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L'INSTITUT  
DE LA VIE

1968 No 18

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DE L'INSTITUT  
DE LA VIE

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Siège: 89, B<sup>o</sup> Saint Michel, Paris V<sup>e</sup>  
Téléphone: 033-94-86  
*Périodicité:* trimestrielle

Prix du numéro: France 5 F. Étranger 6 F  
Abonnement: France 18 F. Étranger 22 F  
Conditions spéciales aux membres de  
l'Institut de la Vie.  
Renseignements au Siège.

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CONFÉRENCE INTERNATIONALE  
PHYSIQUE THÉORIQUE ET BIOLOGIE  
Versailles 26-30 juin 1967

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## DISCUSSIONS

B. B. LLOYD: You alluded, Dr. Halberg, to the periodicity of liver glycogen and you also talked about Claude Bernard's earlier statement of liver glycogen being sometimes very low (in fact, I think, sometimes zero) and sometimes very high. Has this periodicity anything to do with the nervous system and is it related to Bernard's work on *piqûre* diabetes.

F. HALBERG: Thank you kindly, Dr. Lloyd, for providing me with this opportunity to comment on Claude Bernard in relation to the study of rhythms. Let me refer you in this connection to comments made earlier [1]. To the thinker [2], not to mention Bernard the demonstrator of experimental procedure [3], the significance of physiologic timing should have been apparent. Yet if one scrutinizes his writing about the conditions of a "continuous life", one finds only somewhat ambiguous remarks such as the following:

"The phenomenon of nutrition is accomplished in two times (he probably means two stages), and these two times are always separated one from the other by a period of more or less long duration, which (duration) is a function of a variety of circumstances" (. . . dont la durée est fonction d'une foule de circonstances) [2].

At 52 years of age, the active investigator recognizes explicitly the "milieu intérieur variable" (sic); in 1856, the editors of the *Journal de l'Anatomie et de la Physiologie* announce [4] that Mr. Claude Bernard will soon publish an introduction to the study of experimental medicine. They do so in a footnote to an article by Claude Bernard himself, written under the title "Of the diversity of animals subjected to experimentation" and—what is more important in our context—"Of the *variability* (italics mine) of organic conditions in which they (the animals) present themselves to the experimenter":

"M. Claude Bernard doit publier prochainement une 'Introduction à l'étude de la médecine expérimentale', 1 vol. in-8 de 400 pages. Nous sommes heureux d'offrir à nos lecteurs un extrait de ce livre, qui est un exposé de doctrines présentant le tableau complet des faits et des idées que le professeur a développés dans son cours de médecine au Collège de France et dans son cours de physiologie générale à la Faculté des sciences, depuis ses dernières publications de 1859." [4].

This footnote states that they (the editors) are happy to offer to their readers an "extract" of the book prepared by its author. The material in the article then reflects what the active (rather than senescent) Bernard himself regarded as his most important experience. Of primary interest in the same context remains

Bernard's emphasis that "one must keep in mind not only the variations of the cosmic external milieu, *but also the variation of the organic milieu* (italics mine), i.e., that of the actual state of the organism".

Claude Bernard writes further that one might be in great error in assuming that it suffices to experiment on two animals of the same species in order to obtain identical experimental conditions and suggests that *the physiologic conditions of the internal milieu manifest an extreme variability* (italics mine); that, at a given moment, such variability introduces considerable differences into the results from experimentation on animals of the same species that appear to be identical.

He states further that "more than anybody else," he has insisted on the need to study these different physiologic conditions and to have demonstrated that they are the essential basis of experimental physiology. He points out that one must admit in fact that, in a given animal, the vital phenomena vary (sic) only according to precise and determined conditions of the internal milieu.

Students who evaluate rhythms as the elements of an organism's time structure will hasten to agree with such statements much more readily than students of "constancy". From appropriate statistical work one can indeed specify circadian system phases "dans lesquelles il y a *toujours* du sucre et d'autres conditions dans lesquelles il n'y en a *jamais*"—as will become apparent from fig. 1 for the case of glycogen, if not sugar, in mouse liver. Liver glycogen contents of, say, intact ad libitum fed animals continue to be presented by prominent biochemists without a qualification of the sampling time in terms of rhythms. However, the best chemical procedure will yield results that in such instances are physiologically difficult to interpret or actually misleading (fig. 1). One can cite Claude Bernard further in this connection:

"... Pour le moment, je veux uniquement appeler l'attention des expérimentateurs 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." [4].

Furthermore, in his *Phenomena*, on page 114, [2] Bernard indicates that constancy presupposes the "self-perfecting" of an organism in such a fashion that the external variations are at each moment *compensated and balanced*; the "higher animal", rather than being indifferent to the external world, is in a *close and wise relationship* with it, in such a fashion that animal equilibrium results from a continuous and delicate compensation established by the most sensitive of balances.

The temptation is great to read "rhythm" into "equilibrium", and to attribute to the aged Bernard a hint of the close and wise interactions ("... étroite et

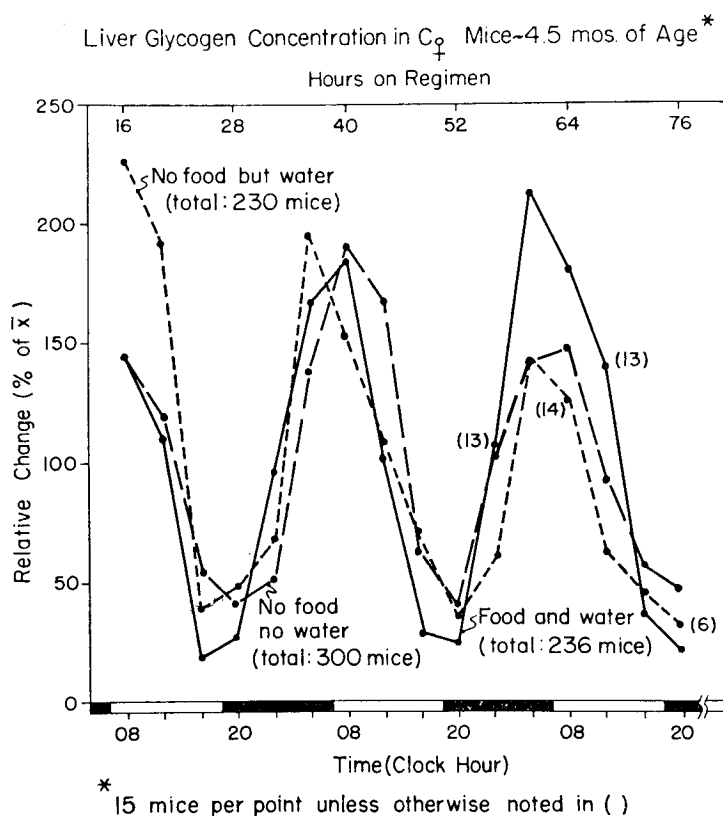


Fig. 1

savante relation . . .”) in which the cycles of a terrestrial environment are “anticipated” by rhythmic organisms evolved on earth. This would probably be no more than wishful thinking—as far as can be judged from Bernard’s writings. It is difficult further to reconcile the remarks on a “close and wise interaction” with the concept of a *shielding* of the organism from the environment by an internal milieu that is constant. Indeed, it is important at this symposium to emphasize that shielding by a basic constancy currently credited to Bernard must not be confused with the view of basic rhythmic physiologic interactions underlying a superficial constancy or, rather, a limited variability. In the former view, rhythms remain secondary or even trivial considerations as to both interpretation of body function and experimental method. In the latter view, rhythms become primary basic features of temporal integration and adaptation in organisms.

It is probably because Claude Bernard gave precedence to shielding over rhythms that throughout his work on diabetes and glycogenesis there is not a single reference indicating precautions taken to control the rhythm in liver glycogen.

To turn to Dr. Lloyd's question as to whether Bernard's work on the "piqûre diabétique" relates to periodicity, one might suggest that Bernard failed to recognize such a relation. In his *Leçons sur Le Diabète et La Glycogénèse Animale* (Baillière, Paris 1877), Bernard recalls how he arrived at the piqûre after describing its effects; he writes that he had the preconceived idea to augment hepatic secretion and sugar production "en excitant les origines du pneumogastrique, comme j'avais, dans d'autres circonstances, augmenté la sécrétion salivaire en excitant les origines de la cinquième paire." (p. 370). The most prominent periodicity of this variable remains ignored until the explicit statement by Erik Forsgren in 1927 that liver glycogen undergoes about 24-hour periodic changes that are partly independent of nutrition. For a discussion of historical features to Bernard's piqûre and to subsequent work, interested individuals can be referred to a book by Jakob Möllerstrom, *Das Diabetesproblem: Die rhythmischen Stoffwechselforgänge*, (Thieme, 1943).

- [1] F. Halberg, Claude Bernard, referring to an "extreme variability of the internal milieu" in *Claude Bernard and Experimental Medicine*, (Shenkman, Cambridge, Mass., 1967) 193-210.
- [2] C. Bernard, *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux* (Baillière, Paris, 1885).
- [3] L. Binet, (editor), *Claude Bernard, Introduction à l'étude de la médecine expérimentale* (Paris, Les Chefs-d'Œuvre Classiques et Modernes, 1963).
- [4] C. Bernard, De la diversité des animaux soumis à l'expérimentation. De la variabilité des conditions organiques dans lesquelles ils s'offrent à l'expérimentateur. *J. Anat. Physiol. Homme Animaux* 2 (1865) 497-506.

P. DEJOURS: Deux questions à M. Halberg: Pouvez-vous expliquer pourquoi il est plus difficile d'aller d'Ouest en Est que dans la direction opposée? En ce qui concerne les oiseaux migrateurs entre l'hémisphère Nord et l'hémisphère Sud, que devient leur cycle circadien? Existe-t-il un déphasage de 180 degrés lorsqu'ils passent l'équateur?

F. HALBERG: We are searching for the answer to Dr. Dejour's question, which gains greatly in importance in this day and age of global travel not only for passengers but also for the aviator and finally for all of those concerned with work hygiene. Short reflection will lead one to recognize that to adapt following rapid eastward travel, one must advance a rhythm, whereas after a westward flight, one adapts by a delay of rhythm. These two adaptations are distinctly different. It appears that the circadian system has some "polarity", as demonstrated for the rat in my text-fig. 4. The same point is also made by fig. 2 for man. This figure summarizes studies done in cooperation with Dr. Walter Nelson and Dr. Erhard Haus of our laboratory at the University of Minnesota. It shows for the variables and subjects studied a much more rapid delay of rhythm as compared to an

advance. Another question concerning the relative ease of adaptations after transmeridian travel relates to the extent of transient frequency and phase desynchronization during such adaptations. From fig. 2 it also appears that while the adaptation following a flight from east to west is faster, the transient desynchronization among certain urinary variables during this relatively short shift time is greater than that occurring in connection with an advance of rhythms.

Such problems of chronophysiology relate perhaps to accident prevention. Certainly the avoidance of performance decrements by manipulating the adaptation of rhythms in the physiologically most favorable fashion compatible with logistic needs can be expected to lower the accident rate following changes in routine.

PHASE-SHIFTS OF THE HUMAN CIRCADIAN SYSTEM AS A RESULT OF 2 INTERCONTINENTAL FLIGHTS, GAUGED BY URINARY EXCRETION RATES\*

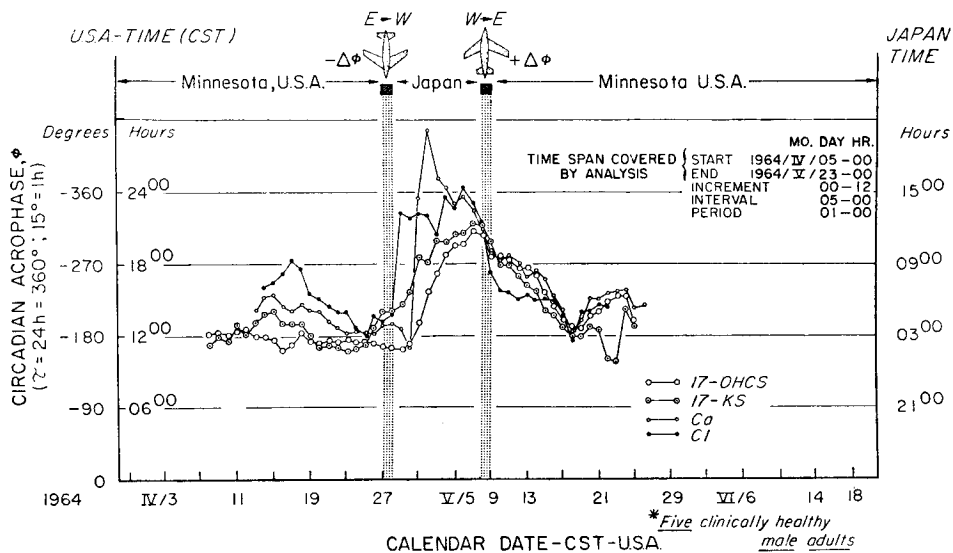


Fig. 2

With such problems in mind, Dr. Fessard, Dr. Reinberg and a number of us in other parts of the world intend to assign to the facilitation of rhythm shifts required by transmeridian flight or by odd work routines a prominent place in a chronobiologic project now planned within the framework of the International Biologic Program.

Concerning Dr. Dejour's second question, it would be desirable indeed to telemeter the rhythms of an arctic tern while this animal flies from one Pole to the other. I wish I knew how long this flight takes on the average, and I plead guilty to not having studied classical papers such as those of Bertil Kullenberg

(Über Verbreitung und Wanderungen von vier Sterna-Arten, *Arkiv. Zool.* **38 A** (1946) no. 17 and Finn Salomonsen (Migratory movements of the arctic tern (*Sterna Paradisaea* Pontoppidan) in the Southern Ocean; Danske Videnskabernes Selskab, *Biol. Meddelelser* **24** (1967) 1.

If the flight from Pole to Pole takes weeks rather than a few days, there may be a slow adjustment to the local setting, as has been reported for the case of sea travel by man. In 1905, Gibson reported an apparent adjustment of the timing of the body temperature rhythm—day by day and coincident with the shifting of the routine—during sea travel from the United States to the Philippine Islands and back. This author, who studied two individuals, describes for both of them a “somewhat limited daily range of variations” in body temperature after arrival in Manila, the Philippines (R. B. Gibson: The effects of transposition of the daily routine on the rhythm of temperature variation; *Amer. J. Med. Sci.* **129** (1905) 1408). During a sea voyage from Melbourne, Australia, Osborne in 1908 found that throughout the journey the evening maxima of the body temperature rhythm followed local time (C. Osborne: Body temperature and periodicity; *J. Physiol.* **36** (1908) 34–41). Conceivably, the arctic terns adjust their circadian rhythms in the case of slow travel across latitudes and longitudes as do human beings. Finn Salomonsen’s fig. 2 on page 7 of the above mentioned publication indicates that some terns, at least, follow a transmeridian path during part of their inter-polar journey as champion long-distance migrants, a circumstance kindly indicated to me by Mr. David Cline of the Museum of Natural History of the University of Minnesota.

For information on rhythmicity in arctic birds, two reviews by Donald S. Farner are quite pertinent: Role of extreme changes in photoperiod in the annual cycles of birds and insects, *Federation Proc.* **23** (1964) 1215–1220; and Circadian systems in the photoperiodic responses of vertebrates in *Circadian Clocks*, Proceedings of the Feldafing Summer School, Ed. J. Aschoff (North-Holland, 1965) p. 357–369. Further research may well be aimed at evaluating the time course of any circannual rhythm adaptation during 5 or 6 inter-polar round-trips made by an arctic tern in consecutive years.

H. MARGENAU: I have a question or two, Sir. The first one is this. Is anything known about the physiological or chemical mechanism which carries these rhythms? I am particularly interested in those rhythms which one might call active, which originate within the organism itself and are not tied to the surroundings.

The second question which I might voice at the same time concerns the interesting paper by Dr. Lindauer. I would like very much to know whether there is any knowledge or whether there are any conjectures as to how bees become aware of a magnetic field?



M. LINDAUER: In a few years, so we hope, this question can be answered by experimental data. Theoretically a magnetic field can affect the organism (the sensory cells or the neurons):

- 1) by generation of electromotive force in moving conductors (a honey bee performing the waggle dance is a fairly fast moving conductor);
- 2) by the force exerted upon moving charge carriers (Hall-effect);
- 3) by force or torque exerted on para- and diamagnetic particles.

The voltages resulting from the effects can be very small only (in case 1  $\approx 0,1 \mu\text{V}/\text{cm}$ ); however Lissmann and Machin have shown that "electric" fish can sense voltage gradients as low as  $0,03 \mu\text{V}/\text{cm}$ .

F. HALBERG: In the mammal, adrenocortical steroids represent the mechanism of certain circadian rhythms but not of others. Corticosteroids are, of course, entities that are chemically well defined, recognized physiologically as well and amenable to anatomical localization as to their site of production. Removal of the adrenal gland in man and mouse or Addison's disease—a condition associated with adrenocortical insufficiency—resulted in the obliteration of a circadian rhythm in blood eosinophil cells, at Michigan [1] in Utah [2] as well as at Minnesota [3]. Dr. Azerad, Dr. Reinberg and Dr. Ghata in France, over a decade ago, demonstrated that circadian rhythms in the urinary excretion of certain electrolytes also are obliterated as group phenomena in human adrenocortical insufficiency [4].

Aussi pour le cas de l'incorporation du radiophosphore dans les phospholipides hépatiques le cortex adrénalien semble être un mécanisme critique [5]. Nous avons été un peu troublés lorsque nous avons étudié les rythmes du marquage de l'ADN, il y a dix ans. Nous avons trouvé que les rythmes dans le métabolisme de l'acide désoxynucléique persistent après une adrénalectomie bilatérale. Alors il y a des différents mécanismes endocriniens et cellulaires pour différents rythmes circadiens. En passant du mammifère au microorganisme, on signale un travail intéressant qui a été fait par Sweeney et Haxo sur l'acetabularia [6]. Dans ce cas on peut couper les "rhizopodes" de base et enlever ainsi le noyau. Dans ce travail de Sweeney et Haxo et autres, vous avez la continuation d'un rythme circadien photosynthétique, en l'absence des acides nucléiques formes et connus [7, 8]. Finalement, on peut démontrer avec les méthodes modernes de rythmométrie des rythmes mêmes pour *E. coli* [9].

- [1] Mechanisms of diurnal eosinophil rhythm in man, *J. Lab. Clin. Med.* **45** (1955) 247.
- [2] The diurnal variation of blood leucocytes in normal and adrenalectomized mice, *Endocrinology* **58** (1956) 365.
- [3] Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases, *J. Lancet* **71** (1951) 312; Eosinophil rhythm in mice: Range of occurrence; effects of illumination, feeding and adrenalectomy. *Am. J. Physiol.* **174** (1953) 313–315.
- [4] Disparition du rythme nyctéméral de la diurèse et de la kaliurie dans 8 cas d'insuffisance

- surrénale, *Ann. Endocrinol. Paris* **18** (1957) 484-491; See also Rythmes des fonctions cortico-surréaliennes et systèmes circadiens, in: *Symp. int. sur la Neuro-endocrinologie*, (L'Expansion Scientifique Française, Paris, 1966) 75.
- [5] F. Halberg, H. Vermund, E. Halberg and C. P. Barnum, Adrenal hormones and phospholipid metabolism in livercytoplasm of adrenalectomized mice, *Endocrinology* **59** (1956) 364.
- [6] B. M. Sweeney and F. T. Haxo, *Science* **134** (1961) 1361.
- [7] E. Schweiger, H. G. Wallraff and H. G. Schweiger, Endogenous circadian rhythm in cytoplasm of acetabularia: Influence of the nucleus, *Science* **146** (1964) 658.
- [8] E. Schweiger, H. G. Wallraff and H. G. Schweiger, Über tagesperiodische Schwankungen der Sauerstoffbilanz kernhaltiger und kernloser *Acetabularia mediterranea*. *Z. Naturforsch.* **19** (1964) 499.
- [9] F. Halberg, and R. L. Conner, Circadian organization and microbiology: variance spectra and a periodogram on behavior of *Escherichia coli* growing in fluid culture, *Proc. Minn. Acad. Sci.* **29** (1961) 227.

K. MENDELSSOHN: There is of course a rotation of the polarization axis and the thing that is observed is a polarization. It would be a small effect, but biological indicators are sensitive.

H. C. LONGUET-HIGGINS: I have two observations to make. First, an observation on the proposal of Dr. Mendelsohn. He must be thinking of the Faraday effect, because he referred to the rotation of the plane of polarization of light by a magnetic field. This is all very well for radio waves passing through the ionosphere, where you have free electrons with a large mean free path. But for light the effect is quite negligible, and very difficult even to detect. One must then suppose that the earth's magnetic field produces a variation, in the plane of polarization of the light from the sky, of several degrees, because the error that the animals seem to make is of the order of 5 or 10 degrees. This is what your hypothesis seems to imply; but quite possibly I have misunderstood your original suggestion. That is my first point.

The second point is quite different; it concerns the speculations raised by the question which you brought up, Mr. Halberg. I have been trying to think of a reason why it is, apparently, easier to slow down a biological rhythm than to speed it up. These rhythms must arise from metabolic processes, and one may imagine that some of these processes take place with the maximum possible speed, if one thinks of the processes which have been described to us by Dr. Thomas of Denmark. If they do indeed go at maximum speed, it will be very difficult to hurry the rhythm of the overall cycle. One may take as an analogy a person playing a scale on the piano as fast as he can; it will be easy for him to slow down, but quite impossible to go faster.

F. HALBERG: There are examples of a different "polarity" of the circadian system. For instance, Dr. Aschoff found in certain birds that an advance of circadian

rhythms occurred faster than a delay [1]. He obtained similar results even in a human being studied in his bunker for possible differences in shift time following advances and delays of schedule [2]. Nonetheless, in our hands a number of human subjects studied after intercontinental flights, and rats, undergo much faster delays of their circadian rhythms investigated thus far, as compared to advances. All I am trying to say is that too broad a generalization may be premature. Furthermore, a number of rhythms have been found to be accelerated in disease. Thus, in human cancer we encounter what might be interpreted as a clear acceleration revealed by variance spectra [3].

This "speeding up" comes to the fore if one takes serial biopsies every two hours from human cancers and analyzes these data thereafter by variance spectra, as was done for the research by Dr. Tähti and Dr. Voutilainen of Finland. From such analyses it becomes apparent that in a number of human cancers a circadian component may no longer be demonstrable. The so-called "ultradian" component predominates, indicating that indeed one can "accelerate" the rate of one cycle in 24 hours; one may do so of course by "napping" as well. Nonetheless, from studies carried out in the isolation of caves on mature healthy men, one might be tempted to agree with the suggestion that we are indeed running with maximal frequency, if we keep in mind that under such conditions sleep-wakefulness at least on occasion changes from a primarily circadian phenomenon to a prominently infradian one. Some such isolated subjects sleep once in 48 hours rather than once in 24 hours, but many more individuals will have to be studied for more variables and under additional conditions before the most interesting polarity commented upon by Dr. Longuet-Higgins can properly be discussed.

Nous sommes de tels poltrons que nous n'osons pas mettre de côté les données sûres que nous avons pour nous lancer un peu dans l'inconnu, mais je suis d'accord avec vous: il est très probable que les opinions de M. Longuet-Higgins sont une bonne hypothèse à l'état actuel des informations.

Par les travaux français de M. Michel Siffre avec Alain Reinberg et Jean Ghata et aussi par les études de John Mills en Grande-Bretagne, on peut démontrer, au moins pour l'état de veille et de sommeil—ce qui n'est pas un bon index pour le métabolisme que dans ces conditions on trouve dans quelques sujets une transposition, au moins partielle, de la variance de la région spectrale circadienne avec des cycles d'environ 24 heures ( $\pm 4$  h) à la région spectrale infradienne (notamment, des fréquences d'environ un cycle en 48 heures).

Ceci s'aligne très bien sur votre proposition: que la veille/sommeil sinon le métabolisme représente un oscillateur qui fonctionne à l'état maximum. Mais comme nous l'avons discuté en haut, pour le cas des cancers humains, on peut trouver un déplacement de fréquence dans la direction opposée, notamment une accélération très claire, révélée par des spectres de variance [3]. Donc, en jugeant sur le cancer, nous constatons que nous pouvons "accélérer", dans une notable proportion, par rapport au rythme d'un cycle en 24 heures.

Ceci nous ramène à des questions de base, qui ont à faire avec l'activité cellulaire comme aussi avec l'organisme global. On trouve donc à tous les niveaux de l'organisme une structure temporelle dont les composantes pourraient être dans une relation des harmoniques—dans le sens physique plutôt que musical. Ainsi on peut élaborer sur les idées originales de M. Fessard en ce qui concerne la spontanéité des rythmes.

- [1] J. Aschoff, R. Wever, Resynchronisation der Tagesperiodik von Vögeln nach Phasensprung des Zeitgebers, *Z. Vergleich. Physiol.* **46** (1963) 321.
- [2] J. Aschoff, Adaptive cycles: their significance for defining environmental hazards, *Int. J. Biometeorology* **11** (1967) 255.
- [3] M. Garcia Sainz and F. Halberg, Mitotic rhythms in human cancer, reevaluated by electronic computer programs—evidence for temporal pathology, *J. Nat. Cancer Inst.* **37** (1966) 279.

L. TISZA: I would like to ask Dr. Lindauer how long does it take for bees transferred from the Northern hemisphere to the Southern hemisphere to readjust their north sense orientation? Do you know something about it?

M. LINDAUER: After the translocation experiment we have tested the bees every second week. Only after the 40th day they had readjusted their orientation to the new situation. The result is different however if we let hatch bees in an incubator and then raise them without sun (the bee colony is put in a cellarroom by artificial illumination). After 4 weeks I took the bees out of the cellar into the field. They were unable to use the sun as compass, they used it on the first 3 days just as it would be a fixed light point on the sky. After the 5th day however (the bees had absolved 500 collecting flights for a goal in 200 m distance) all collectors had *learned*, how fast and in what direction the sun moves across the sky. They had changed from the simple "angle orientation" to the true "compass orientation".

K. MENDELSSOHN: Since we now seem to have a little time left, allow me to reply shortly to Dr. Longuet-Higgins. I have no wish to be dogmatic about my suggestion that the magnetic rotation of the axis of polarization is the operative mechanism occurring in the bee. It only occurred to me that it may be a possible explanation. Of course, the effect is a small one but the relationship involved is not necessarily a linear one. There are cases where a small external field can, in a suitable substance, affect the direction of a very much larger internal field. This means that the field registered in the sensing mechanism could be of the order of a kilogauss rather than one gauss.

F. HALBERG: Si j'ai bien compris votre question, M. Lindauer (nous en avons parlé en aparté): vous avez un renforcement temporel de la mise en condition. Donc, le comportement dépend de la structure temps comme étant un certain

nombre des programmes et engrammes de M. Fessard. Pendant très longtemps, la structure temps a été considérée comme étant imprimée par l'extérieur et persistant de l'intérieur. Les études et les données dont on dispose maintenant, au niveau cellulaire, à celui des processus inter-cellulaires, ou même au niveau du comportement de l'organisme global, nous permettraient de supposer que certaines "fréquences" existent dans l'organisme dès la naissance.

Tout comme l'enfant humain, sur auto-demande, aura un déplacement ultradien vers circadien en fonction de son âge pour l'alternance veille-sommeil, les abeilles ont peut-être également un rythme circadien. Il sera bien important d'étudier les abeilles en libre cours. Peut-être est-il possible de mesurer le bruit dans une ruche comme une fonction de temps? Cette mesure paraît simple, mais peut-être est elle compliquée par la possibilité qu'un nombre indéterminé d'abeilles n'est pas synchronisé avec la population entière étudiée. Naturellement il serait bien préférable d'étudier une fonction de l'abeille elle-même, laquelle peut être évaluée par une analyse longitudinale, individuelle. Ceci permettrait de savoir si une mise en condition quelconque—pour la menthe, dont vous avez parlé—est un réflexe conditionné, qui est superposé à une formation temporelle circadienne de base de l'abeille.

Tout ceci se rapproche de la théorie de M. Fessard: il y a un programme, le programme s'écoule. A tel ou tel moment, l'organisme est exposé à une stimulation; il commence à apprendre et ce qu'il va apprendre dépend aussi de sa phase au moment de la stimulation. Puis, ce qu'on a appris doit être reproduit; il faut consulter cette information avant d'agir—comme déterminant d'une action orientée de l'organisme. Les tâches de tenir en réserve ("storage") et de reproduire ("retrieval") l'information sont probablement, au moins en partie, une fonction du système circadien.

The interesting studies described by Dr. Lindauer reemphasize a discussion of long standing as to the extent to which rhythms relate to learning and vice versa (the extent to which learning depends upon an organism's time structure). Some features of a rhythm's synchronization have indeed been compared to conditioned reflexes. Thus Maizelis [1] described as a "cause" of spontaneous changes in motor activity the formation of positive condition reflexes to the time and environment in which muscular work was performed. Few will question the suggestion that conditioning contributes to our time structure, consisting of a number of programs and engrams as discussed by Dr. Fessard. Our "memory traces" when they are being registered also may well be timecoded. This circumstance should not lead one to presume that conditioning usually brings about the rhythm at the outset; instead, the rhythm may well be innate and a determinant of conditioning. Several lines of evidence demonstrate indeed that conditioning can be determined by the stage of a circadian rhythm in which the conditioning procedure is applied—the work of Charles Stroebel at the Institute of Living in New Haven, Connecticut being a case in point [2]. Dr. E. Bünning in Tübingen

had once reported that a disturbance at a certain stage of the rhythm, I believe in leaf movement, might reappear in a fashion similar to a "memory trace" on subsequent days with a predictable timing.

To turn back to Dr. Lindauer's most interesting observation, the time may be ripe to study a relatively easily measured yet pertinent variable, the noise of a bee hive or, to avoid confounding from at least a partial lack of inter-bee synchronization, preferably some function that can be measured longitudinally on individual bees, under as constant conditions as possible. If, then, from such work a period desynchronized from both a 24-h solar and a 24.8-h lunar day could be found and the experiment with peppermint and honey described by Dr. Lindauer could then be repeated, one might have at least some tentative information toward the question whether the conditioning described by him represents simply a conditioned reflex or some kind of reinforcement of training, or whether there is indeed a "program" preexisting for the timed conditioning of such stimuli within the organism as suggested at this meeting by Dr. Fessard.

- [1] M. R. Maizellis, Time and Conditions of Performance of Muscular Work as Factors of Organization of Diurnal Periodicity, *Bull. Exp. Biol. Med.* **45** (1958) 526.
- [2] C. Stroebel, Behavioral aspects of circadian rhythms, *Comp. Psychopathology* (1967) 158.

P. O. LÖWDIN: There is one rhythmic phenomenon which I think is very intriguing. It's the phenomenon of sleep. Do you care to comment?

F. HALBERG: One of the interesting features of sleep is its multiple frequency structure. At the moment a good deal of work revolves around what is called „fast" sleep or REM (rapid eye movement) sleep. The seven or eight hours of behavioral sleep (diagnosed on the basis of relative quiescence with our eyes closed) are modulated by rhythms of say  $\sim 1.7$  hours in a number of functions and perhaps in association with dreaming. It seems pertinent that in this day and age, molecular biology has already aimed at elucidating relations between dreams and molecules, at a symposium held recently at the Massachusetts Institute of Technology. Ultradian frequencies of human sleep become behaviorally overt by day as well as by night in patients with narcolepsy, a problem so rigorously studied by Pierre Passouant of Montpellier.

At the other extreme of the frequency components of spontaneous sleep one finds in some cases of human isolation the infradian component with one cycle in about 48 hours. What seems to be most important with respect to Dr. Löwdin's question may well be the recognition that whereas in human isolation sleep may change from a circadian to an infradian frequency, the adrenal cortical cycle may maintain a primary circadian rhythm. Thus the contention by many classical physiologists that most, if not all, bodily changes along the 24-hour scale are determined by sleep can be ruled out by results from studies by Michel Siffre

*et al.* covering several months and allowing the "self-selection" of a different frequency for sleep than for the adrenal cortical cycle [1].

This is not to say, however, that the time relations between these two functions are random. Temporal integration indeed can be achieved on more than one frequency, and such integration is particularly favored by the circumstance that the frequency of sleep-wakefulness "demultiplies" to one-half that of the adrenal cycle—as noted by us in the study on a human subject isolated for several months.

Pendant le sommeil l'enregistrement des mouvements de l'œil, l'électromyogramme, le pouls et la respiration complètent les études électroencéphalographique du sommeil rapide et il y a déjà des méthodes puissantes pour analyser de telles données si on fait l'enregistrement directement sur bande magnétique [2, 3]. Il sera très intéressant d'enregistrer chez les sujets ambulatoires et sains quelques unes de ces variables pour voir les équivalents d'un composant ultradien pendant la veille aussi bien que pendant le sommeil.

- [1] M. Siffre, A. Reinberg, F. Halberg, J. Ghata, G. Perdriel and R. Slind, L'isolement souterrain prolongé, Etude de deux sujets adultes sains avant, pendant et après cet isolement, *Presse Med.* 74 (1966) 915.
- [2] D. F. Kripke, C. Clark and J. A. Merrit, A system for automated sleep analyses and physiological data reduction, Document ARL-TR-68-12, August, 1968.
- [3] N. Cartwright, D. F. Kripke and P. Cook, Statistical reduction of handstaged sleep analysis. Document ARL-TR-68-5, June, 1968.

G. CARERI: If there exists a periodicity in sleep, then suppose an organism take a sleep out the periodicity. Is there any phenomenon you could detect in this siesta which is different from the phenomenon observing in sleep, because you break the periodicity?

F. HALBERG: Rather than necessarily "breaking" a circadian periodicity by taking a "siesta" one may simply accentuate and/or prolong a physiologic phase of a presumably innate ultradian rhythm (with a frequency much higher than circadian). The ultradian rhythm can be gauged by the telemetered intraperitoneal temperature, among other functions.

Si vous chronométrez la température intra-péritonéale par un détecteur physiologique placé dans l'abdomen d'un rat, et même si l'animal se trouve dans une ambiance sous contrôle aussi absolu que possible (parce que la température d'ambiance est contrôlée ( $\pm .5^\circ\text{C}$ ) et les bruits, dans la mesure du possible—on ne peut pas toujours faire les choses parfaitement!) vous trouvez des changements en température corporelle qui couvrent en quelques heures une différence aussi grande que  $2^\circ\text{C}$  que nous ne pouvons absolument pas expliquer dans le sens d'une "réponse" à des facteurs connus. Ce sont des changements ultradiens.

Voici donc un rythme circadien avec une modulation par l'élément ultradien. Et vous arrivez à ceci: la sieste est peut être simplement l'expression du rythme

ultradien "approfondi". On trouve des rythmes, ou pararythmes, ultradiens avec des fréquences peu définies qu'on peut démontrer par des spectres de variances aussi dans la concentration du sang en hormones corticostéroïdes, même dans l'effluent de la surrénale cannulée [1]! Je ne sais donc pas ce que la sieste fait à notre physiologie, mais nous faisons beaucoup plus de siestes que vous ne le pensez.

Concerning uncertainties in experimentation, one would have to do so from both theoretical and practical viewpoints. Dr. Fessard's thoughts on the role played by a transducer between stimulus and response included reference to an observer-effect upon the phenomenon being observed, i.e., reference to the equivalent of a biologic uncertainty relation to be considered in the context of Heisenberg's thoughts [2]. In rhythmometry there are in addition many practical points to be considered, including the effect of the interval between consecutive samples upon data analysis on the one hand and upon the subject himself on the other hand. In this connection it is indeed a considerable step forward to dispose of electronic computer programs allowing the analysis of data obtained at unequal intervals whereby certain kinds of interval artifacts are prevented and, what is no less important, the human subject is allowed undisturbed sleep [3]. The only condition for such analyses at unequal intervals is that the density of the data during most of the span be not too drastically different. Of course, for certain tasks other than performance tests, continuous physiologic monitoring—eventually from birth to death—may well be feasible by transducers that, thanks to NASA, already are within the "state of the art".

By achieving such goals, we could obtain for any physical examination at any time of our choice information on the preceding 60 or 80 (or more) circadian cycles in a given monitored variable of interest—body temperature, heart rate or other. Such a desideratum is no more than what we require for the evaluation of high frequency rhythms in current medical practice. More specifically we evaluate heart rates on the basis of 60 or more cardiac cycles. Thus, it seems only fair to require more than a single sample spotcheck and, in some cases, data covering more than a single cardiac cycle, for an assessment of a circadian modulation of the heart rate. However because such monitoring of heart rates and of other pertinent functions such as blood pressure is currently still expensive the student of rhythms will have to demonstrate that such information on certain rhythms with medial or low frequency is worth collecting.

- [1] J. H. Galicich, E. Haus, F. Halberg and L. A. French, Variance spectra of corticosteroid in adrenal venous effluent of anesthetized dogs, *Ann. N.Y. Acad. Sci.* **117** (1964) 281.
- [2] F. Halberg, Chapter on "Medizin" in *Jahrbuch der Internationalen Hochschulwochen des Oesterreichischen College*, (Igonta Verlag, Salzburg, 1946) 336–351.
- [3] F. Halberg, M. Engeli, C. Hamburger and D. Hillman, Spectral resolution of low-frequency, small-amplitude rhythms in excreted ketosteroid; probable androgen-induced circaseptan desynchronization, *Acta Endocrinologica Suppl.* **103** (1965).



B. B. LLOYD: I have a friend who does experiments on himself from time to time. He was measuring his body temperature day and night about twenty years ago. In the course of one day he was sitting by the gas fire, a primitive sort of heating still used in England, at 6 o'clock in the evening until he sweated profusely. The next evening at 6 o'clock, not sitting by the gas fire, he found that his body temperature went down. I would regard this as an example of a memory and of an anticipation in a homeostasis.

One more point. I think you do see Cheyne-Stokes breathing in a dog if you lengthen the path between the lungs and the carotid body, and fiddle the gaseous atmosphere—a feedback loop showing oscillation rather than being critically damped.

F. HALBERG: Dr. Lloyd considers a feed-back loop in relation to Cheyne-Stokes breathing—indeed in one of the several interesting rhythms of the respiratory system. In discussing such feed-back models for high frequency rhythms, one does not as a rule encounter the dangers and limitations so characteristic of feed-back considerations in the domain of rhythms with medial and low frequencies. The following remarks will be restricted to physiologic phenomena in the latter domain—adrenal physiology, cortical as well as medullary, being a case in point. Much work is being done, in the adrenocortical field in particular, without identifying the stage in which a given “feed-back study” is carried out—despite the demonstration of, for instance, a reproducible circadian rhythm with a large amplitude in adrenocortical reactivity to ACTH, *in vitro* as well as *in vivo*. Also ignored is the added feature that *in vivo*, in the C-mouse, adrenal reactivity to relatively unspecific stimuli such as saline also is circadian rhythmic and that furthermore such rhythms in the reactivity of the adrenal cortex to saline solution on the one hand and to ACTH on the other hand are out of phase with each other. Equally pertinent rhythms in pituitary ACTH content and in the corticotropin releasing factor, CRF, of the hypothalamus also are usually ignored, with the mistaken tacit assumption that whatever one does in terms of a “feed-back study” at one time will be reproduced at any other time. Today, successes in electronics have attracted biologists to an often uncritical transfer of comments out of context, i.e., to an application of control system theory to “biologicals”, just as if they were fully inert materials. Thus we repeat the mistakes of a few centuries ago. For instance, so great were Newton's successes and his consequent influence that so distinguished a physiologist as Borelli was prompted to a mechanistic thinking that apparently led him to explain all of digestion by mechanical friction.

The foregoing comments are directed not against a consideration of control mechanisms in physiologic work but rather against the prevailing custom of ignoring rhythms while one does so, whereby mistaken conclusions may be drawn concerning controls. Feed-back considerations then are a useful “scaffold”

for physiologists and should prompt him to evaluate rhythms more rigorously and quantitatively and to search for the underlying factors, rather than misusing them as legitimate excuses for studying rhythmic variables without any consideration for time structure.

L. ROSENFELD: I am not sure that I understand your standpoint. It seems to me that scaffolding, as you call it, is not an obstacle to building a house, but on the contrary helps to do it.

F. HALBERG: We need a scaffolding. However, we must not mistake the scaffolding for the house and move into or onto it. For instance, homeostasis has been a scaffolding for some, and unqualified feedbacks or "stress reactions" were a scaffolding for others. I have used feedback models [1] myself but only as a transition to the specification of physiologic phenomena as they relate to chemical compounds in anatomical locations. A simple black box approach ignoring all anatomical, physiological and biochemical rhythms has unfortunately led to the practice of assessing presumed stress reactions or feedbacks at some single time point convenient only to the experimenter. This approach ignores results such as those indicating a rhythmic change as drastic as the difference between death and survival occurring in response to the identical agent and as a function solely of timing [2]. Such results on the hours of changing resistance dramatize the need to, first and foremost, control the stage of a rhythm in work on an organism's frequency structure and, second, use endpoints from rhythmometry as gauges of physiologic "responses".

Pertinent in this connection are not only studies on rodents [2, 3] but also the important French studies of Alain Reinberg on men [4].

- [1] F. Halberg, E. Halberg, C. P. Barnum and J. J. Bittner, Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine, in *Photoperiodism and Related Phenomena in Plants and Animals*, Ed. Robert B. Withrow, Ed. Publ. No. 55 of Amer. Assoc. Adv. Sci. Washington (1959) 803-878.
- [2] F. Halberg, Organisms as circadian systems; temporal analysis of their physiologic and pathologic responses, including injury and death, in: Walter Reed Army Institute of Research Symposium, Medical Aspects of Stress in the Military Climate, April, 1964, 1-36.
- [3] L. E. Scheving, Circadian rhythms in susceptibility of rodents to nicotine and amphetamine, AAAS, Washington, Resume in *J. Amer. Med. Assn.* **199** (1967) 33.
- [4] A. Reinberg, The hours of changing responsiveness and susceptibility. *Pers. Biol. Med.* **11** (1967) 111.

H. C. LONGUET-HIGGINS: I cannot see, speaking as an outsider, that there is anything inconsistent between thinking that it is good to investigate the cyclic phenomena in the cell, or in the body, or in the organism, and thinking that it is also good to investigate the non-cyclic processes. Living things are very complicated; one must pay attention to many different things. To stress the value of

your own studies is not necessarily to imply that other sorts of studies are not also valuable.

F. HALBERG: Dr. Longuet-Higgins emphasizes of course that there are many approaches to any one biologic problem. Let me hasten to agree and apologize if I gave the impression that rhythmometry is a panacea for any and all problems. Nonetheless, it is difficult to accept "homeostatic" work on rhythmic variables when, as is customary, the identification of the stage of a rhythm in the variable used for sampling is altogether ignored. Knowledge of the stage of the circadian rhythm may be as desirable for a variable such as corticosteroid as is the statement on whether a blood pressure measurement is systolic or diastolic. Our interpretation of a blood pressure determination of, say, 100 mm Hg will be drastically different as a function of whether it is diastolic or systolic; accordingly one may distinguish a hypertensive patient from a hypotensive subject. Quite clearly the Reverend Stephen Hale must have been aware of the difference between diastole and systole when he measured blood pressure directly by attaching a glass tube to the artery of a horse and observing how a column of blood rose and fell with the heartbeat. According to Dr. Leonard Wilson, Borelli had already discussed the change in pressure occurring with each contraction of the heart and had reported that with the systole the aorta stretched and subsequently relaxed. Circadian changes in serum corticosteroid or liver glycogen are no less drastic. Furthermore, the research work on yeast by Kendall Pye and Britton Chance, who regretfully could not be present, shows "ultra-ultradian" trains of oscillations with relatively very little damping once trehalose, a carbohydrate, is added to the medium. [1]

Thus, many aspects of metabolism are rhythmic and yet biochemical journals continue to publish papers by molecular biologists, endocrinologists and others, who ignore any and all periodicity in variables previously shown to undergo large amplitude rhythms amenable to rigorous standardization (e.g., murine liver glycogen). However to remedy this situation is hardly the major task for students of rhythm. Many of us are lead to study a given rhythm because we encounter it as a source of variation that must be controlled, yet in my opinion the primary tasks of the chronobiologist *in statu nascendi* lie on an entirely different plane.

We can raise new questions as to body function in health and disease—whether our interest relates to 1) transmeridian dyschronism after an intercontinental flight, to 2) the hygiene of shift workers, to 3) the failure of rhythmic integration in a psychotic patient, or to 4) the rhythm alteration found in a cancer patient. Such rhythm alterations could constitute determinants of a performance decrement, a resistance deficit or of an actual disease process. If indeed rhythms should prove to play an important role in such processes, sound biophysical theory for the ubiquitous and nontrivial spectral structure of organisms will become yet more desirable. Meetings such as the present one may contribute

to better human performance and health by fostering such theoretical developments—that can be anticipated from the interaction between physicists and biologists and that can hardly fail to have eventual applied value.

[1] K. Pye and B. Chance: Sustained sinusoidal oscillations of reduced pyridine nucleotide in a cell-free extract of *Saccharomyces carlsbergensis*, *Proc. Nat. Acad. Sci.* **55** (1966) 888.

A. REINBERG: Dans son analyse, portant sur les phénomènes rythmiques des nerfs et des muscles, M. Fessard a dit que l'activité rythmique apparaît comme une des propriétés fondamentales de la matière vivante. Nos connaissances actuelles permettent de généraliser cette notion à tous les systèmes vivants. Il apparaît en effet que des processus rythmiques peuvent être mis en évidence à tous les niveaux d'organisation:

1) des organismes uni-cellulaires jusqu'à l'homme y compris,  
2) à tous les niveaux d'organisation chez un être vivant, l'homme par exemple ou les mammifères supérieurs. Qu'il s'agisse de fonctions globales physiologiques ou biologiques, de groupe d'organes, de tissus, de cellules et même de fonctions sub-cellulaires, l'existence de rythmes a été prouvée objectivement. Par exemple: rythmes de l'incorporation du  $P^{32}$  dans le D.N.A. et le R.N.A. des cellules de divers tissus.

3) l'analyse objective de ces rythmes, réalisée suivant des processus de calculs électroniques mis au point par M. Halberg et ses collaborateurs de l'Université du Minnesota, permet de donner une estimation des différents paramètres et de caractériser ces variations périodiques. Il s'agit en particulier de la période ou de la fréquence des phénomènes, de leur amplitude et de leur relation de phase. C'est ainsi que, pour une même fonction physiologique (le rythme cardiaque, l'excrétion urinaire des 17 cétostéroïdes, la température par exemple), des analyses spectrales ont pu être réalisées permettant d'estimer les différentes périodes suivant lesquelles chacune de ces fonctions peut se manifester. L'étude des rythmes biologiques introduit donc une nouvelle dimension dans l'analyse des phénomènes vivants: le temps. Les résultats acquis permettent déjà de discuter les phénomènes sous leur aspect chrono-biologique et chrono-physiologique. Ces recherches ouvrent de nouveaux chapitres qui sont: la chronopharmacologie, la chronopathologie et la chronothérapie.

