemple, on peut télémétrer les séries de valeurs de la température centrale d'un organisme libre dans ses mouvements, c'est à dire, non perturbé par la présence d'un harnais portant des électrodes et des fils. En outre, les chercheurs eux-mêmes et tous leurs assistants, ne sont plus strictement liés à un laboratoire même si on a besoin de mesures individuelles à des horaires déterminés (fig. 4).

Signalons que le transfert des mesures biologiques télémétrées peut se faire directement de l'instrument récepteur à la calculatrice utilisée pour leur analyse, grâce à l'intervention d'un second type d'instruments et/ou de programmes modernes.

8. "Surveillance en marche" (analyses "as you go")

La troisième aide méthodologique moderne repose sur l'emploi d'une série de programmes spéciaux destinée, d'une part, à l'analyse objective et rapide

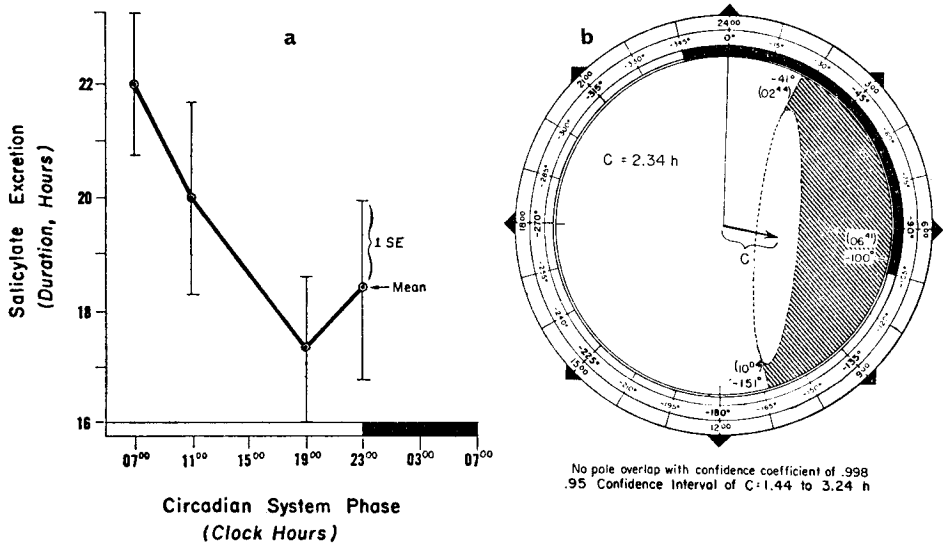


Fig. 13. Even the duration of salicylate excretion depends on the time when the drug is administered. a: Time plot of data (analyzed by cosinor); b: Cosinor summary of circadian rhythm in duration (hours) of salicylate excretion of healthy mature human beings receiving a standard dose of the drug at several different times along the 24-h scale [34].

par des calculatrices électroniques de données télémétrées, et d'autre part, à l'étalement graphique des séries de résultats fournis. Ces programmes représentent un progrès majeur dans l'étude de la structure temporelle. Leur valeur a été comparée d'ailleurs à celle du microscope dans l'étude de structures cellulaires [4]. L'étalement graphique des analyses, sous forme de spectres, de cosinors, etc.... peut être réalisé, à partir de mesures effectuées à des intervalles de temps prédéterminés, d'une façon telle que les résultats des analyses constituent un "moniteur automatique" des fonctions physiologiques étudiées. (figs. 4 et 18) [23, 24]. On peut aussi prévoir des analyses destinées à la recherche, aussi bien qu'à une application médicale, telles que les résultats étalés fournissent, en temps utile, des "ordres" correspondant à la nécessité d'un changement du milieu ou à l'administration de certains traitements, etc....

De cette façon et au moment désiré par l'expérimentateur—à certains temps choisis a priori ou qui se sont révélés être intéressants sur la base de critères vérifiés par l'application des programmes destinés à l'analyse séquentielle ("surveillance en marche" (as you go))—on parvient à des interprétations faciles et objectives et aux décisions expérimentales ou thérapeutiques qui sont, pour cette raison, utiles. Cette combinaison d'instruments pour 1) la télémétrie, 2) le transfert automatique des données, 3) les analyses électroniques immédiates et 4) l'étalement séquentiel des résultats correspond à la double nécessité d'avoir un moniteur à décision automatique et une aide diagnostique. Grâce à ces moyens, un progrès méthodologique a pu être réalisé dans l'étude des rythmes de fréquence moyenne, exactement comme on commence déjà à utiliser ces mêmes techniques pour la surveillance des cardiaques menacés de défaillance. Par l'emploi de tels moyens, la détection et la quantification des éléments rythmés, de structure temporelle pour plusieurs fréquences ont déjà été possible, pour plusieurs fonctions physiologiques—résultat qui nous amène à la conception d'un spectre étendu de rythmes qui va, d'une part, de la fréquence de mille cycles par seconde, dans l'activité électrique des gymnotidés, jusqu'à, d'autre part, un cycle couvrant plusieurs années tel qu'on le rencontre dans des populations d'organismes (fig. 19).

9. Échantillonnage des séries d'informations temporelles; ergodicité

Actuellement, l'échantillonnage longitudinal n'est pas toujours possible, ou immédiatement réalisable, notamment pour les rythmes de basse fréquence—à part quelques exceptions d'une valeur inestimable (fig. 19b). *L'échantillonage longitudinal* porte sur les résultats obtenus chez un sujet pendant un espace de temps, T, beaucoup plus long que la période τ du rythme étudié. L'échantillonage *transversal* porte sur des résultats obtenus, à partir d'un groupe de sujets aussi homogène que possible, pendant un espace de temps relativement court ($T \leqslant 2\tau$). Ce schéma expérimental, très largement utilisé, peut servir à caractériser un rythme circadien ou un système de rythmes circadiens, pourvu que les sujets soient

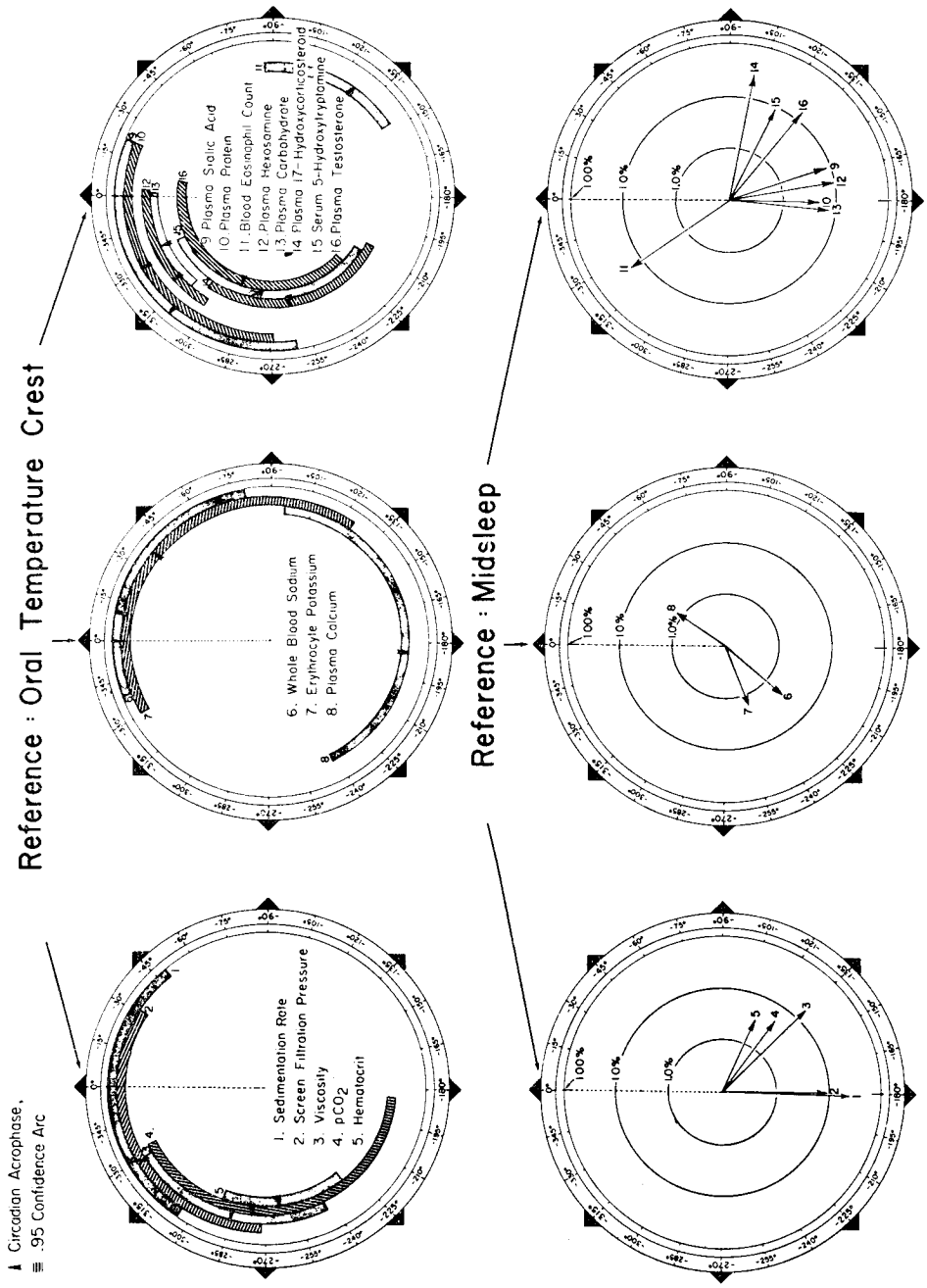


Fig. 14a. Rhythms in man's "milieu interieur constant". Cosinor summaries of circadian rhythms in human blood.

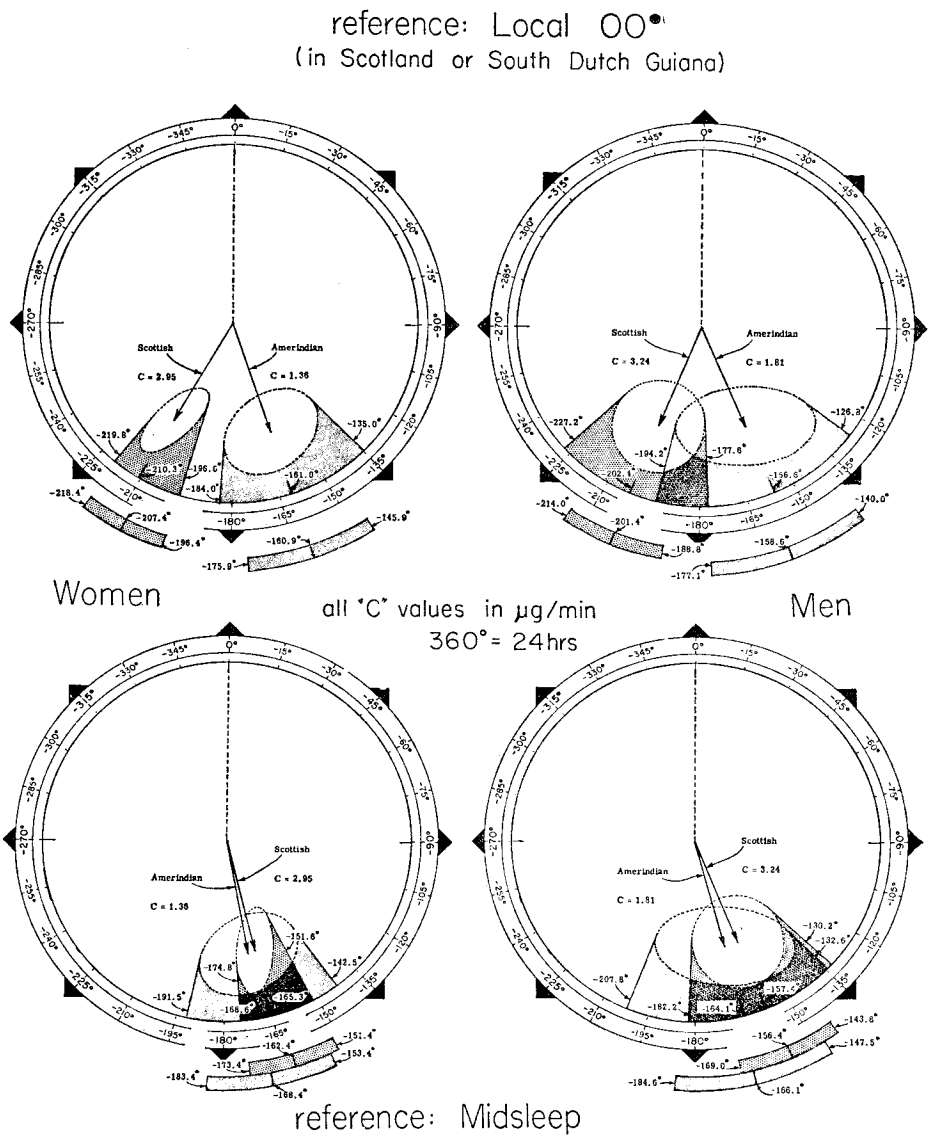


Fig. 14b. CIRCADIAN ACROPHASE (AVERAGE CREST TIME OF RHYTHM), ϕ, OF 17-HYDROXY-CORTICOSTERIOD EXCRETION BY MEN AND WOMEN FROM SCOTLAND AND SOUTH DUTCH GUIANA (AMERINDIANS); SUMMARIES OF EIGHT COSINORS PROVIDING IN CIRCULAR DISPLAYS AN AMPLITUDE (C)-WEIGHTED ϕ AND OF EIGHT ϕ-ESTIMATIONS, DONE WITHOUT C-WEIGHTING, SHOWN AS ARCS OUTSIDE THE CIRCLES. LOCAL MIDNIGHT, 00°°, IS USED AS PHASE REFERENCE FOR DISPLAYS IN THE TOP HALF OF THE FIGURE; MIDSLEEP SERVES AS PHASE REFERENCE FOR DISPLAYS AT THE BOTTOM.

In a cosinor display, ϕ is the angle formed by a vector with the phase reference shown as 0°. The amplitude, C, is usually shown by the length of a vector. In the figure, precise values for C

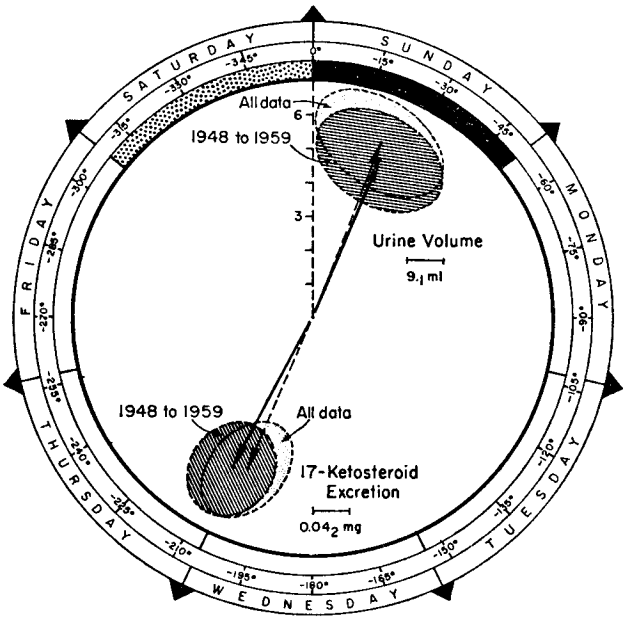


Fig. 14c. Cosinor summary of the circaseptan rhythms in 17-ketosteroid excretion and urine volume of a healthy man. Rhythms in both variables were synchronized with societal 7-day routine during all of the time span analyzed (urine volume) or during most of that span (17-ketosteroid).

Fig. 14b (*continued*).

are given next to each vector but intergroup differences in C are ignored in order to facilitate a comparison of circadian acrophases. Radii drawn tangent to an error ellipse—representing a 95% confidence region for the C-weighted ϕ—delineate the 95% confidence arc for the acrophase. Intersects of the same ellipse with the amplitude vector and with a prolongation of this vector provide the confidence interval for amplitude. Outside the circular cosinor displays, C-unweighted ϕ estimates also are shown as lines in the center of the corresponding shaded 95% confidence arcs.

When local midnight is used as phase reference, the ϕ's of rhythm computed (with C-weighting) by cosinor or without C-weighting for Scottish and Amerindian women are different. The 95% confidence arcs (upper left) do not overlap. The C-unweighted ϕ estimates suggest an intergroup difference for groups of men as well as for women from the two populations—so long as local 00°° is used as ϕ reference (upper half of figure).

In the lower half of the figure, midsleep is used as phase reference. All acrophases, whether or not computed with C-weighting, are very similar and their 95% confidence arcs overlap. An adjustment in phase reference thus not only eliminates a spurious intergroup difference but also provides an objective, quantitative index of a rhythm's timing in different populations. A statistically significant difference in the distribution of the acrophases from the two populations was not detected by an F-test; this result does not indicate of course that no such difference existed.

effectivement synchronisés de manière similaire. L'échantillonage *hybride* intéresse un nombre inférieur de sujets et une durée d'observation supérieure à ceux d'un échantillonage transversal.

En prenant comme exemple le rythme de la température corporelle de l'Homme, l'estimation de l'acrophase circadienne (Φ) et de ses limites de confiance pour une sécurité de 95 p. 100 (exprimées en degrés, par un angle de phase compté à partir du milieu de l'espace de temps correspondant au sommeil) donne les résultats suivants: –200° (limites de –186° à –216°) dans une étude longitudinale portant sur un même sujet pendant 34 jours; –210° (limites de –188° à –252°) dans une étude hybride: 4 sujets étudiés chacun pendant 3 jours; et –199° (limites de –181° à –220°) dans une étude transversale de 11 sujets étudiés chacun pendant 24 heures.

10. Conclusions

Afin de mieux définir les variations physiologiques d'un sujet sain, ou pathologiques d'un malade, on est maintenant en mesure d'apprécier la structure temporelle aussi bien que la structure spatiale à plusieurs niveaux de résolution. Que l'on ait à faire au résultat microscopique d'une structure histologique ou à

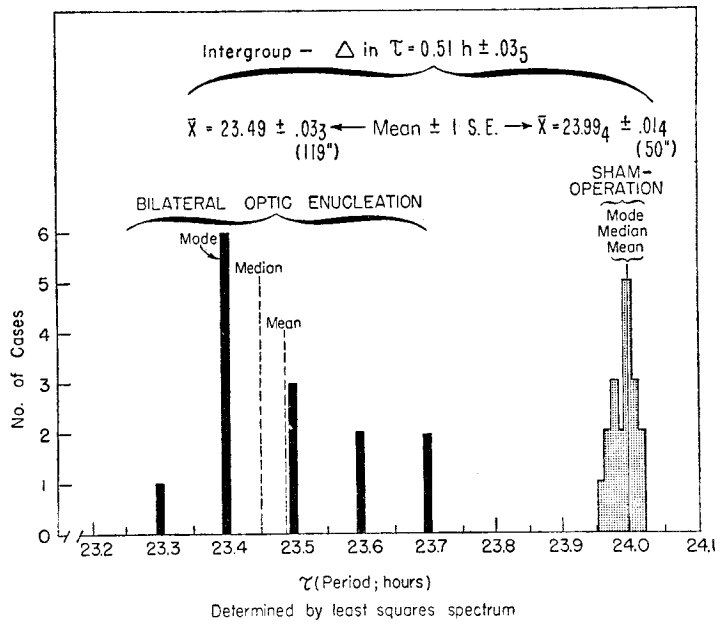


Fig. 15. Summary of circadian period estimates on two groups of mice, originally described at the 4th Conference of the International Society for the Study of Biological Rhythms held in Basel, Switzerland, September 18–19, 1953 [cf. 20].

la résolution par l'ordinateur de la structure spectrale (rythmes), les constatations "macroscopiques" et "microscopiques" peuvent être intégrées afin d'obtenir des informations plus complètes. Le fait important demeure que cela peut être réalisé sans nécessairement mettre sur le même plan ces constatations elles-mêmes, lorsqu'elles sont faites à deux niveaux différents de résolution. L'utilisation de l'œil nu répond à une nécessité importante et le recours à la méthode microscopi-

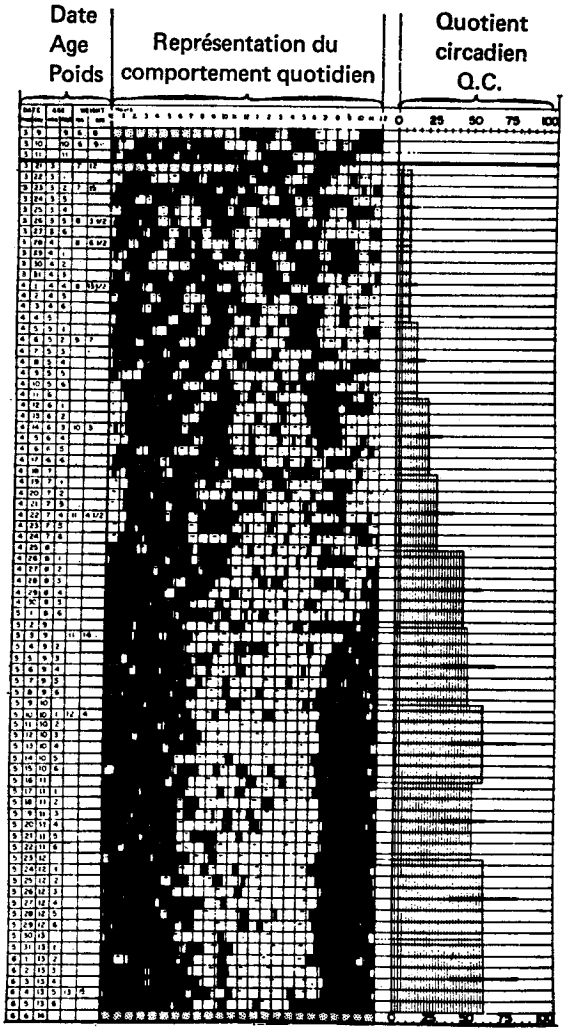


Fig. 16. Behaviour-Day Chart of a healthy male infant, raised on "self-demand", visualizing problems encountered in quantifying certain rhythms and near-rhythms. Circadian quotient gauges in this case the extent of prominence of the circadian rhythm as a function of age.

■: Sommeil; □: Veille; —: Alimentation;
harcelé: Q.C.; ——: limites de confiance de Q.C.

que par l'ordinateur à un dessein complémentaire—le résultat "microscopique" étant souvent plus satisfaisant en pathologie morphologique classique spatiale et, peut-être, dans un proche avenir, en chronopathologie.

D'un point de vue historique, l'importance "de la variabilité des conditions organiques" a été reconnue par Claude Bernard [38]:

> ". . . Pour le moment, je veux uniquement appeler l'attention des expérimenta-
> "teurs sur l'importance qu'il y a *à préciser les conditions organiques* (sic), parce
> "qu'elles sont, ainsi que je l'ai déjà dit, la seule base de la physiologie et de la
> "médecine expérimentale. Il me suffira, dans ce qui va suivre, de me borner
> "à des indications, car c'est à propos de chaque expérience en particulier qu'il
> "s'agira ensuite d'examiner ces conditions, aux trois points de vue physiologique,
> "pathologique et thérapeutique" . . .

Claude Bernard, qui sur ses vieux jours, décrivait la constance du milieu intérieur, a aussi écrit, en pleine maturité scientifique qu'il y a des conditions physiologiques "dans lesquelles il y a *toujours* du sucre et d'autres conditions dans lesquelles il n'y en a *jamais*" [dans le foie] [37].

Par cette remarque expérimentale, Claude Bernard a souligné l'importance d'une structure temporelle. Les facteurs déterminants de cette variabilité ont fait récemment l'objet des mises au point, des revues et des travaux originaux nombreux [39–63].

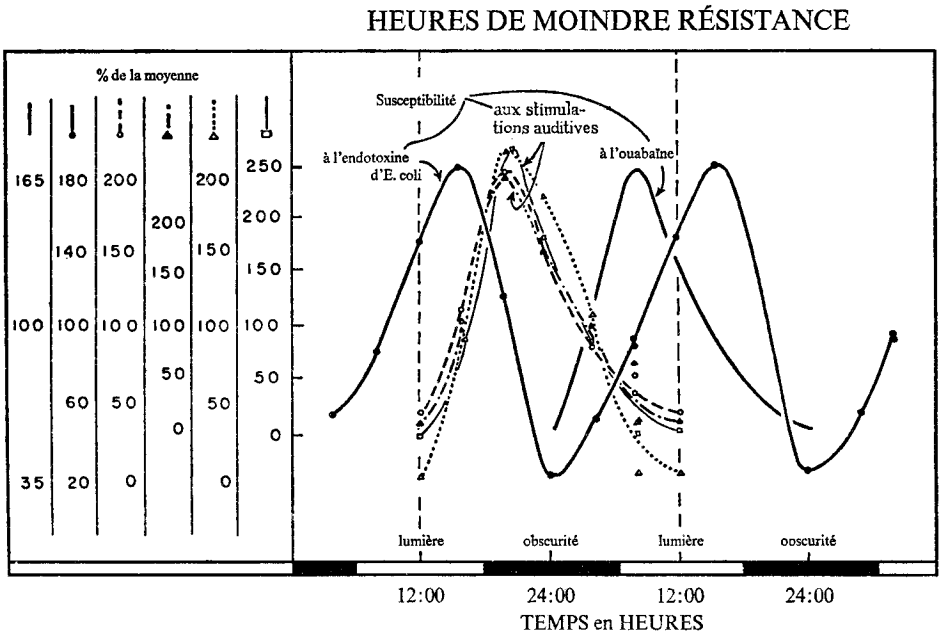


Fig. 17a. Hours of changing resistance [2].

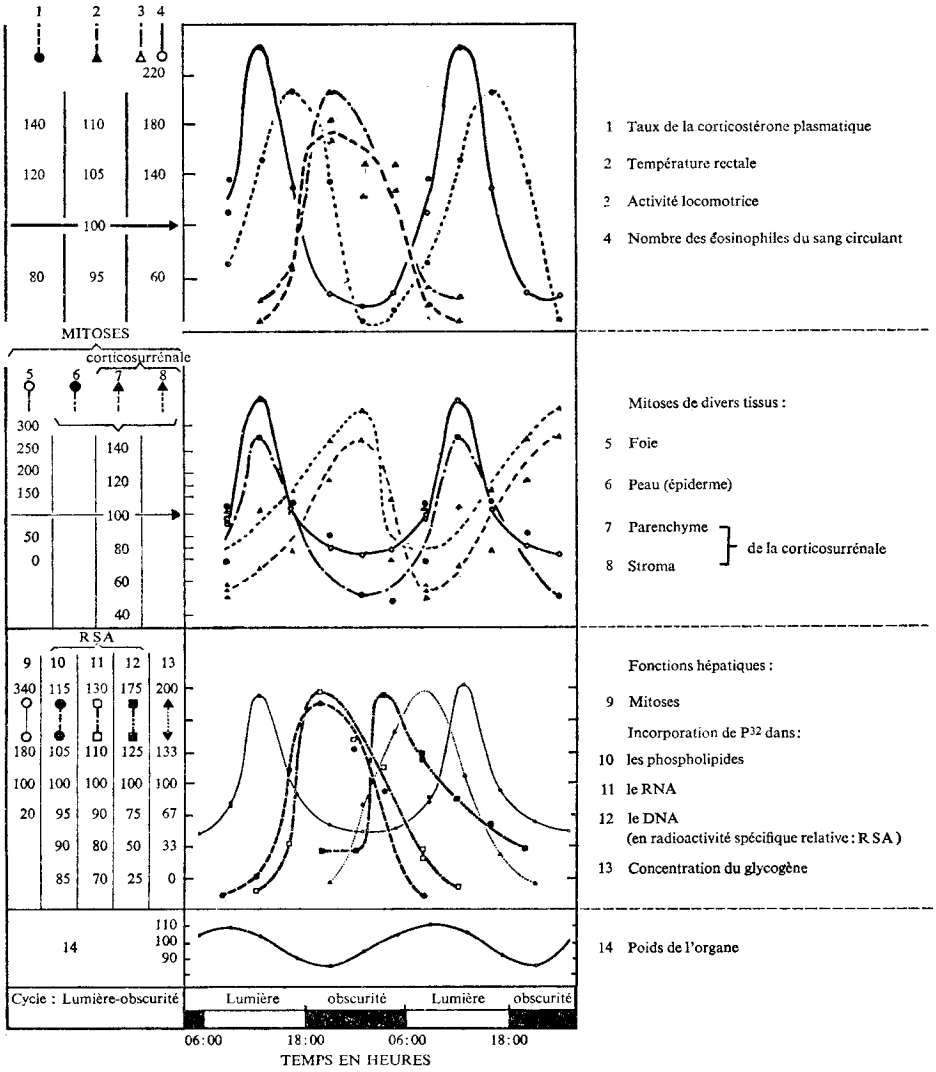


Fig. 17b. Circadian rhythms at different levels of organization [2].

En ce qui concerne la physiologie humaine, les informations récentes, peut-être les plus intéressantes, ont été apportées par Alain Reinberg *et al.* à propos des "heures de moindre résistance humaine". Par ailleurs, l'équipe composée d'Alain Reinberg, de Jean Ghata, de Michel Siffre et de nous-mêmes a mis en évidence la persistance des rythmes endocriniens, de fréquence moyenne, chez l'Homme adulte sain, pendant l'isolement souterrain prolongé, sans synchroniseurs connus. Ces résultats sur l'Homme peuvent être comparés à ceux d'expériences de désynchronisation externe réalisée chez des Souris, (fig. 20). Chez l'Homme, comme

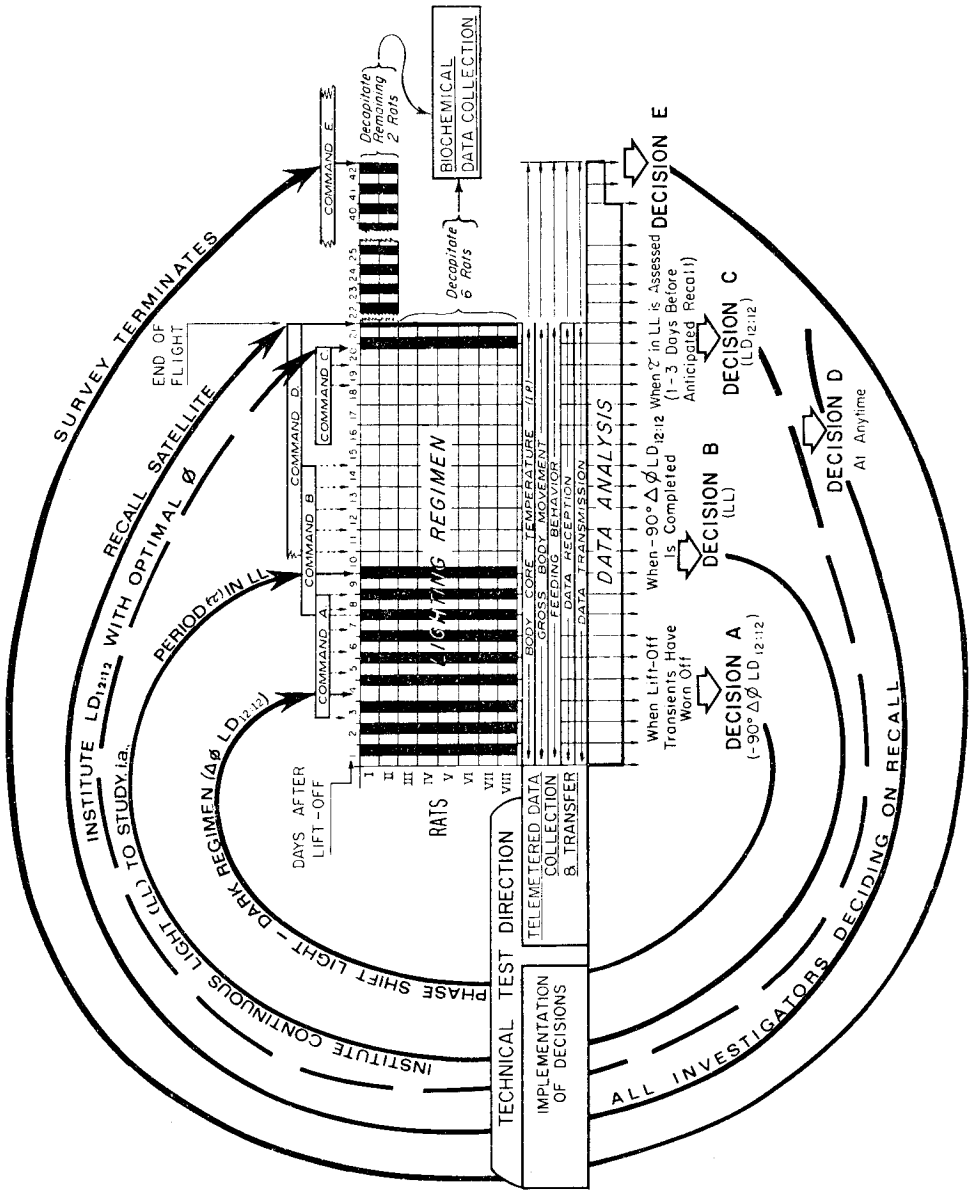


Fig. 18. Sequential decisions originally intended for as-you-go analyses during a Biosatellite survey of rhythms. The same as-you-go analyses may be useful for assessing rhythm characteristics in experimental work on Earth and conceivably in the clinic. Schedule developed in cooperation with Dr. Walter Nelson and Dr. Walter Runge of the Chronobiology Laboratories, Department of Pathology, University of Minnesota.

chez la Souris, on trouve que la distribution des temps internes de certains rythmes désynchronisés correspond à ce qu'on rencontre lors d'un état de synchronisation par un cycle social de 24 heures. (Chez l'Homme, les contraintes horaires imposées par sa vie sociale constituent les éléments du synchroniseur le plus puissant).

D'un autre côté, c'est grâce aux recherches des écoles des Professeurs J. Benoit et I. Assenmacher que le cycle circannuel du diamètre testiculaire du Canard et sa persistance pendant plusieurs années dans l'obscurité complète (sans synchroniseur connu) a pu être mis en évidence. Dans ce climat français de physiologie spectrale, j'espère que j'aurai, quant à moi, dans un avenir prochain comme par le passé, beaucoup de nouvelles choses à apprendre. Je remercie tous ces amis pour l'occasion qui m'a été donnée de coopérer avec eux.

11. Spéculations

Certains aspects de la structure biologique temporelle représentent des adaptations évolutives des organismes aux variations cycliques prédominantes de leur milieu (structure temporelle de l'environnement) plutôt que, seulement, des adaptations "personnelles" réacquises (p. ex. "apprises") par chaque individu, à chaque génération.

Dans une perspective de l'évolution des espèces, les variations périodiques de l'environnement, prédominant sur presque toute la surface du globe terrestre, furent de nature géophysique—principalement et essentiellement, parmi ces variations, on doit prendre en considération les changements relativement sûrs des alternances de lumière et d'obscurité suivant différentes échelles de temps: circadienne (environ 24 h.) et circannuelle (environ 1 an). Tandis que la période circannuelle est considérée comme constante, la période circadienne a probablement changé au cours de l'histoire terrestre. Les textes élémentaires d'astronomie [67, 68] font état de l'augmentation lente et graduelle de la durée du jour (ralentissement de la rotation de la Terre sur elle-même) et aussi, à part cela, de variations annuelles, et autres, actuellement non-prévisibles avec précision. Il est tentant de considérer, compte-tenu des données qui précèdent, que la fréquence circadienne la plus élevée enregistrée jusqu'ici par des procédés tels que le spectre de variance et le périodogramme a été trouvée au voisinage d'un cycle par 21 heures—dans le comportement de *l'Escherichia coli* [66]. Nous avons discuté ailleurs [64] la possibilité, actuellement très hypothétique, d'une géochronométrie et d'une biochronométrie de l'évolution par la datation, au moyen de la période des rythmes. La date à laquelle une espèce a évolué pourra être reflétée, en partie, par la période circadienne observée lorsqu'un organisme donné est maintenu dans le cycle lumière-obscurité à la fréquence la plus haute compatible avec une synchronisation rythmique. L'obstacle principal à cette entreprise de datation est qu'une telle hypothèse n'est, au mieux, que partiellement correcte; autrement dit, le τ

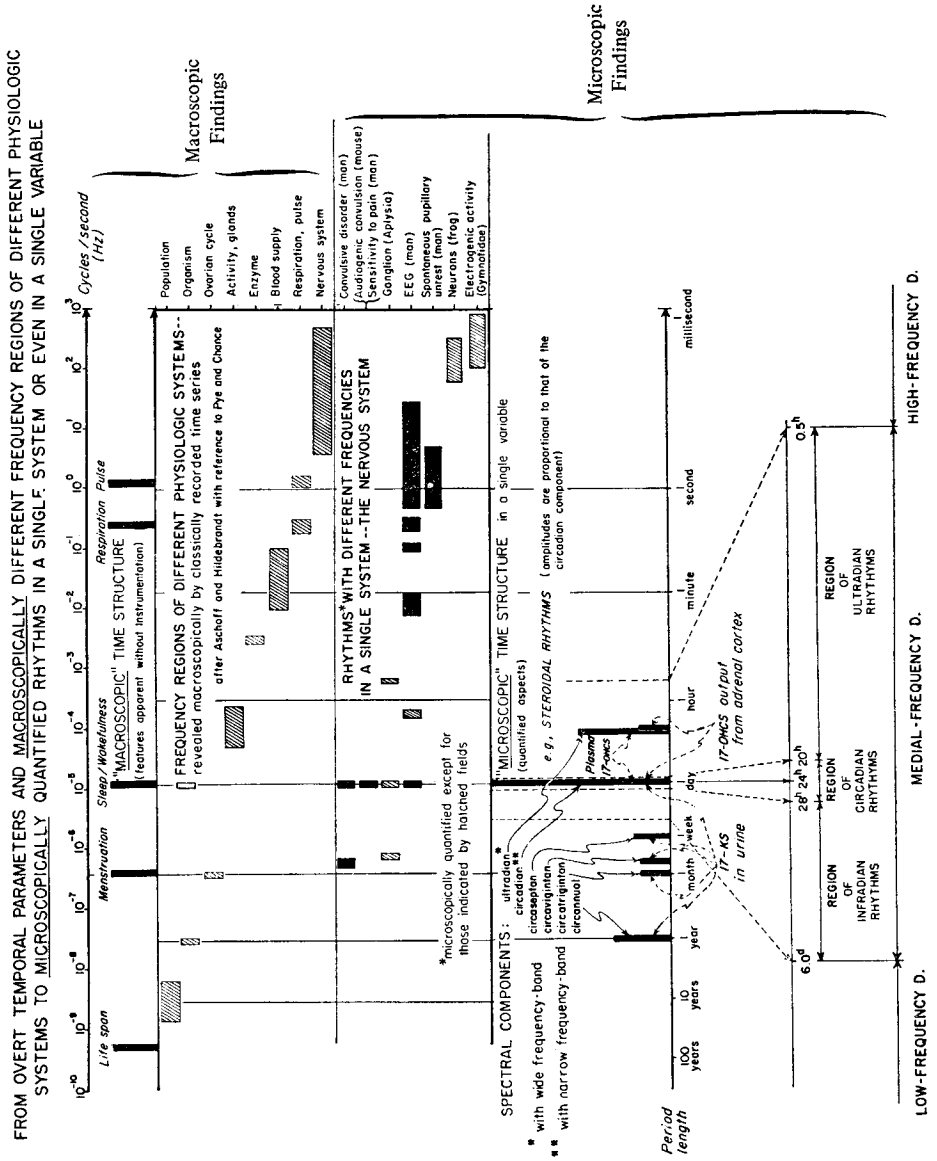


Fig. 19a. Spectral components of different scope—including those recognized by primitive man (top).

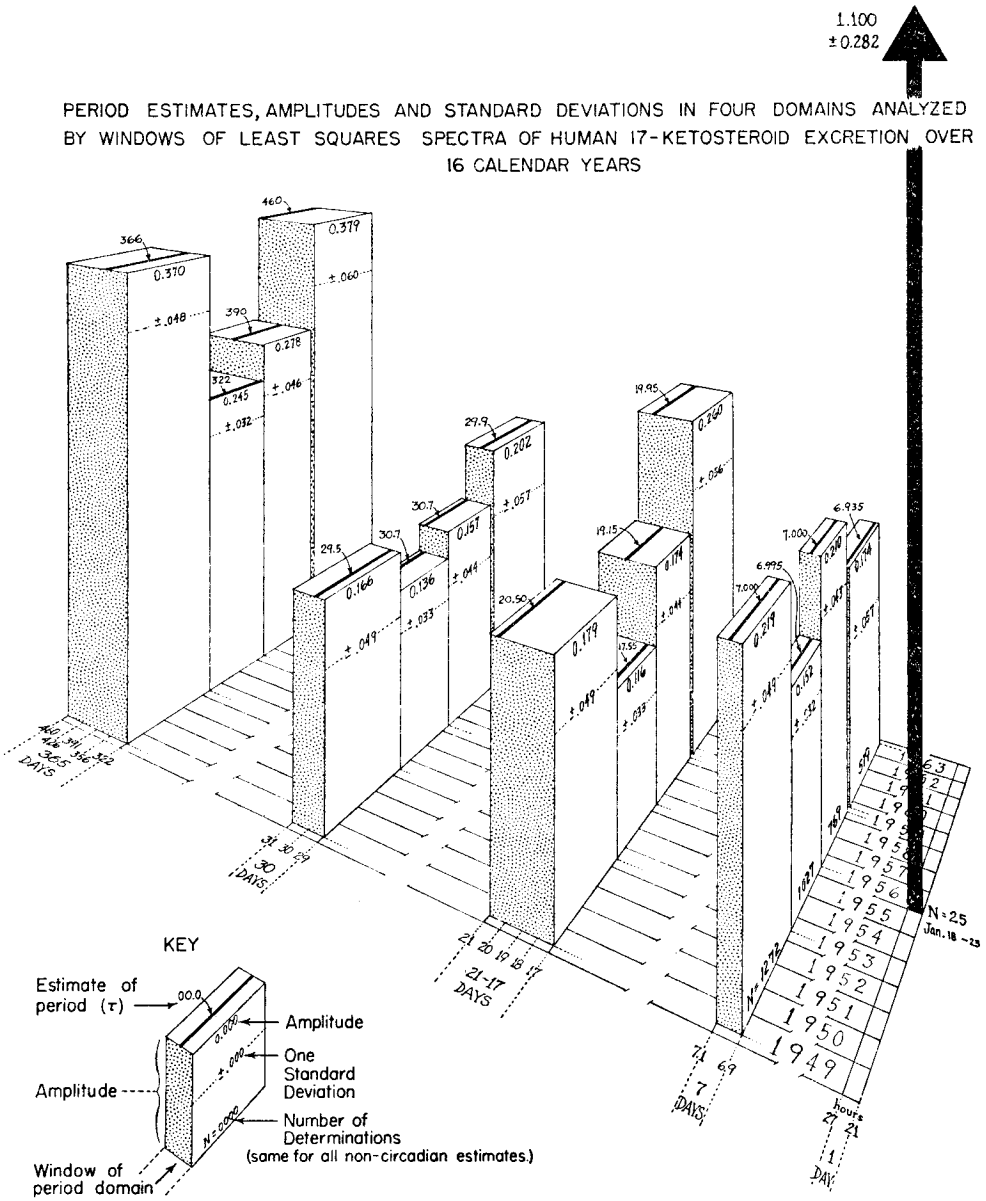


Fig. 19b. Reproducibility from one data section to the next of certain rhythms with a relatively low frequency, in the 17-ketosteroid excretion of a healthy man. Arrow in heavy print visualizes certain comparable estimates for the much more prominent circadian rhythm of urinary 17-ketosteroid excretion [10; cf. also 72 and 73 for circannual rhythms].

circadien dépend, en partie, de la rotation de la Terre sur elle-même au moment où une espèce terrestre a évolué. Peut-être trouvera-t-on aussi, pour des autres rythmes biologiques, une dépendance similaire, vis à vis de la longeur du jour lunaire, à l'époque du "passage" d'une espèce du milieu aquatique au milieu terrestre—si un tel passage s'est réellement produit [79–81]. De plus, la localisation spectrale d'une bande de fréquence(s) circadienne(s) dépendra de l'histoire du système après son évolution "primordiale"—par exemple, après la transition d'une forme aquatique à une forme terrestre. Au cours de cette histoire, on peut admettre que, par leur reproduction sélective, certaines mutations aléatoires ont persisté parce qu'elles possédaient une valeur d'adaptation positive. On peut donc envisager une meilleure adaptation à des bandes de fréquences circadiennes géophysiques, si les bandes circadiennes des organismes se sont allongées – peut-être plus rapidement que le ralentissement de la Terre elle-même—spéculation qui aurrait pour conséquence qu'aujourd'hui les périodes circadiennes peuvent être plus longues que la période terrestre de 24 heures.

12. Conclusions et résumé

Les résultats présentés suggèrent qu'il y a de nombreuses exceptions à la constance présumée des organismes, que nous considérions l'Homme ou les unicellulaires. La structure biologique comme la structure physique est *spatio-temporelle*. La structure temporelle dynamique d'un organisme, à plusieurs niveaux de résolution, est le complément de sa morphologie [82–84] de sa structure spatiale, plus statique; la structure temporelle comprend un spectre de rythmes de différentes fréquences ainsi qu'il apparaît à la fig. 19. Elle montre le début de la métamorphose depuis a) une analyse exclusivement "macroscopique" de la périodicité, qui consistait seulement en une inspection de la représentation, étalée en fonction du temps, de processus biopériodiques, jusqu'à b) la rythmométrie "microscopique", qui est l'étude complémentaire de la structure biologique temporelle et qui permet de représenter une variable physiologique par son amplitude ou sa variance étalée en fonction de sa fréquence, sa période ou sa phase.

La morphologie spatiale est couramment définie par une exploration fructueuse et toujours plus profonde de la cyto-architecture. Les chercheurs en biologie moléculaire, en microscopie électronique et en biochimie joignent leurs efforts dans leurs tentatives de localiser le groupement et l'interaction de différentes molécules en diverses parties d'une cellule donnée. Cependant, toute analyse complète des relations géométriques, dans une structure spatiale, peut avoir (et ce sera un jour une condition indispensable) pour complément une exploration concomitante et, à nouveau, toujours plus profonde des aspects temporels de l'organisation biologique spectrale comme le montrent les figs. 19a et 19b. Une telle entreprise dépend du progrès des techniques de télémétrie en biochimie aussi

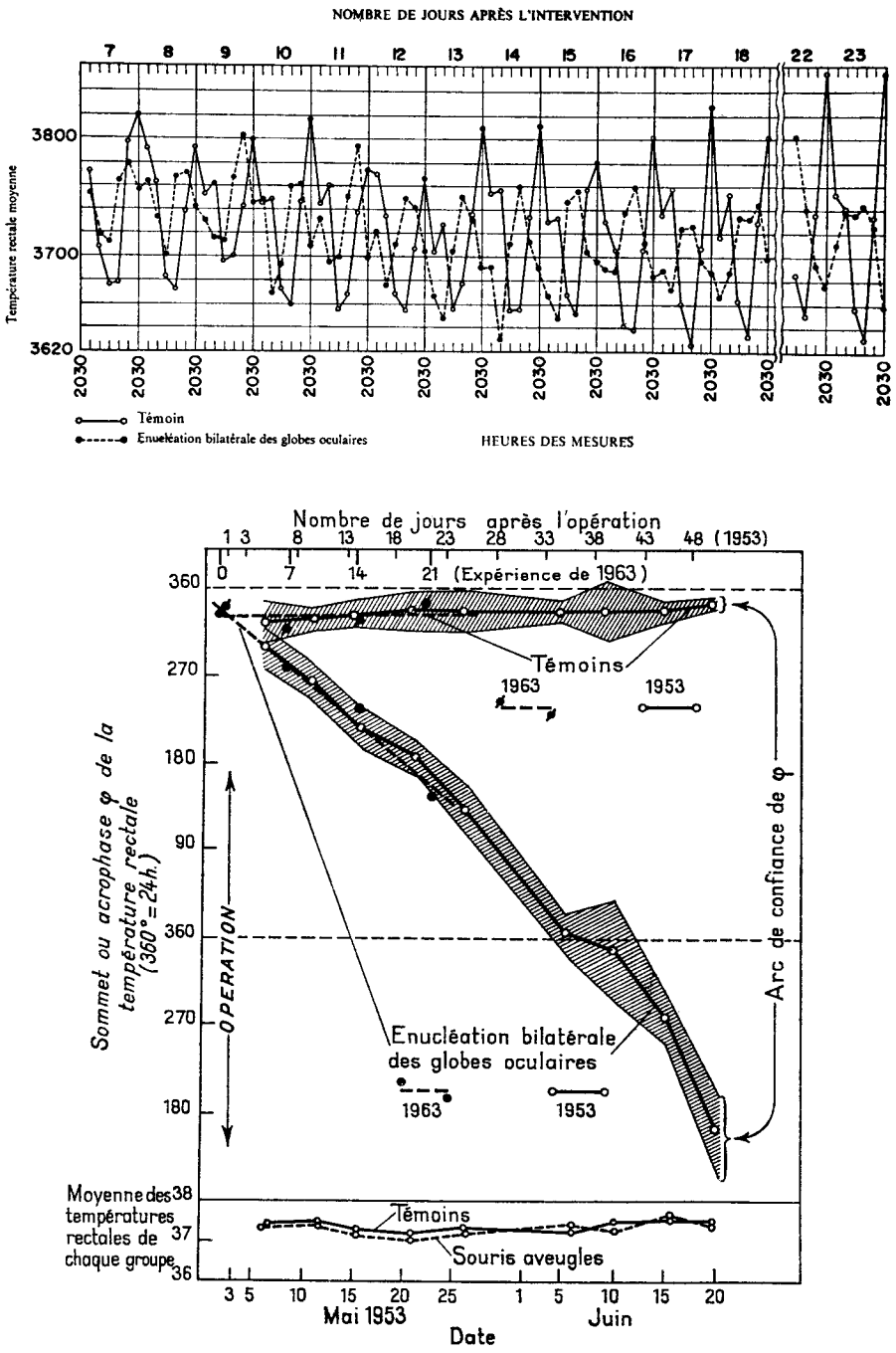


Fig. 20a. Circadian desynchronization following blinding and its reproducibility in studies carried out a decade apart [cf. 2].

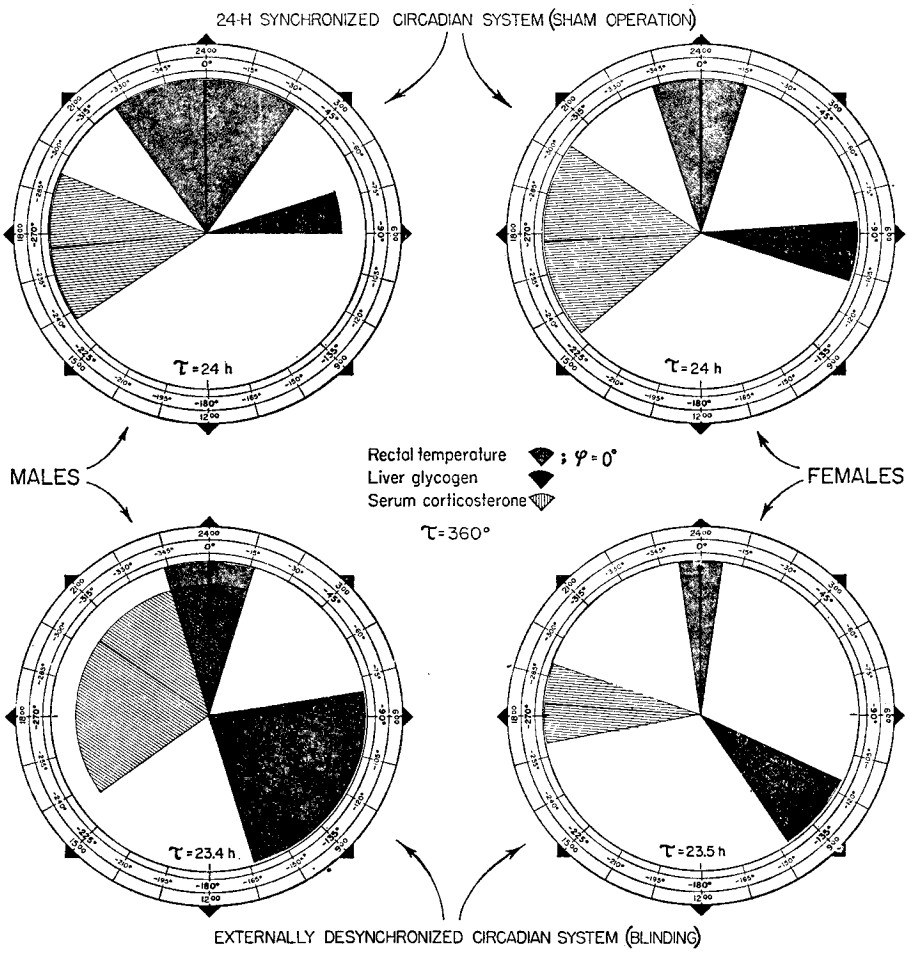


Fig. 20b. Lead in circadian acrophase of serum corticosterone in relation to the rectal temperature acrophase—in the 24-hour-synchronized (top) or externally desynchronized (bottom) circadian system of the mouse.

bien qu'en biophysique. Les séries temporelles de mesures collectées grâce à ces techniques, ou suivant des méthodes plus usuelles, peuvent dès maintenant faire l'objet d'analyses numériques spéciales, celles-ci étant devenues réalisables du fait des progrès des calculateurs électroniques. Par ces méthodes, les rythmes sont susceptibles d'être détectés, isolés, définis et développés sous la forme quantitative d'entités statistiques reproductibles.

Chaque rythme, qui à l'origine représentait, peut-être, les résultats de pressions adaptatives et de reproduction sélective en réponse à la localisation géophysique—rythmes circadiens, p. ex.—s'est adapté aussi, sans besoin dès lors de changement de fréquence, aux aspects temporels d'une niche socio-écologique de l'habitat.

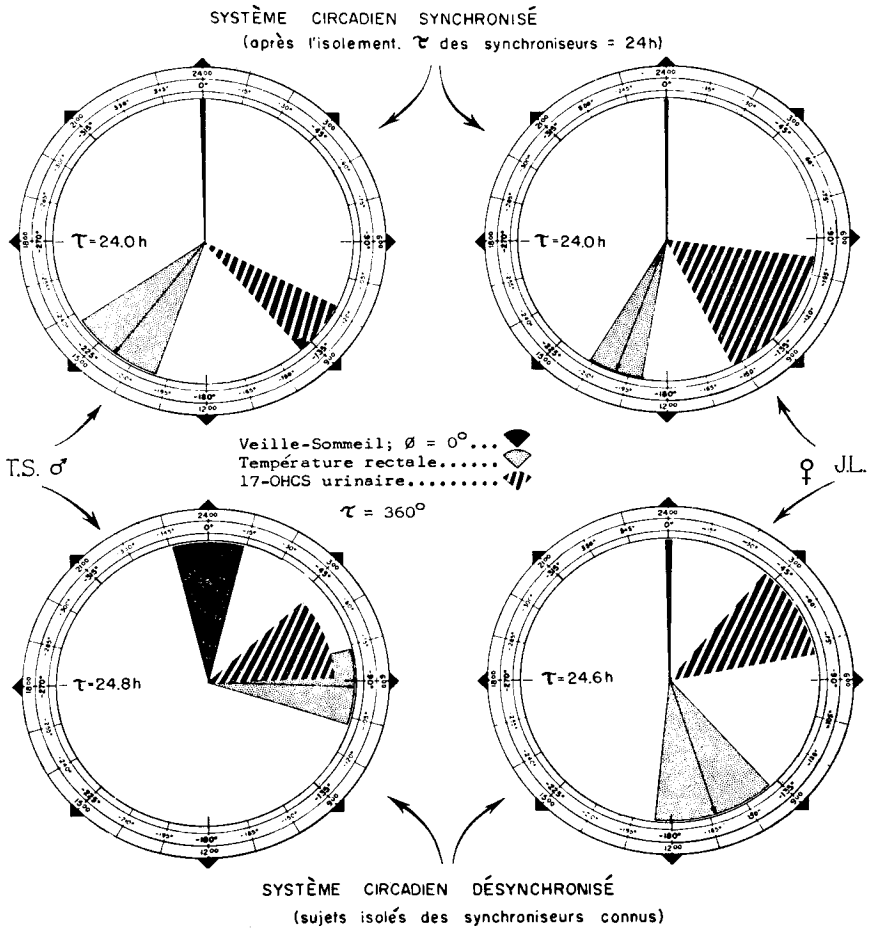


Fig. 20c. Lead in circadian acrophase of urinary 17-hydroxycorticosteroid excretion in relation to the rectal temperature acrophase—in mature human beings, under conditions of synchronization with a societal routine on top and under conditions of isolation at the bottom.

Pour certaines composantes de la structure spectrale temporelle, nous ne connaissons pas de contre-partie géophysique de même fréquence; tel est le cas des rythmes d'environ une semaine. Cette composante spectrale circaseptidienne a été récemment mise en évidence à partir d'une série longitudinale de l'excrétion quotidienne des 17—cétostéroïdes couvrant 16 années consécutives (fig. 19b).

Qu'un rythme donné ait, ou non, sa contre-partie dans l'environnement, un phénomène prévisible de cette nature représente un des aspects de l'intégration temporelle de l'organisme, en dehors de leur rôle dans l'adaptation à l'environnement. Dans l'un et l'autre des cas—intégration ou adaptation—nous avons à faire à un phénomène qui rend possible une division du travail dans le temps. D'un côté, une division temporelle du travail peut être achevée par l'évolution

d'une composante spectrale, avec une fréquence donnée, pour différentes fonctions physiologiques qui y sont reliées; les différences de phase parmi de tels rythmes physiologiquement interdépendants, et ayant une fréquence similaire—la même fréquence, en moyenne—représentent l'ordre temporel dans ce cas. Des différences de phase, entre les rythmes détectés par des méthodes isotopiques de marquage du RNA, du DNA et pour le taux des mitoses hépatiques des souris constituent un exemple d'un tel ordre, c'est à dire d'une telle organisation.

D'un autre côté, les organismes ont acquis, probablement au cours de l'évolution, pour leurs diverses fonctions, des bandes de fréquence spécifiques et différentes en quelques manières: certaines tâches doivent être exécutées plus fréquemment que d'autres. Dans le spectre des fréquences, il y a des grosses différences entre les rythmes d'un cycle par jour et les rythmes ayant une fréquence d'un cycle par environ un mois ou environ un an. Une de ces différences est réduite par la survenue, entre autres, d'une composante spectrale circaseptidienne—peut-être l'intégration temporelle est-elle ainsi facilitée.

Il reste la possibilité de la découverte éventuelle par les géophysiciens, de variations périodiques de l'environnement suivant le spectre des fréquences que le biologiste rencontre—lorsqu'il étudie les aspects chronobiologiques, *inter alia*, de la neurophysiologie ou de la circulation (dans le domaine des fréquences les plus hautes) aussi bien que les aspects métaboliques, reproductifs et autres (dans les domaines des rythmes des basses ou des moyennes fréquences). Les informations à la fois plus approfondies, plus détaillées et plus objectives concernant de tels phénomènes nous conduisent à la *chronobiologie* qui réunit comme spécialisations:

(1) la chronophysiologie – Elle répond à l'étude des facteurs physiologiques (nerveux, endocriniens, métaboliques etc.) qui déterminent les caractéristiques temporelles d'un processus biologique; (2) la chronopathologie – Elle répond à l'étude des altérations de ces caractéristiques temporelles déterminant un état pathologique ou résultant de celui-ci (psychose, cancer, endocrinopathie etc.); (3) la chronotoxicologie qui répond à l'étude des variations rythmiques prévisibles d'effets non-désirables ou dangereux provenant d'agents chimiques, physiques ou autres, y compris les poisons, les substances polluantes et les surdosages thérapeutiques; et l'étude de tels effets non-désirables sur les rythmes biologiques; et (4) la chronopharmacologie – Elle répond à l'étude des effets d'une drogue sur ces caractéristiques temporelles, effets qui varient de manière prévisible et cyclique en fonction du temps.

Les informations plus générales sur l'organisation temporelle constituent le domaine ou se rencontreront dans l'avenir, comme à cette réunion, le physicien, le biologiste et le medecin—pour analyser en commun les principes qui ont conduit à transformer l'adage: *Qui habet tempus habet vitam* en: *Qui habet structuram in tempore habet vitam*, formulation nouvelle qui condense les acquisitions de la physiologie statistique appliquée aux séries de mesures temporelles plutôt qu'aux mesures isolées et non situées dans le temps.

11. Speculations

Certain aspects of biologic time structure may represent evolutionary adaptations of organisms to the predominant cyclic variations of their environment (to the temporal structure of the environment) rather than merely the "personal" adaptations reacquired (e.g., "learned") by each individual in each generation.

Along an evolutionary scale the pertinent environmental periodicities prominent over most of the Earth's surface were geophysical—first and foremost among them the relatively reliable cyclic changes in the alternation of light and darkness, changing along several time scales, circadian and circannual. Whereas the circannual period of the environment is regarded as being on the average more or less constant, the circadian period has probably changed systematically in our terrestrial history. There also is evidence, cited even in elementary astronomy texts [67, 68], to indicate a gradual long-term increase in day length—by virtue of a slow-down of the Earth's rotation around its axis.

It is tempting to consider in this connection that the highest circadian frequency detected thus far in a biologic system not synchronized by a cycle of alternating light and darkness and quantified by procedures such as the variance spectrum and the periodogram has been found to be in the neighborhood of 21 hours. For the behavior of *E. coli* [66] one might consider the as yet highly speculative possibility of evolutionary biorhythm dating, on the basis of the length of the desynchronized circadian period detected in currently living forms—an endeavor extending the geochronometry and biochronometry that has become possible by comparisons of circadian growth lines in living and fossil marine invertebrates.

The date at which a species evolved might be reflected at least in part by its circadian period observed when the contemporary form of this organism is maintained in a cycle of light and darkness of the highest frequency no longer compatible with the rhythm's synchronization. An obstacle to such an extrapolation made for the rhythm dating of evolution is that such an hypothesis can at best be only partially correct. The circadian frequency of a terrestrial organism can depend only in part upon the speed of the Earth's rotation around its axis at the time when the "ancestors" of this form of life evolved. Conceivably, one may find for certain other organisms originally evolved in an aquatic milieu a similar dependence of their biologic rhythms upon the length of the lunar day at that epoch of the Earth's history when the counterpart of the contemporary form might have "resettled", if indeed it did, on terra firma [79–81].

In any event, the spectral location of a band of circadian frequencies will also depend upon the history of a given biologic system after its early evolution. For example, changes in the circadian period resulting from certain random mutations might have persisted through selective reproduction because they had positive adjustment value. One could indeed envisage a better adaptation of organisms to the prevailing geophysical circadian frequencies via a lengthening of the organism's circadian band.

Conceivably such a lengthening of the organism's circadian period occurred more rapidly than the lengthening of the geophysical day resulting from the slow-down of the Earth's rotation around its axis. For this reason, perhaps, circadian bands that are longer than the terrestrial period, now 24 hours, are encountered in a number of organisms under several conditions. Can it be that an overshoot lengthening of the biologic circadian period occurred because organisms are so structured in time that, according to different lines of evidence, it is easier for several contemporary life forms [71] to delay a rhythm than to advance it?

12. Summary and conclusions

The foregoing evidence suggests that there are many exceptions to the presumed constancy of organisms, whether we consider unicellulars or man. Biologic structure is *spatiotemporal.*

An organism's dynamic structure in time complements at several levels of resolution the more static morphology in space. Temporal structure includes a spectrum of rhythms with different frequencies, as sketched in the abstract fig. 19. This figure conveys the beginning metamorphosis from a "macroscopic" periodicity analysis, consisting of the inspection of bioperiodicity in time displays, to a "microscopic" rhythmometry of the biologic time structure.

Spatial morphology is currently being resolved by the successful and ever deeper probing into cyto-architecture. The molecular biologist joins hands with electron-microscopists and biochemists in their attempts to localize the arrangement and/or interaction of different molecules in various parts of a given cell. Yet any analysis of geometric relations in spatial structure can be complemented by a concomitant and again ever deeper probing into the temporal aspects of biologic spectral organization, as indicated in figs. 19a and 19b. This undertaking depends upon the advancement of biochemical as well as biophysical telemetry techniques. Time series collected by such techniques or by more conventional means already can be subjected to special numerical analyses rendered practicable by the advent of electronic computers [2]. By such methods rhythms become amenable to isolation, resolution and display as reproducible statistical entities.

In any event, what originally represented, perhaps, a result of adaptive pressures and selective reproduction in response to the geophysical setting—circadian rhythms, for example—adapted also, with no need thus far for a change in frequency, to temporal aspects of a socio-ecological habitat niche. For certain components of the spectral time structure, we do not know of a geophysical counterpart with the same frequency. A case in point is an about-weekly rhythm. This circaseptan spectral component has recently been documented from a longitudinal series consisting of values for the daily 17-ketosteroid excretion—a series covering about 16 consecutive years (figs. 19a and 19b).

Whether or not a given rhythm has an environmental counterpart, such predictable phenomena represent features of the organism's temporal integration, quite apart from their role in environmental adaptation. In either case —integration or adaptation—we are dealing with a phenomenon which makes possible a division of labor in time. On the one hand, a temporal division of labor can be achieved by the evolution of a spectral component with a given frequency in several related physiologic functions; *differences in phase* among such physiologically interdependent rhythmic phenomena with similar frequency represent the temporal ordering in this case.

On the other hand, organisms also have acquired, presumably, in the course of evolution, somewhat specific and *different frequency bands* for their diverse functions: some tasks have to be done more frequently than others. In this spectrum of frequencies there are sizeable gaps between rhythms with one cycle per day and rhythms with a frequency of one cycle in about one month or in about one year. One such gap is reduced by the occurrence of a circaseptan spectral component—perhaps temporal integration is thus facilitated. The possibility remains that eventually environmental frequencies may be discovered by the geophysicists for those frequencies that we biologists encounter whether we study chronobiologic aspects of neurophysiology in the highest now known frequency domain or whether we evaluate rhythms in the domain of medial or low frequencies.

Such information leads us to the fledgling field of chronobiology, with several subspecialties: a) *Chronophysiology*—study of physiologic factors underlying biologic temporal characteristics, e.g., adrenal, renal and metabolic factors underlying the phase-shifting of rhythms following transmeridian flights; b) *Chronopathology*—study of alterations in biologic temporal characteristics as 1) functions of disease, and, what seems more important, as 2) determinants of disease; c) *Chronotoxicology*—study of undesired or harmful effects from chemical, physical or other agents, including poisons, pollutants and overdoses of drugs 1) upon biologic temporal characteristics, and 2) as a function of biologic timing; d) *Chronopharmacology*—study of drug effects 1) upon biologic temporal characteristics and 2) as a function of biologic timing. Thus, by improving the toxic-therapeutic ratio of a drug, added benefit may be derived from conventional

therapy, e.g., a reduction of side effects. More important is the possibility of finding new therapeutic applications for conventional drugs (e.g., ACTH for resynchronization) or of developing new drugs, against the background of any data indicating that rhythm alteration such as circadian desynchronization contributes as a determinant of disease.

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