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BIOLOGICAL CLOCKS: ENDOGENOUS CYCLES SYNCHRONIZED BY SUBTLE GEOPHYSICAL RHYTHMS

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Organismic sensitivity to atmospheric electromagnetic forces, together with usually asymmetrical phase-response systems, have enabled a simple unified explanation of the biological clocks and their timed rhythms. The clocks, endogenous cycles synchronized by subtle geophysical Zeitgeber, are always accurate. The rhythms, freely phase labile, autophase in "constant conditions" to generate a host of overt frequencies.

1. Introduction

There have always been two defensible, but alternative paradigms concerning the nature of biological clocks with impossibility to date of eliminating either one. Viewing the history of the two concepts we find that both have been entertained intermittently for more than a century. The popularities of both have waxed and waned. The most widely popular at present is that the clocks are autonomous physicochemical cellular mechanisms (Van den Driessche, 1975; Njus, Sulzman and Hastings, 1974; Ehret and Trucco, 1967). Perhaps somewhat less appreciated is that the clocks may depend upon continuous organismic-environmental interactions (Brown, 1960, 1969 and 1972). Both now appear probably to be cooperating in the clock phenomenon.

In terms of the first hypothesis the daily clocks are presumed almost never to be completely accurate, yet almost incredibly so for biological systems over such relatively long time spans. Their inaccuracy is expressed by the various periods (seldom more than about 15% away from 24 hr) of observed free-running circadian rhythms in the absence of all Zeitgeber changes; 24-hr cycles of obvious Zeitgeber such as light are needed to assure their proper synchronization with the solar day. In terms of the second hypothesis, the clocks have quite understandably perfect accuracy, with the apparent inaccuracy of their timed rhythms attributed to phase lability with regular daily self-phase-shifts or autophasing.

External dependence of the clocks was supported for many years by such scholars as Nobel Laureate chemist Svante Arrhenius (1898), the famous plant physiologist W. Pfeffer (1907), and most recently had been supported by me and my associates. The present widespread acceptance of autonomous biological timing began early in this century when W. Pfeffer (1915) finally felt compelled, in terms of all knowledge then available to him, to conclude that each organism must independently contain its own timer for its own circadian rhythm. Of great importance for his conclusion were the diverse free-running periods with every individual organism having its own. This view which has largely dominated the field to the present reached its zenith in the early 1960's. About this time, two extremely important discoveries were made and areas of research opened. During the period 1958 to 1963, there was demonstrated to be (1) continuous interaction between organisms and geophysical electromagnetic fields at strength levels even relatively recently deemed theoretically impossible, and (2) the existence of a peculiar asymmetrical phase-response system in organisms operative in phase-shifting and phase-determining the rhythms. Indeed, had Pfeffer had these two major contributions available to him in 1915 he would probably not have felt compelled to accept the then popular concept of autonomous internal timing, as he reluctantly and belatedly did, and the nature of the mysterious clocks would have remained an open question.

People pressed to offer the arguments favoring the presently widely accepted internal cellular hypothesis have been forced to admit essentially as Hastings (Brown, Hastings and Palmer, 1970) did, in concluding a chapter on such evidence, "It is true that the biochemical investigations which I have outlined fall short of dealing with the endogenous-exogenous question in an authoritative way. But the considerations which I have outlined leave me and many of my colleagues with the conviction that biological systems can function autonomously as "clocks", ..." Indeed, it is presently impossible to develop an unambiguous case accounting for biological rhythms either in terms of fully autonomous clocks or of fully exogenous ones. The answer is becoming ever clearer that both are involved. Let me describe some newer information that has supported the concept that the timing includes involvement of both endogenous and exogenous elements. Such a timer could free us at once from the need of continuing to attempt to account for all of the multifold extraordinary characteristics of the circadian and other geophysically correlated rhythmic phenomena exclusively in terms of hypothetical, wholly internal oscillators.

2. Absence of constant conditions

An alternative hypothesis* to autonomous internal clocks began to take form in my mind in 1953. Extensive ongoing studies of the rhythmic system of fiddler crab color changes in darkness had led us to report concurrent persistent, accurate, temperature-independent 24-hr solar-day and 24.8-hr lunar-day cycles. Accurate 24 hr periodicity can always be suspected of being timed by subtle environmental variations which are a consequence of the influences of man on many subtle and pervasive aspects of the ambient physical environment. Lunar-day ones are, however, quite clearly free of such suspicions, and yet the lunar variations seemed just as accurate and persistent as the solar-day ones. Our break with the vast majority of clock scholars began when we moved in the direction of attempting to account for the great apparent precision of the cycles, emphasizing the lunar-related ones to avoid the suspected 24-hr period, while the others turned their attention to the non-24-hr free-running circadian ones in constancy of all Zeitgeber fields, both postulating these to reflect the independent internal clock periods, and to avoid any 24-hr artifact.

It was our view at that time that if such accurate periods of clock-timed rhythms as exhibited by the crabs could occur in even a single species, then the diverse, variable, and unpredictable free-running circadian periods were unlikely to represent directly the actual periods of the clock. We believed that they must have some alternative explanation. We felt, further, that they must in some manner be dependent on a clock system, fully temperature-independent over at least a large temperature range, of the same sort the crabs possessed. It seemed improbable that other plants and animals, especially the homoiothermic birds and mammals, would have been saddled during their evolution given a significantly less precise timing system than that of the poikilothermic crabs. Indeed, the latter speculation has appeared to be true. Free-running circadian rhythms of rats (Brown, Shriner and Ralph, 1956; Brown and Terracini, 1959), hamsters (Brown, 1965; Brown and Park, 1967) and even man (Aschoff, 1967), among other species, have been described to show rather strong propensity for adopting rather precise lunar periodicities when free-running in the absence of dominating Zeitgeber.

About 1953, evidence was also beginning

to suggest strongly that the organisms were, in our carefully regulated "constant conditions", still steadily receiving subtle information from their atmospheric environment. This evidence, never contradicted, was the discovery of numerous cross-correlations with meteorological, and other variations of factors and specific parameters of them, from which the organisms were fully screened (Brown, 1962, 1968). The interpretation compelled presumption of responsiveness of living things to unidentified fluctuating forces of the atmosphere.

It seemed evident in the light of the foregoing that it was theoretically possible for an organism to derive accurately the geophysical periods from its environment, periodicities obviously with full temperature and drug independences. If through some unidentified means frequency transformations could be effected, cycles deviating slightly and in varying degrees in their periods from any natural geophysical ones could be generated within the organisms. I stated in 1957 (Brown, 1957) that such cycles would be indistinguishable from fundamental, purely endogenous oscillations of the same period. Two years later I introduced the term, autophasing (Brown, 1959), or self-phasing, for such a theoretical basis for the "free-running" circadian periods. This term was very disquieting for the concept of autonomous internal timing. It was indeed a pivotal term for an alternate view for genesis of free-running rhythms. The term autophasing was introduced, coincidentally, the same year that Halberg (1959) coined the term circadian to emphasize that the rhythmic periods, in Zeitgeber constancy, were rarely exactly 24 hr. This was a fact that the concept of autophasing was proposed to explain. However, circadian became quickly applied to the clocks themselves, consistent with the autonomous biological oscillator concept which arbitrarily assumed the internal clocks and rhythms to have the same periods. The question of the two hypotheses, namely whether or not the biological clocks depend for their stability upon the subtle environmental rhythms, was at that time arbitrarily decided by most workers in the field in favor of what was felt to be the simpler of the two hypotheses, namely private timing by relatively inaccurate clocks inside each individual organism. Such inaccuracy was also believed at that time to establish that the clocks were fully independent of all environmental rhythms.

3. Endogenous and exogenous components of clocks

The expression biological clock places the clock mechanism within the organism. The clocks are investigated through the diverse processes which exhibit periodic fluctuations. It has become commonplace to investigate the rhythms that are observed in unvarying conditions of all obvious factors of the environment, conditions under which the clocks have been presumed to be running free. These controlled factors include all those that have been demonstrated to be able to alter the phase relations of the rhythms with respect to the environmental cycles. All of the investigated rhythmic processes involve components derived during a development directed by the genome. It has become customary to presume the observed periods of the rhythms to be those of the clocks, and properties of the rhythmic cycles to be properties of whatever the timer involved. The observed biological fluctuations comprising the rhythms are clearly endogenous. The rhythmic changes, however, while clock-dependent are not generally believed to be the clocks.

The observed cycles may be altered in many ways without interference with their timing system. Cycle amplitudes may be altered by light level (Brown and Hines, 1952), the cycle form may reflect specific past environmental events (Renner, 1955; Bünning, 1956), and these alterations may persist within timed rhythms when the stimulus producing them is no longer provided. That the cycles have some means of timing which is independent of all local-time geophysical rhythms was shown long ago by geographic translocation studies on crabs transported from Massachusetts to California (Brown, Webb and Bennett, 1955) and honey bees from Europe to New York, and New York to California (Renner, 1955, 1959). The free-running character of numerous rhythms also indicated that the observed periods and forms of the cycles did not reflect directly any external atmospheric ones. Either the rhythms, or a rhythm-clock complex, were freely phase-labile with respect to all local-time environmental cycles. The geographic translocations established that the organisms were able to bridge with at least moderate accuracy a period of several hours within a day through independent internal timing unless, of course, some exogenous source of a 24-hr period on Greenwich or universal time such as some components of the earth's electromagnetic complex were operative.

The possession by organisms of a 24-hr cyclic component, exogenous in character, and on universal time has become increasingly suggested as we have learned more concerning orientational capacities in both space and time. The extraordinary ability to continue measurement of the 24-hr period during eastward and westward travel, while most remarkable, is theoretically capable of being explained in terms of a fully independent internal clock. But a most remarkable sense of geographic space known as the map sense by which an organism is apprised of the direction of geographic displacement from home in the absence of every obvious cue cannot be so simply explained. Yet, this capacity appears to be one of the most accurate. To do this last, more information is clearly essential. Needed is the kind of information that would be provided by the concurrent presence of a very stable Greenwich chronometer operating along with an informational input concerning local-time celestial relationships of the sun, moon, and stars. Such a combination has served well for man to provide him with the means for establishing his longitudes and latitudes accurately. Could it be that living creatures can obtain subtle exogenous information concerning universal daily cycles to use in conjunction with knowledge of celestial relationships obtained through perception of electromagnetic correlates providing local solar-, lunar-, and sidereal-day information?

A principal natural atmospheric parameter contributing universal time to the earth is the periodic electrical charge in the highly conducting ionosphere. This charge, which averages about 300,000 volts higher than the earth's surface, displays such a 24-hr universal-time variation which is quite large in amplitude. A test for a correlated biological rhythm could be made by simultaneously obtaining the solar-day variations in a single species under carefully controlled conditions of obvious factors at a large number of equidistant points around the earth at any given latitude. With 12 synodic months of daily data the lunar- and sidereal-day components would be essentially randomized and any statistically significant residual modulation of the mean solar-day cycles with longitude would reflect an extant 24-hr universal-time component.

The known electric-field sensitivity of life is, as will be described later, probably adequate to play such a role. A discovery of this character would be of inestimable importance not only in helping to account for an accurate and stable sense of time for terrestrial creatures but could provide that last, still missing, piece of information required to enable an explanation of the still completely unresolved map sense. The organisms would have a complete sense of space. Homing pigeons even when translocated the thousands of miles between Europe and North America remain aware of the directions of their displacement (Wallraff and Graue, 1973). The map sense seems to be intimately related in some manner to an exogenous origin of timing. This is indicated, among other means, by modulations in the homing activity or in home directional bias by other natural geophysical periodicities including the month and year (Gronau and Schmidt-Koenig, 1970).

The free-running rhythmic periods were found to be experimentally alterable to small degrees by changing light and temperature levels and by a few chemical substances. The free-running period, other factors equal, was also genome-determined. These last facts were interpreted to strengthen further a case for autonomous internal timing of the rhythms. Clearly, there appeared to be, at very least, a substantial endogenous contribution to biological rhythms including their timing within cycles themselves. A carefully examined short term internal sense of time in man studied by Hoagland (1933) was described to be influenced by temperature as were biological oxidations in general. However, Q_{10} in the conventional kinetic range would be quite inadequate to account wholly for the timing mechanism of the biological clocks. We don't know the degree of temperature independence of the internal timing component within most cycles. It seems quite improbable, however, that its degree and stability over a broad temperature range could account adequately for the known very small Q_{10}'s, sometimes as low as 1.00, of the clocks.

Those whose working hypothesis has been that the clocks were fully autonomous biophysicochemical oscillators have sought such oscillators but so far without success. Much more exciting, however, are proposed hypothetical models for a biological clock system. These models have so far been proposed for only the circadian cycles. One model is rooted in actions of polycistronic chromosomes, chronons, whose synthetic activities are postulated to require very close to a day to complete (Ehret and Trucco, 1967). A moderate degree of temperature independence is achieved by postulating that physical diffusion with its low coefficient was the limiting factor for the process. More recently, the cell membrane and ionic diffusion has been modelled as a circadian clock (Njus, Sulzman, and Hastings, 1974). The known temperature compensating activity of the lipids of the fluid mosaic membrane, among other things, gave credence to this model. But both of these model types still fall short of providing all of the reported known properties, including long-term stabilities and greater temperature independence, of the rhythm-clock complex. One or both of these models, however, may perhaps become established as an essential component of an endogenous contribution to the clocks of life. Such models are being tested for their validity by every available means.

At the same time, however, other kinds of findings have been reported. Stoppel (1916) found that the rhythms of bean seedlings correlated with the cycles of electrical conductivity of the atmosphere. Oysters, crabs, rats, hamsters, and chicks were reported to synchronize to the periods of atmospheric solar or lunar cycles in the absence of every ordinary cue. Was it conceivable that information steadily received by living things from the environment was in some manner contributing significantly to the observed, astounding rhythm stabilities? Period measurement could theoretically be independent, but phase synchronization could not be. Subtle Zeitgeber were indicated. We felt that this largely neglected aspect of organismic-environmental interactions through pervasive geophysical fields needed intensive attention. If the organisms that had been presumed to be in constant conditions were still in a rhythmic physical environment to which they could be shown to be sensitive, then this, we believed, could comprise a critical element for biological timing. The view of the vast majority of investigators who were then in the clock field was that the clocks did not depend in any manner on such exogenous cycles and any findings in this area would be irrelevant to the clock problem.

Those seeking external contributions to clock-timing have made many novel and exciting contributions. They have discovered a responsiveness of the organism to atmospheric fields that previously had been thought to be theoretically impossible. A fantastic non-thermal responsiveness to the extraordinarily weak electromagnetic fields of the atmosphere has been disclosed and some characteristics of the biological reactions to them have been outlined. Responsiveness itself exhibits fluctuations with the natural geophysical periods. Indeed, apparent phase-response systems to pervasive elements apparently involving responsiveness to the geomagnetic field have been reported (Brown and Chow, 1976). This phase-response system appears to possess simultaneously two elements, a stronger one which is phase-labile and follows phase shifts in the circadian cycles by light, and a weaker one which remains phase-locked to the clock hour of the solar day. Phase-response variations were also found for lunar daily and monthly periods, as well as annual. These all suggest a role of atmospheric electromagnetic fields as synchronizers.

The progress that has been made by scholars seeking external sources of timing as well as by scholars searching for an internal clock have been substantial. These two rapidly expanding areas of investigation, both searching for the mechanism of biological timing, should cooperate in their attack against the very difficult problem. The clocks of life probably depend in some manner on the combined roles of internal factors and subtle external Zeitgeber. It seems improbable that the rhythmic solar, lunar, and annual rhythms persisting cycle after cycle with their observed regularities could persist as they have been described in individual living creatures steadily deprived of all information about the external environment, or solely as a passive response to geophysical cycles. The living system actively interacts with these environmental cycles. Persons investigating biochemical and physiological processes within the organisms usually see only the components of endogenous rhythms; those searching for exogenous contributions to the timer can discover only these latter. The objective of each group appears to have been to account wholly for the biological clock phenomenon in terms of its own findings.

A semantic confusion exists in the literature regarding the biological clock. Experimental disruption of a specific overt circadian rhythm within an individual commonly results in a published conclusion that the organism's biological clock has been destroyed or stopped. Instead, such results actually indicate only that a normal essential link in an intraorganismic chain of processes between clocks and effector system responsible for the specific rhythm has been disturbed or broken. The evidence suggests that the clocks of the organism are present in every tissue and cell, clocks that continue to run though dissociated from any given specific activity. Other kinds of studies of the "arrhythmic" organisms usually disclose their ongoing activities.

4. Geoelectromagnetic responsiveness

One major kind of contribution relevant to the clock problem made since 1958 has given support to the concept of subtle environmental synchronization of the endogenous clock mechanism. First was the demonstration of electromagnetic-field perception. This was established for fishes by Lissman and Machin (1958). It was followed up by a series of studies in our laboratories from 1959 onward (Brown, 1971) on responses of a spectrum of kinds of living things to a variety of parameters of the ambient atmospheric electromagnetic fields. Organisms demonstrated that they could clearly derive environmental information from extremely weak magnetic, electrostatic, and background radiation changes but only when the strengths remained very close to the natural ambient ones to which the organisms seemed especially adapted. The responses varied with time of day, phase of moon, and time of year and with geographic vector direction of the applied experimental fields.

All these findings supported a concept that the organisms possessed the capacity to utilize the subtle recurring variations comprising the physical cycles in their environment as synchronizers for the clocks timing genetic metabolic patterns or of events that transpired in the course of their lives. Recognizing the insoluble ambiguity enveloping the clock problem produced by the temporal dimension of the organisms' four dimensional environment, the temporal one was removed by asking the organism "clock" questions and obtaining answers by its responses to a two-dimensional horizontal geographic grid (Brown and Park, 1967). Experiments, for example, with planarian worms on such a plane surface established first that the worms could distinguish among compass directions in the absence of all obvious cues. Experiments with weak bar magnets next established that an important cue by which the directions were identified was the horizontal magnetic vector.

Secondly, rotating horizontally by 180° a light relative to the earth's natural magnetic vector, or rotating an equivalent experimental magnetic field relative to the fixed directional light, were found to phase-shift abruptly a monthly rhythm of light response of the worms by 180°. In short, the worms could be induced, by altering its magnetic field relative to an overt factor, to do at full moon what they did before the rotation at new moon. A changing direction of light in relation to magnetism was in some manner determining the phase-setting of a monthly variation in the animals. Third, and finally, the worms appeared not only capable of identifying the geographic direction of an imposed light stimulus by subtle means but they could associate this information with respect to the compass grid of subtle parameters and "remember" this relationship as an internal biological behavioral modification for at least 5 to 10 min.

Since in the space-time continuum the 360° compass grid of subtle geophysical factors can be expected to vary systematically within the 360° daily temporal cycles, it could therefore be presumed that the organism could encode the 24-hr daily patterns produced by the subtle field changes with either, or both, of daily genetic patterns and of experienced events of concern to its livelihood. The encoded information could become a portion of a phase-labile circadian pattern.

The continuum of space and time, with such common denominators for organisms as magnetism for the two, merges the problems of orientation of the activities of organisms within the two domains. The perceptions dealing with the two domains, in other words, appear to share common parameters. It was suggested earlier in this account that the map sense points to the probability of an exogenous universal-time 24-hr cycle. Homing and navigation may employ clocks and celestial cues, but are able to occur without either (Keeton, 1971; Wiltschko and Wiltschko, 1972). The detailed characteristics associated with each specific compass direction differ systematically as the sun and moon alter their relationships to the compass plane as the earth rotates. The conditions are restored, in varying measures, for each earth rotation relative to each of these celestial bodies. They are restored more fully with the period of the synodic month when both sun and moon essentially repeat their relationships. And evidence points increasingly to an annual modulation. And currently the clocks of life have been expanded to include not only lunar tidal, but also monthly and annuals ones, all with "circa" periodisms and with common properties.

The basic problem of the clocks seems unlikely to involve, therefore, only closed-system time-series oscillations which are independent of all spatial considerations of the rotating and sun-orbiting earth as most clock scholars have recently assumed to be true. Instead, those natural periodic time-series variations reflecting the recurring environmental cycles appear to be probably an important contributor to the clock periods. Most biologists have assumed that any exogenous periods must be of constant length when, instead, the physical periods are actually of systematically and slightly varying lengths. Orbits of celestial bodies are not circular nor are speeds of the celestial bodies constant. The lunar-correlated ocean tides show much greater systematic variations in periods, their frequencies modulated by harmonics effected by local topography as well as by interactions with solar-day tidal clocks. The demonstrable responsiveness and responses of organisms to the vector fields of the pervasive electromagnetic forces of their environment and their properties, have been largely ignored by supporters of the independent oscillator concept of the clocks, despite the fundamentally electromagnetic character of life itself.

Many other reports that have followed, have continued to demonstrate extraordinary perceptive capacities of organisms to their natural weak environmental fields, and properties of the responding systems. The sensitivities have been shown to enable perception of only a few gamma changes in the earth's 50,000 gamma magnetic field (Lindauer and Martin, 1968; Stutz, 1971). Perception of electric fields down to the order of 0.01 μV/cm (Kalmijn, 1971) have been proven as well as perception of strengths and vector directions of hard gamma (Cs^{137}) radiation (Brown, 1963 and 1971) very close in strength to the natural ambient background radiation fields.

The responsiveness to very weak electromagnetic fields has even been shown to be involved in normal behavioral responses. European robins can identify by magnetism their migratory direction (Wiltschko and Wiltschko, 1972), direction finding by homing pigeons includes a use of geomagnetism (Keeton, 1971; Walcott and Green, 1973), sharks locate prey by sensing the very weak electric fields the prey projects into its environment by its physiological activities (Kalmijn, 1971). Eels have been found to be so sensitive that they should be able to find their way about the oceans by sensing the electric fields generated as the ocean currents move relative the geomagnetism (Rommel and McCleave, 1972). Groups of seeds absorbing water in neighboring glass or plastic dishes can interact (Brown and Chow, 1973), mutually altering their uptake rates. That organisms are in some manner adapted to the unidirectional rotation of the earth has been disclosed by the discovery of non-equivalence of slow clockwise and counterclockwise rotation (Jones, 1960). Evidence has been disclosed that this involves a response to motion relative to the geomagnetic fields (Brown and Chow, 1975).

5. Subtle clock Zeitgeber

Clearly organisms have adequate sensitivities to perceive the natural periodisms in ambient fields of numerous pervasive parameters of the atmosphere and waters of the earth effected by the relative motions of earth and sun, and even of the much weaker ones related to the moon. Nowhere on the earth, whether at the essentially fixed geographic poles or at the rhythmically moving magnetic poles, in the deepest cave or the abyssal ocean depths, would the environment be devoid of all of them. At the very crudest level the relatively large daily variations in the whole complex host of electromagnetic parameters could serve as subtle Zeitgeber for the 24-hr period to all organisms on the earth. Similarly the other major clock periods comparably have available subtle external Zeitgeber. And probably through "stimulus" filtering for the more regular periodic parameters, the detailed precision of the subtle Zeitgeber rhythms in the course of evolution become vastly expanded even beyond what we now know.

It is of great significance that a precise 24-hr period among the diverse circadian frequencies has been noted to behave as a "special" one in the temperature influences on periods (smallest) and in variance of periods (smallest). Also, this period occurs as the "free-running" one, especially in darkness, more frequently than any other circadian period. Whether or not this last is due to man created, or to natural, subtle pervasive fields this phenomenon has led to widely increasing general recognition of an action of subtle and pervasive atmospheric parameters on period and phase synchronization of biological rhythms when playing this role. Indeed, the common operation of subtle Zeitgeber is now widely acknowledged. There are a number of experimental reports of mean 24-hr components persisting with moderately reproducible forms even in organisms with rather regular free-running cycles with periods differing significantly from 24 hr. I have referred to these, generally, as geophysically-dependent oscillations, organismic responses to pervasive, subtle, environmental variations. Their existence was predictable from the early discovered correlations with meteorological factors from which the organisms were screened.

We can conclude on many such experimental grounds, that an environmental daily timing source, possibly even including one on universal time, is always available to our organisms in all their experimental set-ups and, indeed, has been present throughout their evolution. This is true even when there is apparently no entrainment of the much larger amplitude free-running or phase-shifting cycles. Would natural selection have left such a source of the natural periods lying fallow when timing of the natural periods was tremendously advantageous for organisms? Would the selective process have evolved instead through some means still fully unknown and only hypothetical, a less accurate "clock", incredibly complexly regulated (Bünning, 1974) inside each organism, indeed in every cell of it? Even an enucleated cell can have a circadian rhythm (Schweiger, Walraff and Schweiger, 1964). Aware of the essential perfection of the selective process enabling organisms to capitalize on every subtle factor, I would postulate not. And what other kind of timing system could provide circadian (Bryant, 1972) and monthly variations (Brown and Chow, 1973) in dry seeds not undergoing significant basic biochemical changes. Indeed, the need of a temperature-independent component underlying the regulating systems of life, constitutes itself a question of fundamental theoretical and philosophical importance.

Other exciting contributions made within the past year or two further suggest exogenous contributions to the rhythmic systems of organisms. A. Rothen (1974) has reported a circadian rhythm in a nickel-coated glass slide, presenting evidence that this was produced by a parallel rhythm in a low-energy cosmic radiation from the sun, excludable by 3.5 cm of lead. Planarian worms were earlier shown to be able to distinguish strengths and directions of very weak, hard gamma radiation emanating from a Cs_{137} source (Brown, 1963). Three very suggestive reports have been made independently over the past year that very small circadian variations in weak experimental magnetic fields produced by Helmholtz-coil systems can entrain and even determine phase relations in circadian bean sleep movements (McBride and Comer, 1975), can entrain rhythms in mice (Gribble, 1975), and entrain a free-running circadian rhythm in birds (Bliss and Heppner, 1976). These reports describe results that would have been impossible to contemplate seriously before 1960. Could the atmospheric periodisms of geomagnetism be steadily entraining the clocks of life?

We have seen that although thought-provoking contributions are being made to possible clock machinery by biologists with their efforts oriented towards explorations at the molecular level, correspondingly important discoveries on the rhythms are also concurrently being made at the organismic level. The extremely low kinetic thermal energies of atmospheric fields that are involved currently appear probably to preclude their direct influences at the molecular level. Perhaps, however, the effects of atmospheric electromagnetism at more organized levels in cells such as the omnipresent membranes of cells and cellular elements can, in turn, regulate molecular phenomena which participate in the clocks.

Regulation is the most fundamental and difficult problem faced by scholars in life sciences. Cause and effect are often so intermingled and interdigitated that their identities have not been dissociated. This problem is especially acute and extremely difficult at the level of the continuum of pervasive electromagnetic fields between comparable ones in the organism and in the physical environment.

But the continuity of rhythmic systems from relatively simple purely physical ones such as the atmosphere and nickel-coated glass slides, through relatively inactive biological systems such as dry seeds, to the actively metabolizing organism suggest that the ultimate basis of the rhythmicity and clocks made its appearance before the active vital processes of life.

Adequate evidence is now available to demand an expansion of the present concept of an endodiurnal organization, to become an endosolunar one for terrestrial life. Life appears to be geared to its geophysical environment as a dual endogenous rhythmic system with its two briefest major cyclic periods differing in length by only about 50 minutes. These two periods appear to be functionally interrelated within the living system (Webb and Brown, 1965). Either a 24.0- or a 24.8-hr cycle appears able to induce its collateral cycle. The two periodicities are phase-shifted together by altered light cycles. Entrainment of the 24-hr cycle by either subtle geophysical Zeitgeber or light Zeitgeber permits the presence of the slightly longer-period collateral cycle. Experimental entrainment of the 24.8-hr cycle by a light cycle of the same period carried a collateral cycle 50 min longer (about 25.6 hr). Subtle geophysical entrainment in constant conditions of light and temperature of a solar-day genetic pattern by a pervasive, exogenous, 24.8-hr one, appears able to induce a collateral cycle of 24.0 hr. This last period has been reported under these conditions for the rat by Brown and Terracini (1959). Turtles (Brown and Press, unpublished) and quahogs (Thompson, 1974) have been found to exhibit 23.2-hr cycles. A postulated means by which the two closely similar cycles are associated was suggested by Brown (1962) in terms of an approximate endogenous measurement of the 50-min difference. Such a dual-period internal organization of protoplasm would be anticipated to enhance tremendously the capacity of the living system normally to synchronize to the two closely similar cycles of the physical environment.

Recently Hoshizaki (1974) reported both lunar-day and solar-day periodicities in primates. A complex of these two periods, 24.0 and 24.8 hr, appears to form a fundamental temporal component in the rhythmic complex of these homoiothermic organisms.

Endogenous cycles are postulated to constitute a dynamic biological receptive system for entrainment by the fundamental solar-day and lunar-day subtle and pervasive atmospheric periodisms, which serve as subtle Zeitgeber, to provide accurate clocks to living systems. The same subtle Zeitgeber may under some circumstances extend their influence and entrain clock-dependent biological rhythms normally responsive to fields of obvious Zeitgeber such as light. However, under most conditions the genetic patterns constituting the rhythms are dominated by the obvious Zeitgeber such as light or other factors which adaptively determine the rhythm phase or generate circadian free-running. This hypothesis of the nature of biological clocks finds added support by its general applicability, in principle, to those biological clocks timing the longer cycles such as the semi-monthly, monthly, and annual circaperiodisms. Despite their longer periods, properties of these latter rhythms are being found increasingly to resemble those of the circadian and circatidal ones.

6. On the origin of 'circa' periods

A fundamental issue of the clock problem can be couched as a question. Are the free-running periods really reflecting inaccurate clocks, ones which must be reset during each cycle to the normal local-time environmental cycles by obvious Zeitgeber, or are the clocks exogenously synchronized, hence far more accurate, with the diverse free-running periods generated by a frequency transformation inside each organism when in fields of unvarying obvious Zeitgeber? This question is of more than academic interest. The question determines the direction of research on the clocks whether these are pursued by molecular or organismic biologists. For example, the role of a phase-response system for obvious parameters including light and temperature can be viewed in opposite manners, depending upon one's working hypothesis. It operates either to reset steadily inaccurate endogenous clocks to environmental time, or generates in experimentally controlled environments the diverse and variable free-running periods by frequency transformations from accurate exogenously-synchronized clocks. Either view is tenable and permits useful roles for clock-timed rhythms. The former view would appear to imply more disruption for the living system in the normal rhythmic environment than does the latter, in that it calls for larger or smaller daily phase-shifts.

How could a clock system accurately synchronized to the physical cycles give rise to what superficially seems to be an inaccurate clock system, its inaccuracies slightly amplified by a sensitivity to the levels of the constant light and temperature, and by the genome? This last I believe was a consequence of the kind of a phase-resetting process that, on the one hand, while adaptively freeing the organisms' behavior from slavery to an external timing system, would, on the other hand, in the critically controlled conditions of constancy of all Zeitgeber fields in the laboratory, spawn by a self-resetting process, or autophasing, diverse free-running periods intensively studied by most investigators as indicative of the postulated inaccurate periods of their biological clocks.

A major discovery for biological clocks occurring about the same time as responsiveness to the ambient electromagnetic fields, was the existence and varied odd forms of asymmetrical phase-response systems of organisms. The first clear indication of a daily rhythm in the phasing capacity of light had been suggested by Webb (1950), followed shortly by evidence offered by Brown, Fingerman, and Hines (1954) that the phase-shifting in an individual in response to light changes could occur in either of two directions, phase advances or delays. The final break-through was made in a beautifully executed study by DeCoursey (1960), using flying squirrels. The finding, termed a circadian phase-response variation, was quickly seized upon by everyone as an essential component for phase shifting and phase setting of the rhythms and, for the internal timer advocates, daily correction of the postulated autonomous clocks as well. It became incorporated into both the endogenous and exogenous concepts of biological clocks, emphasizing their ambiguity.

Phase-response curves to light have been found wherever they have been appropriately sought. A tremendous range in their forms and phase relations to the light-dark cycles has been described. Phase-response curves to another Zeitgeber, temperature, have also been described and are probably equally widespread though more difficult to investigate because of the strong kinetic effects of this factor. Indeed, there is reason to presume that phase-response systems occur for every environmental stimulus that can, when periodic, serve to set the phases of a rhythm.

For the external timer advocate, the phase-response system provided the probable means predominating in frequency transformations from the accurate, environmentally synchronized clock cycles to yield the host of 'free-running' frequencies being observed in all laboratories. Nocturnal flying squirrels, for example, exhibited characteristically an asymmetrical phase-response cycle usually with the dominant portion, phase delay to light, occurring over time of activity onset with a lesser maximum in phase advance occurring over time of termination of activity. This assured that activity would commence near the beginning of the daily period of darkness. For diurnal vertebrates such as finches the dominant portion would be anticipated usually to be phase advance to light occurring nearer morning activity onset with a lesser maximum phase delay in afternoon. This would assure that the activity would commence close to daybreak. The former animals have been described to have in continuous light free-running cycles typically longer than 24 hr, and the latter animals to have their free-running cycles shorter than 24 hr: These last were, of course, the algebraic expectations of when animals were held in unvarying light, the cycles were resetting each day in response to the illumination interacting with the successive portions of the inherently asymmetrical phase-response systems.

As I have spelled out elsewhere (Brown, 1972), phase-response variations of organisms, while tremendously variable, are typically asymmetrical between the phase delay and advance portions of their cycles. They would, therefore, be expected in constancy of Zeitgeber fields to provide small day-by-day phase shifts either to earlier or later times depending upon the direction of their functional asymmetry at the time. Every individual would show its own period since the detailed forms of the phase-response cycles are known to vary among individuals (DeCoursey, 1960; Natalini, 1972). Natalini went onward to describe a relationship in kangaroo rats of the individual's form of its phase-response variation to that individual's free-running periods, providing support for the autophasing concept. More recently, in a carefully designed and executed experiment, Avery (1974), working with rats, concluded that the free-running period in light is predominantly a consequence of autophasing to the constant light field. This was the parsimonius conclusion drawn from a demonstrated highly significant correlation between the lengths of phase-shifting transients in response to an inverted light cycle and the free-running period in continuous illumination of the same intensity.

These last studies have also substantiated a hypothesis that differing rates of phase-shifting accounts for hitherto unexplained small influences of levels of illumination on the 'free-running' periods. Brighter lights would be expected to produce greater daily shifts away from 24-hr. All this too, is quite consistent with our general knowledge that free-running periods longer than 24 hr tend to lengthen, and free-running periods shorter than 24 hr to shorten, as the illumination gradually increases (Aschoff, 1960). The usually very small, but still statistically significant, differences from 24 hr that commonly continue in total darkness can be postulated to be a consequence of phase-labile rhythms autophasing to the unvarying field of some other and weaker Zeitgeber such as temperature. Light is the dominant but not the sole Zeitgeber, and the circadian cycles are usually completely phase-labile relative to any postulated exogenous subtle Zeitgeber rhythm.

The reported small effects of differences in temperature levels upon the period yielding small Q_{10}'s but ones differing statistically significantly from 1.00 may result from Q_{10}'s even within the conventional kinetic range of 2 to 3 for the short total time during the circadian periods that the cycle was truly running free from a 24-hr fully temperature-independent exogenous-endogenous clock. Q_{10}'s could also be spurious, generated by autophasing to temperature. The genotype, and all other factors including earlier treatments and conditions, and chemicals such as D_2O and cycloheximide, that can alter a free-running period can be interpreted to be either modifying the form of the phase-response curve or influencing its normal phase shifting activity.

All deviations of rhythm period from their natural environmental correlates are, in terms of the concept of endogenous-exogenous clocks, a consequence of phase-shifting or autophasing of the endogenous rhythm. The recurring genetic patterns comprising the overt cycles timed by the clocks are postulated to have a completely phase-labile relationship with the underlying basic clock cycles. They may be reset cycle after cycle relative to the clock to yield overt cycles either longer or shorter, or may maintain any fixed constant phase relation to it, depending on the organism and the environmental conditions. Such lability obviously allows biological rhythms to play their many well-known adaptive roles in nature.

This concept of the origin and general nature of biological rhythms and clocks, incorporating all the reported findings, tremendously simplifies the problem of explaining the essential temperature- and drug-resistant clock properties while allowing, indeed theoretically accounting simply for the relatively small changes effected by these factors. With this suggested direction for the solution of the clocks of life, all the remaining related problems of the rhythmic system appear to conform more easily to the conventional rules of physics, chemistry and physiology. The clock itself, quite unbiological in several of its properties, introduces us to another, but especially difficult, new chapter in the physiology of perception of, and of responses to, the subtle and pervasive atmospheric fields. The organism and its physical environment appear to merge intimately for the timing of life.

Conclusions and summary

The hypothesis of joint exogenous-endogenous clocks of life which is supported in this article has been rendered probable by a number of relatively recent discoveries. These include importantly (1) the many evidences for an endogenous, geosolunar timing system and suggestive models that can provide at least significant temperature-compensation, (2) the remarkable reported sensitivities to the electromagnetic forces of the environment which are able to furnish the geophysical periods, (3) the peculiar kind of asymmetrical phase-response systems providing a natural mechanism for intraorganismic frequency transformations to generate the multiplicity of free-running periods, and (4) the capacity of an organism to associate and 'remember' obvious events in relation to points at which they occurred within a specific geophysical cycle identified exclusively by the subtle geophysical information. It is postulated that the geophysical rhythms can serve as subtle, pervasive Zeitgeber providing the stability and remarkable temperature and chemical independence of the clock periods.

The common presence of accurately timed cycles of geophysical lengths without obvious cue, and discovery of common parameters indicating to organisms both spatial and temporal orientations, have immensely strengthened the case for a concept of the clock phenomenon resulting from interaction of the organism with its environment. In terms of this concept all the well-known properties of the circadian systems as well as those of longer periods such as the month and year are accountable. Solved most simply are the toughest problems, the tremendous stability of the periods over long intervals. Also, the small alterations of the lengths of free-running periods in unvarying Zeitgeber fields effected by different light intensities, temperature levels, a few chemicals, and differing genomes can be incorporated readily as influences of these factors on the autophasing of phase-labile genetic cycles. The astounding relative constancy of the free-running periods, even over months or years, becomes much easier for most biologists to credit in terms of clocks synchronized to the widely recognized, inexorable atmosphere cycles generated by relative motions of earth, sun, moon, and stars rather than timed exclusively by still hypothetical autonomous biological oscillators whose proposed mechanism displays such non-biological characteristics.

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References

Arrhenius, S., 1898, Die Einwirkung kosmischer Einflusse auf physiologische Verhultnisse, Skand. Arch. Physiol. 8. 367–416.

Aschoff, J., 1960, Exogenous and endogenous components in circadian rhythms. Sym. Quant. Biol., Cold Spring Harbor 25, 11–28.

Aschoff, J., 1967, Human circadian rhythms in activity, body temperature and other functions. Life Sciences and Space Research (North-Holland Publ. Co.) pp. 159–173.

Avery, D.L., 1974, The relationship of light-dark reversal transients and free-running rhythms. Int. J. Chronobiol. 2, 223—232.

Bliss, V.L. and F.H. Heppner, 1976, Circadian activity rhythm of house sparrows, Passer domesticus, influenced by near zero electromagnetic field, Nature (in press).

Brown, F.A., Jr., 1957, Biological Chronometry. The general nature and significance of the problem, Amer. Nat. 91, 129—133.

Brown, F.A., Jr., 1959, Living Clocks, Science 130, 1534—1544.

Brown, F.A., Jr., 1962, Extrinsic rhythmicality: A reference frame for biological rhythms under so-called constant conditions, Ann. N.Y. Acad. Sci. 98, 775—787.

Brown, F.A., Jr., 1963, An orientational response to weak gamma radiation, Biol. Bull. 125, 206—225.

Brown, F.A., Jr., 1965, Propensity for lunar periodicity in hamsters and its significance for biological clock theories, Proc. Soc. Exp. Biol. Med. 120, 792—797.

Brown, F.A., Jr., 1968, "Endogenous" biorhythmicity reviewed with new evidence, Scientia 103, 245—260.

Brown, F.A., Jr., 1969 A hypothesis for extrinsic timing of circadian rhythms, Canad. J. Bot. 47, 287—298.

Brown, F.A., Jr., 1971, Some orientational influences of non-visual, terrestrial electromagnetic fields, Ann. N.Y. Acad. Sci. 188, 224—241.

Brown, F.A., Jr., 1972, The "clocks" timing biological rhythms, Amer. Sci. 60, 756—766.

Brown, F.A., Jr. and C.S. Chow, 1973, Interorganismic and environmental influences through extremely weak electromagnetic fields, Biol. Bull. 144, 437—461.

Brown, F.A., Jr. and C.S. Chow, 1973, Lunar-correlated variations in water uptake in bean seeds. Biol. Bull. 145, 265—278.

Brown, F.A., Jr. and C.S. Chow, 1975, Non-equivalence for bean seeds of clockwise and counterclockwise magnetic motion: A novel terrestrial adaptation. Biol. Bull. 148, 370—379.

Brown, F.A., Jr. and C.S. Chow, 1976, Uniform daily rotation and biological rhythms and clocks in hamsters. Physiol. Zool. (in press).

Brown, F.A., Jr., M. Fingerman and M.N. Hines, 1954, A study of the mechanism involved in shifting of the phases of the endogenous daily rhythm by light stimuli, Biol. Bull. 106, 308—317.

Brown, F.A., Jr., J.W. Hastings and J.D. Palmer, 1970, The Biological Clock: Two Views (Academic Press, New York) 94 pp.

Brown, F.A., Jr. and M.N. Hines, 1952, Modifications in the diurnal pigment rhythm of Uca effected by continuous illumination. Physiol. Zool. 25, 56—70.

Brown, F.A., Jr. and Y.H. Park, 1967, Synodic monthly modulation of the diurnal rhythm of hamsters. Proc. Soc. Exp. Biol. Med. 125, 712—715.

Brown, F.A., Jr. and Y.H. Park, 1967, Association-formation between photic and subtle geophysical stimulus patterns — a new biological concept. Biol. Bull. 132, 311—319.

Brown, F.A., Jr., J. Shriner and C.L. Ralph, 1956, Solar and lunar rhythmicity in the rat in "constant conditions" and the mechanism of physiological time measurement, Amer. J. Physiol. 184, 491—496.

Brown, F.A., Jr. and E.D. Terracini, 1959, Exogenous timing of rat spontaneous activity patterns, Proc. Soc. Exp. Biol. Med. 101, 457—460.

Brown, F.A., Jr., H.M. Webb and M.F. Bennett, 1955, Proof for an endogenous component in persistent solar and lunar rhythmicity in organisms, Proc. Nat. Acad. Sci. USA 41, 93—100.

Bryant, T.R., 1972, Gas exchange in dry seeds: circadian rhythmicity in absence of DNA replication, transcription and translation, Science 178, 634—636.

Bünning, E., 1956, Die physiologische Uhr. Naturwiss. Rundschau. 9, 351—357.

Bünning, E., 1974, Critical remarks concerning the Q_{10} values in circadian rhythms, Int. J. Chronobiol. 2, 343—346.

DeCoursey, P.J., 1960, Daily light sensitivity rhythm in a rodent, Science 131, 33—35.

Ehret, C.F. and T. Trucco, 1967, Molecular models for the circadian clock. I. The Chronon concept, J. Theoret. Biol. 15, 240—262.

Gribble, R.E., Jr., 1975, The effect of extremely low frequency electromagnetic fields on the circadian biorhythms of common mice, Chronobiologia, Suppl. 1, 24—25.

Gronau, J. and K. Schmidt-Koenig, 1970, Annual fluctuation in pigeon homing, Nature 226, 87—88.

Halberg, F., 1959, Physiologic 24-hr periodicity: general and procedural conditions with reference to the adrenal cycle, Z. Vitamin-, hormon-, und Fermentforschung 10, 225—296.

Hoagland, H., 1933, The physiological control of judgments of duration: Evidence for a chemical clock, J. Gen. Psychol. 9, 267—287.

Jones, R.L., 1960, Response of plants to uniform daily rotation, Nature 185, 775.

Hoshizaki, T., 1974, Biorhythms of a nonhuman primate in space, in: Chronobiology, L.E. Scheving, F. Halberg, and J.E. Pauly (eds.), (Igaku Shoin Ltd., Tokyo).

Kalmijn, A.J., 1971, The electric sense of sharks and rays, J. Exp. Biol. 55, 371—383.

Keeton, W.T., 1971, Magnets interfere with pigeon homing, Proc. Nat. Acad. Sci. 68, 102—106.

Lindauer, M. and H. Martin, 1968, Die Schwereorientierung der Bienen unter dem Einfluss des Erdmagnetfeldes, Z. vergl. Physiol. 60, 219—243.

Lissman, H.W. and K.E. Machin, 1958, The mechanism of object location in Gymnarchus niloticus and similar fish, J. Exp. Biol. 35, 451—486.

McBride, E.L. and A.E. Comer, 1975, Effect of magnetic fluctuation on bean rhythms, Chronobiologia, Suppl. 1, 44—45.

Natalini, J.J., 1972, Relationship of the phase-response curve for light to the free-running period of the kangaroo rat, Dipodomys merriami; Physiol. Zool. 45, 153—166.

Njus, D., F.M. Sulzman and J.W. Hastings, 1974, Membrane model for the circadian clock, Nature 248, 116—120.

Pfeffer, W., 1907, Untersuchungen über die Entstehung der Schlafbewegungen der Blattorgane. Abh. sächs Akad. Wiss., Math-Physik., Kl. 30, 257—472.

Pfeffer, W., 1915, Beiträge zur kenntnis der Entstehung der Schlafbewegungen. Abh. säcks Akad. Wiss., Math-Physik., Kl. 34, 1—154.

Renner, M., 1955, Ein Transoceanversuch zum Zeitsinn der Honigbiene, Naturwiss. 42, 540.

Renner, M., 1959, Über ein weiteres Versetzungs-experiment zur Analyse des Zeitsinnes und der Sonnernorientierung der Honigbiere, Zeitschr. vergl. Physiol. 42, 449—483.

Rommel, S.A., Jr. and J.D. McCleave, 1972, Oceanic electric fields: Perception by American eels? Science 176, 1233—1235.

Rothen, A., 1974, Circadian activity of a nickel-coated glass slide used for carrying out immunologic reactions at a liquid-solid interface, Biophys. Jour. 14, 987—989.

Schweiger, E., H.G. Walraff and H.G. Schweiger, 1964, Über tagesperiodische Schwankungen der Sauerstoffbilanz kernhaltiger und kernloser Acetabularia mediterranea, Z. Naturforsch. 19B, 499—505.

Stoppel, R., 1916, Die Abhangigkeit der Schlafbewegungen von Phaseolus multiflorus von vershiedenen Aussenfaktoren, Z. Bot. 8, 609—684.

Stutz, A.M., 1971, Effects of weak magnetic fields on gerbil spontaneous activity. Ann. N.Y. Acad. Sci. 188, 312—323.

Thompson, I., 1974, Biological clocks and shell growth in bivalves, in: Growth Rhythms and the History of the Earth's Rotation, G.D. Rosenberg and S.K, Runcorn (eds.), (Wiley-Interscience, N.Y.).

Van der Driessche, T., 1975, Circadian rhythms and molecular biology, BioSystems 6, 188—201.

Walcott, C. and R.P. Green, 1973, Orientation of homing pigeons altered by a change in the direction of an applied magnetic field, Science 184, 180—182.

Walraff, H.G. and L.C. Graue, 1973, Orientations of pigeons after transatlantic displacement, Behavior 44, 1—35.

Webb, H.M., 1950, Diurnal variations of response to light in the fiddler crab, Uca, Physiol. Zool. 23, 316—337.

Webb, H.M. and F.A. Brown, Jr., 1965, Interactions of diurnal and tidal rhythms in the fiddler crab, Uca pugnax, Biol. Bull. 129, 582—591.

Wiltschko, W. and R. Wiltschko, 1972, Magnetic compass of European robins, Science 176, 62—64.