of Raney nickel, followed by H_2S treatment of the nickel to release the small amount of xanthine. 8-Methylmercaptoxanthine of 34,900 count min^{-1} $\mu mole^{-1}$ (*14*) was thus reduced to xanthine, which, after chromatography on 10 ml of Dowex 50, had a specific activity of 35,500 count min^{-1} $\mu mole^{-1}$ (*15*). Similarly, 8-chloroxanthine of 99,000 count min^{-1} $\mu mole^{-1}$ (*16*) could be catalytically hydrogenated over Pd-C to yield xanthine of 79,000 count min^{-1} $\mu mole^{-1}$. Additional evidence for the identity of the 8-chloroxanthine was obtained in an experiment with $^{36}Cl^-$ (*17*). An injection of 2.3 mmole of $Na^{36}Cl$ and 20.3 mmole of 3-hydroxyxanthine was administered to rat A. By 6.5 hours the urine contained 5.7 percent of the $^{36}Cl^-$ at a dilution of only 1 : 5.5, which corresponds with the fact that most of the radioactive chloride was still extracellular during that period (*18*). From a longer column (see Table 2), the $H^{36}Cl$ was eluted as an initial sharp peak, the trail of which obscured the 8-chloroxanthine radioactivity peak. When 660 count min^{-1} of radioactivity from the 8-chloroxanthine peak was rechromatographed on paper with NH_4Cl, 40 percent clearly accompanied the carrier 8-chloroxanthine and somewhat more accompanied the sodium chloride (Table 2). The low activity of the 8-chloroxanthine suggests that it was formed from intracellular chloride, as would be expected.

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References and Notes

1. The more common "carcinogenic" should be restricted to agents that induce carcinomas. Oncogenic is an inclusive term for agents that induce any form of neoplasia. H. Martin, *Arch. Surg.* **7**, 534 (1963); P. Rous, *Cancer Res.* **27**, 1919 (1967).
2. K. Sugiura, M. N. Teller, J. C. Parham, G. B. Brown, *Cancer Res.* **30**, 184 (1970).
3. G. Stöhrer and G. B. Brown, *J. Biol. Chem.* **244**, 2498 (1969).
4. A. Myles and G. B. Brown, *ibid.*, p. 4072.
5. G. Stöhrer and G. B. Brown, *ibid.*, p. 2494.
6. U. Wölcke and G. B. Brown, *J. Org. Chem.* **34**, 978 (1969).
7. U. Wölcke, W. Pfleiderer, T. J. Delia, G. B. Brown, *ibid.*, p. 981.
8. U. Wölcke, N. J. M. Birdsall, G. B. Brown, *Tetrahedron Lett.* **10**, 785 (1969).
9. J. A. Miller and E. C. Miller, *Progr. Exp. Tumor Res.* **11**, 273 (1969).
10. P. D. Lotlikar, J. D. Scribner, J. A. Miller, E. C. Miller, *Life Sci.* **5**, 1263 (1966).
11. J. R. DeBaun, J. Y. Rowley, E. C. Miller, J. A. Miller, *Proc. Soc. Exp. Biol. Med.* **129**, 268 (1969).
12. C. M. King and B. Phillips, *Science* **159**, 1351 (1968).
13. M. N. Teller, G. Stohr, H. Dienst, *Cancer Res.* **30**, 179 (1970).
14. ε (283 nm; 2*N* HCl) = 15.8×10^3; determined on reference samples.
15. ε (260 nm; 1*N* HCl) = 9.2×10^3; determined on reference samples.
16. ε (278 nm; 0.1*N* HCl) = 11.9×10^3; R. K. Robins, *J. Org. Chem.* **26**, 447 (1961); J. B. Lloyd, *Chem. Ind.* **1963**, 953 (1963).
17. Obtained from the Oak Ridge National Laboratory, 11.7 μc per mole. For determination of ^{36}Cl, the channel ratio technique was used.
18. K. Nagao, *Osaka Shiritsu Daigaku Igaku Zasshi* **8**, 221 (1959).
19. We thank E. Corbin for assistance, I. Wempen, P. H. Stahl, N. J. M. Birdsall for suggestions on the chemical reductions, and U. Wölcke for a sample of 8-methylmercaptoxanthine. Supported in part by the American Cancer Society grant P-295, PHS grant CA 08748, and AEC contract AT(30-1)-910.

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Neuroendocrine Control of Ecdysis in Silkmoths

Abstract. *An adult moth sheds its pupal skin only during a specific period of the day. The brain is necessary for the synchronization of this behavior with the environmental photoperiod. This function is fully preserved when all the brain's nervous connections are severed or when a "loose" brain is transplanted into the tip of the abdomen. By appropriate experiments it was possible to show that the entire mechanism is brain-centered. The components include a photoreceptor mechanism, a clock, and a neuroendocrine output. The clock-controlled release of the hormone acts on the central nervous system to trigger a species-specific behavior pattern which culminates in ecdysis.*

Lepidopterists have long been aware that the escape of butterflies and moths from their old pupal cuticle occurs at a certain time of the day which is characteristic of the species (*1*). The three silkworms used in this study, *Hyalophora cecropia, Antheraea polyphemus,* and *A. pernyi,* proved to be no exception. When developing moths were placed in a photoperiod, the subsequent ecdyses, which were recorded by means of a 48-hour kymograph (*2*), were confined to a specific "gate" (*3*), the time of which depended on the particular photoperiod regimen (*4*). Figure 1 (left) shows the ecdyses of moths that were exposed to a regimen consisting of 17 hours of light per day (17L : 7D). The gates of the three species differed in respect to both time and width. The "clock" which determines the time of ecdysis also has a circadian component. Thus, when developing Pernyi were transferred into constant darkness from a 17L : 7D regimen, the subsequent ecdyses continued to occur synchronously with gates at 22-hour intervals after lights-off (*4*). In addition, an examination of the kymograph records (Fig. 1, right) showed that ecdysis is immediately preceded by a period of hyperactivity (the emergence behavior), the pattern of which was also species-specific. The present report describes a brain-centered neuroendocrine mechanism by which ecdysis is synchronized with a light-dark regimen.

The preliminary stage of this study involved an examination of the role of various cephalic nerves, ganglia, and endocrine organs in the ecdysis of silkmoths (*5*). The operations were performed primarily on diapausing pupae of the Cecropia moth (*6*); the animals were then allowed to begin adult development, and about 3 weeks later the effect of the surgery on the ecdysis of the resulting moths was ascertained (*7*). Sectioning of the optic nerves and circumesophageal connectives or extirpation of the compound eye anlagen, the subesophageal ganglion, the frontal ganglion, or the corpora allata and corpora cardiaca was inconsequential. All the operated moths emerged within the normal Cecropia gate, that is, between 1 and 9 hours after lights-on in the 17L : 7D regimen. By contrast, when the brain was removed, the moths were no longer influenced by light. Both brainless Cecropia and brainless Pernyi emerged randomly throughout the day and night (Fig. 2B). The removal of the brain somehow interrupted the chain of events leading from the reception of light to ecdysis.

It is important to note that the brain functioned correctly even after its connections to the eyes (*8*) and the ventral nerve cord were severed. That being so, the implantation of a brain into a brainless animal should restore the competence to synchronize emergence with photoperiod. To test this hypothesis, "loose-brain" moths were prepared by removing the brain from the head of each pupa and immediately reimplanting it into the tip of the abdomen. These animals proved to be fully able to respond to the photoperiod regimen. The emergence of "loose-brain" moths was gated and occurred at about the same time as that of unoperated moths (Fig. 2C). Also, as with unoperated animals, the ecdysis rhythm of "loose-brain" moths was free-running in continuous darkness (*9*).

In order to synchronize the emergence behavior with photoperiod, a minimum of three components are nec-

essary, namely, a photosensitive system for the reception of light cues, a timing mechanism to measure the hours after lights-off or lights-on, and a mechanism to trigger the emergence behavior. The removal of any one of these components could give an apparently random emergence under the conditions of these experiments. What, then, is the role of the brain? In order to determine whether the adult brain was the site of the photosensitivity for the ecdysis rhythm (*10*), the brain was removed from each of the 20 Cecropia pupae. In ten pupae it was reimplanted into the head; in the other ten, into the tip of the abdomen. Each pupa was then placed in a hole in an opaque diaphragm which separated two photoperiod chambers (*11*). The anterior end of each pupa was exposed to a 12L : 12D regimen (photophase from 21:00 to 09:00, E.S.T.) and the posterior end was exposed to the reciprocal regimen (photophase from 09:00 to 21:00, E.S.T.). Therefore, with the exception of the brains, all pupae were exposed to the same light-dark conditions. The photoperiod which the brain "saw" determined the time of ecdysis. The moths which had their brains in their abdomens emerged early in the day "seen" by the posterior ends. Similarly, the moths which had their brains reimplanted into the anterior ends emerged during the day "seen" by the head end. These results clearly showed that the brain was photosensitive.

In order to show that the brain contains the ecdysis clock, brains were interchanged between Cecropia and Pernyi pupae (Fig. 2D). The brainless Cecropia which received implants of Pernyi brains emerged primarily at the time characteristic of Pernyi; so also, brainless Pernyi with Cecropia brain implants emerged at the normal Cecropia time. In short, the timing of ecdysis was characteristic, not of the brainless host species, but of the species which donated the brain. Thus, the moth brain also has the clock which determines the timing of ecdysis relative to the photoperiod. The circadian function of this clock most probably also resides in the brain.

It is of interest that the brainless Cecropia which had an implant of a Pernyi brain emerged at the time characteristic of Pernyi but displayed the emergence behavior of Cecropia (as in Fig. 1). The host-specific behavior pattern was also evident in brainless Pernyi which had Cecropia brains.

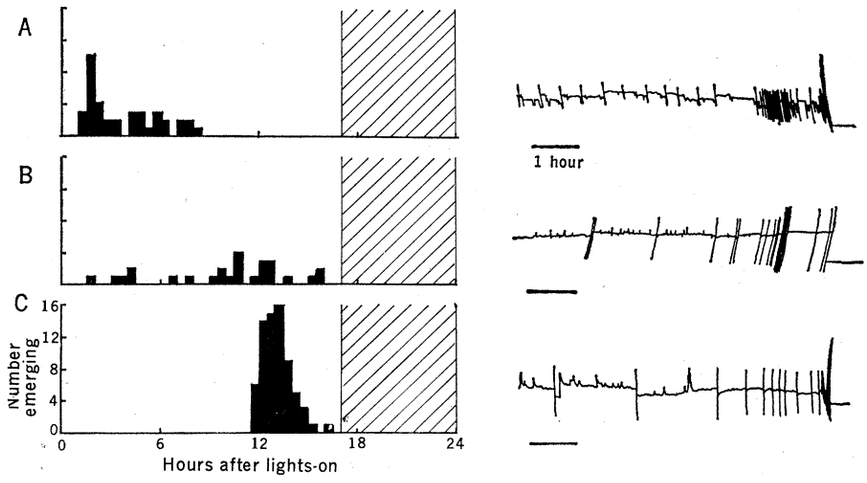

Fig. 1. The distribution of ecdyses in a 17L : 7D photoperiod regimen (left), and tracings of the kymograph records of activity accompanying ecdysis (right). (A) *Hyalophora cecropia*; (B) *Antheraea polyphemus*; (C) *A. pernyi*.

Thus, the brain is not responsible for the behavior pattern, but rather serves to synchronize it with the photoperiod. Since it can do this even while loose in the abdomen, it must exert its control through hormonal means. Moreover, from the experiments which involved the interchange of brains between Cecropia and Pernyi, we learn that this hormone is neither species- nor genus-specific. The existence of an ecdysis hormone is further supported by the fact that homogenates of the brains of pre-ecdysis Pernyi moths (in the last day before emergence) induced ecdysis at any time of the day when injected into developmentally mature individuals. Such injections were invariably followed by the onset of emergence behavior and the eventual ecdysis about 1½ to 2 hours later. The injection of an equal volume of Ringer solution or of a homogenate of the adult abdominal ganglia did not have this effect. In order to differentiate between the effect of this hormone and the "brain hormone" that controls growth and metamorphosis (*12*), we tentatively suggest the name "neurotropic ecdysis hormone."

It is important to emphasize that the varied operations did not substantially affect the ecdysis behavior itself. The species specificity and the stereotyped nature (*13*) of the behavior indicate that it is prepatterned into the thoracic

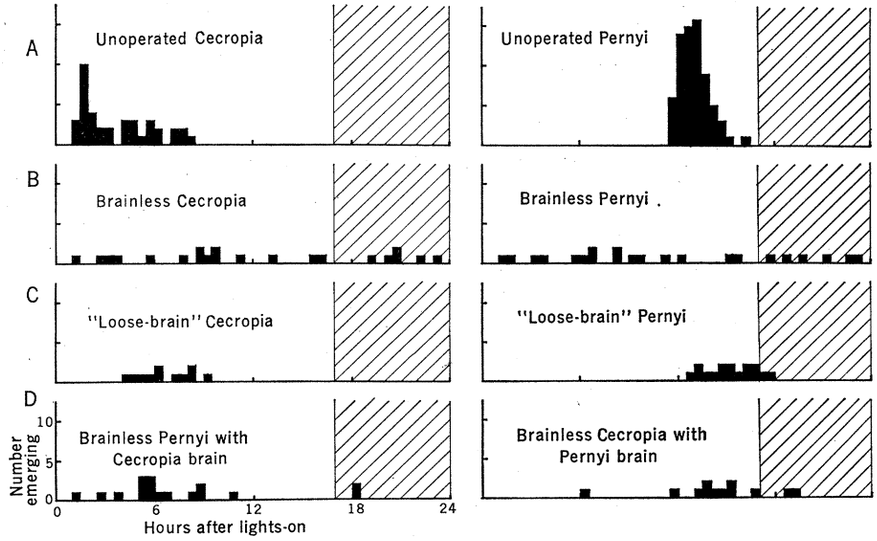

Fig. 2. Ecdyses of Cecropia and Pernyi moths in a 17L : 7D photoperiod regimen, demonstrating the effects of brain removal, the transplantation of the brain to the abdomen, and the interchange of brains between the two species.

or abdominal ganglia. The release of the hormone serves only to elicit this pattern. Thus, we see that adult ecdysis involves two separable systems. The first is comprised of prepatterned nervous information and does not involve the cephalic ganglia. The second is completely brain-centered and couples the emergence behavior to the environment through a hormonal link.

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References and Notes

1. A. S. Packard, *Mem. Nat. Acad. Sci.* **12** (part 1), 1 (1914); W. J. B. Crotch, *A Silkmoth Reader's Handbook* (Amateur Entomologists' Society, London, 1956).
2. Pupae were placed ventral side up in individual plasticine cradles. A cotton thread was attached with melted wax to the tip of the abdomen of each pupa and the animals were placed at 26°C in a room programed for the appropriate photoperiod regimen. One to 2 days prior to ecdysis, each preparation was attached by means of the thread to a lever that wrote on a smoked drum which revolved once every 2 days.
3. Gate refers to a period of the day during which developmentally mature adults are "permitted" to undergo ecdysis [C. S. Pittendrigh, *Z. Pflanzenphysiol.* **54**, 275 (1966)]. The position and width of this gate is shown by the ecdysis peak.
4. J. W. Truman, in preparation.
5. For general reviews on the control of insect rhythms see: S. K. Roberts, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965); J. Brady, *Nature* **223**, 781 (1969). By means of a histological correlation of changes in nuclear volume of the neurosecretory cells of the brain and the cells of the corpora allata, an interaction of these two organs has been postulated in the control of the *Drosophila* ecdysis rhythm [L. Rensing, *Science* **144**, 1586 (1964)].
6. For the techniques of operating on silkworm pupae see: C. M. Williams, *Biol. Bull.* **90**, 234 (1946); *ibid.* **116**, 323 (1959); H. A. Schneiderman, in *Methods in Developmental Biology*, F. H. Wilt and N. K. Wessels, Eds. (Crowell, New York, 1967).
7. Unlike the situation found in cockroaches [D. Bodenstein, *J. Exp. Zool.* **129**, 209 (1955)], extensive outgrowth of nerves from cut connectives did not usually occur in these preparations. Apparently during metamorphosis the neural lamella heals over the cut ends; unless the sectioned ends are in close proximity, regeneration of connections does not occur. Representatives of each group were autopsied to assure that regeneration had not occurred.
8. Prior work with an eyeless mutant of *Drosophila* has shown that the eyes are unnecessary for the entrainment of the ecdysis rhythm by light [W. Engelmann and H. W. Honneger, *Naturwissenschaften* **53**, 588 (1966)].
9. J. W. Truman, in *Proceedings of the Symposium on Biochronometry* (Friday Harbor, Washington, 4–6 September 1969), M. Menaker, Ed. (National Academy of Sciences, Washington, D.C., 1970).
10. The photosensitivity of the pupal brain has been demonstrated for the photoperiodic termination of pupal diapause in the Pernyi silkworm [C. M. Williams and P. L. Adkisson, *Biol. Bull.* **127**, 511 (1964)].
11. The chamber used for this experiment was the same as used in the study of Williams and Adkisson (*10*).
12. C. M. Williams, *Biol. Bull.* **90**, 234 (1946); ———, in *Insects and Physiology*, J. W. L. Beament and J. E. Treherne, Eds. (Oliver and Boyd, London, 1967).
13. In Cecropia the behavioral sequence always had three distinct phases: a period of hyperactivity, followed by a similar period of reduced activity, and finally a short burst of hyperacitvity which culminated in ecdysis. The duration of the entire sequence was 72 ± 9 minutes ($N = 52$).
14. Supported in part by NSF grant GB-7966 to L.M.R. and by a NSF predoctoral fellowship to J.W.T. We thank Professor C. M. Williams for helpful discussions during the investigation and the preparation of the manuscript.

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Reassembly of Living Cells from Dissociated Components

Abstract. *Combining the techniques of nuclear transplantation and cytoplasmic transfer, dissociated amoeba nuclei, cytoplasm, and membranes were reassembled to form viable amoebae. The techniques of cell reassembly appear to be sufficiently adequate so that any desired combination of cytoplasm, nucleus, and membrane can be assembled into living cells.*

Interest has developed in the possibility of synthesizing living cells and in reassembling living cells from isolated cell components. In 1965 Price proposed that such syntheses be made an American national goal (*1*). After participating in a symposium on the experimental synthesis of living cells (*2*), we decided that we had the means to carry out the reassembly of *Amoeba proteus* from its major components: namely nucleus, cytoplasm, and cell membrane. We have now shown that new viable amoebae may be produced with the membrane of one cell, cytoplasm from one or more other cells, and nucleus from a third cell. These three components are nonviable individually, and when any of them is missing the reassembled cell cannot live.

The basic procedures of reassembly are (i) removal of the nucleus from an amoeba, (ii) removal of the cytoplasm to the extent that the remaining part cannot survive even when a nucleus has been inserted, (iii) injection of desired cytoplasm to refill the above, and (iv) insertion of a nucleus.

The first two steps can be carried out either by (i) enucleation and removal of cytoplasm by micrurgical methods (*3*) or by (ii) centrifugation of cells at high speed. In a single cycle with the use of micrurgical methods involving removal of cytoplasm and nucleus and refilling the membrane with cytoplasm from another cell, only about 75 percent of the original cytoplasm can be withdrawn. On refilling with cytoplasm, the volume can be brought to about 75 percent of the original. Thus a single cycle results in a cell, the cytoplasm of which still consists of one-third of the original cytoplasm; however, two cycles would reduce this to one-ninth.

Table 1. Results of reassembly experiments. Letters A, B, and C represent different strains used in an experiment. In actual experiments, five strains (D, P, Q, S, and G) were used, but they were grouped together for simplification. Suffixes n, c, and m denote nucleus, cytoplasm, and cell membrane, respectively. Superscripts indicate the source of a component, for example, in the experiment with $A_n' + A_c'' + A_m'''$ the superscripts indicate that the nucleus, cytoplasm, and membrane came from three separate cells of strain A.

Combination	Cells (No.)		Clone (No.)	Viable (%)
	Studied	Dividing		
$A_n' + A_c'' A_m''$	200	172	170	85
$A_n' + A_c'' + A_m'''$	58	48	43	74
$A_n + B_c' B_m'$	168	72	0	0
$A_n' + A_c'' + B_m$	244	82	2	<1
$A_n + B_c + C_m$	22	4	0	0

For removal of nuclei and cytoplasm by centrifugation, amoebae are layered over 10 percent Ficoll (*4*) and centrifuged first for 5 minutes at 30,000*g* and then for 30 seconds at 40,000*g* at 4°C. The centripetal portions of the cells contain little particulate cytoplasm, except for colored lipid droplets, and are nonviable even after renucleation. The subsequent refilling with cytoplasm and renucleation (the last two steps above) are performed by micrurgical methods in both cases.

Where cytoplasm, membrane, and nucleus are all obtained from cells of the same strain, reassembly is relatively easy and 80 percent of the reassembled cells are normal amoebae, behaving and reproducing so as to be indistinguishable from cells of the original clone (Table 1, experiment 2). However, when one or two of the three components are from different strains, only a small proportion of the reassembled amoebae form viable clones. The great majority are able to maintain normal functions only for a limited period, sometimes dividing not more than four times (Table 1, experiments 3–5). It is likely that the failure of these cells to form clones is partially due to the newly discovered interstrain lethal factors (*5*). However, that we have already obtained