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Circadian (about Twenty-four-hour) Rhythms in Experimental Medicine [*Abridged*]

Professor Franz Halberg
(*University of Minnesota, Minneapolis, U.S.A.*)

Before the end of the last century, Patrick Manson's discovery of microfilarial periodicity was reported in London. Among many British contributions to the field of rhythms with periods of about twenty-four hours one may single out as a spectacular one the study by Mottram (1945) who found that mice painted with benzpyrene developed far more tumours in the ensuing weeks when the paint was applied at midnight than when it was applied at noon. More extensive is the work of Bullough on mitotic rhythms. Other British speakers at this meeting will discuss their own continuing series of specific basic contributions to the rapidly developing field of physiologic rhythms. My remarks will bear only in general terms on the scope of circadian rhythms in experimental medicine. These rhythms will be viewed in the context of other physiological frequencies. The significance of the circadian component at different levels of organization will be discussed, with reference to the potential value of pertinent information in experimental medicine. Documentation must be restricted to a few illustrations, reviewed elsewhere in the context of work by others (Halberg & Howard 1958, Halberg *et al.* 1959, Halberg 1960, Ross Laboratories 1961, Halberg & Panofsky 1961, Ungar & Halberg 1962).

Before turning to my subject, I must cite the story of an earlier meeting. When, according to Manson-Bahr & Alcock (1927), Patrick Manson's

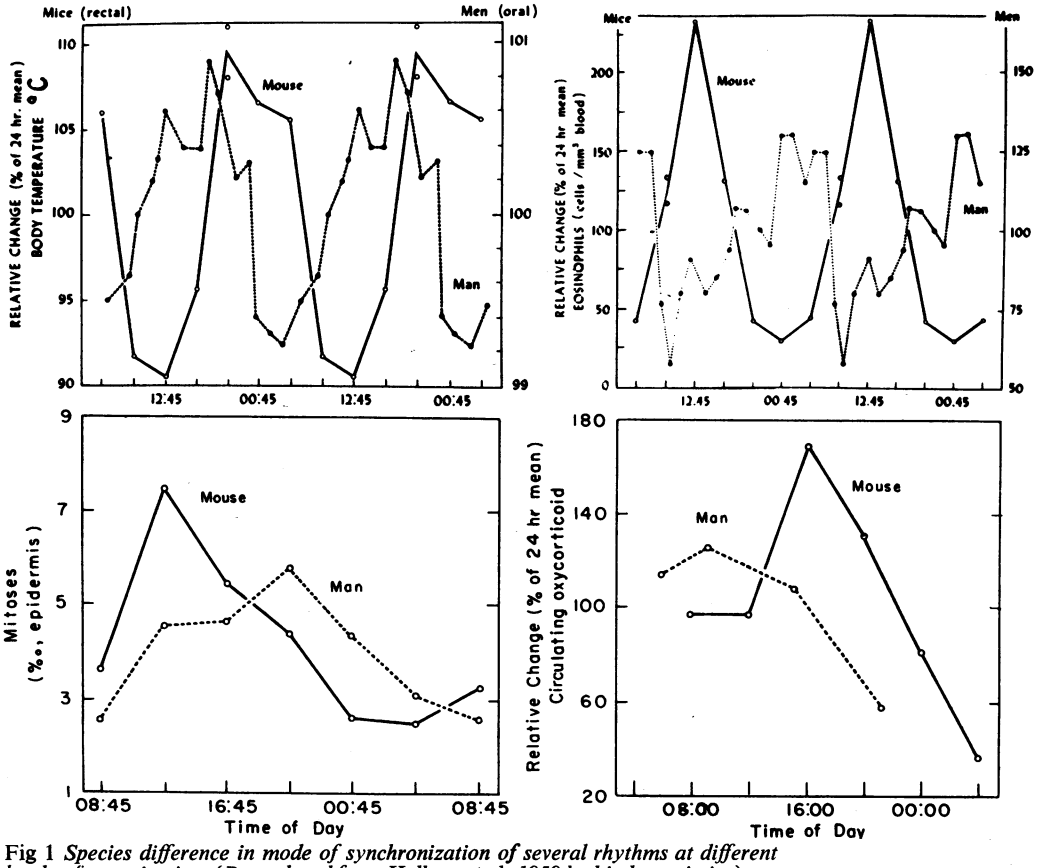


Fig 1 *Species difference in mode of synchronization of several rhythms at different levels of organization. (Reproduced from Halberg* et al. *1959 by kind permission)*

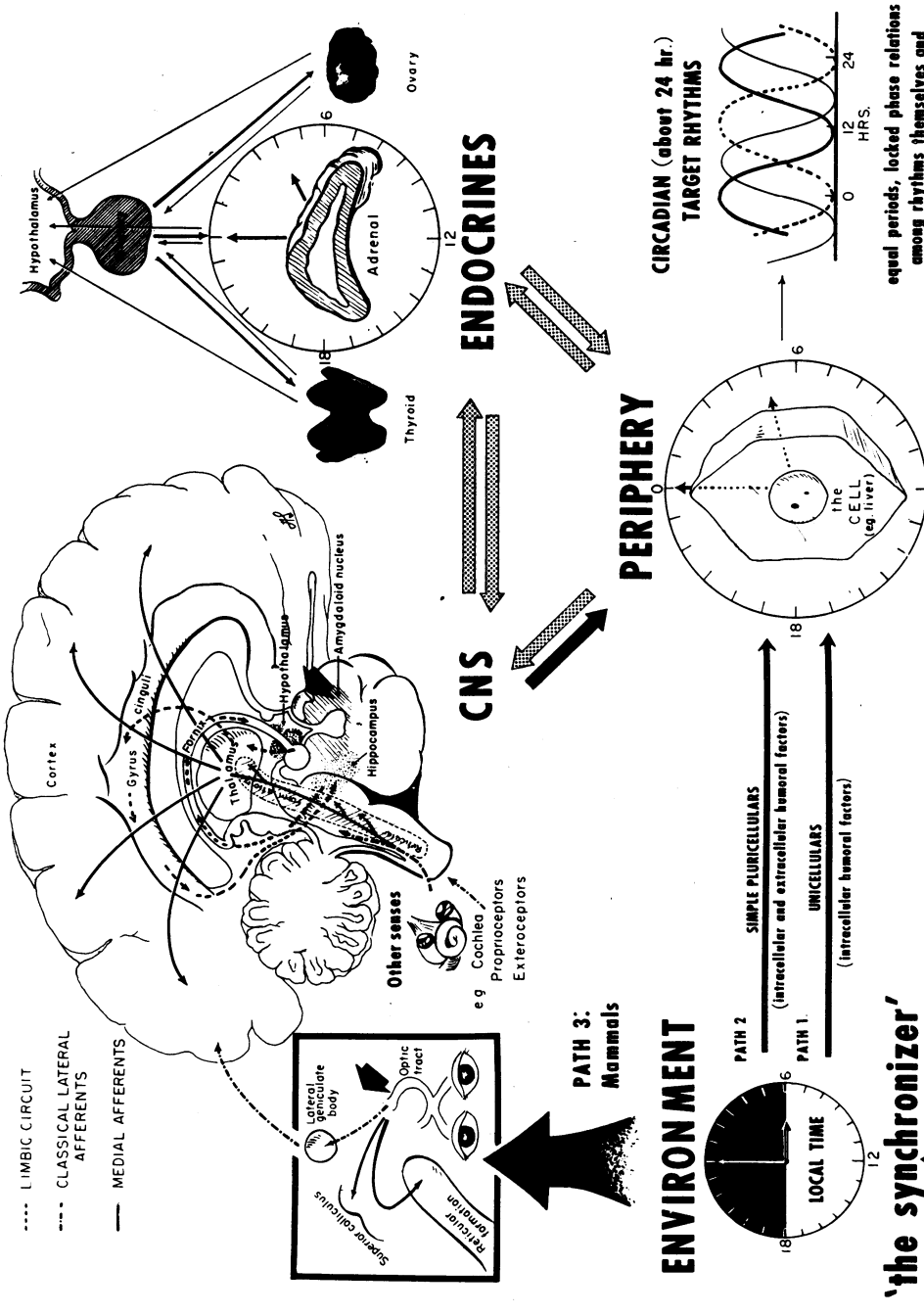


Fig 2 *Sketch of factors and pathways known or hypothesized to participate in frequency synchronization among circadian rhythms themselves, as well as in synchronization between rhythm(s) and environmental synchronizer(s). Note that the adrenal is a prominent endocrine control of certain circadian rhythms but it is not sketched as their sole governor. Cf. Halberg* et al. *(1959) for a more detailed discussion. (Reproduced from Halberg 1960 by kind permission)*

discovery of the nightly emergence into the blood of *Wuchereria bancrofti* microfilariæ was first made known in a scientific club in London, a Gratiano (from 'The Merchant of Venice') then present apparently raised the question whether the microfilariæ carried watches. This jester inadvertently heralded our decade of biological clocks, yet the biological measurement of time has until now remained an unsolved problem and it must remain beyond the scope of my remarks. From the viewpoint of experimental medicine, however, circadian rhythms can be treated as entities in their own right – as parameters of the organism's time structure acquired by evolution on earth.

Circadian systems: Cells, tissues, organs, organ-systems or organisms, or groups of these entities, may be viewed as circadian systems if they exhibit rhythmic changes with about 1 cycle a day. Among many other species, man and mouse constitute circadian systems. The data summarized in Fig 1 are presented as circadian charts obtained under conditions of twenty-four-hour synchronized circadian system analysis.

Phase-shifting: By manipulating the routine used for such synchronization, the external timing of circadian rhythms, i.e. their timing as to the local clock hour, may be shifted along the twenty-four-hour scale. This applies to functions at different levels of mammalian organization. Phase-shifting of circadian rhythms can be accomplished by a change in the institutional routine of human subjects or by the reversal of the lighting regimen in the experimental animal room.

Free-running: Circadian rhythms persist under conditions of constancy in physical environmental factors, as far as such conditions can be achieved on earth. Tests of their persistence are now being planned in extraterrestrial space as well. On earth organisms maintained, for instance, in constant darkness or constant light show average circadian periods that usually deviate from exactly twenty-four hours by minutes or by a few hours. We have detected such periods in blinded mice – in their rectal temperature, blood eosinophil and pinnal mitotic rhythms – and have tentatively called these periods free-running ones, by analogy to a free-running oscillator (for review *see* Halberg *et al.* 1959). We know of no exact environmental counterparts to such periods. This does not imply that the organism, an open system, is no longer affected by environmental influences, periodic or other, when it exhibits non-twenty-four-hour circadian periods in one or other function. The free-running period does suggest, however, the operation of intrinsic mechanisms in the maintenance of an organism's circadian periodic behaviour. Free-running circadian rhythms are also of methodological interest since, for example, the peak of a given rhythmic function with such a period will occur at a different time each day.

Mechanisms: A circadian periodic sequence of related cellular processes is governed in the mammal by adrenal and other, juxtaposed and superimposed, controls (Fig 2). Adrenal factors are essential in the maintenance of some circadian rhythms of man and mouse, but other circadian rhythms appear to be maintained in the absence of the adrenals (Halberg *et al.* 1959).

Hormone effects: The detection of an effect of pituitary growth hormone *in vivo* or the response of the mouse adrenal to corticotrophin, *in vitro* (Ungar & Halberg 1962) as well as *in vivo* (Haus & Halberg 1960), depends upon the time relations among the organism's circadian rhythms, i.e. the circadian system phase.

The adrenal cycle is a basic physiologic entity preparatory to daily activity. In the absence of stimulation other than daily routine, a circadian

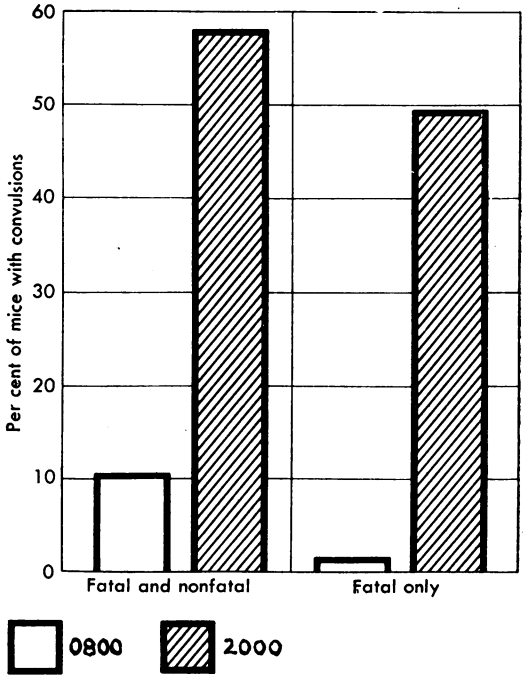
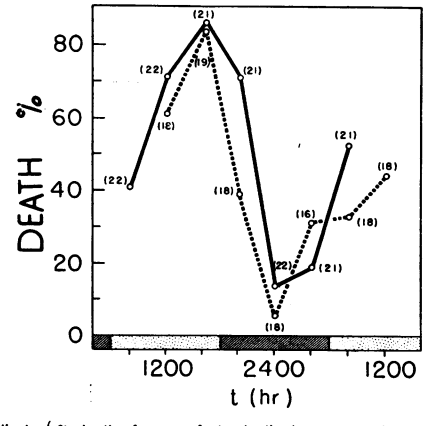


Fig 3 *Unequal number of convulsions and of deaths from convulsions of comparable mice after exposure to identical auditory stimulation at different stages of eosinophil rhythm. (Reproduced from Halberg & Howard 1958 by kind permission)*



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 4 *Physiological circadian periodicity and the end-point 'death from an endotoxin'. (Reproduced from Halberg 1960 by kind permission)*

periodic rise in corticosteroids of blood precedes the onset of motor activity, in men and mice. Three stages of this cycle have thus far been recognized in inbred male C mice, by the time relations among the peaks of the circadian rhythms in (1) pituitary ACTH content, (2) spontaneous adrenal activity gauged by serum and adrenal corticosterone levels, and (3) adrenal reactivity to ACTH. Peak (2) lags behind peak (1) by about 6 hours and leads peak (3) by about 12 hours (Ungar & Halberg 1963). The basic nature of the adrenal cycle is attested by its persistence under conditions that obliterate the œstrus cycle, even in mice deprived of all food and water (Galicich *et al.* 1963).

Resistance to injury: A mammal's circadian time structure at the moment of exposure to injury can predictably tip the scale between death and survival – following the administration of agents ranging from physical ones such as noise (Fig 3) to bacterial endotoxins (Fig 4) and even drugs. As a temporal concept the hours of diminished resistance seem to be well documented and may be aligned with the morphological counterpart, the loci of diminished resistance.

Variance spectra: Circadian functional integration and adaptation provides but one component to the broader spectrum of physiological frequencies. This component and others may be quantified by periodograms and variance spectra (Blackman & Tukey 1958). In the latter technique the total variance of a set of measurements is resolved into a series or spectrum of component variances, one for each unit frequency. In the absence of a rhythm the variance is more or less evenly divided over all frequencies; a peak in the variance at one or more frequencies indicates the presence of a corresponding number of biological rhythms. The probability of such peaks appearing in the data by chance can be calculated (for further detail *see* Halberg & Panofsky 1961). Figs 5 and 6 summarize halting steps in using changes in circadian components of body temperature as a gauge of disease and/or drug effect – in the absence of fever or hypothermia.

Conclusion

Organisms evolved on earth represent circadian systems. Study of such systems calls for the definition of pertinent temporal parameters that provide the essential control in any work on functions demonstrated to be rhythmic. In this connexion an indication of time of day of sampling, in clinical tests or laboratory work without fur-

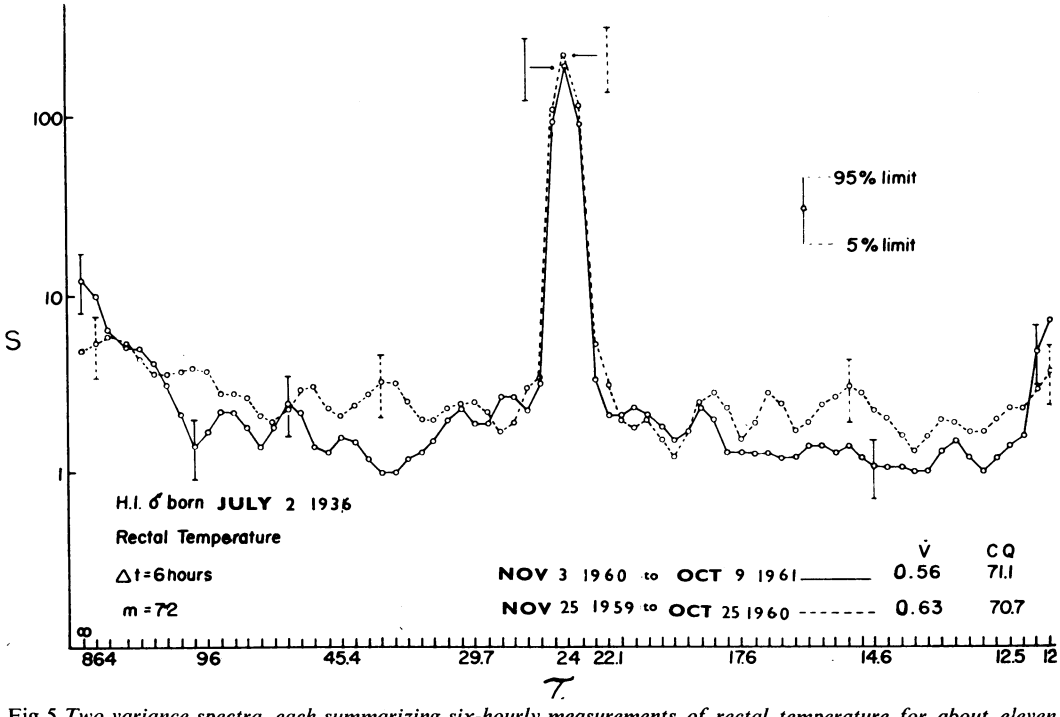


Fig 5 *Two variance spectra, each summarizing six-hourly measurements of rectal temperature for about eleven months on the same male human subject, reveal the remarkable stability of the circadian component from one year to the next.* V *is the variance;* S=*variance/unit frequency;* τ=*period in hours;* CQ *is the circadian quotient, which indicates the proportion of the variance associated with the circadian rhythm;* m *determines the resolving power in spectral analysis. (For details* see *Halberg & Panofsky 1961; reproduced therefrom by kind permission)*

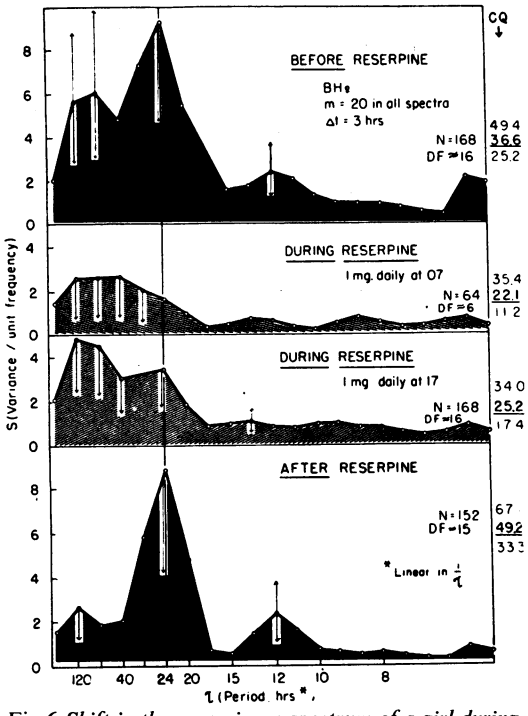


Fig 6 *Shift in thermovariance spectrum of a girl during two periods of reserpine administration (hatched). Shift occurs in the absence of fever or hypothermia. Circadian component is prominent in spectra on top and bottom (before and after reserpine administration) but the variance is spread out into adjacent frequency domains during reserpine administration (middle).* N, *number of observations;* DF *degrees of freedom.* CQ, *circadian quotient.* m *determines resolving power in spectral analysis (for details see Halberg & Panofsky 1961). (Reproduced from Halberg 1962 by kind permission)*

ther qualification, is not a sufficient precaution since circadian rhythms may be phase-shifted or free-running.

Resistance to injury from a variety of agents and the effect of certain drugs and hormones depend significantly upon circadian system phase.

Variance spectra provide end-points of potential use to the experimental pathologist and pharmacologist by revealing the significance of changes in the circadian component as well as other aspects of a broad domain of physiological frequencies, in health and disease.

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Dr Frederic C Bartter & Miss Catherine S Delea
(Clinical Endocrinology Branch, National Heart Institute, National Institutes of Health, Bethesda, Maryland)

Circadian Aspects of Human Adrenal Function

All measures of adrenal cortical function for which appropriate information is available show circadian fluctuations whose magnitude may be very large relative to the absolute value at any given time. It is perhaps superfluous to emphasize the importance of such circadian fluctuations at this symposium, but neither adequate experimental design nor correct interpretation of laboratory results is possible without estimates of circadian components. The changes resulting from circadian fluctuations in the indices of adrenal function often exceed those resulting from the stimulus under study in a given experiment; the variance of data from which circadian components have not been removed may be so great that distinctly abnormal values are included in the 'normal' range.

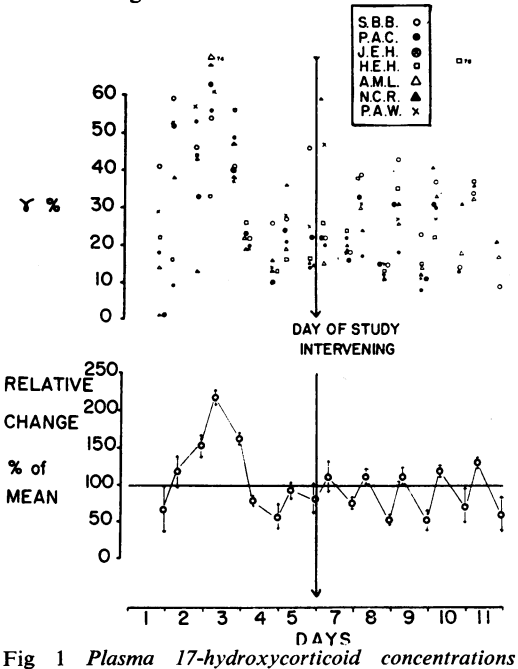

Fig 1 *Plasma 17-hydroxycorticoid concentrations determined daily at 0715 and 2315 in 7 normal female subjects. At the bottom of figure the same data are plotted as relative change, per cent of mean*