cent inhibition was produced by $10^{-4}M$ concentrations of either anthramycin (I), epianthramycin (II), or anhydroanthramycin (III). The nitrile (IV) retains significant inhibitory activity, while the other analogs tested (V to IX) are inactive.

The results of these structure-activity studies indicate that the ability of anthramycin and its derivatives to act as chemosterilants in houseflies correlates closely with the inhibitory effects of these compounds on the RNA polymerase of *E. coli*. In both assays, only anthramycin (I) and the closely related derivatives II, III, and IV are active. Analogs in which the phenolic function is methylated (V), the aniline nitrogen is acetylated (VI), the carbinol-amine function is replaced by an amide group (VII), or in which the conjugated side chain is absent (VIII and IX) are devoid of the sterilizing effects of anthramycin. The activity observed with compounds II and III was not unexpected, since the three forms are in rapid equilibrium with anthramycin (I) in aqueous solution (*14*). The other active analog (IV) differs from anthramycin in that the primary amide group is replaced by a nitrile function.

If the cytotoxic activity of anthramycin against animal cells and bacteria is correctly attributed to the interaction of this antibiotic with DNA [see (*10*, *11*)], the chemosterilant effect on adult flies may also relate to binding of DNA by this alkaloid. This interpretation is consistent with an effect on gene expression, which is usually responsible for chemosterilant activity. Selective effects on the male are of special interest for purposes of insect pest eradication, but further studies are needed to precisely define a molecular basis for the chemosterilant action of anthramycin.

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28 June 1971

Synchrony and Flash Entrainment in a New Guinea Firefly

Abstract. *Fireflies can duplicate both faster and slower rhythms of artificial light. Since the interval between the pacer signal and the firefly's flash of the next cycle approximates the firefly's normal free-run period, it is suggested that the pacer signal resets the flash-timing oscillator in the brain, thus providing a mechanism for synchronization.*

Males of many species of fireflies flash spontaneously in a regular rhythm. Each flash is preceded by a volley of action potentials detectable in the main nerve trunk (*1*). Experiments with ablation and local excitation indicate that the neural timing mechanism (timer) which controls periodic flashing is in the brain. Flashing can be both enhanced and inhibited by appropriate photic or electrical stimulation of the eye (*1–3*), but little is known about how the rhythm is generated. As have others (*4*), we shall postulate that the timer normally behaves as a spontaneous "relaxation oscillator" (*5*) in which excitability increases progressively to a threshold or triggering level at which the system discharges, returns to baseline excitability, and begins its next cycle.

In parts of the tropical Orient there are species of fireflies in which the males habitually congregate in large swarms and flash in unison. Many hypotheses as to how the individual timers are brought into step have been advanced (*6*), but the synchronization is only partly understood. In the Thai species *Pteroptyx malaccae* Buck and Buck (*7*) found the interval between earliest and latest individual flashes in a communal flash to be only about 30 msec, whereas the minimum delay for flash generation, even by neural stimulation near the light organ, was 55 to 80 msec. During a mass flash, therefore, the fireflies cannot be responding to each other directly. Rather, it was suggested, each firefly lengthens or shortens his next interflash period according to whether he had flashed earlier or later than the average during the previous concerted emission. It was thought, in other words, that each firefly must be capable of distinguishing flash sequence and of controlling the period of his endogenous timer (*8*).

The recent *Alpha Helix* Expedition to New Guinea (*9*) gave us opportunity to study firefly synchrony with high-speed multichannel recording. We describe here only the most basic form of entrainment, that of single males responding to rhythmic flashes of artificial (pacer) light, and in only one species, *Pteroptyx cribellata* (*10*), which has a normal or free-run period of close to 1000 msec at 25°C. The firefly was

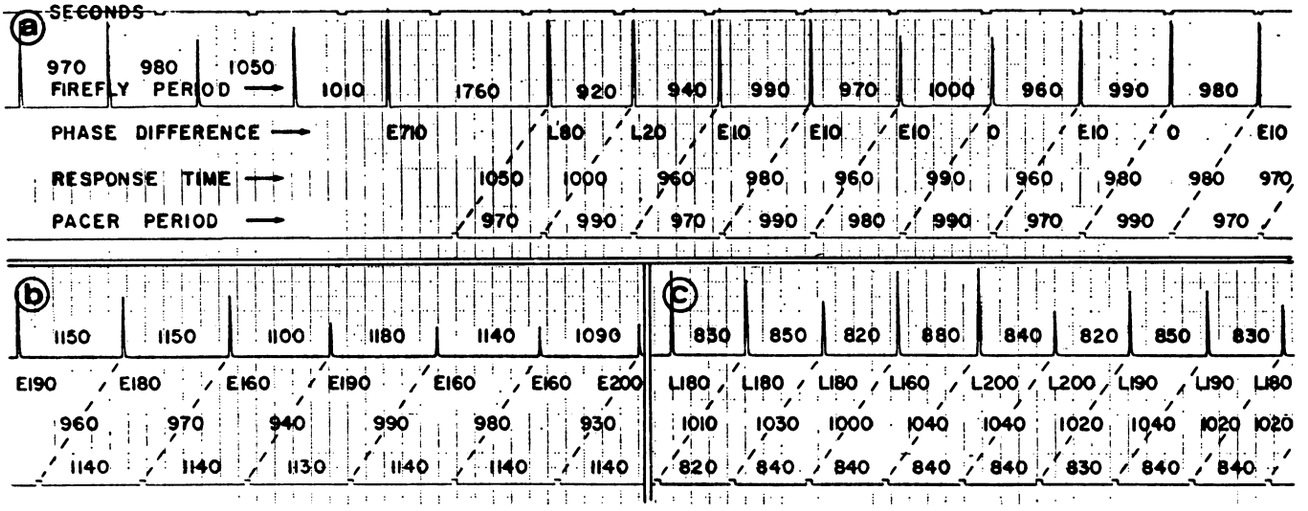

Fig. 1. Pacings of a Cape Hoskins *Pteroptyx* male firefly at equal, slower, and faster rhythms. In the phase difference rank, *E* means that the firefly flash was early with respect to the corresponding pacer flash; *L* means the firefly flash was late. Firefly flashes have been retouched to darken trace. (a) Pacer equals 980 ± 0.9 msec. The firefly record starts with last 4 of 17 successive free-run flash periods (mean = 982 ± 13 msec). The pacer was introduced 710 msec into the fifth cycle. Subsequent firefly interflash periods, phase differences, and response times averaged 982 ± 2, 5.7 ± 1 (early), and 976 ± 2 msec, respectively (44 cycles). (b) Pacer equals 1136 ± 1 msec. Entrained firefly periods, phase differences, and response times averaged 1136 ± 12, 203 ± 10 (early), and 936 ± 11 msec, respectively (16 cycles). (c) Pacer equals 838 ± 1.3 msec. Entrained firefly periods, phase differences, and response times averaged 837 ± 5, 192 ± 2 (late), and 1032 ± 4 msec, respectively (44 cycles). Means are given with standard errors.

anchored to a wax pallet without injury. Square pulses (60 msec long) of white light from a Sylvania R1166 glow modulator lamp, controlled by a Grass S-4 stimulator, were conducted to the eye by means of fiber optics (*2*). The associated flashes of the firefly were detected by a photomultiplier and recorded together with the pacer trace on a dual-channel penwriter chart. Several thousand free-run and paced cycles were measured from 14 fireflies.

Figure 1 gives excerpts from records of pacing of a firefly at frequencies equal to, slower than, and faster than his free-run rhythm. The firefly channel of Fig. 1a shows the last 4 of a series of 17 free-run flashing cycles that averaged 982 msec, then a conspicuously long cycle in which a pacer with an average period of 980 msec was introduced, and then the first 8 of 44 successive cycles of entrainment to the pacer (that is, 8 cycles with a 1 : 1 association of firefly flashes and pacer signals and in which the average firefly period approximated the pacer period). Figure 1b gives 6 out of 16 cycles of steady-state entrainment to a pacer averaging 1136 msec, and Fig. 1c presents 8 out of 44 cycles of pacing at an average period of 838 msec. These runs illustrate the firefly's ability to duplicate both longer and shorter pacer periods.

The most important conclusion from the entrainments shown in Fig. 1 is that the interval between each pacer signal and the firefly flash of the next cycle approximates the animal's normal free-run period. We call this interval the response time and have indicated it by diagonal dashed lines in the figure. Over the complete records sampled in Fig. 1 the response times averaged 976, 936, and 1032 msec during pacings at 980, 1136, and 838 msec, respectively. None of these response times differed from the spontaneous flash period prior to pacing (982 msec) by more than about 5 percent. Hence the response times seem independent of pacer periods, whereas the corresponding average firefly interflash periods (982, 1136, and 837 msec) are clearly determined by the pacer periods. The relative constancy of the response time and its approximation to the free-run period are illustrated in Fig. 2 for 28 experiments on the three males that duplicated the widest range of pacer periods.

The response time is not the physiological latency (minimum possible interval) between stimulus (light in the eye) and response (flash). That interval is only of the order of 200 msec (*11*). In responding, therefore, the firefly adds a large delay component, presumably in the central nervous system.

The evidence that the response time includes central delay and that it approximates the normal repetition period of spontaneous flashing suggested that each pacer signal might be overriding the endogenous control of flashing and resetting the timer to the start of its cycle (*12*). Thus, looking at Fig. 1a with the relaxation oscillator model in mind, one can imagine the firefly's endogenous interval timer having reset itself normally 150 to 200 msec before the fifth flash and then having built up excitability until it was interrupted by the first pacer signal and set back again to the start of its cycle. The timer would then be expected to resume cycling and, not being overridden this time by a pacer signal, to evoke a flash after the normal average delay of about 980 msec. The first response time (1050 msec) was in fact somewhat long, but thereafter the values agreed well with expectation.

The apparent resetting of the timer after the firefly had flashed, by postponing the predicted next flash, lengthened the first paced cycle drastically (to 1760 msec, Fig. 1a). With pacer cycles longer than the free-run period this sort of period lengthening prevails throughout the series. When paced at 1136 msec, for example, every cycle was lengthened by about the difference between the pacing and free-run periods (Fig. 1b). Pacer cycles shorter than the free-run period can have the analogous effect of shortening each interflash period (Fig. 1c), although here the mechanism is not so obvious since the pacer affects not the firefly flash that follows immediately but the second. A reasonable explanation of this effect is that, although the pacer does reset the central timer, the more peripheral neural

processes that eventuate in the immediately following flash are already under way and cannot be halted.

There are, of course, limits to the pacer frequencies that the fireflies can track. On the short-cycle side, none of the multiply tested animals (Fig. 2) bettered 838 msec, and the shortest pacer cycle that any other male could follow more or less regularly was 813 msec, or about 20 percent shorter than his free-run period. With pacing periods shorter than 800 msec fireflies either stopped flashing entirely or broke away from the pacer and resumed free-run flashing. This result is consistent with the evidence that the flash-eliciting process may occupy up to about the last 200 msec of the free-run period and with the consequent prediction that pacing signals introduced earlier than about 800 msec into the timer cycle, if effective at all, would reset every successive cycle before a flash could be triggered. Failure to entrain to fast pacers is not due to physiological inability to flash at the required frequency since *Pteroptyx cribellata* can produce discrete flashes at frequencies of up to at least five per second when mechanically disturbed.

Toward the low-frequency (long period) part of the pacing range the results were more variable. Although the Cape Hoskins firefly responded to one pacer presentation 710 msec late in his cycle (Fig. 1a, fifth cycle) he failed to entrain consistently to cycles longer than 1263 msec (Fig. 2). The Navuneram specimen, in contrast, showed quite respectable entrainment up to pacer periods of 1600 msec.

In well-entrained series the firefly's interflash interval that immediately followed the final paced cycle was of free-run length. A 31-cycle pacing series of the Navuneram firefly at 2117 msec proved interesting in that it consisted of a regular alternation of longer (mean, 1119 ± 9 msec) and shorter (mean, 994 ± 8) firefly periods. The former were those in which the pacer signal occurred, and are thought to represent cycles reset by stimuli arriving at about 120 msec into the period, whereas the latter are unpaced periods. Similarly, a less regular 23-cycle pacing at 4200 msec gave a series consisting of one longer (reset) period followed by three shorter (free-run) periods (means of 1236 ± 41, 998 ± 8, 982 ± 11, and 989 ± 19 msec). The postulated resetting thus occurs cycle by cycle, with the firefly reverting immediately to his free-run period when there is no pacer input. This conclusion was confirmed on an as yet unidentified synchronizing firefly (free-run period 1014 msec) in which 46 single pacer signals were intruded at random times during several hundred cycles of free-run flashing. When the signals, which were well distributed through the cycle, fell between 800 msec and the end of the cycle they did not affect the immediately following flash, but those falling between 10 and 780 msec into a firefly interflash period lengthened that period by approximately the time into the cycle, with the next period being then only about 1000 msec long.

It might be expected that a rhythmic biological event that is entrained to an artificial pacer should occur simultaneously with the pacing signal. In fact that is what is ordinarily meant by "synchrony." As Figs. 1 and 2 show, *Pteroptyx cribellata* fireflies flash simultaneously only with pacers of equal period. With all longer and shorter pacer periods they lead or lag, respectively, the (concurrent) signal. By specifying that the underlying mechanism of entrainment to both faster and slower pacers actually involves a single fixed-delay response to the signal of the previous cycle, the timer-reset hypothesis supplies a consistent interpretation for lack of simultaneity between pacer and firefly (*13*).

Although the fit of the firefly data to the hypothesis seems quite impressive for behavioral experiments, there are some small but statistically significant departures. Almost all response times were slightly shorter than the free-run periods, and the Hoskins values were disproportionately long at the short end of the pacer range and short at the long end (Fig. 2). The fact that such discrepancies can find reasonable possible explanations within the framework of timer-resetting (*14*) strengthens the evidence that the minimum hypothesis provides a parsimonious model for neurally mediated entrainments in which (i) phase lock occurs in the first cycle of pacer presentation, (ii) entrainment is

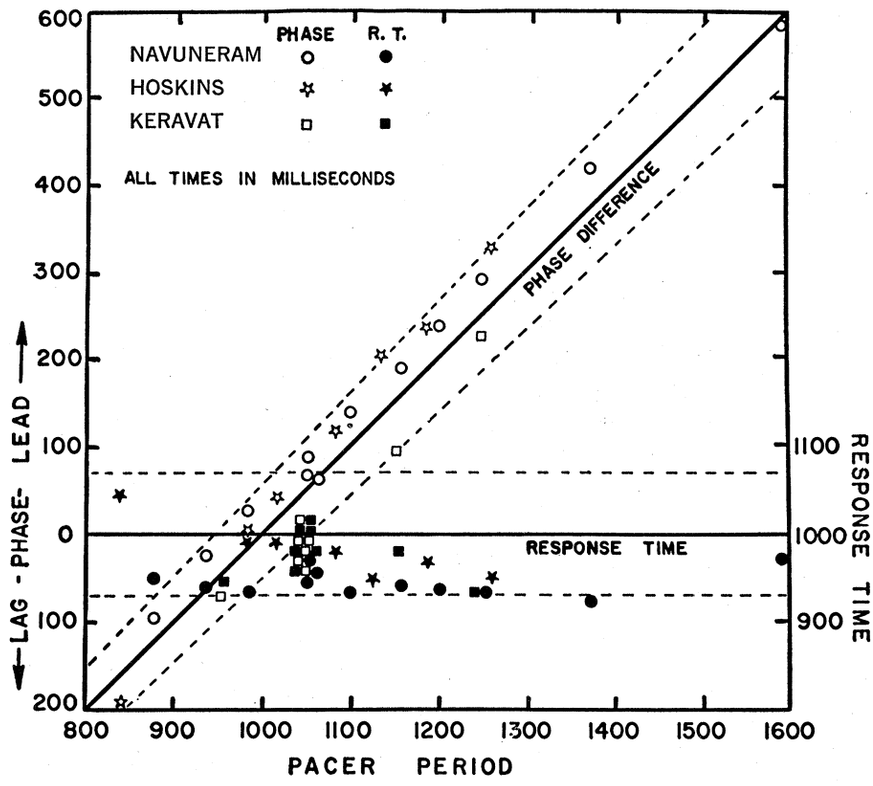

Fig. 2. Pacer cycle length in relation to phase difference with firefly (left axis) and to response time (right axis). Data were taken from three *Pteroptyx cribellata* males from different localities on New Britain (7 pacings of Cape Hoskins specimen, 12 pacings of Navuneram Village specimen, and 9 pacings of Keravat specimen). All data were normalized to a free-run period of 1000 msec and are based on 887 paced cycles, with no point representing fewer than 13. Heavy lines are theoretical linear regressions of phase differences on pacer cycle length (oblique line) and response time on pacer cycle length (horizontal line), assuming each reset cycle exactly equals that individual's free-run period. Broken lines delineate ± 5 percent limits for phase difference and ± 7 percent limits for response time. The Navuneram phase data have a 0.997 correlation coefficient for linearity and a slope of 0.992.

cycle by cycle, (iii) the biological event coincides with the pacer signal only when the respective cycling frequencies are equal (Fig. 1a) (*15*), and (iv) phase leads and lags occurring with unequal pacers approximate the respective differences between pacing and free-run periods. Further, the timer-resetting idea, whether in the restricted format which our best firefly data appear to approach as a limit, or modified to cover different oscillator behaviors (*14*), offers the following persuasive advantages over most previously suggested mechanisms for animal synchronization (*16*). First, it accommodates entrainment to both faster and slower pacers in a single mechanism (*17*). Second, it explains not only the fact of consistent phase differences between pacer and animal during entrainment but their magnitudes and directions. Third, it provides for entrainment to a wide variety of foreign rhythms without requiring (*7*) that the animal be able to discriminate the sequence of his act in relation to the acts of neighbors in the synchronizing community. Finally, it allows the animal to duplicate a variety of pacer cycles without actually changing the intrinsic period of its endogenous timer.

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8. Ability to change flash frequency has also been suggested by N. Baldaccini, V. Fiaschi, and F. Papi [*Monit. Zool. Ital.* **3**, 239 (1969)].
9. We thank the University of California, San Diego, for support, travel, and facilities on the expedition. J.B. thanks the American Philosophical Society (Penrose Fund grant 5017) and the National Geographic Society for technical support. J.F.C. had additional support from ONR contract N00014-69-A-0200-8006 and from the University of California Faculty Research Fund. F.E.H. received additional support from a faculty grant, University of Texas.
10. The *Pteroptyx* fireflies studied were from three localities on New Britain, Territory of Papua and New Guinea: Navuneram Village and Keravat, 10 miles apart, and Cape Hoskins, 100 miles to the southwest. By present criteria all belong to the same species. We thank Lesley A. Ballantyne and Miriam R. McLean for identifying the fireflies investigated. Taxonomic details will be found in L. Ballantyne and M. McLean, *Trans. Am. Entomol. Soc.* **96**, 223 (1970).
11. The minimum conduction plus organ excitation delay, measured during strong electrical stimulation of the brain, and presumably bypassing any central delay, varies from 70 to 110 msec in various species [(*1–3*) and present work]. Stimulation by light in the eye usually adds in the order of another 100 msec, hence almost certainly involves more than just visual processing delay.
12. We have no direct evidence of what "resetting the endogenous timer" means in terms of the neuroeffector control mechanisms of flashing. However, in another insect, the roach, the oscillator that controls the circadian rhythm of locomotion has been localized in the optic lobes of the brain, and the eye has been shown to be the organ by which the oscillator is entrained by light [J. Nishiitsutsuji-Uwo and C. S. Pittendrigh, *Z. Vergl. Physiol.* **58**, 1, 14 (1968)].
13. Phase differences between pacer and timer during entrainment have been observed in a variety of other biological systems, for example, crustacean heart [D. M. Maynard, *Biol. Bull.* **109**, 420 (1955)], circadian rhythms [C. S. Pittendrigh and D. H. Minis, *Am. Nat.* **98**, 261 (1964); A. T. Winfree, *J. Theoret. Biol.* **28**, 327 (1970)], and pacemaker neurons [D. H. Perkel, J. H. Schulman, T. H. Bullock, G. P. Moore, J. P. Segundo, *Science* **145**, 61 (1964)]; however, we defer comparisons until the full presentation of our findings.
14. Time relations during resetting depend strongly on the model chosen for the relaxation oscillator. In the minimal or "ideal" situation, in which the timer charges at a linear rate throughout its cycle, triggers always at a fixed level of excitation, and discharges in all-or-none fashion, response time must equal the free-run period exactly, and leads and lags in steady-state pacing must be symmetrical with respect to the free-run period. However, response times shorter or longer than the free-run period could result if the pacer discharged the timer respectively less or more completely than the normal endogenous process or at a slower or faster rate. Similarly, linear phase versus pacer plots (Fig. 2) with slopes different from 1.0, or with inverse or curvilinear relations, can be accommodated readily. Examples of such modified resettings in other firefly species will be given elsewhere.
15. With a pacer period equal to the free-run period it is sometimes not possible, except in the introductory transient, to be absolutely sure that the firefly is actually entrained since equal rhythms could stay in association for a long time by chance. There can be doubt also with pacer cycles shorter than the free-run firefly period (unless there are several different pacers with different apparent lags, as in Fig. 2) because entrainment with constant lag could represent a series of direct sequential responses rather than successive resettings [see figure 17B in (*4*)]. In this connection, the name "paced," applied by Buck and Buck (*7*) to synchrony involving minimum latency triggering by a premature flash, should be renamed "led" synchrony to distinguish it from the present hypothesis of pacer action.
16. Many of the older theories (*6*) can also be questioned for various a priori reasons (*7*) or for lack of precise measurement. For example, a visual observation of exact synchrony in fireflies is of dubious value in relation to possible entraining mechanisms in view of the report that the human eye cannot, under field conditions, distinguish asynchrony between flashes closer together than about 110 msec (*8*).
17. T. J. Walker [*Science* **166**, 894 (1969)], studying entrainment of tree crickets to series of artificial chirps, found the response chirps earlier than the pacer chirps when the pacer rhythm was slower than the cricket's and later when faster. He reported that crickets synchronize "by responding to the preceding chirp of their neighbors" but concluded that the period shortening ("S response") and period lengthening ("L response") are due to qualitatively different mechanisms.

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Hemispheric Asymmetry of Electrocortical Responses to Speech Stimuli

Abstract. *In a group of normal adults, averaged cortical evoked responses to natural speech stimuli were recorded from scalp electrodes placed symmetrically over the two cerebral hemispheres at frontal, Rolandic, and temporoparietal leads. The amplitude of the most prominent component was consistently larger in left hemisphere derivations, with the major hemisphere difference observed in the temporoparietal records. These electrophysiological measures may be sensitive indicators of hemispheric specialization of function.*

It has long been recognized that the neural structures of the left cerebral hemisphere play a dominant role in the mediation of human language. The major source of evidence has come from patients with localized cerebral lesions. Language impairment is much more likely with left hemisphere involvement than with lesions of comparable size, nature, and locus on the right. Over a century ago Paul Broca demonstrated that aphasia was associated with lesions restricted to the third frontal convolution of the left hemisphere. Serious and lasting language defects occur when lesions involve the gyri surrounding the posterior tip of the Sylvian fissure. This region has been considered by some authors as the indispensable speech cortex (*1*). One of the posterior speech regions, namely, the classical area of Wernicke, is significantly larger by gross anatomical measurement in the left hemisphere (*2*).

This report concerns the applica-