Circadian Rhythms and the Circadian Organization of Living Systems

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The writing of this paper has been influenced by strongly held convictions. This does not concern the validity of the theoretical scheme it offers; it concerns the need at this juncture in the study of "daily" rhythms for bold and explicit theory formation. We are beset rather than blessed with an enormous number of observations about a great diversity of organisms that range from unicellulars through African violets to man. Moreover, the fact that a majority of these observations is highly fascinating is itself a danger—the common danger threatening the biologist of mistaking acquisition of more fascinating facts, and more concrete detail, for analytic progress. To make progress analyzing circadian rhythms we must perceive what the problems are—or rather state what we take them to be—and proceed with accumulation of new information only as it tests, and alas probably eliminates, theory. The low life-expectancy of any detailed explanatory scheme in this field is no reason to eschew theory formation altogether. On the contrary, I take it there is an infinity of facts, relatively few of which are necessary for an understanding of general principles; and that the function of theory is not only, or even principally, to state one's best estimate of those principles as to minimize the error in improving that estimate through discovery of new fact. The applied mathematicians and physicists from whom we are seeking models and analogs wish to know not all the facts but the significant facts. And one can rebut the likely objection that which are significant is something known only in retrospect by insisting that present judgment on the matter is necessary to proceed. Theory formation, then, involves judgments—especially in a loosely defined field; judgment on what the problems are; and judgments on which are the most pertinent of all the available observations. It is, in brief, a tool for what remains to be done. At any rate it is in this spirit that the present essay has been written; I have chosen for emphasis those facts I think are significant.

I have discussed everything in this paper so many times with my colleague, Victor Bruce, that I am uncertain where many ideas came from; several I know came from him. This, however, is not to imply that he endorses all my interpretive ventures. I am indebted also to Ewald Pauming and Dorothy Minis; to my students Drs. Burchard, Roberts, and Menaker, and Messrs. Swade, Plumlee, Weiss, Tobin, and Golden—they have all contributed to the experimental results used here. And finally I take pleasure in expressing deep gratitude to Professors Aschoff and Bunning in whose laboratories I recently spent several profitable months while enjoying a Guggenheim Fellowship.

I owe a special debt to Mr. Swade for permission to reproduce and interpret some unpublished records of his on arctic mammals; it goes without saying that he cannot be held to my views of their meaning. His work and all the other experimental results from our laboratory reported here were made possible by funds from several sources; from the Eugene Higgins Trust; the National Science Foundation; the Office of Naval Research; and the Air Force Office of Scientific Research.

CIRCADIAN RHYTHMS; THE EMPIRICAL GENERALIZATIONS

Table 1 is a summary of major empirical generalizations about circadian rhythms. A general treatment of these rhythms might follow either of two leads afforded by the list. One would be the functional significance and physiological implications of their temperature-compensated period. This has been the approach Bruce and I have taken in several other discussions [1, 2, 3, 4, 5]; it is the approach which was stimulated initially by regarding daily rhythms as clocks [1]. Another is afforded by those generalizations concerning the ubiquity of circadian rhythms, their existence in both single and multicellular systems, the fact they are self-sustaining, and, finally, that they are always innate. For what these statements challenge is the attitude that "daily rhythms" are in some vague sense no more than appropriate responses to daily change in the environment; that they are, as it were, secondary adaptations superficial to the main physiological architecture of the organism.

What the generalizations listed, in fact, imply is the flat converse: that circadian rhythms are inherent in and pervade the living system to an extent that they are fundamental features of its organization; and to an extent that if deranged they impair it. They suggest, indeed, that circadian rhythms present a major problem for general physiology of the type Needham [6] had in mind in stating that—"the organization of living
systems is the problem, not the axiomatic starting point of biological research.”

This is the approach that has been adopted here. In developing the evidence and implications of a pervasive circadian organization a start is made through the experimental results which forced Bruce and me to abandon the view that the rhythm of adult emergences in a Drosophila culture was controlled by a single physiological oscillation.

THE 2-OSCILLATOR MODEL FOR THE DROSOPHILA ECLOSION RHYTHMS

It has been shown in earlier papers [4, 7] that transients develop in the Drosophila rhythm when this

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<th>Table I. Empirical Generalizations about Circadian Rhythms</th>
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<td>The following abbreviations used in this table have also been adopted for brevity in later sections of the text: LD; light-dark cycle, which can be further specified as, e.g., LD 12:12 to denote a cycle of 12 hours light and 12 hours dark. LL; constant light. DD; constant dark. CR; circadian rhythm. ( \tau_{\text{FR}} ); period of an environmental or circadian rhythm, measured in hours. ( \tau_{\text{DD}} ); period of a rhythm free running in constant light and constant temperature. ( \tau_{\text{LL}} ); period of a rhythm free running in constant dark and constant temperature. ( \phi ); phase of a rhythm. ( \Delta \phi ); phase-shift.</td>
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<tr>
<td>References given to the generalizations listed are intended only as convenient guides to the extensive literature bearing on each of them; they are, specifically, not intended as indications of the original or even principal authority concerned.</td>
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I: CR's are defined as those biological rhythms whose \( \tau_{\text{FR}} \) is an approximation to the period of the Earth's rotation [3, 4, 44, 46]

This remains the most powerful, though by no means, the only line of evidence justifying III, below.

II: CR's are ubiquitous in living systems [4, 47]

This holds in the systematic sense of kinds of organisms, and the physiological sense of kinds of functions. The emphasis in the literature on rhythms of, say, locomotion and leaf movement reflects only ease of assay for these “superficial” phenomena; rhythms of DNA synthesis, e.g., exist but are less easily followed routinely.

III: CR's are endogenous in the living system [3, 44, 47, etc.]

This generalization is universally accepted; but one laboratory [48] retains some complex qualifications, and would object to the generalization unless so qualified (See [48 and 54] and Professor Brown's question in the discussion following this paper.)

IV: CR's are usually (if not always) self-sustaining oscillations [4, 47, 51]

This is clearly the case in animals; some plant rhythms damp out but it is still not fully clear that this implies real damping of individual cell rhythms or merely their desynchronization which imposes an overt aperiodicity on the whole organism [51].

V: CR's are innate [1, 4, 47, 49, 50]

They are not learned from or impressed by the environment as so much of the older and even comparatively recent literature has suggested. In those systems that are aperiodic if raised from egg or seed in constant conditions, periodicity is elicited by a single (non-periodic) stimulus that in Drosophila may be only a 3000 sec. flash of light [1, 55].

VI: CR's occur autonomously at both cell and whole-organism levels of organization [4, 47]

They have not yet been sought sufficiently at levels lower than the cell for us to know whether they occur (autonomously) even there.

VII: \( \tau_{\text{FR}} \) is characterized by a remarkably small variance in a freerunning sequence of cycles; the underlying system displays remarkable precision [4, 32, 52]

Observed standard errors of the period may be less than 2 minutes per day.

VIII: \( \tau_{\text{FR}} \) is not a fixed characteristic of the individual organism; it is open to spontaneous and induced shifts within a range of values [4, 32, 52, etc.]

The limits of this range may (but are not proved to) be characteristic of the individual.

IX: Some species differ clearly from others in the range of realizable \( \tau_{\text{FR}} \) values [46]

There is a suggestion that in nocturnal species the range (measured as \( \tau_{\text{DD}} \) values) is biased below 24 hours; in diurnal species above 24 hours. In some species the range fully spans 24 hours (see Fig. 7, this paper).

X: \( \tau_{\text{FR}} \) may show after-effects of the regime immediately preceding the steady-state freerun being studied

Evidence for this new generalization is presented later in this paper.

XI: \( \tau_{\text{FR}} \) is so slightly temperature-dependent that it is proper to emphasize its near-independence of temperature [4, 53]

The known \( Q_{10} \)'s range from -0.9 to -1.2. This feature suggests the near-independence reflects a compensation achieved by a several-component system; and the temperature-compensation is taken by most workers to reflect functional significance of the system as a “clock” [4]; but see [48 and 54].

XII: \( \tau_{\text{FR}} \) is light-intensity dependent [44, 46]

There is evidence of a fairly strong further generalization which I propose to call Aschoff's Rule. This can be summarized by \( \tau_{\text{LL}} > \tau_{\text{DD}} \) in nocturnal animals; \( \tau_{\text{LL}} < \tau_{\text{DD}} \) in diurnal animals.


Circadian Organization

Table 1.-Continued

XIII: CR's are ENTRAINABLE by a RESTRICTED CLASS OF ENVIRONMENTAL PERIODICITIES [4, 9, 46]

Light and temperature cycles are the dominant entraining agents (Aschoff uses the term zeitgeber [46]), and in many species probably the only agents. There are many pertinent subsidiary generalizations concerning limits of entrainment (narrower in more complex organisms), etc., which are fully treated in [9]. The present writer remains unconvinced by, but must note here, claims of Professor Brown [48] that unknown geophysical cycles are "sensed" by organisms and, in fact, somehow explain the facts summarized by generalizations VII and XI.

XIV: THE PHASE OF A FREERUNNING CR CAN BE SHIFTED BY SINGLE PERTURBATIONS IN THE LIGHT AND/OR TEMPERATURE REGIMES [3, 4, 32, 33, 53]

The character of the Δϕ response is a function not only of intensity and duration of the perturbing signal, but—especially of the phase at which the CR was perturbed.

XV: TRANSIENTS ALWAYS PRECEDE ATTAINMENT OF A NEW STEADY-STATE [3, 4, 7]

This is true whether the former steady-state was disrupted by a single perturbation or by a Δϕ in the entraining cycle.

XVI: CR'S HAVE SO FAR PROVED SURPRISINGLY INTRACTABLE TO CHEMICAL PERTURBATION [40]

But see [41] and later discussion in this paper.

is perturbed from its freerunning steady-state by single signals of light and temperature. Transients are defined as those intervals between peaks of eclosion that are different from that recurrent interval which defines the period of the steady-state. The striking differences between light- and temperature-induced transients originally prompted the 2-oscillator scheme Bruce and I developed for Drosophila; it has since been extended to explain many other features of the system.

The essential feature of this model is its assumption that the physiological controls immediately underlying eclosion: 1) are themselves autonomously oscillatory (the B-oscillation in our 1959 paper); and 2) distinct from the light-sensitive (A-) oscillator that serves as pacemaker for the organism. The light-insensitive B-oscillation is, in a sense, a peripheral system; it is coupled to and phased by the A-oscillation and probably relies on this entrainment for the temperature compensation that characterizes the system as a whole and derives from the pacemaker. The data on transients and other effects generalized by the model imply the B-oscillation is temperature sensitive, probably in its coupling to A-and certainly in the sense that it can be directly entrained (independently of its coupling to A-) by temperature cycles. (Figures 1 and 2.) The data imply, further, that there is some feedback of B to A, and it is slight.

Whether light- or temperature-induced, the transients reflect the motion of the B-oscillator. The light-induced transients lead to a new steady-state whose phase is both clearly different from the previous one and fully determined by the phase of the previous light signal (Fig. 1): the light signal resets phase in the A-oscillation, and the observed transient marks the motion of B as it regains phase with the pacemaker (Fig. 2). When the temperature-induced transients subside, there is a trivial phase-shift (-2 hours) in the new steady-state, and what shift there is bears no relation to the phase of the perturbation that induced the transients (Fig. 1): the temperature step-down affected the coupling of B to A, and the temperature dependent period of B is temporarily manifest but disappears as B regains its coupling to A which was nearly insensitive to the temperature change (Fig. 2).

The alternative to the present model is that a single oscillator, responding differentially to light and temperature, underlies the overt rhythm and hence that its motion is reflected by the transients. Experiments summarized by Fig. 3 discriminate between the alternatives. The test is made possible by a detailed knowledge of the system’s response to single 12-hour light signals (Fig. 1); those beginning before subjective dawn reset phase via an advance; those falling after subjective dawn reset phase via a delay. Subjective dawn is that point in a DD rhythm whose normal phase in an LD cycle coincides with the dark/light transition. The two alternative models predict radically different positions for subjective dawn while the system is in a temperature-induced transient. The figure shows that the prediction from the 1-oscillator scheme fails; and the prediction from the 2-oscillator scheme is remarkably precise. A signal coincident with the predicted subjective dawn causes no reset; signals falling after it reset by delays; the one signal preceding it resets by an advance.

Either light or temperature, as a periodicity, can entrain the rhythm to a particular phase. In terms of the model, light achieves its ultimate entrainment of the B-oscillation and hence of the overt rhythm only via entraining A to which B is coupled. Temperature directly entrains B and in bringing the whole system to equilibrium must do so via feedback of B on A. We have studied the simultaneous action of entraining cycles of light and temperature including the effect of regimes in which the phase of these two agents is systematically altered from the normal. The normal phase relation (that of the field) is when dawn falls somewhere near the low point of the temperature cycle; teleonomically [8] we should expect the two cycles to impose a similar phase on the system when in that
FIGURE 1. Light-and temperature-induced transients in the eclosion rhythm of *Drosophila pseudoobscura*. (Reproduced from [7].)

Each horizontal row of points in A and B represents medians of *Drosophila* eclosion peaks in individual cultures. The cultures had previously been in a light-dark cycle with 12 hours of light and 12 hours of dark, and the time scale shown (which is the same for all three figures) represents hours elapsed since the last dawn of this 24-hour cycle. The seven cultures represented by A were in complete darkness for the time shown, and the temperature of each culture was dropped from 26°C (temperature at which the cultures were raised) to 16°C at the time shown by the heavy diagonal line a-a, between hours 24 and 48. The diagonal line between hours 96 and 126 does not represent a second series of signals; it is included only to mark the phases of the signals given 72 hours earlier. Vertical guide lines are given at 24-hour intervals after the last seen “dawn.”

Each of the thirteen cultures represented by B was exposed to a single 12-hour light signal beginning at the time shown by the heavy diagonal line (between hours 24 and 48) and was subsequently left in complete darkness; as in A, the second diagonal (hours 96-120) is for comparison only and does not indicate a second series of signals. The absence of data after hour 120 in B is due to the fact that the experimental cultures involved had completed all eclosion by this time.

The stippled area in A and B is included to facilitate comparison of the ultimate phase-shifts caused by the temperature and light signals. The open and solid arrows on B and C direct attention to the existence of delays (long-period transients) and advances (short-period transients) in both the observed and calculated results.

1C gives a calculated behavior for the light-induced transients; it is derived from a mathematical approximation for the 2-oscillator scheme given in [7].

In 1-A there are noteworthy features to cultures ii and iii. There is no peak between hours 48 and 72; the peak at 72 hours was twice the average size clearly being a compound of two including the “missing” peak. The latter, then was forced into a phase-jump of the type analyzed in Fig. 6, and discussed in the text.
**Figure 2.** 2-oscillator interpretation of the Drosophila eclosion rhythm. Upper figure: The steady-state in DD and constant temperature. Middle figure: A temperature-induced transient caused by temperature step-down from 26°C to 16°C at the point (TS) indicated. Lower figure: Response to light signal of 12-hour duration beginning at point (LS) indicated. Solid circles are medians of the eclosion peaks. The saw-tooth and square-wave form of A and B oscillations is pure convention. SD, SD*, etc., are the subjective dawns in the cultures. They are determined by the last seen dawn of the prior LD 12:12 regime. They are set to correspond with the downstroke of the A-oscillation’s saw-tooth form. The SD’s of the steady state are carried through the figure for comparison with the perturbed cultures. The eclosion peaks at the extreme right mark the steady-state phases of the control (C) temperature-perturbed (T) and light-perturbed (L) cultures.

**Figure 3.** Tests to discriminate between 1- and 2-oscillator interpretations of temperature-induced transients. I: The 2-oscillator interpretation of the steady-state; cf. Fig. 2; A-oscillation is saw-toothed. Three successive eclosion peaks shown. II: The 2-oscillator interpretation of the temperature-induced transient. III: Implications of a single-oscillator interpretation. IV: Experiments to decide where the subjective-dawn is during the temperature transient. Heavy lines on the "histories" of cultures 2, 3, 4, 5, 6, and 8 are 12-hour light perturbations.

φC: Phase of control
φ Reset: Phase of steady-state induced by the light-resets. See text.

rhythm that reflects this conflicting control. As the low point of the temperature cycle is steadily moved to the right relative to a fixed light regime, the overt rhythm follows it up to a point about 15-16 hours from dawn when a discrete phase jump ensues (Fig. 4). Of the 360° of conceivable phase relative to the light cycle, only 180° is realizable; there is a 180° zone of forbidden phase relations. Identical effects have now been found in Euglena by Bruce [9] and in the cockroach by Roberts [10].

Drs. Wever and Aschoff have pointed out to me that this result could be explained in terms of a single oscillator driven simultaneously by two entraining cycles; its phase would respond to phase conflict between the drivers in the way we observe; there would be only 180° of allowed phase. However, the 2-oscillator scheme not only explains the 180° phase jump equally well, but seems uniquely fitted to accommodate the further experiments shown in Fig. 5. The data have been given elsewhere [5] in their raw form. In the plot given here the median of each peak is plotted as a point, and successive days are plotted one below the other. There are four cultures involved. Each is driven by light and temperature cycles and in each the degree of phase conflict between the cycles differs. The steady-state phases of cultures I and II lie just to the left of the forbidden zone; those of III and IV just to the right. In cultures II, III, and IV there are minor peaks of activities each day lying on the other side of the forbidden phase zone from that on which the major peak lies.

In Fig. 5 (middle section) the light cycle is discontinued after day -2, and the transient approach of the 4 rhythms to new phase is obvious. They move to the phase dictated by the persisting temperature cycle. This result would be expected on either a 1- or oscillator interpretation. But not so the results in the lower section of the figure. Here both light and temperature cycles are discontinued on day -2. The phase of the rhythms does not remain where it was, nor does it move to some compromise between that of
the former light and temperature cycles. It moves to the phase of the light cycle. This behavior suggests that during the steady-state imposed by conflicting light and temperature same system strictly followed the phase of the light cycle: its phase was registered in the flies, for when all external control was removed the system resumed the phase of the light cycle. This, of course, conforms with the earlier results on transients after single temperature signals: the feedback of B in A is slight and when displaced to an abnormal mutual phase A imposes its phase in B.

The phase relations of any coupled oscillator system involve 180° of forbidden phase, for were the driven system to lie in that zone it would be transferring energy into, not drawing it from, the driver. And there are further observations in Drosophila that again make this the preferred interpretation for the phase-jump just discussed. These further facts are summarized in Fig. 6, redrawn from Fig. 4 in my 1954 paper [1] in which their significance was not seen. A 10° temperature step-down occurs in a freerunning DD rhythm at hour-zero, which is subjective dawn as defined earlier; in the experiment shown it occurs at SD-2. Two results ensue: one is the transient elongation of the next interval prior to the eclosion peak labelled E-3; E-3 is delayed 12 hours. The second result is that a slight phase shift (-2 hours) shows up in the subsequent steady-state. This means the subjective dawn (SD-3 in the figure) was delayed only 2 hours unlike the eclosion peak, E-3. In the lower figure an identical temperature step-down occurs at hour-zero, but in a culture that continues to be entrained by an LD cycle. The light cycle checks the phase shift of subjective dawn: the phase of the ultimate steady-state is normal. But the light fails to prevent the delay of E-3. Indeed
Figure 6. Evidence of a forbidden and an allowed (AZ) zone of phase relations for the *Drosophila* eclosion rhythm. See text.

This delay is further increased by almost 12 hours (180°) as E-3 is forced over to the region of the next dawn (SD-4). This is a very strong fact and immediately yields to the demands of the 2-oscillator scheme with its implicit zone of forbidden phase relations. In Fig. 6 the vertical lines marked SD register the phase of the A-oscillation. In the DD freerun the subjective dawn (SD-3) slips two hours to the right as implied by the phase of the subsequent steady-state; and consequently the delay of E-3 still leaves this peak just within the allowed phase zone (AZ) relative to the A-oscillation. But the same delay places it to the right of the allowed zone when A is pulled back -2 hours by the light. A phase jump across the forbidden zone is then imposed; E-3 is forced to its further delay. Similar effects are detectable in the details of Fig. 1A, but need not be spelled out here.

Finally it is noted that the postulated 2-oscillator scheme with the implication of restricted phase relations between the A and B components will also explain a singular previously unpublished feature of temperature-induced transients in the *Drosophila* system. Temperature step-downs produce, as noted, large transient delays which are evidently observable only insofar as the induced delay in the B rhythm still leaves it within the zone of allowed phase relations. Temperature step-ups, on the other hand, produce only a negligible transient advance of the peak: advance is not realizable insofar as it would bring the B rhythm into its forbidden zone relative to A.

It may well prove that none of the observations recorded here absolutely and individually demand a 2-oscillator scheme. But many, even individually, elude any obvious one-oscillator treatment; and, collectively, they render the 2-oscillator analysis as highly probable as one can hope for until A and B can be identified concretely.

CIRCADIAN ORGANIZATION; A MULTI-OSCILLATOR SYSTEM

Our main concern is with a broader concept of the rhythm problem which the 2-oscillator scheme for *Drosophila* eclosion suggests. Harker [11] has also been led recently to a 2-oscillator scheme for cockroach activity rhythms, as were Brown and Webb [12] several years ago when studying the rhythm of color change in Uca. The broader implications of a a-oscillator model rest, therefore, on a much wider base than the details of the *Drosophila* case.

If we reject, as reason demands we must, the possibility that in selecting eclosion in *Drosophila*, locomotion in the cockroach, and color-change in Uca, we picked by chance the only system in each organism mediated by a rhythmic component (B-oscillation) distinct from the light-sensitive pacemaker (h-oscillation), we are forced to conclude there must be many distinct oscillatory physiological systems in the individual that are not themselves directly coupled to the light-regime as entraining agent. We are forced, in fact, to abandon the common current view that our problem is to isolate and analyze "the endogenous rhythm," or "the internal clock," and are faced with the conclusion that the organism comprises a population of quasi-autonomous oscillatory systems. The consequences of such a view seem to me so fundamental in any attempt to set our problems in perspective that we should pursue further both its meaning and the evidence for it before proceeding with its implications.

Halberg's extensive writings (see [13], e.g.) reveal that mammals, like men or mice, can display any or all of the following rhythms: locomotion, body temperature, blood sugar, liver glycogen, eosinophil count, adrenal activity, phospholipid synthesis, RNA and DNA synthesis, cell-division, drug-specific sensitivities, etc. And this list is surely limited only by available assay methods, and the time so far invested! It is, then, not a question of whether many rhythms coexist in an organism; that is a matter of simple fact. The problem is the much subtler one of delimiting responsibility for all of them; of recognizing which are autonomous, and which are merely imposed or forced on intrinsically non-rhythmic systems by the controlling activity of a central oscillatory pacemaker.

It is possible that many rhythms do in fact belong in
the latter category. For instance, the known physiological links between adrenal activity and virtually every other aspect of mammalian metabolism could be the basis for regarding whole complexes of rhythms as only forced by the rhythmic activity of these important endocrines; for regarding, in short, only the adrenal as a self-sustaining autonomous oscillator. But our knowledge of all such physiological links in metazoa involving humoral or nervous action is very non-specific as to causal detail, and they (the links) could well be nothing more than channels of coupling information serving for unilateral or mutual entrainment between tissues that are independently oscillatory in their own right.

The latter alternative is the one I believe the facts, collectively, imply. It is supported by some direct evidence from tissue and organ culture. Enderle [14], many years ago, demonstrated a circadian rhythm of growth in Daucus tissue culture; Bunning has claimed [15] the persistence of a circadian oscillation in excised segments of hamster intestine; and there is evidence from several workers [18] that cell-division manifests a circadian periodicity in mammalian tissue culture. Finally, along these lines one notes that some degree of autonomy in the oscillations of individual tissues and organs is virtually demanded by the fact that single cells—at least as protists—manifest fully self-sustaining circadian oscillations which are formally identical with those of multicellular systems [16, 17]. Indeed, unless the protistan cell is radically different in this respect the multicellular system is—literally—a population of autonomous oscillators. And its physiological organization must involve, as a major feature, communication channels whose principal function is not to impose rhythmicity but merely to couple and hence appropriately phase oscillatory activities inherent in the individual subsystems.

The remainder of this section concerns observations on freerunning rhythms of activity and body temperature. None of them demands my inference of autonomy in tissue and organ oscillations. But they all reveal complexities in the rhythmicity of the whole organism that are most easily explained by it; and to this extent they are part of my case for its validity.

We have observed in the past few years a widespread phenomenon (roaches, lizards, mice, hamsters, finches) we call “after-effects.” These are detected in the period of rhythms freerunning in DD. They have been found to follow three kinds of immediate pretreatment. The first is an after-effect of entraining the animal to an atypical period. Figure 7 (lower section) gives data for four male sibling hamsters that were first entrained to a 23-hour day and later to a 25-hour day. After each entrainment they were released into DD where the steady-state DD was found to be larger after the 25-hour day than after the 23-hour day.

The second is an after-effect of constant light which, like entrainment, can modify \( \tau \). Roberts [10] has found that cockroaches exposed to LL lengthen \( \tau \), and this lengthening persists at least in part when the animal goes back to DD. Repeated exposures to LL gave an increasing or accumulative after-effect on \( \tau_{ODD} \) in one individual. Figure 8 is another case in the mouse Peromyscus. Here LL makes for an overt aperiodicity; in other individuals of this species it merely lengthens \( \tau \) [19]. The individual shown was returned to darkness on the indicated day and ran with an extraordinarily long period for DD throughout several weeks. This is clearly an LL after-effect; the animal ultimately reverts to a typically short DD period.

The third category of after-effects has been found following transients. Figure 9 shows freerunning rhythms in a hamster and a finch. In each case the phase of the freeruns is reset by single light signals of a 12-hour duration. In the finch the phase of the signal is such as to cause a reset that is attained by advancing (short-“period”) transients; and when the transients subside the steady-state has a much shorter period than the preceding one. Similar after-effects appear in the hamster figure which includes resets involving both delaying and advancing transients; following the former, \( \tau \) lengthens, following the latter it shortens.
FIGURE 8. An after-effect of LL on the freerunning rhythm of the mouse, *Peromyscus maniculatus*. LL, constant light; DD, constant dark. See text. The data are presented as follows: each horizontal line in the left-hand of the figure is a day's record from the running wheel; activity is indicated by the vertical pen marking on the line; these fuse to give a solid band during intense activity. Successive days are plotted one below the other. The entire record is reproduced on the right, displaced upwards one day, to facilitate visual following of long or short period rhythms that rapidly scan across the 24-hour cycle.

FIGURE 9. After-effects of transients induced by 12-hour light signals in a hamster and a finch. Plotted points are onsets of locomotory activity. In both animals the light signal shifts phase of the rhythm (as defined by the time of activity onset). The new steady-state shows a period \( \tau \) that differs from that \( \tau \) of the prior steady-state; the sign of the difference in \( \tau \) reflects the character (delay, advance) of the transient(s) involved in the reset.

Finally, Fig. 7 (upper section) shows several observed \( \tau_{DD} \) values for each of eleven hamsters. In eight cases two values are plotted as larger circles, one open and one solid. In each case the open circle is the \( \tau \) value observed in a freerun that immediately preceded an entrained steady-state and a second freerun; the \( \tau \) value for the latter is given by the solid circle. Attainment of the intervening entrained steady-state involves transients by which the animal gains appropriate phase with the light cycle. In 4 animals these transients were delays, and in the subsequent freerun \( \tau \) was longer. The intervening entrainment was brief, a matter of days; but it is clear that in spite of the animal overtly equilibrating with the light cycle some "inertial" effect of the transient persisted and imposed itself on the second freerun.

It is clear that if an individual is to be characterized by its \( \tau \)-if, that is, there are genetic differences in the control of \( \tau \)-this characterization must be expressed as a range of realizable \( \tau \) values. The system can be pushed within this range to any one of, presumably, many frequencies where it is stable, at least for a while; its state perpetuates itself.

The only strong conclusion to be drawn from these remarkable properties is that the oscillatory system underlying the overt rhythm of an individual is in no sense simple. No familiar single oscillator behaves like this. The system has no real *eigenfrequenz* determined by a fixed set of parameters. Nor in turning to conceivable properties of a multi-oscillator circadian system is there an obviously unique explanation; but a suggestion may be offered that leads us to other pertinent facts. The observed frequency of the individual must
be a compromise of a spectrum of frequencies that would be individually manifest if the constituent oscillations could escape entrainment from the rest of the system and freerun. The coupling mechanisms that bring about the complex of mutual entrainments must involve discontinuities, making possible a range of realizable system-frequencies.

There are now many cases where a freerunning system gives evidence of comprising more than one component with different characteristic frequencies. The first is afforded by data from Menaker's [20] study of body temperature rhythms in hibernating bats. In the case shown in his Fig. 1, a seven day record, the main pattern of the rhythm is defined by a sharp rise and plateau of temperature once every 24 hours. But there is a clear, lower amplitude peak in the pattern which scans the main pattern at a distinctly lower frequency (~25 hours).

Three other examples come from Mr. Swade's unpublished work on arctic mammals. Two of them concern the effect of constant light on rhythms in mice. The first is the mouse whose LL aftereffect has already been noted (Fig. 8). Its ultimate reversion to a typically short DD frequency involves a remarkable phenomenon. The reversion is neither abrupt nor gradual (both of which qualities have been seen in other cases). It is achieved by developing the more typical frequency as a distinct component in the total activity pattern before the atypical frequency (LL induced) has subsided. For about 10 days two distinct frequencies coexist. The second mouse (Fig. 10) is released from LD into LL on the indicated day. The freerun initially shows the long period (low frequency) characteristic of LL action on nocturnal species. This long period continues until the activity onsets have nearly scanned a 24-hour cycle; it then abruptly changes to a shorter period (high frequency) which remains stable. The remarkable feature is that if the phase of this ultimate short period freerun is extrapolated back, it coincides with the phase of the original LD steady-state. This surely implies that some component in the organism had been running in the short period from the outset of the LL regime; that the other oscillations, proximally controlling activity itself, had broken loose to freerun on their long period but are eventually recaptured into entrainment by the rest of the system; and only recaptured, moreover, when they reached their normal phase relation with it.

This type of behavior is more fully manifest in Fig. 11 for an arctic ground squirrel in LL. The light intensity...

**Figure 10.** Rhythms in a red-backed *Clethrionomys rutilus*. Data presented as explained for Fig. 8. The first 35 days involved an LD 12:12 regime which entrains the animal. Note the very gradual approach, via. transients, to the entrained steady-state. Lights go on and off at hours indicated. Mouse released into LL on 36th day. Long period freerun follows, but switches fairly abruptly to a short period on the 31st day of the freerun. The phase of this latter freerun extrapolates back to the phase of onsets in the prior steady-state. See text for interpretation.
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FIGURE 8. An after-effect of LL on the freerunning rhythm of the mouse, Peromyscus maniculatus. LL, constant light; DD, constant dark. See text. The data are presented as follows: each horizontal line in the left-hand of the figure is a day's record from the running wheel; activity is indicated by the vertical pen marking on the line; these fuse to give a solid band during intense activity. Successive days are plotted one below the other. The entire record is reproduced on the right, displaced upwards one day, to facilitate visual following of long or short period rhythms that rapidly scan across the 24-hour cycle.

Finally, Fig. 7 (upper section) shows several observed TD values for each of eleven hamsters. In eight cases two values are plotted as larger circles, one open and one solid. In each case the open circle is the $\tau$ value observed in a freerun that immediately preceded an entrained steady-state and a second freerun; the $\tau$ value for the latter is given by the solid circle. Attainment of the intervening entrained steady-state involves transients by which the animal gains appropriate phase with the light cycle. In 4 animals these transients were delays, and in the subsequent freerun $\tau$ was longer. The intervening entrainment was brief, a matter of days; but it is clear that in spite of the animal overtly equilibrating with the light cycle some "inertial" effect of the transient persisted and imposed itself on the second freerun.

It is clear that if an individual is to be characterized by its $r$-if, that is, there are genetic differences in the control of $r$-this characterization must be expressed as a range of realizable $\tau$ values. The system can be pushed within this range to any one of, presumably, many frequencies where it is stable, at least for a while; its state perpetuates itself.

The only strong conclusion to be drawn from these remarkable properties is that the oscillatory system underlying the overt rhythm of an individual is in no sense simple. No familiar single oscillator behaves like this. The system has no real $\textit{eigenfrequence}$ determined by a fixed set of parameters. Nor in turning to conceivable properties of a multi-oscillator circadian system is there an obviously unique explanation; but a suggestion may be offered that leads us to other pertinent facts. The observed frequency of the individual must.

FIGURE 9. After-effects of transients induced by 12-hour light signals in a hamster and a finch. Plotted points are onsets of locomotory activity. In both animals the light signal shifts phase of the rhythm (as defined by the time of activity onset). The new steady-state shows a period ($\tau$) that differs from that ($\tau$) of the prior steady-state; the sign of the difference in $\tau$ reflects the character (delay, advance) of the transient(s) involved in the reset.
of the LL regime is increased on the day indicated. Many days later two features become clear: first, the density of activity at the beginning of each circadian band ultimately becomes diluted; second, as this happens the band becomes two-parted. The second part is a separate component moving at a much lower frequency (longer period) than the main band. The freerun of this low frequency component stops when,

**Figure 11.** Freerunning rhythm of the arctic ground squirrel (*Spermophilus undulatus*) in constant light. Data presented as explained in Fig. 8. Intensity of LL is increased on the day marked by the dashed line running across the figure and exceeding its edge. Arrows mark times of replacing burned out lamp bulbs from the bank of lights. See text.
having scanned the cycle of the main component, it regains entrainment in the early part of the main activity band. This is evidently its normal phase when coupled to the rest of the system; the dilution of activity in this region, coincident with the freerun, disappears when the freerun is ended by re-entrainment.

Other fully explicit cases of dissociation of constituent rhythms in the system as a whole are given in Lobban’s [21] paper in this symposium.

THE PHYSIOLOGY OF CIRCADIAN ORGANIZATION; AND ITS BREAKDOWN

The autonomy and potential dissociability of distinct oscillatory components in the circadian system is the crux of the viewpoint, and its implications, I am attempting to develop. The normal temporal organization of the system must, a priori, involve maintenance of identical frequencies among the several components, and maintenance of appropriate mutual phasing. How are these prerequisites fulfilled? There are two answers. First, in the field the system is entrained unilaterally by the external cycles of light and temperature. This environmental entrainment actually fulfills a dual function: (a) it phases the whole system appropriately relative to the external cycle of environmental change; and (b) in so doing it imposes uniformity of frequency on the multiple endogenous oscillations. How far it directly maintains their correct phasing is not clear. This must depend, even in the field, to some extent on their mutual coupling and hence, mutual entrainment. In the absence of external cycles of light and temperature the integrity of the circadian organization must depend exclusively on this mutual entrainment of individual oscillations to maintain both identical frequencies and appropriate phasing. There is no doubt this mutual entrainment of constituent oscillations is commonly sufficiently strong to maintain adequate organization even in rigorously constant conditions of light and temperature. But it is now equally clear: (1) that these constant conditions, which the physiologist so assiduously cultivates, are often detrimental; and (2) that the damage they engender derives from a breakdown of the innate circadian organization. This breakdown is in all probability a consequence in the fly discussed below) is a consequence of their circadian oscillations. If, however, the implant is 12 hours out of phase with the host, the latter develops transplantable tumors in the mid-gut wall which lies below the out-of-phase implant.

THE ACTION OF AN APERIODIC LIGHT REGIME

There is no direct evidence that constant darkness is detrimental to anything but green plants. But there is increasingly strong evidence that constant light (LL) is deleterious at least in some species, and that its action is not due to an excess of light as such but to its effect on the freerunning circadian system.

Several workers, beginning with Arthur and Harvill [24], have noted tissue damage in tomatoes grown in constant light and constant temperature; and similar damage occurs in other plants. Hillman’s [25] study of the problem reveals what is surely the key to the phenomenon: no such damage develops in LL if the plant is simultaneously exposed to a 24-hour temperature cycle. A striking parallel to these results is provided by the response of the Drosophila eclosion rhythm to LL under a constant vs. cycling temperature regime. In LL at constant temperature the period of the rhythm increases to begin with, but the system becomes rapidly aperiodic. However, such LL induced aperiodicity does not develop if a 24-hour temperature cycle is imposed [26].

The importance of the Drosophila data is that they bear directly on the response of a known circadian system to LL in a periodic vs. an aperiodic temperature regime; and invite the following interpretations for both the tomato and the fly: (1) the action of LL is in some sense to disorganize the circadian system; (2) that a temperature cycle can maintain the organized state otherwise disrupted by LL; (3) that the LL damage observed in the tomato (and a different striking LL consequence in the fly discussed below) is a consequence of the circadian system’s disorganization; and (4) that this in fact amounts to a loss of mutual entrainment, and hence of appropriate phasing, among constituent oscillatory subsystems. The damage results, in brief, from a dysphasia of oscillations in the system; and the fact that these, no longer properly coupled, can freerun on their own individual frequencies explains the LL induced aperiodicity of the system as a whole. That LL indeed affects aperiodicity via such an uncoupling of constituent oscillations is both plausible in terms of its
other certain action and demonstrated to occur in a few cases like those illustrated in Fig. 11. The other certainly known action of LL is to change the overt freerunning period of the organism as a whole. But there is evidence that this change in the frequency of the whole system is itself a complex matter. Figure 12 shows the entry of a mouse into constant light. The “period” of the freerunning rhythm lengthens (typical for a nocturnal species) but aperiodicity ultimately develops because the period of activity cutoffs increases less than the period of the onsets. It is as though activity time were determined by the phase angle between two oscillations whose frequencies were differentially dependent on light intensity. It will be noted that, on re-entry to DD, this same mouse begins with a very brief activity duration each day, but this expands gradually; again the onset and cut-off of activity have different frequencies. This increase in activity time persists until the animal becomes aperiodic. One is strongly attracted to the view that having lost (been forced out of) normal coupling and entrainment in LL, constituent oscillations fail to regain entrainment in DD. Thus it is by no means excluded that the light-sensitive system itself may comprise more than one oscillation [26] and that in LL reciprocal effects of light on their period lead to breakdown of even the pacemaker’s (the light-sensitive system’s) own integrity.

It is of course directly to the point that the best, most fully explicit, case one could present showing the freerun of a distinct oscillatory component was in an animal after increase of LL intensity; and that the other cases strongly implying a freerun of dissociated components were similarly in LL.

Green plants are not the only organisms in which LL has been found to impose real ‘damage’. Mr. Swade’s studies of LL action on arctic mice in constant temperature has been hampered by the fact that a majority of them have died within a week of entry in LL. And Highkin, who earlier reported damage in peas developing from a constant temperature regime, now reports here [27] that LL imposes a similar stunting in these plants. Perhaps the most striking feature of Highkin’s study is its indication that this damage is not immediately repaired on re-entry to a periodic regime; it persists, at least as a dauermodifikation, in some succeeding generations.

We have recently encountered a situation in Drosophila melanogaster which at the present state of analysis looks similar. In a deliberate attempt to find a system in animals sensitive enough to uncover detrimental effects due to interference with the innate circadian organization, we have begun several analyses of the effects of periodic vs. aperiodic conditions on the expression of genes with variable penetrance. One of those selected is a recessive allele tu^{e} responsible for melanotic pseudotumors. Figure 13 summarizes results so far available. The homozygous stock has been maintained in LD 12:12 at 22°C. by mass transfer since we acquired it. Several other lines have been established in DD, LL, etc. Figure 13 shows that in the line transferred to constant light the penetrance of tu^{e} fell rapidly; it dropped from 99 per cent to 40 per cent in the first generation; by the fifth generation it was down 20 per cent, and from the twelfth generation onwards to the 24th (at present) it has remained below 5 per cent. Penetrance in the LD control has meanwhile fluctuated from 69 to 99 per cent; and the same is true of a DD line. The drop in penetrance in LL is not reversed, at least immediately, on return of the stock to LD; this has been done twice, and the second return to LD is now in its 9th generation. Clearly the LL conditions have wrought some inherited change. It is noteworthy that the LL line has been crossed to the LD line three times, and on each occasion (Fig. 13) there has been a difference between the reciprocal crosses; and the difference is overwhelmingly significant in the third instance (Gen 39 LD × Gen 22 LL). In each case the penetrance of tu^{e}is lower in those F_{1}′s obtained when the LL parent was female. There is to this extent some evidence of cytoplasmic change, which we were led to look for by the suspiciously rapid drop over the first LL generation. This is further supported by a single experiment of Bruce’s in which the saline supernate from centrifuged homogenates of LD flies was added to the medium of LL cultures. Such supernates increased tumor incidence but not significantly if they were subjected to UV and clearly not if they were autoclaved. This work is progressing, with attention being given to the information needed to disentangle simple effects of LL selection on penetrance modifiers from the more intriguing possibilities of induced cytoplasmic change.

It is clear that if the interpretations of LL action offered here are correct, the severity of its deleterious action will be inversely related to the strength of the mutual couplings among constituent rhythms: individuals whose overt periodicity survives exposure to LL are individuals whose circadian organization resists dissociation, and we should anticipate them to be less sensitive to outright LL damage, and to other subtler modifications like that of tu^{e} penetrance in Drosophila.

**THE ACTION OF AN APERIODIC TEMPERATURE REGIME**

One can trace physiological consequences to an aperiodicity of the temperature regime also. But here the evidence is not so much of outright damage due to constant temperature; it is that performance is improved by a circadian periodicity in the temperature regime. The initial discovery of this effect we owe apparently to Went [28] who demonstrated beneficial action of a daily thermoperiod in tomatoes. Thermo-periodism is now well known in plants but has previously, to my knowledge, never been reported in animals. We have recently detected the phenomenon in
FIGURE 12. Rhythms in a red-backed mouse Clethrionomys rutilus. Data presented as explained in Fig. 8. LD, light cycle entraining rhythm for several weeks; lights on and off at points indicated. The LD behavior is interesting and will be discussed by Mr. Swade elsewhere. LL, constant light freerun; DD, constant dark freerun. Note that even when the DD activity becomes distributed throughout the whole day clear periodicities can be discerned in the component bands. See text.
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Figure 13. The incidence of tumorous phenotypes in a stock of Drosophila melanogaster homozygous for the recessive allele tug. See text. The large circles plotted at generations 16, 20, and 30 are for F1 progenies of LD and LL crosses. The open circles give the values for the cross in which the female was from the LL line; the half black circles for the cross in which the female came from LD. At two points (generations 13 and '23) a subculture from LL was transferred to LD and maintained there for several generations.

We have been examining the penetrance of several sex-linked recessive lethals provided by Dr. E. E. Novitski. These have been balanced in stock against a multiple inversion x-chromosome (y sc4 A-49 sn4 sc8) which is a strong semi-lethal in homozygotes. While we have not enough information on the penetrance of the various recessive lethals, it is already clear that the viability of females homozygous for the balancing chromosome is markedly sensitive to the aperiodicity vs. periodicity of the temperature regime. Table 2 summarizes results from several crosses. In each case the homozygotes are more frequent (as % total females) in the periodic temperature regime than in the aperiodic.

In the same way as LL damage is interpreted here as due to an imposed breakdown of circadian organization, the benefits of thermoperiodism may be interpreted as due to more effective entrainment of the system than a light cycle achieves alone.

DELETERIOUS AND BENEFICIAL EFFECTS OF CHANGE IN FREQUENCY

If maintenance, or failure, of appropriate mutual phasing among constituent oscillatory subsystems is the basis of thermoperiodic benefits and LL damage, we could well have predicted two other types of observation which workers in the Earhart Laboratory in Pasadena have reported. These “predictions” would have stemmed from the postulate, made earlier, that one role of the light-sensitive pacemaker is to maintain (or contribute to maintenance of) appropriate phase relations in the rest of the system, and to do so by virtue of entraining them. But as with all entrainment, the pacemaker can only achieve this end if its frequency is close enough to that of the other constituents: there are limits to entrainment as Bruce [26] has emphasized.

Table 2. The Viability of a Semi-lethal Homozygote in Drosophila melanogaster in Relation to the Periodicity of the Temperature Regime

<table>
<thead>
<tr>
<th>Cross No.</th>
<th>Temperature</th>
<th>Heterozygotes</th>
<th>Homozygote(s)</th>
<th>Homozygotes as per cent Total Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PT</td>
<td>249</td>
<td>34</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>383</td>
<td>9</td>
<td>2.3</td>
</tr>
<tr>
<td>II</td>
<td>PT</td>
<td>387</td>
<td>67</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>438</td>
<td>37</td>
<td>7.8</td>
</tr>
<tr>
<td>III</td>
<td>PT</td>
<td>358</td>
<td>31</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>409</td>
<td>15</td>
<td>3.5</td>
</tr>
<tr>
<td>IV</td>
<td>PT</td>
<td>351</td>
<td>41</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
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<tr>
<td>V</td>
<td>PT</td>
<td>383</td>
<td>31</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>294</td>
<td>12</td>
<td>3.9</td>
</tr>
</tbody>
</table>
It follows that the competence of the light-sensitive system to entrain the rest of the system should be impaired by either of two circumstances: (1) by an external light cycle that in turn entrains the \( \tau \) of the pacemaker too far from that of the non-light-sensitive oscillations; and (2), holding the light cycle fixed, by changing the level of a constant temperature regime and thereby also changing the freerunning frequency of the temperature-dependent, non-light-sensitive oscillations too far from that of the pacemaker.

Effects interpretable in both these ways have been reported from the Earhart Laboratory. Highkin and Hanson [29] reported damage to plants which, although they experience the same total light as others, receive it in periodicities whose \( \tau \) deviates too far from 24 hours. Went first uncovered the second effect in African violets (Saintpaulia) and others have since done so in other species [30]. The growth of the plant, measured at a particular constant temperature, is a function of \( \tau \) in the prevailing light cycle. This again is not confined with total light received: over a long period light constitutes 50% of each cycle no matter what the \( \tau \). This is essentially the Highkin and Hanson result; what is added is that the optimum \( \tau \) of the light cycle is a function of the temperature. At lower temperatures the optimum \( \tau \) is longer; at higher temperatures it is shorter. In present terms this means that the action of the light-sensitive pacemaker on the rest of the system is stronger the closer its frequency approximates the (slightly temperature-dependent) frequency of the rest of the system it must entrain.

**Other Predictable Effects**

If the interpretation developed above is correct, we should expect two other sources of damage to the circadian system which to my knowledge have not been looked for.

First, systems driven by simultaneous light and temperature cycles should be sensitive to the phase angle between these entraining agents, for (on hypothesis) they drive distinct components of the system. Where the light and temperature cycles are nearly \( 180^\circ \) out of phase (cf. Fig. 4) they should induce, in severe form, that dysphasia of constituent subsystems I have assumed underlies the lesions induced by LL and by abnormal light cycles, etc.

Second, we may also expect that such dysphasia will develop in experimental organisms subjected to large and abrupt phase-shifts of the environmental entraining agent. We do this in resetting hamsters abruptly by 5 or 6 hours; and it is imposed on man nowadays who abruptly shifts the phase of his circadian system when flying from say, New York to Paris, or vice versa. The meaning Bruce and I have given to overt transients implies the system is in temporary and partial dysphasia so long as they last. In mammals they may last for weeks. It remains to be seen whether any stress or even damage is actually imposed by this type of phase-shift which the system has never been called upon by natural selection to accommodate.

**THE PHYSIOLOGY OF THE LIGHT-SENSITIVE PACEMAKER**

On the face of it, entrainment seems a simple affair; an endogenous oscillation, coupled to it by appropriate sensory inputs, “follows the light cycle”; but, in fact, as Bruce has shown us, the mechanism of entrainment is far from understood. Its discussion here has two purposes: (1) to emphasize recent progress in the comparative study of the action of single light signals and the response curves so obtained; (2) to outline a functional interpretation of these response curves that leads to a general qualitative theory of entrainment.

It is convenient to begin by listing the facts that any complete theory of entrainment by light must explain, (1) The theory must be compatible with the known action of light revealed by the response of freerunning rhythms to single signals applied throughout the cycle; (2) It must explain how the freerunning circadian period is brought into precise match with that of the earth’s rotation; (3) How in so doing an adaptively appropriate phase is maintained relative to the changing pattern of day-length and, thus, to the whole external cycle of environmental change. This problem of phase control specifically involves the obvious differences between nocturnal and diurnal species: as spring advances the phase of activity-onsets in the former must be delayed each day to follow sunset delays; while, in the latter, it must be advanced to follow dawn. In the autumn the converse relation holds.

(4) It must be compatible with the facts concerning the freerunning periods of circadian rhythms. These include: (a) the weak generalization that \( \tau_{DD} \) is commonly less than 24 hours in nocturnal species and more than 24 hours in diurnal species; (b) that in some species (e.g., hamster), however, the range of \( \tau_{DD} \) among individuals is wide, falling on both sides of 24 hours; and (c) that \( \tau_{DD} \) is in some sense labile, or plastic, as discussed earlier in this paper.

(5) It must be compatible with the known action of LL on \( \tau_{FR} \) which is summarized by what I suggest we call Aschoff’s Rule; Aschoff [44, 45, 46] is responsible for much of the pertinent data as well as for recognizing the generality involved. The rule states that \( \tau_{LL} > \tau_{DD} \) in nocturnal species but \( \tau_{LL} < \tau_{DD} \) in diurnal species. There are some exceptions to this rule (e.g., Drosophila), but it is far stronger than that in (4a) above, and certainly strong enough to demand explanation.

(6) The theory must also clearly accommodate the fact that there are limits to the value of \( \tau \) which entrainment can enforce; and

(7) Finally, it must be compatible with the phenomena of frequency demultiplication.
The Comparative Study of Response Curves for Single Light Signals

Several workers [3, 4, 5, 7, 31, 32, 33, 34] have recently focused attention on this subject. The assumption has been that the response to such non-periodic perturbations should be a simple phenomenon in terms of which entrainment by periodic signals might be clarified. The most encouraging feature of the results is the recurrence of a particular pattern of responses in widely different species from unicellulars to mammals. This pattern is summarized in Fig. 14 for several types of signals applied to Drosophila pseudoobscura. The data for 12-hour and 4-hour signals have been published before [3, 4, 7]. They are replotted here in a way that facilitates comparison with the new data for very short flashes (≤1000 sec.), and for other species. The response to a signal applied at a particular phase in the free-running rhythm (abscissa) is plotted as the phase-shift induced (ordinate). This shift may be attained in either of two ways: by long (“delaying,” or phase-lag) transients, or by short (“advancing,” or phase-lead) transients. This difference in response is incorporated in the curve by the convention of plotting shifts attained by delays as positive values and those attained by advances as negative values.

In addition to those for Drosophila the figure includes curves for the hamster and the flying squirrel; the former is taken from an unpublished study [31] by Dr. Burchard in Princeton, and the latter from DeCoursey’s [32, 33, 34] extensive recent analysis of Glaucomys. The figure shows that in all species the sign and magnitude of the response is a function of the phase at which the endogenous oscillation was perturbed; there is a clear switch-over from phase-delays to phase-advances in the subjective night. The comparison of the species cannot be pressed too far at present; it would be confounded by differences in signal intensity, duration, and criterion of response. Thus, DeCoursey’s curve for Glaucomys is based on lo-minute flashes at 0.5 ft.c., and the response is measured by the magnitude of the first transient following stimulation, whereas the curves from our laboratory use ultimate phase-shift after transients have subsided and involve several signal durations none of which is 10 minutes. In spite of these current complications there remains a strong qualitative convergence: all 3 species show both advances and delays, and in all 3 the switch occurs in the subjective night. Bruce and Pittendrigh [35] have reported the same pattern from Euglena; it is evident in Hastings and Sweeney’s [36] analysis of Gonyaulax, and in Ehret’s [37] of Paramecium. Roberts [10] found evidence of it in cockroaches, and other unpublished work in Princeton has detected it in a lizard and a finch. We have, then, a new and major similarity to add to the list of these properties of circadian rhythms which encourages the view that they indeed present us with general problems.

A Theory of Entrainment by Photoperiod

The meaning of the response pattern lies in the mechanism of entrainment. It is of course recognized that we must ultimately explain it in terms of the detailed physiology of the stimulus-response system,
and the structure of the light-sensitive oscillator(s); at present this is not possible. We can, however, already perceive its functional significance for entrainment, and attempt the outlines of a qualitative explanation for many of the seven listed generalizations which any complete theory must accommodate.

The functional significance of the response curve in Glaucomys has already been noted by DeCoursey [32]. Combined with the innate $\tau_{DD}$ which is typically less than 24 hours in this nocturnal animal, the curve guarantees entrainment with adaptive phase control (Fig. 15). If a flying squirrel is set at any atypical phase relative to an LD cycle, it will automatically regain adaptive phase and stay there; if set to the right of its normal phase, the combined action of $\tau_{DD} < 24$ and of light falling on the advance section of the response curve drives it back to the left. On regaining normal phase it remains there because an equilibrium is developed between an advance effect due to the innately fast $\tau_{DD}$ and a delay effect due to the light impinging on the delay section of the response curve. Displacement to the left is followed by the converse; it brings the delay part of the curve into the light and forces a gradual shift to the right which is arrested only when the delay part of the curve is moved out of the light, or so far out that its action is balanced by.

The essential feature of this interpretation is that the entrained steady-state represents an equilibrium between the $\tau_{DD}$ and the action of light which can be either an advancing or a delaying effect. It follows that in a typical diurnal species (like a bird or lizard) in which $\tau_{DD} < 24$ hours, an equilibrium will also develop if it has a response curve of the general type discussed here; and that at equilibrium the advance section of the response curve must lie within the light period to offset the daily delay due to $\tau_{DD}$. When these typical $\tau_{DD}$ values obtain, both diurnal and nocturnal forms shift phase appropriately as photoperiod changes. Its expansion in the spring forces the nocturnal species to the right, following sunset, because the photoperiod now embraces more of the delay curve; conversely it appropriately forces the diurnal species to the left (following dawn) because more of the advance curve is covered.

DeCoursey also noted that the action of LL on Glaucomys could be understood in qualitative terms from the response curve for that species, $\tau_{LL} > \tau_{DD}$ in the flying squirrel, following Aschoff’s Rule; and one cannot avoid the conclusion that this increase in $\tau$ is related to the delay section of the response curve greatly exceeding the advance section both in range and especially in amplitude. This interpretation of the action of LL on $\tau$ can, moreover, be extended to what we should expect of a diurnal species. $\tau$ in diurnal forms shortens in LL; this would imply that in them the advance section of the response curve must exceed the delay section. And this is precisely what is demanded for good phase control; a diurnal species should have the converse of the Glaucomys (Fig. 15) curve.

_Drosophila pseudoobscura_ is, however, a diurnal species and does not possess the hypothetical response curve of a diurnal species inferred from this line of interpretation: its advance and delay curves are nearly equal. Indeed the area under the delay curve slightly exceeds that under the advance curve. This is, however, an encouraging exception because _D. pseudoobscura_ also violates Aschoff’s Rule in that $\tau_{LL}$ slightly exceeds $\tau_{LD}$; and to this extent it is an exception that “proves” the present interpretation of Aschoff’s Rule. The detailed form of the _Drosophila_ curves, which DeCoursey found in conflict with her analysis of Glaucomys, points up additional aspects of the whole problem.

It is obvious from the _Drosophila_ curve for brief flashes that any usual photoperiod (8-16 hours) will embrace both the advance and delay sections of the curve. The equilibrium during entrainment must therefore involve (in addition to any effect from $\tau_{DD}$) effects from both sections (advance and delay) of the response curve. In such cases, where both sections of the curve always fall in the light, the role of $\tau_{DD} > 24$ in the equilibrium may be trivial; the equilibrium probably depends almost entirely on the interaction of the morning advance and the evening delay caused by the light. One may well expect that in those species where the $\tau_{DD}$ values are loosely distributed on both sides of 24 hours entrainment and phase control will be of this type, thus explaining why the generalization (4a) listed earlier is weak.

The explanation of _Drosophila_ entrainment in terms of the net action of the photoperiod is a flat reversal.
of the earlier position of our laboratory [1, 2, 3, 4, 5], in which we supposed the single dark/light transition at dawn was the effective entraining signal. This contradiction has led us to re-examine the bases of our earlier view; they have proved wrong. It was based on a poorly executed experiment summarized in Fig. 2 of a previous paper [5]. The several cultures were all raised in LD 12:12 and switched to the various photoperiods studied only on the day before data collection began. The erroneous conclusion that phase was nearly insensitive to photoperiod was founded on the fact that—as one only now realizes and can perceive in the old data—the cultures never got out of transients in the five days of observation. Recent experiments (Fig. 14) prompted by the present interpretation of the response curve have shown that the phase of the Drosophila eclosion rhythm is in fact strongly dependent on photoperiod.

The fact that a long duration signal (like 4 or 12 hours) embraces parts of both the advance and delay sections of the flash curve in Drosophila is surely responsible for the quantitative differences among the three response curves for this species given in Fig. 14. But the complexity of the system, even superficially, is greatly increased by this dual action of a single signal, and until we know how advances and delays are effected, and how they interact, we cannot predict, even qualitatively, what the net action of a long signal should be. This dual action also imposes obvious difficulties in the way of using phase-shifts to measure an action spectrum.

Two possibilities immediately suggest themselves on how light acts, and since one of these is immediately dismissable, at least for Drosophila, it is worth noting them: (1) light exerts some continuous action on one of the fundamental parameters of the oscillation, in a way analogous to a continuing change in the resistance of an electronic oscillator throughout a substantial fraction of its cycle; (2) the light is an effective signal only as it comes on and goes off. In this view the 2 transition (dark/light and light/dark) act as more or less discrete external perturbations. The known response of several metazoan photoreceptor preparations provides a clear concrete model for this. The onset of a long light signal elicits an “on” discharge which rapidly decays; the preparation then remains silent, though illuminated, until light is discontinued when an “off” discharge is produced.

The continuous action approach has some attractive features if one assumes the net action of a long duration light signal is approximated by summing the areas under those sections of the response curve it embraces. This approach yields computed effects for 12 and 4 hour signals that are plausible estimates of the observed responses; one gets an excellent model of phase control along the lines discussed earlier; and summing areas under the advance and delay sections for Drosophila and Glaucomys “predicts” a net action of LL compatible with Aschoff’s Rule. But the hypothesis must be rejected in view of several recent observations from our laboratory which Bruce [9] has noted in his discussion, and also because of an experiment summarized in Fig. 17 that was performed to test the hypothesis. Nineteen separate cultures of Drosophila were raised in LD 12:12 and ultimately allowed to freerun in DD. Each culture, however, received a different light signal on the last day before release into DD. All 19 saw dawn at the same time (that which obtained throughout their previous LD regime). But in each culture the light was discontinued at a different time. The final photoperiod was thus different in each case. According to the continuous action hypothesis the several photoperiods should have exerted a different net action on the rhythm which is estimated by summing the areas under the advance and delay sections of the response curve covered by each photoperiod. While this approach is clearly at best a very rough estimate, it does lead to clear qualitative predictions. For example, a 21-hour photoperiod should shift the phase of the DD free-

**Figure 16.** The phase of the Drosophila eclosion rhythm as a function of photoperiod. The upper figure plots the distribution (under various photoperiodic regimes) of eclosion throughout the day. The dawn of each photoperiod is synchronized as a reference point for discussion of phase. Each curve is normalized to the same area and based on mean values at each hour of the day from a 5 day run in steady-state. The numbers on each curve indicate the duration (in minutes or hours) of the entraining photoperiod.

The lower figure, right of the break, replots the same data; the phase of the rhythm (on the 24-hour abscissa of the square) is characterized here by the arithmetic median of the distribution (solid circle). The ordinate of the square is the photoperiod of the entraining signal.

The lower figure, left of the break, replots the same data; the phase of the rhythm (on the 24-hour abscissa of the square) is characterized here by the arithmetic median of the distribution (solid circle). The ordinate of the square is the photoperiod of the entraining signal.
Figure 17. Experimental evidence for rejection of the continuous-action hypothesis of light effects; and that the light-dark transition ("sunset") of any photoperiod greater than 12 hours is an absolute phase-giver for the subsequent steady-state. All cultures studies were raised in LD 12:12 (see text).

The left hand square in the figure has a W-hour ordinate and a 24-hour abscissa. The former measures the final photoperiod the cultures experienced before release into DD. From the response curve for flashes, plotted on the same abscissa that measures the photoperiod, one estimates what total phase change (delay or advance) the final photoperiod is expected to impose on the ultimate freerunning steady-state. The qualitative expectation is given by the dashed line on the right hand square. Note the change in the phase curve expected at 19 hours as further increase of photoperiod begins to add an advance effect.

Observed phases of the steady-state are plotted as solid circles; and clearly force rejection of the continuous action hypothesis.

running rhythm to the right but not as far to the right as an 18-hour signal: the 21-hour photoperiod includes a substantial advance effect missing in the 18-hour signal. The results of the experiment are extremely clear: the hypothesis is wrong. Any increase of photoperiod beyond 12 hours causes a phase-shift to the right, and the new phase is always $\pi X \rightarrow 24 + 15$ hours after the last light/dark transition. This transition (as other experiments have implied) is thus an absolute phase-giver provided it is not followed by a dark/light transition within the next 24 hours. When this happens the transitions interact; and the steady-state phase resulting from their interaction is a sensitive function of the phase angle between them. It is, in short, a function of the photoperiod. We need to know precisely how the transitions act and interact before we can rigorously relate the response curve for long-duration signal to that for flashes, and derive a general quantitative theory of entrainment that explains the dependence of phase on photoperiod.

THE GENERAL PROBLEMS

"Why this absurd concern with clocks, my friend?" Walter de la Mare. "The Winged Chariot."

Wilhelm Hufeland, a German physician, wrote a book in 1798 on the art of prolonging life. He advised among other things that one should heed the evangelist John Wesley who is evidently responsible for the old jingle "Early to bed, early to rise, makes you healthy etc." Hufeland had, one likes to feel, an insight into our problems: for apart from this tortuous implication that he suspected irregular entrainment would stress the circadian system, he has two sentences, quoted by Thienemann [38], that are an excellent caption for much recent discussion: "Die 24-Stundige Periode.... Sie ist gleichsam die Einheit unserer natürlichen chronologie." His assertion here has been well sustained by the brilliant work of later German naturalists-of von Frisch, of Kramer, and their students-to whom we owe the truly wonderful discovery that bees and birds (and now many other metazoa) can measure the time of day with an inner clock whose basic motion is a 24-hour oscillation. It is true that many biologists before 1950--including Pfeffer, Bunning, Kalmus, and Kleitman in particular-recognized there were first rate problems in the phenomena of daily rhythms. But the literature of the last ten years fully attests the key role played by the discovery of time-compensated sun-orientation in reviving and reformulating the interest attaching to them. As Bruce and I noted elsewhere [4] the evolutionary biologist refuses to suppose "clocks" appeared suddenly, de novo, in arthropods and verte-
brates. He looks for simpler precursors and, even in ignorance of Hufeland’s suggestion, turns to daily rhythms as potential time-measuring oscillations. This explicit reformulation of rhythms as clocks was mainly responsible for establishing, in the last ten years, the temperature-compensation of their period as a real generalization: it was deliberately sought as a functional prerequisite of a good clock.

To some of us who were attracted to circadian rhythms by this line of thought the phenomenon of temperature-compensation seemed the outstanding problem; it challenges the usual emphasis on the temperature dependence of cellular processes. To some workers it continues to pose the major riddle and has evidently been partly responsible for encouraging Brown to seek its explanation in terms of control by unknown geophysical periodicities. It was also partly responsible for encouraging Bruce and me [2, 4] to have, initially, a too-sanguine hope that biological clocks all had the same basic mechanism. One felt the mechanism for temperature-compensation must have been so hard-won by early cells that having once acquired it living systems retained it in all later evolutionary development. Both lines of thought are surely wrong. Burckhardt’s [39] recent paper on temperature relations of a stretch receptor in the crayfish reminds one that many aspects of the living system must be temperature-compensated; it maintains its organization as such over a wide temperature range. One has had no right to take for granted the proposition that neurally transmitted information is probably frequency modulated; not, at any rate, if one worries over temperature-compensation. For unless the spontaneous frequency of a sense organ is compensated for temperature like the stretch-receptor Burckhardt studied, it will be useless for anything except temperature sensing. The point is not that temperature-compensation fails to pose a first-rate problem; it is that circadian rhythms a priori cannot be and are empirically known not to be unique in this respect. The compensation may be a difficult trick but surely one the organization turns all the time, doubtless in diverse ways, in achieving all sorts of ends.

Their temperature relations therefore merge with those other properties of circadian rhythms, discussed earlier, that focus our search for the general problems in an underlying circadian organization as the real object of our study.

The comparative physiology of circadian rhythms in hamster, *Drosophila*, and *Euglena* obviously concerns systems which in their concrete details are radically different. And the striking formal similarities they possess must owe their origin to convergent evolution imposed by a common demand for an inner temporal order that matches that of the external environment. The magnitude and regularity of the daily change in the environment is another thing one too easily takes for granted. It is, in fact, little wonder that it has clearly been an ever-present and intense selective agent which few-if any-species have escaped; and it remains to be seen how far even arctic, cave, and deep sea forms fully lack circadian systems. There is some reason to suspect we shall find them even there—but this is another hazardous evolutionary prediction. The point I have in mind is that many of these species have comparatively recent ancestors more fully exposed to the effects of the earth’s rotation; and, further, that the circadian organization selection has wrought probably serves broader timing functions than only those of phasing to the external world. Organization in a living system involves time quite apart from the periodicity of the environment, and it may be that the presence of such oscillations in every system one studies reflects the exploitation of the circadian system for a general temporal ordering of constituent subsystems. If so, it may not be easily abandoned even in caves or the deeps where external periodicity has been left behind. And, if true, it also gives its study-specifically that of dysphasia in the system-added meaning. Nevertheless, the fact that the selection for circadian organization must have been so widespread, strong, and of so long a standing, reduces the prospect of a common concrete mechanism to vanishing point.

This is surely true of what are called B-oscillations in the earlier discussion; and in spite of our [7] recent hope that the universal light-sensitive oscillation might provide a common concrete mechanism there is now little reason even for this. When Bruce and I raised this hope it was based on our finding a strongly characterized response pattern in *Drosophila* to single light-signals; on finding it elsewhere; and finally on failing to see adaptive meaning in it. One then guessed he had something that could not be dismissed as another convergence induced by natural selection. But that is clearly not the case if the qualitative theory of entrainment outlined above is valid. The response curve for light is adaptive, and moreover reflection shows that the pattern of the curve (which is all that different species share) has to be as it is: only a morning advance and an evening delay will give a stable equilibrium no matter what the shape of the curve. Thus a morning delay response would continue to shift the phase of the system to the right until either the delay part of the curve were forced into the dark, or the succeeding part of curve (eliciting an advance response) were dragged into the light; only then will the system cease to shift its phase.

There is perhaps a lingering hope that all these caveats to seeking a common concrete system have clear pertinence only to multicellular systems; and that the single cell will have, or be, the common flywheel in all clocks. This remains to be seen. At present all we know is that, formally, protists resemble multicellulurs in their circadian rhythms; and these formal similarities—an innate circadian \( \tau \), temperature-compensation, and response curve to light—are what we know
natural selection would demand irrespective of how achieved. Nevertheless, in taking stock of problems and directions it is sure that work on single cells and simple tissue cultures will provide considerable insights. At present the chemical attack has yielded little in spite of the extensive attempts of himself and others that Hastings [40] has summarized for us. After a similarly long list of negative results Bruce and I [41] have taken some encouragement from finding that the system can be chemically manipulated: Euglena can be phase-shifted by exposure to D$_2$O and when adapted to heavy sater, presumably replacing at least some of their hydrogen by deuterium, the cells freerun on a much lower frequency than they do in H$_2$O. One still knows so little of what this implies that it remains to be seen how far it takes us. Ehret’s [42] indications of nucleotides being involved are more promising in leading to well-known and central features of the metabolic system. Even so, assuming that present indications of their being involved mature to clear proof, the next step is none too clear. One suspects the search for a discrete clock which the cell “has” may prove fruitless; that the search was founded in the first place on too loose a use of language. One is entitled to say only that the cell “is” a clock, for he has no assurance yet that any lower level of organization (mitochondria, for example, remain unstudied in this respect) can autonomously sustain a circadian oscillation. The suggestion, of course, is that again the problem goes back to organizational features. The individual cell’s rhythm is compensated for temperature, and if the explanation of this feature does lie in the properties of an organization, as such, we shall hunt in vain in the cell for an isolable timepiece. But to abandon—at least on this account—search for meaning in Hifield’s statement that a 24-hour period is the unit of biological chronology would be to despair of the biologist’s real problem. This, as Needham asserted, is the problem of organization.

Many years ago Binning published the suggestion [43] that the photoperiodic effect was mediated by what one then called the endogenous rhythm; and he added to this basic suggestion a subsidiary hypothesis as to how it did so. This latter postulated the existence of a distinct scotophil (dark-loving) section of the rhythm. A photoperiodic effect, like flower induction, was triggered or not according as to whether light fell, or did not, in the scotophil. This suggestion was made, I believe, before the photoperiodic problem was being explicitly discussed as a “clock” problem. Today we take the implicit time-measurement (of night- or day-length) as reason to speak of the “inner clock” involved. It is not pertinent here to pursue the fact that Binning’s hypothesis has met with very little favor among fellow botanists; to wonder why zoological students have never even considered it at least in their published work; nor to attempt its evaluation in the light of the existing data. My point in raising the issue is to make a necessarily brief attempt at greater explicitness in suggesting that a circadian organization as such (not a discrete physical entity) is the time-measuring thing underlying the diverse phenomena that have been responsible for our using the word “clock” in the first place. Photoperiodism is simply the easiest to tackle for such an explication; the bee’s zeitgedachtnis and the “clock” involved in sun-orientation are even remoter prospects for analysis.

The attempt is based on several features of Figs. 4 and 16 in this paper. First, Fig. 16 reminds us, as Aschoff [44] has shown in more detail, that the steady-state phase of the circadian system is a strong function of the photoperiod. This was not clear for Drosophila previously; and it is fair to comment that in discussing Binning’s hypothesis other students of rhythms have failed to emphasize sufficiently this obviously central point. Thus it is fact—not a matter for discussion—that the circadian system does measure photoperiod; the conclusion that its phase is a “measure” of photoperiod has precisely the same logical status as the conclusion that flower-initiation or diapause-interruption is a “measure” of photoperiod. It is only the vagary of convention that excludes the phase control of rhythms from that motley of phenomena labeled as photoperiodism, sensu strictu.

The only real issue in the debate over Binning’s hypothesis is whether the individual organism uses more than one device to estimate the same environmental parameter it exploits for several different ends. In proposing it probably does not, one is only voicing again the essential feature in Binning’s proposition of 1936. But I would prefer to avoid the special hypothesis of a scotophil and reformulate the essential proposition as follows: photoperiodic effects (sensu stricto) are aspects of the mechanism of entrainment of circadian systems; that the entrained equilibrium of the system is a characteristically different state for each photoperiod; that above or below a critical photoperiod sharp discontinuities in the response mechanism are responsible for imposing different states on the circadian system. On one side of the critical photoperiod phase relations among constituent oscillatory elements allow a particular reaction sequence to proceed; on the other side of that period widely different phase relations keep this metabolic pathway closed.

Returning to Fig. 16: The striking dependence of phase on photoperiod breaks down at the suggestive value of 16 hours. Beyond that photoperiod phase begins to shift back closer to dawn. But the changed response involves more than the system’s “phase” in any simple sense of this word. The adequacy of entrainment begins to fail, and already at 20 hours one sees the beginnings of that aperiodicity which is complete under constant light. After release into DD the freenmning system also indicates that entrainment...
by light involves more than merely establishing phase of an oscillation whose form or state is invariant under diverse photoperiods: spectacular transients (their duration varying with photoperiod) precede the new steady-state whose phase differs substantially (and characteristically for each previous photoperiod) from the prior steady-state. The latter in fact is some dynamic equilibrium attained by the multi-oscillator system responding to opposed perturbations-advance, delay-at the dawn and sunset transitions.

There is now a special significance attaching to our earlier conclusion that the entraining signals of the light cycle are the transitions in morning and evening. Change in photoperiod is a change in the phase angle between these signals. We know that when two entraining signals are coupled to and drive an oscillatory system the latter’s phase is not only sensitive to the phase angle but its response will necessarily involve sharp discontinuities of the type exemplified in Fig. 4. Indeed this figure can serve as a model to illustrate the possibilities involved. It plots the phase of fly and roach rhythms as a function of the phase angle between a light and temperature cycle; and the essential feature is a phase-jump of 180° when that phase angle exceeds a critical value. A chemical oscillation in the cell coupled directly or indirectly to dawn and dusk as opposing drivers will respond in the same way: on passing a critical photoperiod, the phase-jump imposed on this oscillation would constitute the closing of a switch that opened up previously impossible metabolic pathways. For, clearly, a reaction sequence can not proceed if an essential constituent is displaced from others by a 12-hour gap; and it will proceed if this gap can be closed. In such a model the clock is not an entity; the time-measurement (that of a fixed interval-timer) is executed by the responses inherent in the dynamics of the circadian system.

We may yet be confronted with a somewhat wry situation: the student of rhythms protests he has no common mechanism (in the concrete sense) to give his field the unity he would like; the student of insect photoperiodism asserts his system (involving eyes and endocrine glands) bears only a superficial, functional resemblance to that of flower-initiation by photoperiod. And yet both may be wrong in the sense that there are common mechanisms-built of different concrete parts—in circadian systems and photoperiodic effects everywhere. These general mechanisms inhere in the principles whereby constituent oscillatory subsystems are coupled and mutually entrain each other; how in so doing temporal organization is maintained within the organism; how the system as a whole is coupled to the multiple periodicities of the environment; and how critical phase-angle conflicts in the action of the latter can be exploited at least for interval-timing. In brief the prospect of a common mechanism to incite us is slim only if we are too preoccupied with the concrete and neglect our real business of elucidating organizational features of the living system.

REFERENCES


**DISCUSSION**

**Brown:** I would like to caution about confusion of facts with hypotheses. Despite frequent claims, there is no logically defensible proof that the clocks underlying circadian rhythms possess a timing system, a self-sustaining oscillation, which is independent of a continuous inflow of periodic information from the geophysical environment. Such proof has been precluded by the fact that one can never establish through negative evidence alone that nothing on the outside provides essential timing signals. One is not justified in making the assumption that circadian oscillations can be a consequence only of being driven by fully independent oscillations of the same frequency. The very danger of this unwarranted assumption becomes especially evident as we learn that there are no means for differentiating between frequency and phase changes. We have been duly impressed during the past two days with the readiness with which phases may be shifted in organisms, and with the fact that free-running periods are functions of the energy levels of the two principal phasing factors, light and temperature. The last suggests an obvious means by which the organism might modulate, or alter, the frequency of any periodic energy inflow through a means closely akin to ordinary response to a stimulus. The conventional, and hitherto highly successful, approach to physiological problems has been to exhaust first all proximal possibilities of cause and effect before retreating to a position of greater autonomy of observed phenomena.

To prove the existence of an intrinsic timing system, we must take a positive approach and ferret out the actual biological timing mechanism. We must show that its operational properties will account for fully autonomous timing and yield rhythms with all the described properties of circadian ones. No one can doubt that an inherited clock-system is present in organisms. But insisting upon a self-timed, or fully autonomous, living
clock, there always lurks the possibility that we are pursuing a ghost.

Pittendrigh: Dr. Brown has made a variety of propositions, only a few of which should be commented on here; to treat them all as fully as I would like would take more space than is warranted. I would only emphasize his last two sentences: apparently we all—Dr. Brown now included—agree “that an inherited clock-system is present in organisms.” The remaining areas of dispute concern the issues in his last sentence. The question of the ghost is simple—either it is an aspect of living organization, or an unknown geophysical variable. My taste in ghosts suggests the latter but, as scientist, I must agree that Dr. Brown may prove right; and as scientist he will doubtless agree he may prove wrong. We both will have some fun in any case.

Sirohi: I would like to emphasize the idea of dawn and dusk which has been brought up by Pittendrigh in this paper. Considered from a photoperiodic angle, dawn and dusk may be regarded as two points determining the length of a light period; the photoperiod begins with dawn and ends with dusk. It may be mentioned here that Pittendrigh did not give due consideration to the length of the photoperiod or light intensity during photoperiod in his earlier postulation for a biological clock model (Gatlinburg Symposium 1957). He, however, made an attempt to interpret results of Went, Hillman, and Highkin in the light of his model. More consideration of photoperiodic flowering response data, which are the basis of Bunning’s hypothesis, should have been included in such interpretations.

Pittendrigh has indicated some very interesting results which indicate that entrainment to different photoperiods (dawn and dusk) have a carry-over effect which can be observed under constant conditions. Recent work in photoperiodism with varying cycles (see Hamner’s paper) has shown that the effectiveness of a particular photoperiod in the flowering response is dependent on the length of the total cycle. I am aware of the fact that eclosion rhythm in Drosophila and flowering rhythms in plants have different characteristics. Nevertheless, photoperiodic response to odd cycles strongly suggests the fact that in order to further elucidate the entrainment phenomenon of rhythms, studies pertaining to these cycles are very important.

I agree with Klotter that a biological clock model should be able to accommodate maximum biological responses involved in rhythmic phenomena. Photoperiodism and flowering response, therefore, should be given a due consideration in any such postulation.

PITTENDRIGH: The interpretation of the Went, Hillman, and Highkin results given by Bruce and me in our 1959 paper [5] was indeed made while we still thought dawn alone was involved in entraining the A-oscillation in Drosophila. Close inspection of our interpretation there will reveal that it is independent of the way the light cycle entrains the A-oscillation. Data on cycles whose \( T \) is not 24 hours form an important basis for all studies—those of Bruce and myself included—attempting to analyze entrainment phenomena.

Harker: There are two questions I should like to ask. First, is there any evidence that Drosophila pupae or eggs are sensitive to changes in light intensity? It is possible that if they are not some of the transient peaks of eclosion are produced by animals which did not receive the light signal.

Secondly, is there any evidence that the “dawn” effect in your photoperiod experiments is actually concerned with a periodic process? I have found that in Periplaneta the onset of light is followed by an inhibition of activity about five hours later, regardless of the length of the light period. This inhibitory effect is not repeated unless there is another “dawn,” that is, it is not periodic. It may affect the activity rhythm of the animal in a number of ways depending on the stage of the activity cycle on which it acts: as a result one appears to get a different type of result with different light periods. I suppose the onset of darkness might affect some animals in the same way; it is even possible that this might be so in Drosophila rather than that it should be affected by the onset of light.

Pittendrigh: The pupae, which are all that are relevant, are known to be sensitive to changes in light intensity: one can entrain the eclosion rhythm with a sine wave oscillation of light intensity that involves no absolute darkness. However, I do not see the relevance of this to the interpretation of transients you suggest here. The transients (Fig. 1B) you refer to were produced by discrete light signals imposed on DD rhythm; the question of change of intensity is surely not involved; it was infinite. In any case one should re-emphasize that the analysis of transients in Drosophila is not obscured by the fact the system is a population. At any rate the transient pattern for a Drosophila culture is precisely that which is so beautifully shown by many single-animal systems like the hamster, etc.

There is more than one line of evidence that dawn interacts with dusk in affecting a periodic process in Drosophila. The strongest is partly covered by Fig. 16; the rhythm enters into transients on being released in DD, and the duration of these as well as the ultimate phase-shift (both properties of the oscillatory system) varies with the phase angle between dawn and dusk. The differences demand the conclusion that dusk was not the only parameter of the light signal involved in entrainment. This is also demanded by the fact that the phases of the freerunning rhythms do not form a straight line unless the photoperiod is greater than 12 hours.

DeCoursey: Dr. Pittendrigh has clearly indicated the plasticity of the freerunning activity rhythm frequency, and the importance of considering the effect of previous
conditions in obtaining response curves of organisms to single perturbations of light. In the study of endogenous rhythms with Glaucomys a similar slight plasticity of the freerunning rhythms of individuals has also been noted. However, in the determination of the response curve of Glaucomys the small deviation of points checked at widely separated periods of time suggest that the response curve had not changed even though the animals had been subjected to various light regimes in a two-year study period.

Ball, Harold J.: The Effect of Visible Spectrum Irradiation in Oncopeltus Fasciatus (Dallas) and Blattella germanica (L.)

In the class Insecta mode of action of light has been investigated in various ways. However, little information concerning the effect of action spectra is available. This is unfortunate because light is one of the most important stimuli which helps to maintain insects in a morphological form most likely to succeed at any given time.

The methods, materials, and apparatus used in these experiments can be found elsewhere [1].

Tests with Blattella germanica (L.)--
B. germanica (L.) nymphs 10-13 days old were exposed daily to “far red” irradiation (750 m\(\mu\)) for a period of 10 minutes. During the remainder of each day the insects were maintained in holding cages under ordinary room light conditions. A control group was maintained similarly under room light. Weekly weighings were made to determine rate of growth.

Results: During the first three weeks of treatment weight differences between the “far red” treated insects and the controls were slight. By the end of the 6th week of exposure all the treated insects were dead. Statistical analysis of the mean weights at the end of the fifth week revealed a significant difference in weight at the 5\% level with the control insects weighing more. Results of a 5 min. per day exposure to “far red” were comparable. In another experiment, roaches maintained under (DD) conditions were significantly (5\% level) heavier at the end of five weeks than were the control insects.

Tests with Oncopeltus Fasciatus (Dallas)--
In general the results obtained using O. Fasciatus were similar to those obtained in the roach experiments. However, red light (630 m\(\mu\)) also produced deleterious results at exposures of 5, 10, and 20 min. per day. Control insects were significantly heavier (1\% level) than either the “far red” or red-treated insects. The red and “far red” light treatments inhibited the nymphal growth rate, caused a significant reduction in the per cent of nymphs reaching imaginal form, and produced adults which weighed less at maturation.

Conclusions: The results of these tests indicate that the longer wave lengths of light, red, and “far red” are especially inhibitory to the species tested. The mechanism responsible for such growth inhibition is a subject for speculation. It has previously been suggested [2] that tissue of the central nervous system is reached by appreciable radiation from the visible spectrum under daylight conditions. It has further been pointed out [1, 3] that the median neurosecretory cells may be the overall controlling tissue for the type of growth inhibition reported here.

REFERENCES