

CURRENT PROBLEMS IN RESEARCH

Living Clocks

The clocks are accounted for as "open systems" depending upon subtle geophysical rhythms.

Frank A. Brown, Jr.

Familiar to all are the rhythmic changes in innumerable processes of animals and plants in nature. Examples of phenomena geared to the 24-hour solar day produced by rotation of the earth relative to the sun are sleep movements of plant leaves and petals, spontaneous activity in numerous animals, emergence of flies from their pupal cases, color changes of the skin in crabs, and wakefulness in man. Sample patterns of daily fluctuations, each interpretable as adaptive for the species, are illustrated in Fig. 1. Rhythmic phenomena linked to the 24-hour and 50-minute lunar-day period of rotation of the earth relative to the moon are most conspicuous among intertidal organisms whose lives are dominated by the ebb and flow of the ocean tides. Fiddler crabs forage on the beaches exposed at low tide; oysters feed when covered by water. "Noons" of sun- and moon-related days come into synchrony with an average interval of $29\frac{1}{2}$ days, the synodic month; quite precisely of this average interval are such diverse phenomena as the menstrual cycle of the human being and the breeding rhythms of numerous marine organisms, the latter timed to specific phases of the moon and critical for assuring union of reproductive elements. Examples of annual biological rhythms, whose $365\frac{1}{4}$ -day periods are produced by the orbiting about the sun of the earth with its tilted axis, are so well known as scarcely to require mention.

These periodisms of animals and

plants, which adapt them so nicely to their geophysical environment with its rhythmic fluctuations in light, temperature, and ocean tides, appear at first glance to be exclusively simple responses of the organisms to these physical factors. However, it is now known that rhythms of all these natural frequencies may persist in living things even after the organisms have been sealed in under conditions constant with respect to every factor biologists have conceded to be of influence. The presence of such persistent rhythms clearly indicates that organisms possess some means of timing these periods which does not depend directly upon the obvious environmental physical rhythms. The means has come to be termed "living clocks."

Autonomous-Clock Hypothesis

From the earliest intensive studies of solar-day rhythmicity during the first decade of this century by Pfeffer (1), with bean seedlings, certain very interesting properties of this rhythm became clearly evident. Pfeffer found that when his plants were reared from the seed in continuous darkness, they displayed no daily sleep movements of their leaves. He could easily induce such a movement, however, by exposing the plants to a brief period of illumination. Returned to darkness, the plants possessed a persisting daily sleep rhythm. The time of day when the leaves were elevated in the daily rhythm was set

by the time of day when the single experimental light period commenced. It was apparent that the daily rhythmic mechanism possessed the capacity for synchronization with the outside day-night cycles while having its cyclic phases experimentally altered by appropriate light changes made to occur at any desired time of day. These alterations would then persist under constant conditions. Since Pfeffer's time, this property has been abundantly confirmed for numerous other plants and animals. The daily rhythms, therefore, exhibit the capacity for synchronization with external, physical cycles while having freely labile phase relations.

A second discovery, also made by Pfeffer, was that the daily recurring changes under constant conditions could occur earlier, or later, day by day, to yield regular periods deviating a little from the natural solar-day ones. Periods have now been reported ranging from about 19 to 29 hours. The occurrence of persisting rhythmic changes under constant conditions, with regular periods of other than precisely 24 hours, clearly indicated that these observed rhythmic periods could not be a simple direct consequence of any known or unknown geophysical fluctuation of the organism's physical environment.

A third fundamental contribution to the properties of the daily rhythms was made by Kleinhoonte (2). While confirming, in essentials, all of Pfeffer's findings, she discovered that the daily sleep movements of plants could be induced to "follow" artificial cycles of alternating light and dark ranging from about 18-hour "days" to about 30-hour "days." When the "days" deviated further than these limits from the natural solar-day period the plants "broke away" to reveal their normal daily periodicity, despite the continuing unnatural light cycles. This observation clearly emphasized the very deep-seated character of the organismic daily rhythm.

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In the light of his demonstration of both phase and frequency lability for sleep rhythm in beans, Pfeffer concluded that the periods of plant rhythms must depend upon an independent internal timing mechanism.

This hypothesis was hotly contested for many years by Stoppel (3) and others. Stoppel found evidence to suggest that a daily rhythmicity related to the known daily rhythm in air ionization was continuously imparted to the organisms under conditions presumed to be constant for them. Her hypothesis was that the organisms in some manner depended upon this environmental periodicity for the maintenance of their persisting regular rhythms, having periods close to solar-day periods.

Temperature and Rhythm

The controversy appeared terminated in favor of Pfeffer's autonomous internal timing hypothesis as a consequence of what was at that time considered to be a crucial experiment. Bünning (4), apparently recognizing that both Pfeffer's and Stoppel's hypotheses were equally tenable, reasoned that if an independent internal cycle-timer were involved, then, like living processes quite generally, the timer should be speeded up (the cycles shorter) or slowed down (the cycles longer) in response to changes in the level of constant temperature at which the organisms were maintained. Results of his experiment with beans actually did show change in rhythm-period length with change in temperature, in the expected direction. Bünning found the change in cycle period to be only about 20 percent for a 10° rise in temperature, in contrast to the usual 100- to 200-percent increase for typical biological processes. However, the fact of temperature-dependence of period length was greatly emphasized, since it was interpreted to prove the existence of an independent cycle-timer. Several studies that followed this demonstration by Bünning confirmed his results; all these investigators, too, greatly emphasized this minute influence of temperature level on period length. We now know, as we shall see later, that this experiment, demonstrating slight temperature-dependence, was not a crucial one; its interpretation is ambiguous, supporting equally the two fundamental clock hypotheses. Throughout the 20-year period during which this

basic question was considered resolved, there were suggestions that some daily periodicities were fully independent of temperature. Beling (5) and Wahl (6) appeared to find this true for the daily time sense of bees, and Welsh (7), for the rhythmic eye-adaptation in crayfishes.

The whole problem of the relationship of daily rhythmicity to temperature became complicated very early by the discovery by Pfeffer, and the detailed confirmation later by Stern and Bünning (8), that temperature changes, like light changes, were highly effective in setting the phase relationships of the labile daily rhythms. Temperature, therefore, was demonstrated to have two possible effects on rhythms, just as it has for certain other biological reactions. One of these is the general kinetic effect, in which the rate of biological reactions is a direct function of temperature level, and the second is the evocation of a specific response to temperature changes as stimuli perceived by organisms.

It was in a climate of contradictions and confusion regarding the relationship of temperature to daily rhythmicity that Webb and I (9) conducted in 1948 an extensive study of temperature relations of daily rhythms, using the fiddler crab, an extremely favorable organism for the study of such rhythms. It was established that in this animal the period of the daily cycles of change in skin color was completely independent of temperature over a 20°C range. The physiological and ecological implications of this fact were emphasized. This study marked the beginning of a period during which students of rhythmicity tended to stress the temperature-independent nature of the phenomenon and to de-emphasize the small dependence. As was clearly apparent from the earliest studies of daily periodicities, and especially after it was apparently demonstrated for the time sense of bees, in 1929, temperature-independence is a highly useful characteristic.

Two Rhythmic Centers

The next important advance in our knowledge of rhythm timing involved the demonstration by Webb and me in 1949 (10) that the observed daily rhythms of special processes, such as color change, were not the basic timers of the rhythms and that these processes were being regulated by a more fundamental, and relatively more stable, un-

derlying rhythmic component. It was shown that two serially coupled rhythmic centers were involved. A shift in phase of the more superficial, observable rhythm, which could be accomplished by some types of light changes, was found not to be permanent under constant conditions. The phase relationships drifted back, over the course of a few days, to the initial relationships, which were being retained by the unshifted, more stable center. By other kinds of light changes it was possible to shift the phase of the more basic center, a shift which now persisted indefinitely under constant conditions. For example, the more basic center could be phase-shifted through 180°, by two or three cycles of illumination by night and darkness by day, to produce a permanent shift. On the other hand, the more labile center could be phase-shifted to a time 6 hours earlier, relative to the basic center, by alternating 12-hour light-dark (6L:6D) cycles with the last 6-hour light period occurring between 6 and 12 P.M. in the natural rhythm, or between 6 and 12 A.M. in a previously inverted rhythm.

This last discovery was especially important for two particular reasons: (i) it removed the basic timer from the observed physiological rhythms themselves, and (ii) it clearly indicated that the organism possessed within itself a mechanism for generating regular periods of overt rhythms deviating from 24 hours, since this was obviously occurring during the few days in which the more basic daily rhythmic center was shifting the more labile, observed center back into phase with itself. It is evident for the color-change rhythm that, for a period of about 5 days during which the basic 24-hour center was bringing the more labile center back into phase with itself, the periods of observed change possessed an average period of about 25.2 hours. It was similarly clear that this 25.2-hour period depended continuously upon a rhythm of a different period—24 hours in this instance—for its maintenance.

Later work by Stephens and me (11) confirmed and extended this two-center hypothesis of rhythm timing, and quite recent work by Pittendrigh and his associates (12) with some other species and processes has reconfirmed the functional separation of a more basic and stable rhythm from the relatively more labile observed rhythms.

The divorcing of the more basic cycle-timer from the observed rhythmic fluctuations of special physiological

processes, and the demonstration that the observed rhythmic periods could deviate at least for several daily cycles from the exact period of the more basic timer, raised, of course, doubt as to what the actual period of the ultimate organismic timing center might be. It also raised some question as to how faithfully the observed physiological periodicities normally reflect the period of such an ultimate timer.

As long as the observed rhythms and the cycle-timers were generally conceded to be one and the same phenomenon, there was clear need for postulating an independent internal clock. However, despite the now widely conceded separation of the basic timer

from the observed rhythmic process, Pfeffer's hypothesis of the presence of an autonomous clock is still retained by the majority of investigators and, in fact, the internal clock is considered to exist as the more stable of the two rhythmic centers. This clock is considered to have a period reflected faithfully by the overt rhythms, once they have stabilized their period under constant conditions of light and temperature. This period is termed the "free-running period," and only under special circumstances is it exactly 24 hours. For color change in the fiddler crab, the period appears to be exactly 24 hours, in darkness, through a 20°C temperature range. For most species,

it is approximately 24 hours; its deviation from 24 hours is usually a function of the level of constant illumination and temperature. As the prevailing view, it is postulated that the more basic center comprises an independent metabolic "clock system" harboring periodisms that are close to, though seldom exactly the same as, the natural geophysical frequencies. These clocks are presumed to be rather precisely temperature-compensated and to be capable of being experimentally reset to any time of day, or of being made to run slightly faster or slower by suitable adjustment of either the constant light or the constant temperature level.

In brief, it is postulated that the liv-

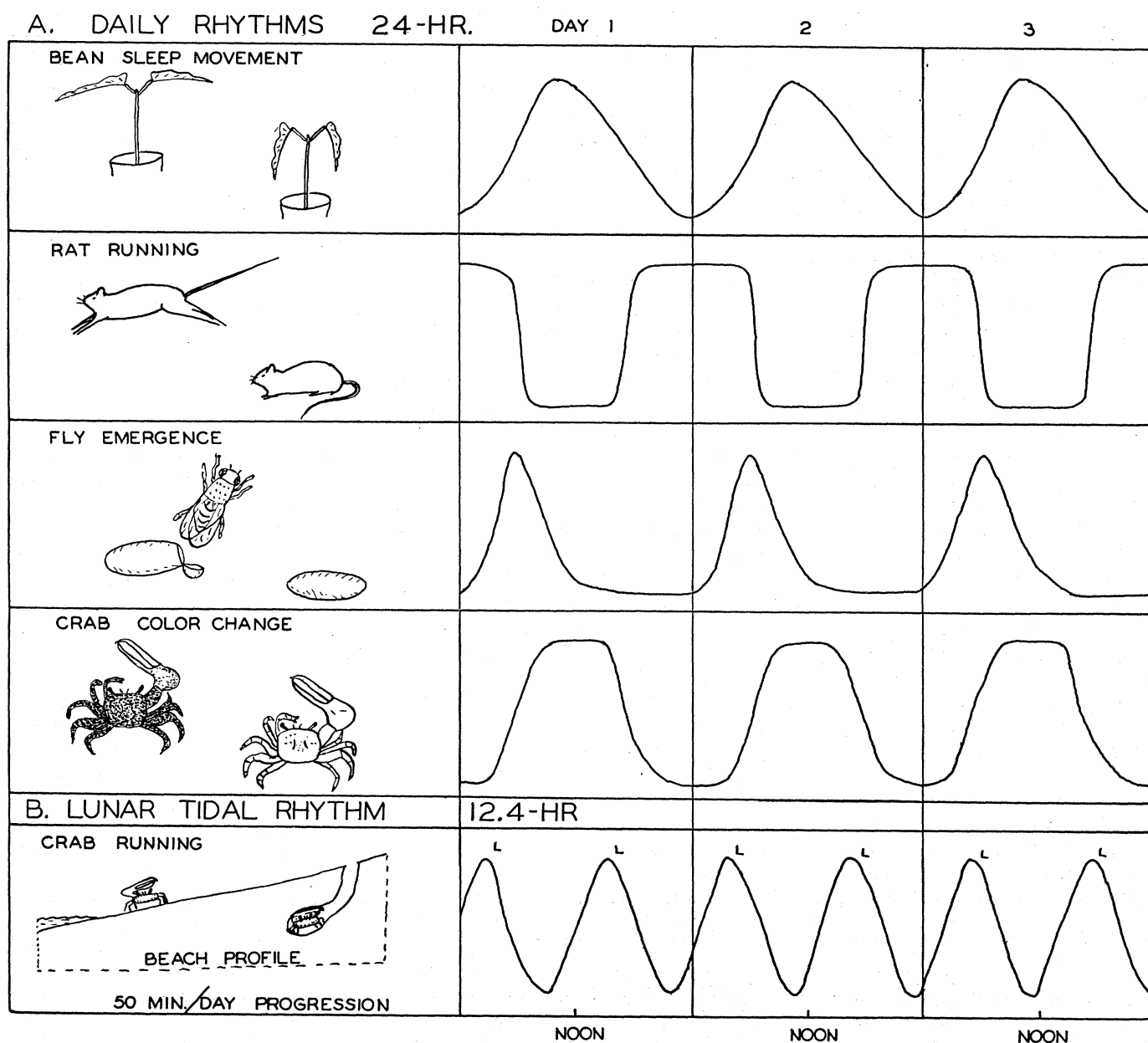


Fig. 1. Examples of rhythmic phenomena experimentally demonstrated to persist under constant conditions in the laboratory, illustrating diagrammatically the natural phase relationships with respect to the external physical cycles. *L*, in the diagram at bottom, indicates time of low tide in the crab's habitat.

ing organism possesses independent, cyclic biochemical systems with inherited natural periods of oscillation. Indeed, it has been shown that, when other factors are equal, period-length in the bean is a characteristic of genetic strain. The periods are presumed to have been derived in the long course of organic evolution under natural selective pressures. The major problems that appear to remain in connection with this hypothesis, are the need for (i) proof that such autonomous clocks actually exist, and if they do, (ii) understanding of the nature of the biochemical mechanisms capable of measuring natural periods even up to a year without any external source of information. In view of an extraordinary immunity of the rhythm period to metabolic poisons, and of the maintained precision of the annual period in dried seeds in the temperature range

-22° to $+45^{\circ}\text{C}$ (13), Bünning has postulated (14) that the internal clock must be predominantly a physical, as opposed to a chemical, mechanism. However, every attempt to characterize such a postulated endogenous clock in terms of our current concepts of possible underlying biophysical or biochemical schemes has emphasized the clock's independence of these schemes. In fact, even the site of the clock in the living thing has been similarly most elusive. It seems to be everywhere, and yet nowhere when we try to localize it.

During the past 6 years the rapidly mounting evidence for a most unorthodox, and even an almost incredible, character of any fully autonomous internal clock in terms of our current knowledge of general physiology rendered it advisable to reconsider the working hypothesis of an independent or "closed system" clock. Also, while being by its very nature incapable of proof, the autonomous-clock hypothesis discouraged search for alternative explanations.

Response to Geophysical Rhythms

Supported at this time is an alternative view, proposed long ago by Arrhenius (15), as to the nature of the more stable, basic timer for the organismic rhythms of approximately all the natural frequencies. Both rational and experimental support are offered for it, and it is shown how biological rhythms are readily accounted for in these terms. This was also Stoppel's general thesis for the timing of daily rhythms. This is the hypothesis that the clock comprises an "open system" and that timing of the periods persisting in so-called constant conditions is derived through a continuous response of the living organism to its rhythmic geophysical environment. It is postulated that both the observed phase and the observed frequency labilities of the rhythms are expressions of the same phenomenon (phase shifting in response to light or temperature), and that these labilities comprise very nice adaptive mechanisms for normal adjustment of the organism to the specific character of the light and temperature changes occurring in nature.

It was suspected that the organism, under constant conditions, was actually deriving its periods in reaction to the environment, first from reports by Stoppel and others of apparent disturbances in the mechanism in otherwise

rhythmic organisms when they were transported to the arctic in summer, or into a salt mine, but especially from an experiment performed with oysters several years ago (16). Oysters transported in light-proof containers from their habitat in New Haven harbor to pans of sea water in a photographic darkroom in Evanston, Ill., gradually rephased their rhythm of shell-opening over a 2-week period from the exact lunar-day time of high tide in New Haven harbor to the exact times of lunar zenith and nadir at Evanston, Ill., the theoretical time of high tides in Illinois were there a coastline. This new phase relationship then remained unchanged through a full month during which the study was continued.

Metabolic Cycles

In an attempt to learn more concerning the fundamental basis of organismic rhythmicity there was initiated about 6 years ago a program of study of metabolic fluctuations in a wide variety of organisms ranging from algae to flowering plants, and from invertebrates to vertebrates. Metabolic rate was selected for study, inasmuch as (i) there was no longer a restriction with respect to the kinds of organisms which could be investigated, and (ii) this seemed as fundamental a phenomenon as could be measured and therefore the process that would be the most likely to reflect any basic living-clock rhythms. For the studies an automatic recording respirometer was used in which the metabolic rates could be measured in organisms hermetically sealed under constant conditions, including constant pressure, for several days at a time without any disturbance. It has now become quite clear that under such constant conditions all living things have continually imposed upon them from their environment metabolic rhythms of exactly the natural geophysical frequencies.

Certain of the studies on fiddler crabs and potatoes have been selected to illustrate the general character of the findings; the basic similarity in results for organisms as widely different as these two—a marine invertebrate and a terrestrial flowering plant—emphasizes the fundamental character of the phenomenon. These, and numerous other studies, suggest that the phenomenon is universal among living things. The average form of the daily metabolic fluctuation in the potato for each of three full years, together with the 3-year mean, is illus-

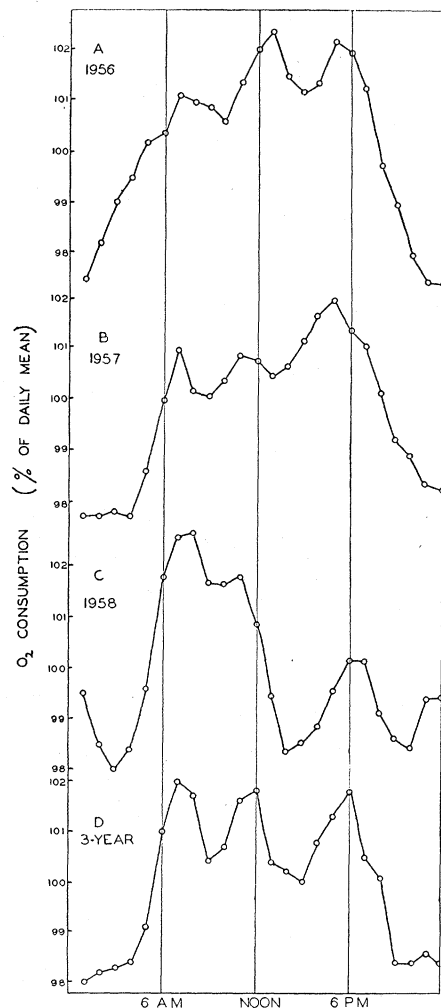


Fig. 2. A, B, and C illustrate the average forms (3-hour moving means) of the daily cycle of O_2 consumption of potato plants under constant conditions during each of three full years of study. D is the 3-year mean cycle.

trated in Fig. 2. Despite change in gross form from year to year, this cycle was characterized by a nighttime minimum, an early-morning rapid rise, and a trimodal daytime maximum. Its average for the 3 years involves about a 4-percent increase from midnight to noon, though this percentage is, day-by-day, statistically significantly correlated with the simultaneous, weather-correlated mean daily outdoor air temperature. The correlation was direct for temperatures below 57°F and inverse for higher ones. Just as outdoor air temperature obviously possesses an annual fluctuation, so is there an unambiguous annual rhythm in the amplitude of the daily metabolic cycles (Fig. 3, *B*), with the major maximum in October and a lesser one in April-May, and with the major minimum in February. There is also a highly significant synodic monthly rhythm in the form of the daily cycles (Fig. 3, *A*); the cycles depressed most at new moon and least at third quarter.

The studies with the fiddler crab have been restricted to summer months during the past 6 years. The day-by-day fluctuations can be clearly resolved into two conspicuous rhythmic components, each involving a 30- to 50-percent fluctuation. One component, a solar-day one, possessed a morning maximum and a late afternoon minimum. The second component, equally large, is a superimposed bimodal rhythm of lunar-day frequency, with maxima occurring at lunar zenith and lunar nadir. The simultaneous presence of these two rhythmic frequencies and their particular phase relationships to the external cycles results in a fortnightly recurring series of daily patterns of O₂ consumption. The form of the fluctuation on any day is a function of the temporal relationship of that day to the time of new moon or full moon. In Fig. 4 are compared, as an example, the average daily cycles that Webb and I (17) found for six consecutive, corresponding days for the two summers 1956 and 1957. Quite comparable would be two series separated by a fortnight; *A5* is a day of new moon or full moon. These two metabolic frequencies of the crab are quite precisely the geophysical frequencies and appear to persist indefinitely.

It can readily be demonstrated that the daily metabolic periods in both the crab and the potato are imposed day by day from the external physical environment. One method has been the following: In Fig. 5 are seen (*A*) the mean summer-month (May-August) daily cycles for 3 years for potatoes, (*B*) a

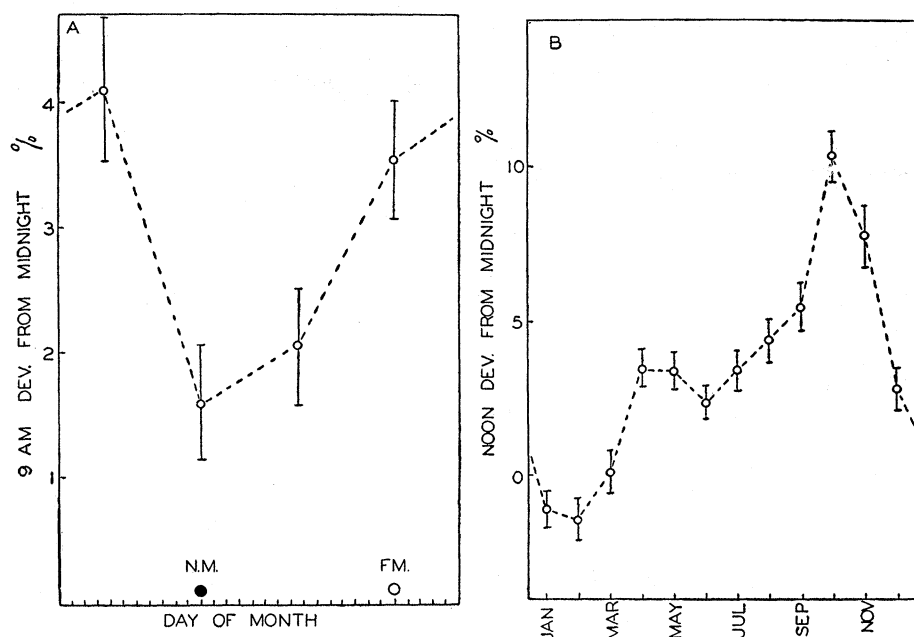


Fig. 3. (*A*) The average percentage increase in O₂ consumption from 1 A.M. to 9 A.M. as a function of four periods centered over each of four quarters of the moon. (*B*) The average percentage increase in O₂ consumption from 1 A.M. to noon as a function of the month of the year during a 3-year continuous study under constant conditions. Standard errors of the means are indicated.

sample summer-month mean daily cycle of barometric pressure, and (*C*) the summer-month mean daily cycle for crabs for 4 years. Far beyond any statistical chance, the 6 A.M. rates of O₂ consumption of both potatoes and crabs are positively correlated with the weather-related deviations in the 2 to 6 A.M. mean rate of pressure change. The higher the rate of morning pressure rise, the greater the 6 A.M. metabolic deviation on that day. The 6 P.M. deviation in metabolism of the potato is negatively correlated with the day-by-day 2 to 6 P.M. rate of pressure change, while in the crab, on the other hand, it is positively correlated. The higher the 2 to 6 P.M. rate of pressure drop on any given day, the lower the 6 P.M. metabolic rate in the crab and the higher the 6 P.M. rate in the potato on that day. Whereas, therefore, both species are deriving daily periods from a pressure-correlated external factor, the potato maintains a high metabolic rate throughout the day by alternating the signs of its 6 A.M. and 6 P.M. correlations. This can be interpreted as an adaptation of the light-dependent plant. The crab, whose adaptive actions are predominantly lunar-tidally regulated, continues with the same sign for both its 6 A.M. and its 6 P.M. correlations. However, the crab appears to possess the capacity to alternate signs like the potato. The bottom curve *D* is the mean daily metabolic cycle for 1 month for crabs whose daily

cycles of color change Webb and I (18) inverted by several periods of illumination by night and darkness by day. Under these circumstances, for the crab the 6 P.M. correlation in, for example, its phase-labile color-change rhythms, is now the physiological equivalent of the former 6 A.M. correlation. The crab, now, like the potatoes, exhibits a maximum rate where there was formerly a minimum. But, interestingly, it simultaneously retains its 6 A.M. positive correlation with pressure change. Thus it appears that the sign of the correlation with the still unidentified rhythmic external factor is, to some degree at least, under organismic control.

In Fig. 6, *A* illustrates for the potato the regressional relationship of the 6 A.M. deviation in metabolic rate with respect to the rate of concurrent morning pressure change. For the crab, in view of the absence through the day of sign change, *B* illustrates both the 6 A.M. and the 6 P.M. deviations relative to the 2 to 6 A.M. and the 2 to 6 P.M. pressure changes, respectively, plotted in one figure. The latter relationship includes data for about 8 months of four summers (1955-58), reduced to the 30 corresponding days of the synodic month. The over-all correlation, 0.67, is good for a biological one. However, some additional, very exciting, information is found to be present in the correlation when it is carefully analyzed. If one removes five points over each, the

first and third quarter of the moon (fifth to ninth days after new and full moons), when the overt cycles of spontaneous motor activity crest over 6 A.M. and 6 P.M. (compare also Fig. 4), the times of day involved in this correlation, the coefficient jumps at once to the relatively high one of 0.86. The points involved are the +’s for P.M. and the X’s for A.M. The coefficient for A.M. alone is 0.62, or for P.M. alone, 0.76. This can only be interpreted as indicating that underlying the weather distortions of the daily cycles of barometric pressure are average parameters of daily pressure change that characterize each day of a fortnight. The crabs appear to be deriving their strikingly reproducible metabolic solar and lunar cycles day by day from a fluctuating external factor whose fluctuations are more independent of the local, weather-

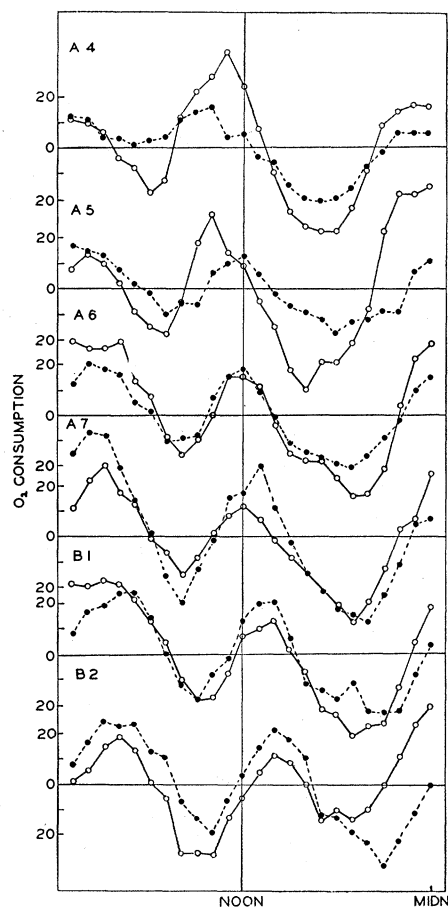


Fig. 4. Comparison of mean daily fluctuations in O_2 consumption in fiddler crabs under constant conditions during the summer of 1956 (solid lines) and 1957 (broken lines) as related to day of lunar month. A5 contains the days of new and full moon. Represented are six consecutive days in the natural synodic month. The ordinate values are deviations from the daily means, expressed in arbitrary units.

related tropospheric turbulences than is the actual, monitored barometric pressure itself.

In other words, in response to these barometric-pressure correlates a group of crabs on any given day of the synodic month—for example, the first day after a new moon—is exhibiting a cycle which, though distorted slightly with the weather of that day, is actually very close to that of the average of enough “new moon +1” days to neutralize the day-by-day random weather distortions. The crabs are behaving as if they were responding to the solar and lunar tides of the calmer “seas” of the upper atmosphere, with this accounting for nearly 80 percent of certain parameters of their measured, regular, fortnightly or semilunar metabolic patterns. Therefore, while their metabolism can be shown to be affected to a small but definite extent by the “wind-battered seas” of the weather-ridden troposphere, the major cyclic periods are being relayed as if from a gentle swell of a relatively calm atmospheric “ocean.”

The mean daily cycle of barometric pressure also exhibits a conspicuous annual modulation in its form. This is particularly evident in the time of occurrence of the afternoon minimum. This gradually drifts from about 7 P.M. in summer to about 2 P.M. in winter. Consequently there is an annual rhythm, involving even a sign change, in the 2 to 6 P.M. rate of pressure change, a parameter with which metabolism of all living things displays a correlation. Correspondingly, an annual modulation in the form of the daily metabolic cycles in potatoes under constant conditions has been demonstrated (19).

It should be emphasized that while exhibiting their pressure correlations the organisms themselves are being maintained under constant pressure, and hence that pressure and the well-known solar and lunar tides of the atmosphere cannot be the immediately effective factor for the organisms. But of tremendous import for the clock problem is the fact that of the several species of plants and animals already studied in our laboratory, all exhibit the same kind of metabolic correlation with the same specific barometric pressure parameters. This has been shown to be true throughout the gamut of living things, from algae to vertebrates.

Another thought-provoking metabolic correlation has been found, that with primary cosmic radiation (20). Leading initially to this discovery was the find-

ing that the daily cycles of several organisms in 1954 were in good measure inverted with respect to those of the same species under similar constant conditions in corresponding months of other years in which they had been studied. The first corresponding apparent inversion in a simultaneous geophysical fluctuation was found to be in cosmic radiation, from data generously loaned by J. A. Simpson of the University of Chicago. In Fig. 7, A and B, it is seen that between the months of July 1954 and July 1955, both the crab and the radiation cycles had essentially inverted, the crab retaining a nice negative correlation with the radiation cycle. For the potato, between the months of May 1954 and May 1955 (C and D in the figure), the metabolic cycle had in-

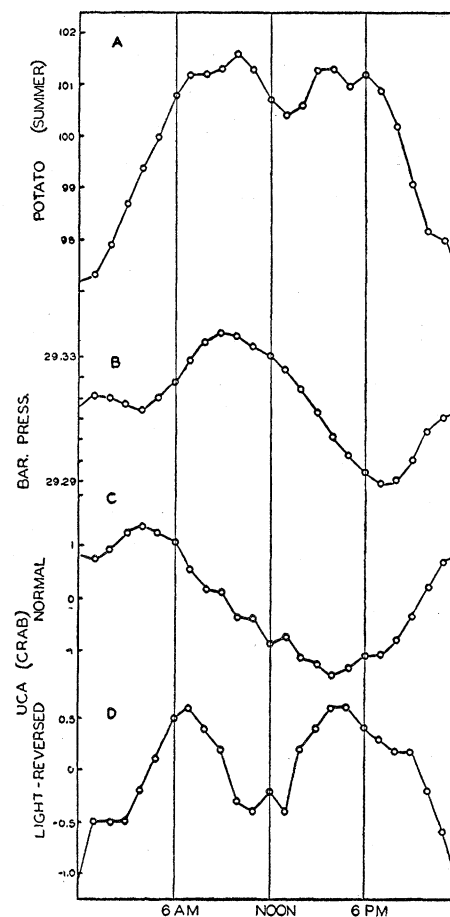


Fig. 5. (A) The mean 3-year, warmer-month (May-August) daily fluctuation in the potato under constant conditions; (B) a typical mean summer-month daily cycle of barometric pressure; (C) the mean summer-month (1955–58) persistent daily cycle of O_2 consumption in the fiddler crab; and (D) the mean daily metabolic cycle for a month in crabs whose physiological cycles were shifted 180° by exposure to illumination for a period by night and darkness by day.

verted for the morning hours only, and, correspondingly, so had the cosmic radiation. The correlation with radiation for the afternoon hours was, for the potato, a positive one for both years. It is interesting to note that for both crabs and potatoes, at the time of morning positive correlation with barometric pressure change, the correlation with the cosmic radiation is negative. The crab, which retains throughout the day the same sign of pressure correlation, continued to mirror-image the radiation throughout the day. The potato, which reverses the sign of its pressure correlation between the morning and the afternoon, is seen to have changed the sign of its cosmic-radiation correlation between these portions of the day.

The mean daily metabolic rate of the potatoes, under constant conditions, also displayed a significant synodic monthly rhythm (Fig. 8, *A*), with the minimum shortly after the new moon, and a clear annual rhythm (Fig. 8, *B*), with the minimum in October-November, during three consecutive years of study. The ranges were 10 and 80 percent, respectively.

It is true that, were one dealing only with regular rhythms of solar and lunar daily, monthly, and annual frequencies, it could conceivably be maintained that such rhythmic events as those described here depended solely upon an inherited "internal-clock" complex which independently duplicated the natural geophysical periodicities. But this clearly becomes highly improbable when, as we have just seen, the organism under so-called constant conditions is informing us through its metabolic fluctuations that it is continuously apprised of even hour-by-hour or day-by-day weather-correlated irregularities in the well-known geophysical rhythms of such factors as outdoor air temperature and atmospheric pressure changes. Certainly no autonomous internal clock can be imagined which would provide us with a continuous accurate record, even hour by hour, of the weather-related random irregularities in natural external physical cycles ranging from the day to the year.

In brief, many such results as those described above clearly indicate that precise mean periodisms of all the natural geophysical frequencies are being impressed from without upon the living system, even under conditions constant with respect to all factors generally conceded to influence such systems.

The Primary Clock

Such evidence, therefore, has proven the existence of a third organismic rhythmic component. The rhythmic fluctuations of this component, dissimilar for the two components previously described, are synchronized with

and have locked phase relations (either 0° or 180°) with external geophysical rhythms. It is postulated that this component provides the organism with reference cycles for all the natural geophysical frequencies. This obviates the necessity for postulating temperature-independent internal clocks for natural

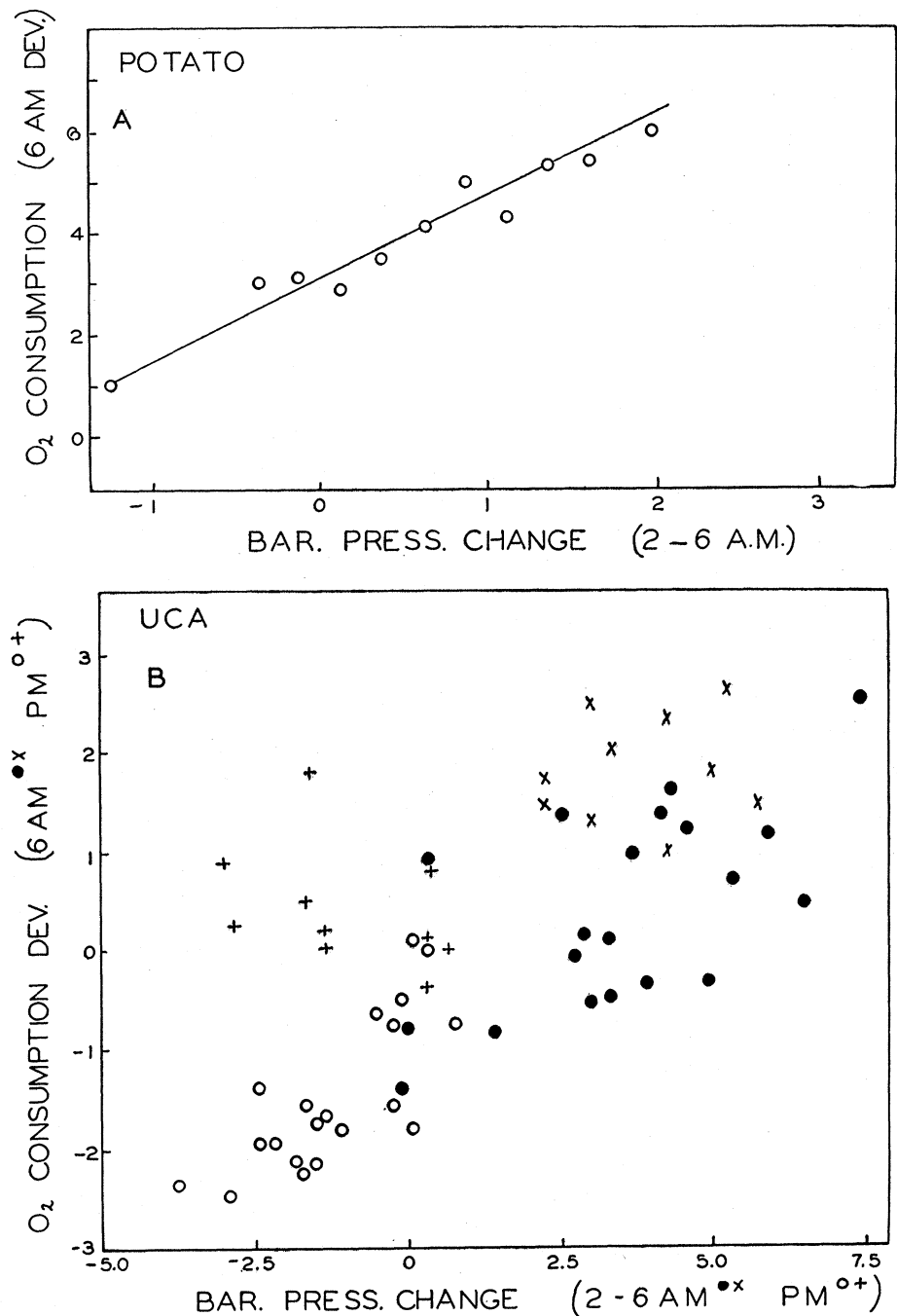


Fig. 6. (*A*) The regressional relationship of the 6 A.M. deviation from daily means in O_2 consumption in the potato, under constant conditions, including constant pressure, relative to the average 2 to 6 A.M. rate of barometric pressure change for the same morning for the warmer months during a 3-year period. (*B*) The regressional relationship of the 6 A.M. and 6 P.M. deviations from daily means in O_2 consumption in the fiddler crab (*Uca* sp.), under constant conditions, relative to the 2 to 6 A.M. and 2 to 6 P.M. mean rates of barometric pressure change, respectively. Data for 4 years, reduced to corresponding days of the synodic month, were used. Symbols + and X are values obtained from the fifth to the ninth days after both the new and the full moon.

periods ranging from the solar day, through the lunar day and month, to the year. It is postulated that this rhythmic component is the primary clock, with the periodicities of the other two organismic rhythmic centers downgraded to secondary and tertiary levels, respectively, and possessing rhythms and periods which are derived ones—derived in reaction between the primary component and environmental factors, including light and temperature. This comprises, in essence, the responder-mediator-indicator (RMI) hypothesis of rhythm timing (21).

Just as the timing of the electric clock depends upon a synchronous motor with a continuous 60-cycle electrical input, so, the evidence suggests, the timing of the living clocks depends upon a continuous inflow of cyclic information of the natural cosmic frequencies. Both clocks, furthermore, may be readily reset in their phases relative to the natural cycles without impairment of the capacity to time individual cycles accurately.

Phase Adjustment to External Cycles

The last matter to be discussed bears upon a probable relationship of the environmentally imposed metabolic rhythms to the observed overt phase- and frequency-labile physiological rhythms of special processes, mentioned in the introduction.

It has been known for many years that overt daily cycles in a wide variety of plants and animals kept under constant conditions of light and temperature may deviate from a precise 24-hour period by as much as plus or minus 4 to 6 hours. For example, in the white rat (22) there were found spontaneous running cycles of 25.25 hours in a constant illumination of 20 lux, and a 24.0-hour cycle in constant darkness. Upon the initial discovery of this phenomenon by Pfeffer, in connection with sleep movements in beans, it was postulated that this represented a corresponding deviation in the period of an internal clock from the actual solar-day period. In fact, this commonly observed characteristic of daily rhythmicity has been advanced as establishing beyond doubt that the internal clock of the living organism is a fully autonomous one.

This deviation from strict, solar-day frequency has been investigated further in more recent years, and it has been shown that there is a general relation-

ship between the intensity level of the constant illumination, ranging from complete darkness to bright light, and the apparent amount of deviation of the period from 24.0 hours (23).

Related to the apparent period length of the daily rhythm is also the level of constant temperature at which organisms are maintained. Usually at some particular temperature, in the normal range for the species, the daily cycles are quite precisely 24 hours in length, but as the constant temperature is adjusted to higher or lower levels, the observed period of the cycle may deviate slightly from the natural daily one as a function of the temperature level. In some species—for example, bean and fruit fly (4, 24)—the period length decreases with increasing temperature; in others—for example, certain algae (25)—the periods increase with increasing temperatures. This relation to temperature has been interpreted by most investigators as a consequence of an imperfect temperature compensation of the postulated autonomous internal clock to the kinetic effect of temperature. In fact, this slight temperature-dependence of the apparent cycle period has been considered by many to be additional proof of the existence of a fully independent daily oscillation in living organisms.

An alternative hypothesis for these deviations in cycle length from 24.0 hours, involving autophasing or self-phasing, was first proposed in 1956 (22). It was postulated that autophasing would be an expected consequence of the demonstration by Webb (26) of a daily rhythm of sensitivity to light as a phase-setting stimulus, together with the demonstration of more detailed characteristics of the phase-shifting mechanism, by me and my associates in 1954 (27). It was reported then that all of numerous observed characteristics of experimental shifting of the rhythm phases in color change in crabs seemed best described by a “6- and 18-hour” daily rhythm of crab response to light as a phase-shifting agent. In the normal adaptive phase relationship to the day-night cycles, there appeared to be a midnight-to-6 A.M. portion of the cycle in which the degree of cycle shift to an earlier time of day was a function of the brightness of illumination to which the animal was exposed during this physiological period. The longer portion of the physiological cycle, extending normally from about 6 A.M. to midnight, was one in which the effect

of illumination change was to phase the cycle in the opposite direction, to a later time of day. This daily rhythm of alternating directions of response to light in rhythm phasing was interpreted as being nicely adapted to enable the animals to retain optimal phase relationships with the natural light cycles.

Under the hypothesis of autophasing, it is postulated that the organism uses its daily rhythmic fluctuation in sensitivity to light to effect a daily shift in its phase relations relative to its environmentally imposed 24-hour periodicity. The manner of action, in general terms, would be as follows: The organism reaching a “light-sensitive” phase in its daily cycle, and encountering the illumination of a constantly illuminated environment, would be given a shifting stimulus whose strength, within limits, would be a function of the level of the illumination. Though physically the light is held constant, in stimulative effectiveness for the organism it is rhythmic as a consequence of rhythms in the organism’s own responsiveness. To use a crude simile, the character of a person’s response to a hot stove is essentially independent of whether the person moves to the stove or the stove is moved to the person. Since it has been demonstrated that the

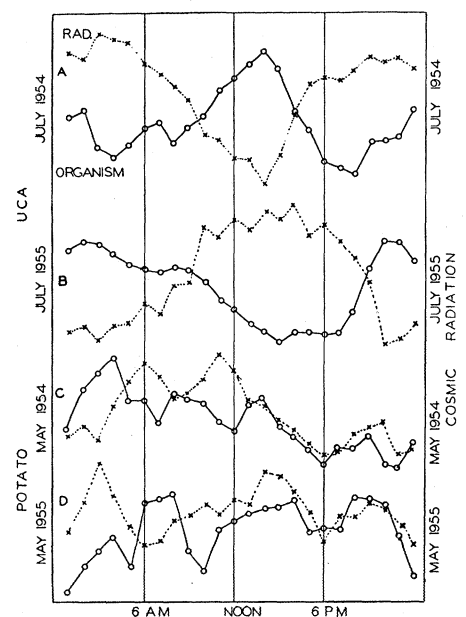


Fig. 7. Comparison of mean daily fluctuations in fiddler crabs (*Uca* sp.) (A and B, solid lines) and potatoes (C and D, solid lines) for periods of a month with mean daily fluctuations of the nucleonic component of cosmic radiation (dotted lines) for the corresponding months of the same years.

phase-shifting by light may be in either direction, depending upon the conditions of illumination, autophasing may give rise to cycles that appear to be either longer or shorter than 24 hours. This may depend upon the character of the daily rhythm of sensitivity of the particular species or individual, and hence may be in part genetic. Under conditions in which there is some perceptible daily cycle in illumination, the shifting would be expected to continue until the daily cycle of light sensitivity was optimally adjusted to the light fluctuation, and this is what is always observed. Under constant illumination, since the illumination is equivalent at all hours of the day, the daily cycle would be expected to shift continually, scanning the solar day time after time. This is what is observed in all appropriately investigated instances.

A quite comparable regular day-by-day shifting would be expected in organisms maintained under constant temperature, since Stephens (28) has demonstrated a daily rhythm of sensitivity to temperature as a phase-shifting stimulus. As temperature deviates from an organismic optimum for some period of the solar day, autophasing would be expected. Also supporting the view that the reported effects of differing constant-temperature levels really operate through the phasing mechanism is the general fact that the more closely a persistent rhythm frequency approximates a natural geophysical period, the more complete is the temperature independence.

This hypothesis for the explanation of deviations in apparent daily-cycle lengths away from 24 hours is consistent with all the observed facts. It even accounts for the hitherto inexplicable observation that changes in energy levels of both constant visible light and temperature within the normal range effect changes of the same magnitude in observed cycle length. The only demonstrated manner in which these two environmental parameters are essentially equivalent for rhythms is as phasing agents. This is also a most rational hypothesis in terms of expected organismic adaptation to the character of light and temperature fluctuations in nature. This characteristic of the mechanism very nicely enables an organism to maintain quite precise 24-hour periodicity in the natural environment despite large day-to-day changes of a weather-correlated nature in the daily illumination and temperature cycles, or

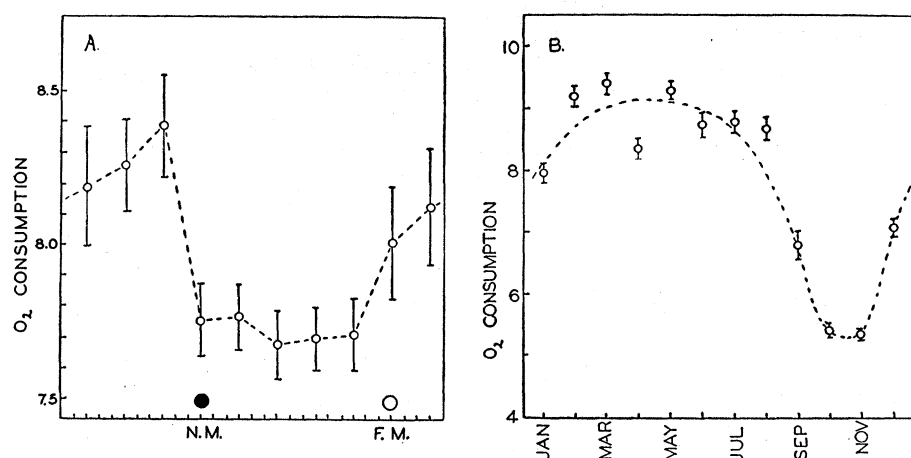


Fig. 8. The mean daily rates of O₂ consumption for 3-day periods through the synodic month (A), and for each of the 12 months of the year (B), for potatoes under constant conditions, during a continuous 3-year study. Standard errors of means are shown.

sudden changes effected by the organism's own locomotor movements carrying it during single days to places of differing illumination and temperature in its habitat.

Since light changes in nature are, unlike abrupt laboratory light changes, very gradual, the organismic cycles are able to be set adaptively to time of solar day with a higher degree of precision and stability than would be the case were the very slowly changing illumination of a single morning or evening twilight the sole means of phase setting. The organism, as it were, employs a recurring 24-hour pattern of light and temperature "testing" to enable it to move gradually into an optimally adaptive phase relationship with the external light and temperature cycles. The farther the organismic cycle from phase adjustment, the greater the daily shift; the nearer to adjustment, the smaller the daily shift. It is presumed that, once set, the cycle periods in nature are maintained with precise mean 24-hour periodicity by continuous 24-hour periodic testing of the two primary phasing environmental parameters, light and temperature. The relative importance of the two would be expected to vary with species.

There is ample reason to believe that both morning and evening changes in the environmental factors are involved in phasing. Since these work antagonistically to one another, there would be no significant change in phase relations in passing from a clear to an overcast day—a change which might occur were the rhythms phased by some absolute threshold for light at a single time of morning or evening.

In terms of this hypothesis the basic clocks are environmentally regulated and of the precise geophysical periods. The deviations of the observed periods of physiological rhythms under constant light and temperature become, instead of errors in a postulated internal clock, a splendidly adaptive means by which the organism gradually "seeks out" the best fits to prevailing external daily cycles of light and temperature. The use of 24-hour cycles of "environmental testing" is clearly a very nice way for the organism to minimize the effects of physiological adaptation to light and temperature which would doubtless render the very gradual daily changes in these two factors difficult to resolve.

Basic Exogenous Timing

Another interesting property of the persistent daily rhythms may be comparably accounted for by basic exogenous timing. It has been known for about 30 years that the daily rhythms of certain processes, such as sleep movements of plants, would follow artificially lengthened or shortened "days" usually up to about 30 hours (15L:15D) and down to about 18 hours (9L:9D). Beyond these limits, the organism would "break away" from its artificially enforced unnatural periods and revert to its natural daily period. Webb (26) found, for example, that with imposed artificial days of 32 hours (16L:16D), the rhythm of color change in crabs displayed an overt 96-hour cycle, the smallest common denominator of the fundamental 24-hour period and the imposed 32-hour periods. In darkness,

the crabs reverted at once to their 24-hour period.

The rhythmic property just described has been interpreted by some investigators as evidence supporting a hypothesis of an inherited, 24-hour oscillatory period in the living organism which could not be strained into periods beyond these observed limits. However, the observation may be accounted for just as simply in terms of the now well-known 24-hour cycles of sensitivity to phasing by light. It is known that after one abrupt phase setting by light-on (or light-off), the organism is sensitive to additional phasing by an opposite abrupt change in illumination only within a restricted range of times following the initial phasing. If the second stimulus occurs either earlier than the limits (that is, if the artificial days are shorter than 18 hours) or later (if the artificial days are longer than 30 hours), the second and immediately succeeding cyclic changes in light fall within periods in the organismic daily rhythm insensitive to phasing by light. Thus, beyond these limits the organism, being unable to phase, would be expected to revert to its fundamental period of 24 hours.

Similarly, the persistence of the natural period of daily and tidal rhythms during rapid east-west transport by airplane can be postulated to be either a consequence of autophasing effected by endogenous aspects of the living-clock system or a result of exogenous timing through a geophysical fluctuation correlated, for example, with atmospheric electrical potential and operating on universal time.

Environmentally Dependent Clock Model

The demonstration that all the natural geophysical periods are being derived from the physical environment by animals and plants even under conditions hitherto presumed to be constant, together with the clear evidence that organisms can use a rhythm of one frequency to derive a rhythm of a different one, eliminates the need for continuing to postulate autonomous internal clocks possessing all the natural periods. Scientifically, such a hypothesis is of a last-resort type, since it is incapable of definitive proof, depending, as it must, exclusively on negative evidence. On the other hand, such an environmentally dependent clock model as that presented here will probably require revisions

from time to time as new properties of rhythmicities come to light. However, short of the demonstration that biological cycles of the natural geophysical periods can still persist in organisms traveling in space well outside of the earth's rhythmic field of influence, there is no logical basis upon which this hypothesis can be rejected.

Furthermore, this hypothesis readily accounts for the hitherto inexplicable immunities of the clocks to temperature changes and to drugs for all natural geophysical frequencies. These last characteristics of the clock are those which are the most unconventional in terms of our current concepts of physiology and biochemistry, and those regarding which no advance in our knowledge of the basic timing mechanism has been made in the half century during which Pfeffer's hypothesis of an autonomous internal clock has been dominant. The present hypothesis reduces the remaining major problems to those upon which, perhaps significantly, most progress has already been made—namely, the mechanism of rhythm phasing, including properties of the coupling systems between the internal rhythmic components, and characteristics of the exogenous information reaching the primary rhythmic component.

In addition, more of the numerous observed properties of the organismic rhythms appear to fall into a consistent framework in terms of this hypothesis than has been the case for any earlier one. This hypothesis also brings, for the first time, the mysterious living clocks out into the open, being far more amenable to investigation and analysis, in terms of our current knowledge in the natural sciences, than earlier "closed system" clock hypotheses. It seems highly desirable to adopt as a working hypothesis a positive, testable approach of this kind.

One of the questions perhaps most frequently asked in reaction to this hypothesis of the nature of the living clocks is the following: How can small imposed metabolic oscillations involving changes of only a few percent be responsible for timing observed biological cycles often involving tremendous energy changes? This, clearly, is no insurmountable problem. In our everyday experience the schedules of railways, airlines, and factories, and even our own daily work patterns, involving relatively huge energy changes, are in fact timed by clocks or watches so low in energy output that a few dust particles may suffice to stop them. The liv-

ing clock system would appear to be another example of a class of physiological phenomena referred to as triggered responses. It is a common biological experience that there need be no fixed relationship between the energies of the stimulus and the response.

In conclusion, it may readily be seen that all the major evidence that has been marshaled in support of an independent internal clock system with periods of close to, but seldom exactly, 24 hours, can quite rationally be interpreted in terms of organismic natural periods precisely corresponding to geophysical periods, from which all other observed periods are derived through phasing as functions chiefly of light and temperature. And the unequivocal evidence at hand that organisms under so-called constant conditions are, indeed, directly deriving these natural periods renders it highly probable that the ultimate period-giver for all the observed persistent rhythms, from the sidereal day to the year, comprises simply a continuing reaction of the exogenously rhythmic living thing with its fluctuating, physical environment (29).

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29. The research program on biological rhythmicity discussed in this article was aided by a contract (No. 1228-03) between the Office of Naval Research, Department of the Navy, and Northwestern University.