ence between the normal and abnormal serums in that a characteristic double-arc "gull-wing" band of precipitation was formed by the inhibitor in normal serum, whereas that in the abnormal serum formed only a single component which corresponded to the fast portion of the normal "gull-wing" pattern (Fig. 2). These differences could not be attributed to the effect of concentration or *p*H and were believed to result from a structural alteration in the nonfunctional protein. Inasmuch as the reaction of identity was found on Ouchterlony analysis, the altered immunoelectrophoretic pattern implies a structural alteration in the nonfunctional inhibitor without deleting antigenic determinants.

Hereditary angioneurotic edema is transmitted as an autosomal dominant characteristic, and studies of pedigrees indicate that patients with the disease are heterozygotes. However, the serums of patients in 10 of the 12 kindreds studied contained only 6 to 25 percent of the normal amount of inhibitor. The deficiency of antigenic inhibitor in these serums results from a defect in synthesis of EI since a study of I^{131}-labeled inhibitor showed that the rate of catabolism of the inhibitor in the patients did not differ from that in the normal subjects (*5*). In the two other kindreds a normal amount of nonfunctional, structurally altered inhibitor is synthesized. The two genetic variants of hereditary angioneurotic edema may be analagous to the two types of tryptophane synthetase mutants of *Neurospora crassa*: one type lacks the enzyme, and, in the other type, enzymatically inactive protein is synthesized and this inactive protein is antigenically cross-reacting (*6*). Possibly synthesis by a heterozygous individual of a nonfunctional, abnormal protein might suppress the regulatory mechanism for the synthesis of normal, functional protein (*7*). Further study of the genetic variant in which a nonfunctional protein is synthesized may confirm the hypothesis.

FRED S. ROSEN
PATRICIA CHARACHE
Children's Hospital and *Department of Pediatrics, Harvard Medical School, Boston, Massachusetts*
JACK PENSKY
Institute of Pathology, Western Reserve University, Cleveland, Ohio
VIRGINIA DONALDSON
St. Vincent's Charity Hospital and *Department of Medicine, Western Reserve University, Cleveland, Ohio*

References and Notes

1. V. Donaldson and R. Evans, *Am. J. Med.* **35**, 37 (1963); V. Donaldson and F. S. Rosen, *J. Clin. Invest.* **95**, 362 (1964).
2. J. Pensky, L. R. Levy, I. H. Lepow, *J. Biol. Chem.* **236**, 1674 (1961); J. Pensky, *Federation Proc.* **19**, 76 (1960).
3. O. Ouchterlony, *Arkiv Kemi* **1**, 43 (1949); J. G. Feinberg, *Immunology* **2**, 346 (1959).
4. J. Hirschfeld, *Nature* **185**, 931 (1960).
5. F. S. Rosen, J. Pensky, P. Fireman, in preparation.
6. S. R. Suskind, C. Yanofsky, D. M. Bonner, *Proc. Natl. Acad. Sci.* **41**, 577 (1955).
7. H. E. Sutton, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, 1960), p. 46.
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Photoreception and Entrainment of Cockroach Activity Rhythms

Abstract. *In the cockroach there are two distinct sets of photoreceptors—the compound eyes and the ocelli—which may function as a sensory input whereby circadian locomotor rhythms are entrained by environmental light cycles. Surgical removal of the ocelli did not interfere with normal entrainment, but covering over the compound eyes effected a loss of entrainment.*

One of the striking features of the circadian rhythmic system is that it can be entrained by light cycles. The word circadian (*circadiem*, about a day) has been widely adopted to emphasize the fact that under constant conditions with respect to light and temperature the periodicity of rhythms in plants and animals, although nearly equal to 24 hours, may be consistently greater or less than this value. For example, the locomotor rhythm of a given animal might show an average period (interval between successive daily onsets of activity) of 23.5 hours instead of 24.0 hours. Such a rhythm is considered to be a "free-running" rhythm—that is, it is not being entained to the frequency of a natural environmental cycle. However, the period of such a rhythm may be entrained to precisely 24.0 hours by regular cycles of light and darkness, for example, 12 hours of light alternating with 12 hours of dark (LD 12 :12). One aspect of this entrainment phenomenon is the question of the site of the sensory input which channels the information about environmental light to the endogenous circadian system. In metazoans with distinct photoreceptors, such as eyes or ocelli, it has generally been assumed that one or more of these act to relay information about the frequency of the light cycle to the circadian system. Recent studies on insects suggest that particular caution should be used in making such an interpretation because, first, there are conflicting statements in the literature and, second, Lees (*1*) found that photoperiodic control of reproduction in aphids is mediated by light falling directly on the pars-intercerebralis of the brain. This latter observation is particularly significant in view of the evidence that circadian rhythms are involved in photoperiodic time-measurement (*2*).

The studies described herein were conducted with the cockroaches *Periplaneta americana* and *Leucophaea maderae*. These insects have two pairs of external photoreceptors: the large compound eyes and a pair of much smaller ocelli lying adjacent to the antennae sockets. Investigators have generally agreed (*3*) that the ocelli augment photokinetic reactions normally mediated by the compound eyes. A wholly new function is explicit in the claim by Harker (*4*) that the locomotor rhythm in the roach, *Periplaneta americana*, is synchronized to LD cycles through the ocelli and not the compound eyes. It should be noted that Harker's results contradict the earlier findings of Cloudsley-Thompson (*5*) which implied, although no supporting data were given, that both sets of receptors must be intact to maintain a synchronized rhythm in *Periplaneta*. More recent experiments on the house cricket, *Gryllus domesticus*, by Nowosielski and Patton (*6*) suggest that entrainment can be mediated by either pair of photoreceptors. In one major respect the results of our studies with roaches lead us to disagree with the earlier reports; namely, we suggest that the sensory input to the circadian system is either through the compound eyes or possibly by direct photostimulation of the brain. Although the evidence does not yet permit a definite choice between these alternatives, it does clearly rule out the involvement of the ocelli.

Our conclusions are based on experiments in which we painted over the photoreceptors, or in some cases removed the ocelli by surgery. After testing various "paint" materials we

found that model-airplane lacquer and bee's wax impregnated with carbon-black were the most effective in omitting light. For observing locomotor rhythms each cockroach was separately housed in a circular cage (*7*) which rotated freely when the animal was active. These cages were kept in temperature-controlled cabinets and their rotations were continuously monitored by a pen recorder activated by a microswitch.

All experimental animals were subjected to LD 12 : 12; the light intensity in these LD cycles never exceeded 275 lu/m^2. Figure 1 illustrates the typical response of an animal in which the compound eyes were painted but the ocelli left exposed. Before being "blinded," the roach was entrained by the LD cycle from day 1 to 19; on day 20 it was removed from its cage and its eyes painted with several coats of black lacquer. When replaced in its cage and again subjected to the LD cycle (day 20 to 50) the insect's rhythm failed to be entrained by the LD cycle. Thus, although the ocelli were exposed and could presumably "see" the light cycle, the roach's rhythm clearly changed to a "free-running" state. On day 50 the paint was removed from the compound eyes and the rhythm was rapidly re-entrained by the LD cycle. On day 68 the ocelli were surgically removed and the rhythm remained entrained until the end of the experiment. Similar tests for ocellar function were repeated on seven individual roaches with identical results. In addition, experiments with 15 animals with their compound eyes covered corroborate the result shown in Fig. 1. In seven of these insects the covering was removed after a free-running rhythm was established and subsequent re-entrainment was always observed. It should be noted that two of the 15 roaches tested never lost entrainment; subsequent examination of these animals revealed that small areas of paint had chipped away from the surface of the compound eyes.

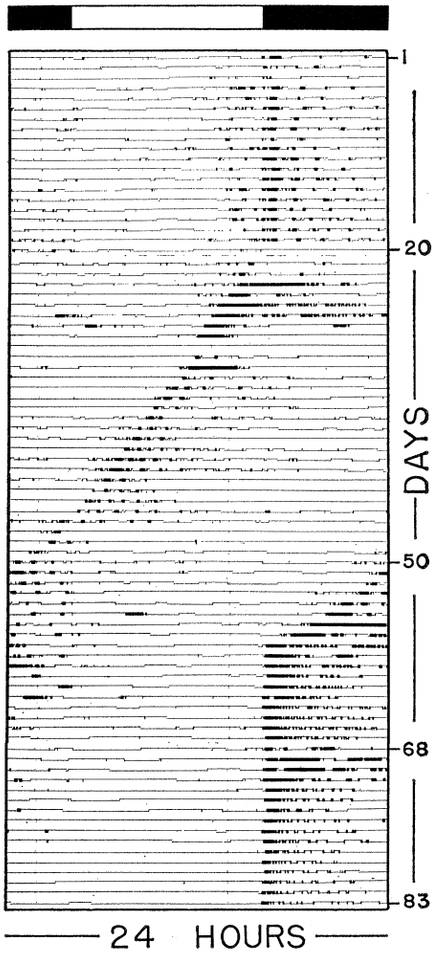

Fig. 1. Record of the rhythm of locomotor activity of a single roach, *Leucophaea*, maintained in a light-dark cycle, LD 12 : 12, for 83 days. On day 20 the compound eyes were painted with black lacquer; on day 50 the paint was peeled off; and on day 68 the ocelli were surgically removed. The position of the light and dark fractions of the LD regime is indicated at the top of the figure.

Since these results differ from those in earlier reports, several comments are warranted. First, none of the earlier experiments demonstrate that an animal's circadian rhythm can be induced to show a free-running period in an LD cycle by interference with a specific photoreceptor. This is a crucial point, since any treatment which interferes exclusively with light input to the circadian system should simply evoke a response typical of that expressed by a normal animal in constant darkness—that is, a free-running rhythm. Also, it should be emphasized that a re-examination of Harker's data reveals that entrainment was *not* lost after the ocelli were painted. Harker's data do show that in animals so treated the phase of the activity is reversed so that the onset of activity begins abnormally at the transition of dark-to-light. Although we have preliminary evidence that similar phase-reversals may occur after the brain has been damaged, no adequate explanation of Harker's observation can be given here. Finally, it must be emphasized that our findings (which strongly implicate the compound eyes as the input to the circadian system) do not rule out the possibility of direct photostimulation of the brain by light transmitted through the cuticle of the head. To ensure adequate exclusion of light in our roaches, most of the head capsule was covered as well as the compound eyes. Consequently, light was being excluded not only from the eyes, but also from much of the brain.

SHEPHERD K. ROBERTS
Department of Biology,
Temple University,
Philadelphia, Pennsylvania

References and Notes

1. A. D. Lees, *J. Exptl. Biol.* **41**, 119 (1964).
2. E. Bunning, *Ber. Deut. Botan. Ges.* **53**, 594 (1937); C. S. Pittendrigh and V. G. Bruce, in *Photoperiodism*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959); C. S. Pittendrigh and D. H. Minis, *Am. Naturalist* **98**, 261 (1964).
3. V. B. Wigglesworth, in *Insect Physiology* (Methuen, London, 1956).
4. J. E. Harker, *J. Exptl. Biol.* **33**, 224 (1956).
5. J. L. Cloudsley-Thompson, *Ann. Mag. Nat. Hist.* **6**, 705 (1953).
6. J. W. Nowosielski and R. L. Patton, *J. Insect Physiol.* **9**, 401 (1963).
7. S. K. Roberts, *J. Cell. Comp. Physiol.* **55**, 99 (1960).
8. These observations were conducted over a period of years; they were begun in Prof. Pittendrigh's laboratory at Princeton University and were completed at Temple University. I acknowledge the following support: Porter Fellowship of the American Physiological Society; funds from the Eugene Higgins Trust allocated to Princeton University; and a research grant from Temple University.

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Conditional-Lethal Mutants of an Animal Virus: Identification of Two Cistrons

Abstract. *Two different temperature-sensitive conditional-lethal mutants of Sindbis virus, an animal virus that contains RNA, have been isolated. When cultured in chick fibroblast monolayers at 42°C, these mutants yield less than 0.05 percent as much virus as does the wild type, whereas at 27°C they grow normally. One mutant appears to be altered in the synthesis of a protein that is produced early in the infection and is required for viral RNA synthesis. The other mutant produces as much infectious RNA as the wild type at 42°C and appears to be altered in the synthesis of a protein produced late in the infection.*

Conditional-lethal mutants have been useful in the study of the genetics and physiology of both DNA (*1*) and RNA (*2*) bacteriophages. With the isolation of temperature-sensitive conditional-