VIII. Biological Clocks[1]

The Functions, Ancient and Modern, of Circadian Oscillations

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DE MAIRAN'S PHENOMENON

ERWIN Bunning has drawn attention to a remarkable, brief note by the French geologist, De Mairan, written in 1729. De Mairan was evidently intrigued by the daily movements, up and down, of the leaves of certain plants. Hoping to elucidate the nature of their environmental causes he took one of these plants, ***Mimosa,*** into a cave where it was free of any daily cycle of light or temperature. To his surprise De Mairan found the daily periodicity of movement persisted in this essentially aperiodic environment. Recognizing the importance of his discovery De Mairan commended the problem to his botanical colleagues. In his closing comments he anticipated slow progress in the matter--not, to be sure, because he held a low opinion of botanists but, he says, because progress in science is wholly dependent on experiment and he presumably foresaw no obvious experimental attack on his surprising discovery.

De Mairan's phenomenon attracted a long and distinguished line

1. This paper is necessarily a brief condensation of the material covered in nearlv four hours of lecture and discussion. The condensation has not treated all sections of the oral presentation equally; the mechanism of entrainment of circadian rhythms by light and the bearing of that mechanism on the problem of photoperiodic time-measurements was discussed at length. It is omitted here. In attempting to give a short written paper some focus I have elected to develop some speculations that were raised only in discussion during the Cloudcroft Seminar.

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of botanists in the 19th Century including De Candolle, Hofmeister, Sachs, Darwin and, especially, Wilhelm Pfeffer. Pfeffer is, of course, far better known for his discovery of osmosis and many other contributions to plant physiology than he is for two books, one in 1875 and the other in 1915, on the persistent daily rhythmicity of leaf movements.

To Sachs in the late 1800's it was already clear that the light cycle of the environment was not forcing any periodicity on the plant, it was only serving to control the timing of a periodicity arising internally from other causes. All of the botanists at the end of the 19th Century were concerned to some extent with what adaptive functions these oscillations served. It was not obvious that any useful purpose was involved at all. Darwin, not surprisingly, was nevertheless confident that some purpose had to exist, the implication being, of course, that to evolve as a result of natural selection some adaptive advantage is necessary.

THE ENDOGENOUS (vs. EXOGENOUS) ORIGIN OF THE OSCILLATIONS

A major theme running through the literature at the turn of the century--and continuing up to 1930--was the possibility that the rhythmicity persisting in constant darkness and constant temperature arose from forces external to the plant; that some unknown Factor-X, an unidentified periodicity in the physical environment, was forcing the rhythmicity. Even today there is still, in fact, one laboratory that remains convinced of the reality of Factor-X. Professor Frank A. Brown, Jr. of Northwestern University has published a long series of studies in which he claims to detect precise 24-hour periodicities in various organic activities (respiration, movements, etc.) that remain phase-fixed to local time. These periodicities (whose reality has been questioned on statistical grounds) he attributes to control by Factor-X. His position has, however, met with considerable criticism in the field. The great majority of workers has long since concluded that De Mairan's phenomenon arises from wholly endogenous, not exogenous, causes.

The evidence for this view derives from various kinds of observation. The principal ones are as follows: The periodicity persisting in constant dark and constant temperature can be stopped by anoxia or low temperature and resumes when oxygen is returned to the system or the temperature is raised again to a level at which metabolism can proceed. The energy on which the oscillation depends is of metabolic origin. When it resumes, after anoxia or low temperature treatment, it does so at essentially the point where it stopped. Implicit in this statement is the important fact, demonstrable with many other techniques, that the phase of the rhythm in constant darkness is wholly independent of local time--that is, of the phase of the earth's rotation and hence of all factors (including the unknown Factor-X) dependent on the earth's rotation.

The most impressive fact in this context is that in organisms in which it can be measured precisely, the period of the rhythm persisting in darkness is not precisely that of the earth's rotation (Figure 1). Proponents of the endogenous nature of these rhythms (including the writer) argue that only by adducing the most cumbersome and unlikely additional assumptions can one explain the origin of, say, a 23 hour and 15 minute period as the product of an (unknown) driving cycle with a period of precisely 24 hours. Franz Halberg has introduced the term circadian (L. *circa, dies*) for De Mairan's oscillations. This term emphasizes the theoretically important discrepancy between their period and that of the earth's rotation. It also obviates the conflict of meanings inherent in diurnal (vs. nocturnal) which has often been used. The phrase "daily rhythms" lacks the precision and implication of Halberg's "circadian rhythm."

Individuals within a species differ, genetically, in their free-running periods--that is, in the period they manifest when uncontrolled by an environmental light cycle. And their periods are also open to some experimental manipulation principally as a result of prior light treatments.

As a matter of fact, it is now unfair to associate Professor Brown with earlier students of Factor-X; he no longer regards Factor-X as the total explanation of De Mairan's phenomenon. He recognizes that the data demand the existence of a periodicity of endogenous origin; his interest in Factor-X focuses on its potential role as a kind of pace-maker that confers precision and temperature compensation on the endogenous oscillation.

The Ubiquity and Pervasiveness of Circadian Oscillations

De Mairan's phenomenon has been, by now, observed in a remarkably broad array of organisms: unicellulars, green plants, and animals at all levels of complexity including man himself. It is a general feature of the physiological organization of living things on this planet. This ubiquity is matched by the diversity of functions within the individual organism that manifest the oscillation. In the unicel-

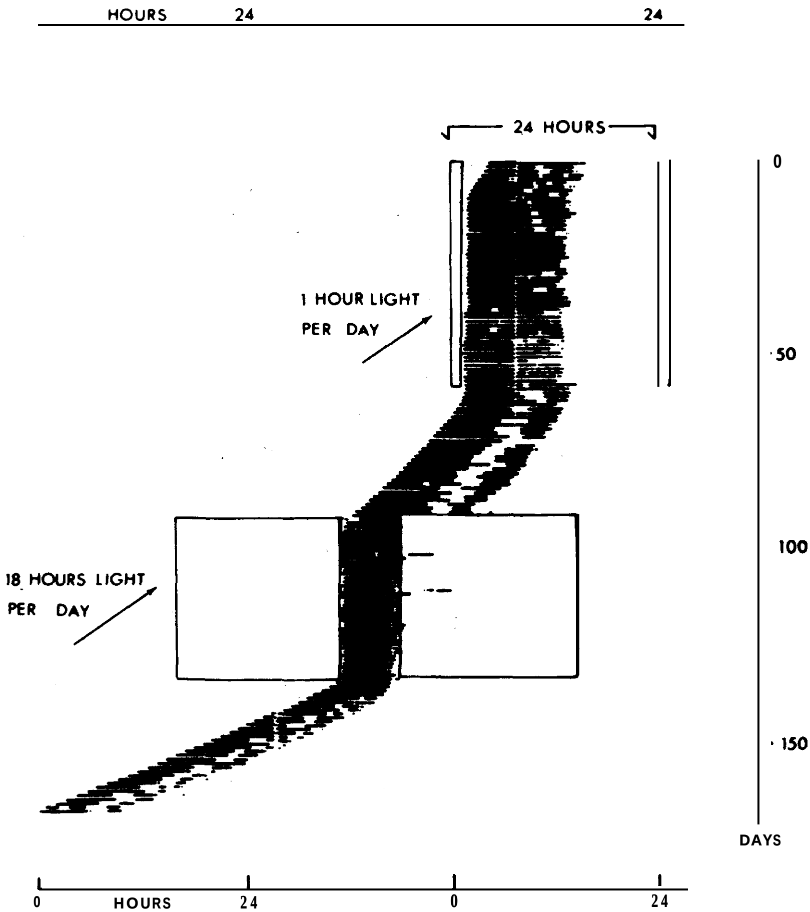

FIGURE 1. **The circadian oscillation of locomotry activity in Peromyscus** ***maniculatus*****, freerunning and entrained. A 1:23 light-dark cycle is imposed from day 0 to day 59. The oscillation is captured into entrainment by day 6. From day 60 to day 92 the rhythm again freeruns in constant darkness. An 18:6 light-dark cycle is imposed from day 93 to day 132 when the rhythm is again allowed to free run. Note the remarkable precision of the period of the freerunning oscillation.**

lular ***Gonyaulax,*** Hastings and Sweeney have found circadian oscillations in two distinct aspects of its luminescent system, in photosynthesis and in cell division; in mice or rats virtually every parameter studied is involved--blood chemistry, liver chemistry, cell divisions, body temperature, susceptibility to X-rays and drugs, and so on. The practical importance of these more recent discoveries in mammals is obvious and immense. A given dose of ***E. Coli*** endotoxin will kill 85% of mice treated at one point in their circadian cycle but only 5% at another (Halberg). Similarly extreme effects have been reported, again by Halberg, for the drug ouabain. Gertrude Stein to the contrary notwithstanding, a rose is not necessarily and unqualifiedly a rose; that is to say, it is a very different biochemical system at noon and at midnight. The phase of the organism's circadian cycle of change is a parameter of major importance the physiologist cannot ignore. Nor can the pharmacologist!

We have found, paralleling Halberg's observations, that the behavior to a fixed stimulus and the temperature tolerance of ***Drosophila,*** is markedly different at different circadian phases. And along with many other laboratories we have found significant differences in the specific activity of a given enzyme system assayed in vitro after extraction in the middle of the subjective day and the middle of the subjective night. In our (Uwo, Nakajima, Townsend and Pittendrigh, ***in press)*** case we found differences in the Michaelis Constant for the system, day and night.

THE PRECISION, INNATENESS AND TEMPERATURE COMPENSATION OF CIRCADIAN OSCILLATIONS

For ease of assay circadian rhythmicity is most conveniently studied by recording some behavioral feature of the whole organism. In mammals, for instance (Figure 1), locomotion lends itself to a very useful assay. Rodents have a curious predilection for exercise on running wheels. The time at which they begin and continue this activity is easily recorded by coupling the running wheel via a microswitch to an operations recorder. Every time the wheel is rotated a pen mark is made on the horizontal lines (24 hour length) in e.g. Figure 1. The onset of their activity in 24 hour cycles of light and dark is precise; in nocturnal species it begins at or near "sunset." When the organism is put into De Mairan's conditions of constant darkness and temperature, the periodicity persists with remarkably clear definition and persists indefinitely. The period of the rhythm, as measured by the intervals between activity onsets, is circadian.

The precision and indefinite persistence of the rhythmicity are among its most striking features. The standard error of the period in some of these free-running rodents may be no more than about a minute. In other words the "error" is of the order 1 in 1000.

The rhythmicity is, moreover, innate to the organism; it is not learned by prior experience (in the individual) of a daily periodicity in the environment. Rigorous demonstrations of innateness have been made in unicellulars, insects and vertebrates.

Perhaps the most surprising property of circadian oscillations is the fact that their period changes only very slightly with considerable changes in temperature. This temperature-compensation of the oscillation is a large topic in its own right. It has played a major role in F. A. Brown's thinking. The difficulty of giving a simple physiological explanation for it led him, in part, to his renewed concern with the possible existence and function of an external physical pacemaker (Factor-X). On the other hand, it was a property this writer inferred should be general if circadian oscillations were fulfilling a general clock-function--a topic to which I shall return shortly.

The Cellular Basis of Circadian Oscillations

A significant result in the last ten years has been the demonstration that De Mairan's rhythms do not depend on the greater complexity of multicellular organization. Single cells manifest the rhythm--in, for example, ***Euglena, Gonyaulax, Paramecium*** and the alga ***Acetabularia.*** Sonneborn and Barnett have studied a remarkable case in ***Paramecium multimicronucleatum.*** "Animals" of this species oscillate from one mating type to another in the course of a single day. From what is known of the genetics of mating type in other species this result is suggestive that a single gene may be undergoing a daily cycle of induction and repression.

Several workers have made attempts--mostly abortive--to strive for further delimitation of the level of organization necessary to sustain a circadian oscillation. In particular the question of whether or not the nucleus or cytoplasm is the site of the driving oscillation has been raised.

Sweeney and Haxo (1961) and Richter (1963) have made some remarkable observations on the famous unicellular alga *Acetabularia* which lends itself readily to such tasks. The localization of the nucleus in one part of a single cell that withstands surgical treatment permits one to ask whether or not the enucleated cytoplasm can sustain a circadian oscillation--in this case, of photosynthetic activity. It can, even for as many as 30 cycles. The ease with which subcellular grafting can be-achieved in this alga permitted Schweiger, *et al* (1964) to show, however, that in a "synthetic" cell consisting of nucleus and cytoplasm placed 180° out of phase, the resultant steady state rhythm of the cell is that dictated by the nucleus. In any case the large amounts of DNA in the extranuclear organization of *Acetabularia* render the capacity of its "cytoplasm" to sustain an oscillation of doubtful general significance.

Many attempts have been made to manipulate circadian rhythmicity chemically; and for the most part these attempts have been singularly unsuccessful. In the absence of labeling the negative results are not too significant; there is no assurance the agents applied entered the cells in significant amounts. But at least some of those who have at tempted the work are impressed with its apparent insusceptibility to chemical control. There are, to be sure, some reports of positive effects but they are not all fully convincing. Hastings appears to have affected phase shifts in *Gonyaulax* with cyanide arsenite and p-chloromercuribenzoate. Bunning has reported data on the effects of colchicine, urethane and alcohol on period length. And Bruce and Pittendrigh found effects of D_20 on both the phase and period of the *Euglena* system. In any case none of these results has proved fruitful of suggestions as to the chemical basis (if any) of the oscillation.

One of the best known studies along these lines is that of Karakashian and Hastings. Using the unicellular (dinoflagellate) ***Gonyaulax*** they measured the effects of several antimetabolites known to affect various steps in the protein synthetic mechanism. The action of actinomycin-D is known to affect the production of messenger RNA-the primary step in the transcription of the inherited message of the cell's DNA. They found, following actinomycin treatment, that the rhythms of luminescence and photosynthesis decayed. This result, surely of considerable interest, is however somewhat equivocal as to meaning. The loss of rhythmicity, as such, by no means demonstrates that the agent responsible has affected the cell's clock; its target could as well be the coupling of the clock (or driving oscillation) to the physiological system assayed.

The only unequivocal demonstrations of manipulation of the clock concern either the period of the phase or a steady-state rhythm.

Strumwasser has very recently given evidence on the action of actinomycin that promises to fulfill these requirements. In a brilliant study of circadian rhythmicity in a single ganglion cell from the mollusk ***Aplysia,*** he has succeeded in making intracellular injections of various agents. The results so far published show a clear phase-shift of the oscillation following injection of actinomycin. At present technical limitations preclude measurement of the rhythm for much more than a single cycle after treatment. We cannot, therefore, be sure that the phase-shift seen in that cycle would persist in the steady-state and thus demonstrate fully that the driving oscillation has been affected and not (again) some coupling mechanism between the driver and the assayed rhythm.

Other suggestions that the nucleic acid systems in the cell are intimately involved in circadian rhythmicity arise from observations by Ehret and, independently, by Sweeney that ultraviolet at 254 $m\mu$ can effect steady state phase-shifts. There are peculiarities in both Ehret's ***(Paramecium)*** and Sweeney's ***(Gonyaulax)*** results that indicate the UV is achieving its effects by a quite distinct route from that of visible radiation.

The Cell and Organism as an Oscillator Entrainable by Light Cycles

In 1957 Bruce and I pointed out that, formally, circadian rhythms are self-sustaining oscillations, and pursued a general comparison between the organism-environment relation and that of two oscillations, one entraining the other. In historical perspective, it is clear that circadian oscillations in the cell and organism are an evolved match to the striking oscillations of the physical environment. Metabolism has evolved an oscillatory time course, and in nature that oscillation in metabolism and behavior assumes a definite phase-relation to the external cycle of physical change. Attainment of that "proper" phase relation is effected--at least principally--by the light cycle. The cellular oscillation couples to the light cycle and is entrained or driven by it. The light cycle, as Sachs long ago recognized, is not ***imposing*** the rhythm. Its action is strictly comparable, mathematically, to the action of one oscillation entraining another, fully-autonomous, self-sustaining oscillator.

Entrainment implies control in two respects. The entrained oscillation assumes the period (or frequency) of the entraining cycle; when, as in the biological case, the entraining cycle is error-free, the error inherent in the entrained oscillation's imperfections are removed. Second, the entrained oscillation assumes a determinate phase-relation to the entraining cycle. The net biological result is clear temporal control; specific events in the circadian cycle occur at particular times in the environmental cycle.

It is, for the most part, an act of biological faith when we go further and say that the end result is execution of given functions at the "right time of day." This is to follow Darwin in his confidence that some adaptive function does, ultimately, attach to such a remarkable piece of organization. To suppose otherwise is, implicitly, to appeal to something other than natural selection as the historical agent of the system.

I will not, in this written version of my contribution, pursue what is now known of the mechanism of entrainment by light. Suffice it to note a few points of general interest. First, light cycles are universally effective in entraining circadian oscillations. In poikilothermous ("cold-blooded") organisms temperature cycles can also entrain but they are probably always less powerful agents, and recent work by Mr. Zimmerman in our laboratory shows this is certainly the case in ***Drosophila***. Second, observations by Halberg, Richter and my laboratory have shown that in mammals the light, in its entraining function, is transduced by the eye; blinded mice, rats and hamsters fail to entrain to light cycles. It is, however, likely that this route is historically secondary. If, as is surely true, the hypothalamus acts as driving center in the system of circadian oscillations within a vertebrate, its coupling to the environmental light cycle will almost necessarily demand an intermediate coupling to a superficial photoreceptor. (It is noted, however, that Ganong's remarkable demonstration of the penetration of the visible into the vertebrate brain stem takes some force from this argument). However, it is equally clear, as experimental fact, that the *Drosophila* circadian system can be entrained without any organized photoreceptor in the larval stage. And several workers (including Lees and

Williams) have demonstrated that photoperiodic induction (which, I shall argue later, is a function of the circadian oscillation) can be effected by the action of light absorbed directly by central nervous tissue. In Paramecium, other unicellulars, and in green plants, the question of an organized "eye" does not arise. They, too, are entrainable by light. The general conclusion is that some molecule in the cell, not specifically devoted to photoreception in the usual visual sense, absorbs the entraining light and is intimately connected with the driving mechanism of the circadian oscillation.

The third point I wish to emphasize about light cycles as entraining agents for circadian oscillations is the general result that the photoperiod in each cycle-the fraction of the period occupied by light-has a major effect on the phase and the waveform (insofar as one can measure or infer this) of the entrained rhythm.

CHRONOMETRY BY CIRCADIAN OSCILLATIONS: CELESTIAL ORIENTATION AND PHOTOPERIODISM

Bruce and I have suggested that the resurge of interest in circadian rhythms since 1950 largely derives from the remarkable studies by Gustav Kramer and Karl von Frisch which showed that birds and bees, respectively, can maintain a given direction throughout the day using the sun as compass. They compensate for the movement of their celestial direction-giver with the aid of an internal 24 hour clock. The experiments supporting these remarkable conclusions are classics of experimental zoology. Hoffman and others have shown that the animals' clock is phased to local time by virtue of being coupled to the environmental light cycle. In starlings Hoffman has shown further that the clock will continue to operate in continuous dim light and proves to be-in these freerunning conditions-a circadian oscillation. Its freerunning period is about 23½ hours.

Kramer's initial demonstration prompted my own reinvestigation of the temperature-relations of the circadian system in ***Drosophila.*** On the hypothesis--then--that circadian oscillations were the evolutionary foundation of Kramer's clock, it seemed to me that to be useful in this respect they should be temperature compensated; and they proved to be so. Since then (1954) temperature compensation has been shown to be a universal feature of circadian oscillations even in single cells (Bruce and Pittendrigh, 1956). And since 1950 time-compensated sun-orientation has been discovered in a remarkably diverse array of metazoa.

It is clear, however, that this spectacular clock function is recent in the history of life; it has exploited already existing circadian oscillations; it does not account for their initial evolution.

Nearly 15 years before the work of Kramer and von Frisch, Erwin Bunning had related circadian rhythms to a quite distinct set of phenomena--those of photoperiodism. Garner and Allard showed, in 1920, that the switch from vegetative to floral growth in some plants was controlled by the number of hours of daylight--the photoperiod --in each daily cycle. Biinning's suggestion in 1936 was that the endogenous "daily" rhythmicity of plants was causally related to this control. He envisaged what we now call the plant's circadian rhythm as consisting of two half-cycles--one photophilic and the other scotophilic. The latter, in its usual phase relation to the environmental day, lies in the nightly dark period. He suggested that as the length of day changed the early scotophil would be illuminated or not, according to season. When illuminated in long day plants the switch to floral initiation was closed; in short day plants illumination of the early scotophil kept the switch open. His hypothesis, translated into the current jargon, was, in fact, that the circadian oscillation of the plant was serving as the clock that effects the time-measurement implicit in photoperiodism; it was the clock measuring the duration of the daily photoperiod.

That hypothesis was a brilliant stroke in its day. It anticipated the current emphasis on the time-measurement as such as the knottiest problem in photoperiodism, and it anticipated the current treatment of circadian oscillations as biological clocks in general. Yet the hypothesis has met with stubborn opposition by students of both plant and animal photoperiodism. That opposition is now weakening and the evidence today leaves essentially no doubt that Bunning's basic proposition is fundamentally correct (cf. e.g. Pittendrigh and Minis, 1964 and Pittendrigh, 1965).

ON THE ANCIENT, OR PRIMARY, FUNCTION OF CIRCADIAN OSCILLATIONS

Time compensated sun orientation is surely a recent development in the history of organisms. And it seems likely that classical photoperiodism is of more recent origin than circadian oscillations in analysis and explanation is not the only nor even the main task of the biologist; but even when he is primarily concerned, as I am, with physiological explanation, he cannot afford to neglect functional issues as touchstones to progress. Living organization is the product of an historical development molded by natural selection whose only concern is in fact functional. It is then possible that by recognizing the possibility of a primary function, so far not recognized, we may be led to useful new avenues of question and casual analysis.

THE LIGHT CYCLE AS THE PRIMARY AGENT OF SELECTION

The relation of the light cycle to circadian rhythmicity is today recognized only as that of its entraining agent, but it is a reasonable speculation that the daily alternation of light and darkness was the historical cause (selective agent) of circadian oscillations in the first place.

This line of thought derives from recalling the prerequisites for organization in a chemical system. The principal of these is that the constituent reactions cannot proceed spontaneously at the prevailing levels of free energy. Thermochemically this means, of course, that the reactions the cell employs involve energy barriers unsurmountable at prevailing temperatures; they proceed only on command which rests with enzymes and ultimately with the nuclear store of information. Little attention seems to have been given--in this general context--to the problem of visible radiation as an energy source that threatens organization.

Of course, the fact is that the majority of the cell's constituents are colorless; and uncontrolled activation by the visible is thus excluded. It may well be that in the history of the cell there has been selection for colorless molecules, but if that is true (and it seems likely) it is a fact that for some functions colorless molecular devices have not been found. The flavins and cytochromes are examples of ubiquitous fundamentally important molecules that are colored--and where color has no detected function.

No attention seems to have been given to the consequences of illuminating those molecules whose color is without obvious function. At any rate it is surely reasonable to consider, at least, the likelihood that some subroutines in the cell's overall tasks are impaired by the activation of molecular piece-parts in the flood of visible radiation to which it is subjected each day. To that extent the routine delegation of some chemical activity to the recurrent darkness of each night would be an obvious escape from the photochemical threat to organization.

A miscellany of otherwise disconnected facts has suggested to me that we should not ignore this line of thought and further that the activities involved may concern the cell's central controls-those of protein synthesis and specification. I noted earlier that there is suggestive but not yet compelling evidence from actinomycin and UV treatments that the nucleic acids are intimately involved in the cell's driving oscillation. There is, too, the long known fact that an enzyme system involved in repairing UV damage to the genetic material absorbs in the visible; this is the phenomenon of photoreactivation. The enzyme concerned is incidentally evidently involved in the mechanism of genetic recombination-at least in bacteria. And the miscellany is completed by the observation of Sulkowski, Slonimski and colleagues that gene induction (in yeast) can be inhibited, at least for some hours, by visible radiation during the transition from anaerobic to aerobic metabolism. It is certain, at least, that part, and perhaps an important part, of the central control mechanism absorbs the visible and is functionally significantly affected by the resulting activation. The possibility arises that one way of coping with this activation is to restrict the steps concerned-perhaps gene induction itself-to the daily dark period.

CIRCADIAN OSCILLATIONS AS SYNCHRONIZING GATES IN THE TIMING OF DEVELOPMENTAL STEPS

I noted some years ago (1954) that the circadian rhythm in ***Drosophila pseudoobscuru*** was functioning as a synchronizing gate for the act of eclosion. In spite of Harker's (1965) recent discussions, this remains clearly true. But recent work by Skopik and me at Princeton demonstrates that the synchronization manifest at eclosion must occur much earlier in pupal development. Harker's data, which she interprets radically differently, show, in our view, the same result. When a circadian oscillation is initiated in ***Drosophila*** at any stage in its development, the remainder of development takes a time to completion that is strictly ***modulo*** the period of the oscillation.

We are currently concerned with the implication that the initiations of new subroutines in development are gated by the circadian oscillation in the system in a manner broadly comparable to that of a master clock in a synchronous computer. In such computers the inputs necessary from a diversity of subroutines--some slow, some fast --are guaranteed to be available by postponing the initiation of the next round until a gate is opened; and that gate, timed by a master clock, occurs with a frequency adjusted to the slowest subroutines. Synchronous computers are, thus, slower than the more elaborate asynchronous devices that can be built, but they are cheaper. All real computers involve a mix of asynchronous and synchronous features. And it is obvious that the organized reading and execution of the cell's DNA is not always regulated by synchronous gates--and certainly not by gates recurring with a period as long as 24 hours. But it is by no means excluded that selection has seized the opportunity of circadian oscillations, especially if they involve a reading of the message, to buy a degree of organization more cheaply than is otherwise possible. There is an obvious appeal in the idea of a temperature-compensated oscillation functioning as a synchronous gating device in a system whose piece-parts are conspicuously temperature dependent. A ***Drosophila egg*** raised at 10° or 28°C yields a fly that is essentially the same; morphogenesis and differentiation are temperature compensated; the reading of the message is temperature compensated.

It is clear that if, in eukaryotic forms, the cell has restricted some light-sensitive step in message reading to the daily dark period, it has also, ***ipso facto,*** instituted a temperature-compensated synchronizing gate. What we are now pursuing in ***Drosophila*** is precisely this idea. The working position is that eclosion is only a special case of a general gating, by the circadian oscillation, of all new gene inductions; inducer may have been available earlier but induction awaits a once daily scan of the message. This view is encouraged for us by Harker's facts which she interprets in a fundamentally different way. She appears almost to deny the existence of oscillations in individual flies and regards the time of eclosion as determined by summing the intervals between earlier developmental steps. It is, of course, trivially true that such summation occurs; eclosion is the end of development. What she ignores is the fact that development times are always ***n*** times the period of circadian oscillation and further that her own data indicate that each new step, like the appearance of eye or wing color, tends to be phase-fixed to the light cycle-and hence to the circadian oscillation.

This frankly speculative excursion into the history and primary functional significance of circadian rhythmicity would be unjustified were it not suggestive of new experimental work. Fortunately, it is. We are, in fact, greatly encouraged in testing the proposition that the timing of new morphogenetic events in mid-development is as manipulable by the phase of circadian oscillation (itself manipulable by brief light flashes) as the terminal event of eclosion itself.

It is not difficult to relate this line of thought to the involvement of circadian rhythms in photoperiodic induction. The change in day length that occurs with season effects a switch in metabolic program --as from vegetative to floral growth. That change demands evocation of a part of the nuclear message otherwise ignored or suppressed. In short, photoperiodic induction must, in last analysis, involve induction of specific genes. The prospect that induction is restricted to a daily scan of the message in each dark period is clearly compatible with the further idea that induction of a given gene lying to wards the end of the scanning sequence is as photosensitive as the inductions Sulkowski et al have observed in yeast cells. There is, of course, evidence now from bacterial studies that any scan of the message--for replication or reading--will begin at a fixed point and proceed linearly down the fixed species specific codon sequence. The meager facts available indicate a scanning time for replication in eukaryotic cells of 6 or 8 hours; it is reasonable to anticipate that a reading scan will take essentially the same time--which is in the right range for measurement of night length.

The scope of the speculation I have ventured here guarantees that much of it will be wrong. Its justification is in part its testability, as I noted. But it is also justified if, in isolating and emphasizing the question of a primary unidentified function for circadian oscillations, the speculation prompts new questions and experiments.