It is interesting to speculate on the mechanism of action of the vWf that would be consistent with a primary role for the carbohydrate moiety. One possibility is that the platelet membrane glycosyl transferases that are capable of transferring single units from one glycoprotein acceptor onto other glycoprotein acceptors actually result in a complex between the enzyme (that is, the platelet enzyme) and the acceptor (that is, the glycosyl residue of the fVIII/vWf protein) (*29*). Perhaps a similar enzymatic activity situated in the subendothelium would permit the vWf to act as a bridge between the subendothelium and the platelet. This type of mechanism may be of more general interest since it may underlie a variety of cell-cell adhesion phenomena (*30*).

HARVEY R. GRALNICK
BARRY S. COLLER
Hematology Service, Clinical Pathology Department, National Institutes of Health, Bethesda, Maryland 20014
YVETTE SULTAN
Hôpital Saint-Louis, Paris, France

References and Notes

1. W. Lehmann, *Acta Genet. Med. Gemellol.* **8** (Suppl. 2), 38 (1959); I. M. Nilsson, M. Blombäck, E. Jorpes, B. Blombäck, S.-A. Johansson, *Acta Med. Scand.* **159**, 35 (1957); E. A. von Willebrand, *Fin. Laekaresaellsk. Handl.* **68**, 87 (1926).
2. E. J. Bowie, P. Didisheim, J. H. Thompson, Jr., C. A. Owen, *Hematol. Rev.* **1**, 1 (1968).
3. T. S. Zimmerman, O. D. Ratnoff, A. E. Powell, *J. Clin. Invest.* **50**, 244 (1971); D. P. Stites, E. J. Hershgold, J. D. Perlman, H. H. Fudenberg, *Science* **171**, 196 (1971).
4. M. D. Howard and B. G. Firkin, *Thromb. Diath. Haemorrh.* **26**, 362 (1971).
5. D. Meyer, C. S. P. Jenkins, M. Dreyfus, M. J. Larrieu, *Nature (London)* **243**, 293 (1973); H. J. Weiss, L. W. Hoyer, F. R. Rickles, A. Varma, J. Rogers, *J. Clin. Invest.* **52**, 2708 (1973).
6. Factor VIII/von Willebrand factor (fVIII/vWf): the term factor VIII/von Willebrand factor denotes the purified material from normal cryoprecipitate having the biologic properties of (i) correcting the coagulation defect in hemophilic plasma and (ii) correcting the defect in platelet retention in a glass bead column and interacting with ristocetin to induce aggregation of von Willebrand's disease platelets or normal platelets made unresponsive to ristocetin.
7. P. A. McKee, J. C. Andersen, M. E. Switzer, *Ann. N.Y. Acad. Sci.* **240**, 8 (1975).
8. H. R. Gralnick and B. S. Coller, *Blood* **46**, 417 (1975).
9. B. Bennett, W. B. Forman, O. D. Ratnoff, *J. Clin. Invest.* **52**, 2191 (1973).
10. G. A. Shapiro, J. C. Andersen, S. V. Pizzo, P. A. McKee, *ibid.*, p. 2198; H. R. Gralnick, B. S. Coller, S. L. Marchesi, *Thromb. Res.* **6**, 93 (1975).
11. M. Legaz, G. Schmer, R. Counts, E. Davie, *J. Biol. Chem.* **248**, 3946 (1973).
12. S. L. Marchesi, N. R. Shulman, H. R. Gralnick, *J. Clin. Invest.* **51**, 2151 (1972).
13. H. R. Gralnick, B. S. Coller, Y. Sultan, *ibid.* **56**, 814 (1975).
14. J. Montreuil and G. Biserte, *Bull. Soc. Chim. Biol.* **41**, 959 (1959).
15. C. Leblond, R. Glegg, D. Eidinger, *J. Histochem. Cytochem.* **5**, 445 (1957).
16. S. Erickson and C. Larsson, *N. Engl. J. Med.* **292**, 176 (1975).
17. G. L. Nelsestuen and J. W. Suttie, *Biochem. Biophys. Res. Commun.* **45**, 198 (1971).
18. A. G. Morell, G. Gregoriadis, I. H. Scheinberg, J. Hickman, G. Ashwell, *J. Biol. Chem.* **246**, 1461 (1971).
19. J. R. Durocher, R. C. Payne, M. E. Conrad, *Blood* **45**, 11 (1975).
20. J. Martinez, J. E. Palascak, C. L. Peters, in *Abstracts of the 5th Congress of the International Society on Thrombosis and Haemostasis* (1975), p. 34.
21. K. F. Mori, *Endocrinology* **85**, 330 (1969).
22. S. Kornfeld, *Biochemistry* **7**, 945 (1968); E. Morgan, G. Marsaglia, E. Giblett, C. Finch, *J. Lab. Clin. Med.* **69**, 370 (1967).
23. W. M. Watkins, *Science* **152**, 172 (1966); C. Race and W. M. Watkins, *FEBS Lett.* **27**, 125 (1972).
24. J. Vermylen, G. de Gaetano, M. B. Donati, M. Verstraete, *Br. J. Haematol.* **26**, 645 (1974); A. T. Nurden and J. P. Caen, *ibid.* **28**, 253 (1974).
25. T. B. Tschopp, H. J. Weiss, H. R. Baumgartner, *J. Lab. Clin. Med.* **83**, 296 (1974).
26. E. A. Jaffe, L. W. Hoyer, R. L. Nachman, *J. Clin. Invest.* **52**, 2757 (1973); *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1906 (1974).
27. L. A. Harker, R. Ross, S. J. Slichtar, in *Abstracts of the 5th Congress of the International Society on Thrombosis and Haemostasis* (1975), p. 56.
28. E. J. W. Bowie, V. Fuster, C. A. Owen, Jr., A. L. Brown, in *ibid.*, p. 322.
29. H. B. Bosmann, *Biochim. Biophys. Acta* **279**, 456 (1972).
30. S. Roseman, *Chem. Phys. Lipids* **5**, 270 (1970); in *Biology and Chemistry of Eukaryotic Cell Surfaces*, E. Lee and E. Smith, Eds. (Academic Press, New York, 1974), p. 317.

31 October 1975; revised 5 December 1975

Insect Photoperiodism: The "T Experiment" as Evidence for an Hourglass Mechanism

Abstract. *"T experiments" demonstrate that the clock controlling termination of larval diapause in* Ostrinia nubilalis *is an hourglass mechanism that measures 8 hours of darkness. Circadian oscillations are not involved in photoperiodic time measurement in this species.*

Many insect species display a seasonal discontinuity in their life cycles (*1*). In temperate latitudes reproduction and development routes are characteristic of spring and summer months. A state of arrested development known as diapause, however, is entered during late summer and early fall, which enables the insects to withstand the environmental rigors of winter. When induction and termination of diapause are controlled by the daily alternation of light and dark in the environment, this is referred to as photoperiodism (*2*).

The central problem in the study of both animal and plant photoperiodism has been that of elucidating the nature of the clock that measures the duration of the day (or night). For 30 years, evidence has been accumulating that circadian clocks are somehow involved in photoperiodism. Circadian clocks are endogenous oscillations that have a period length of about a day when measured under constant conditions of light and temperature. They control the timing of many metabolic and behavioral events in eukaryotic organisms.

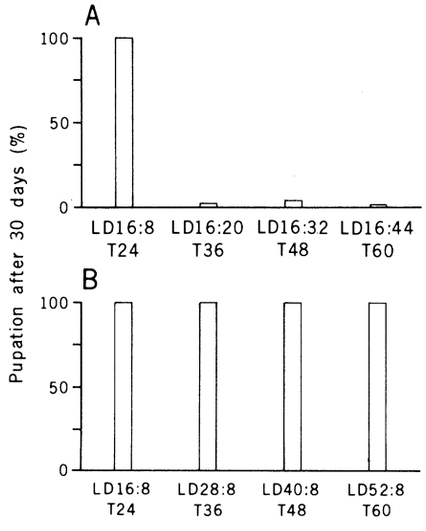

Fig. 1. Percentage of pupation 30 days after transfer from LD 12 : 12 to various T's. In (A), L was held constant at 16 hours and T was varied by extending D. In (B), D was held constant at 8 hours and T was varied by extending L. In both (A) and (B) the data from the four replicates in each light cycle are combined. The sample size is thus 100.

Although an extensive search for a common photoperiodic timing mechanism involving the circadian clock was made in the 1960's, Pittendrigh (*3*) has suggested that a diversity of mechanisms exist. Two different models of the photoperiodic clock, external and internal coincidence, do involve circadian oscillations. A third, however, views the clock as an hourglass or interval timer which measures the length of the night by the accumulation of some unknown metabolic product. Light destroys the product (resets the hourglass). This model of the clock is nonoscillatory in nature.

The experimental paradigm most commonly used to demonstrate that a circadian oscillation is involved in photoperiodic time measurement is referred to as a "T experiment," where T is the period of the light-dark cycle in hours. In a standard T experiment the organisms are exposed to cycles in which a fixed light phase is systematically coupled to dark phases of various durations, thus creating different T's (*4*). When photoperiodic induction rises and falls as a function of T, neither the length of the light phase nor that of the dark phase is responsible for the response. Although the rationale

is too lengthy to pursue here, results from T experiments have been interpreted in terms of the external (*5*) or internal coincidence model (*6*).

Beck (*7*) concluded that induction of diapause in the European corn borer, *Ostrinia nubilalis*, is controlled by an hourglass that measures the dark phase. The optimal light-dark cycle for inducing diapause is 12 hours of light alternating with 12 hours of darkness (LD 12:12; T24). Pittendrigh (*8*) concurred, but in a reanalysis of Beck's data stressed that time measurement occurs most effectively when T = 24 hours, that is, when the circadian system is in what he termed resonance with the light cycle. When borers are raised with T's of other than 24 hours (for example, LD 24:12; T36) the amount of induction drops significantly (100 percent in T24; 65 percent in T36). A replotting of Beck's data by Pittendrigh yielded a circadian surface or topography with a peak at T24. Since Beck's experiments did not extend beyond T40 it was not possible to completely rule out circadian oscillations as the basis for photoperiodic time measurement in this species.

McLeod and Beck (*9*) have demonstrated that termination of diapause in *Ostrinia* is also photoperiodically controlled (optimal photoperiod, LD 16 : 8). Our T experiments show that the type of clock involved in this response resembles an hourglass. Larvae were reared from eggs in LD 12:12 at 30°C on an artificial diet (*10*). After about 22 days, the larvae reach the end of the last larval instar. They cease to feed and enter diapause. The incidence is invariably between 95 and 100 percent. Diapausing larvae were collected and placed in plastic containers (13 by 18 by 5 cm) layered with filter paper that was continuously saturated with distilled water. Twenty-five larvae were placed in each container, and all experiments consisted of four replicate containers. Within a week after the larvae had entered diapause, the containers were placed in lighttight black boxes containing ceiling-mounted 7-watt cool white fluorescent tubes (Sylvania F4T5/D), which were water-jacketed to prevent temperature cycles. The light intensity at floor level was 200 lux. The boxes were ventilated by a small fan connected to a system of baffles; they were kept in a temperature-controlled room at 30°C. Pupation was assayed daily as an indicator of diapause termination. We assume that termination is photoperiodically controlled and that postdiapause morphogenesis proceeds at a fixed rate which, as in *Drosophila* (*11*), is independent of photoperiod. Larval populations maintained at 30°C in LD 12:12 are characterized by high mortality (approximately 70 percent) and a mean time to pupation of about 50 days for the survivors, whereas populations switched to LD 16:8 have a mean pupation time of 14 days and low larval mortality (approximately 10 percent). By examining diapause termination rather than induction, the effects of long T's (for example, T60) can be studied without the added complications of larval feeding and the fact that developing larvae go through several complex stages in their rather brief (22 days at 30°C) developmental sequence before they enter diapause.

We first conducted a standard T experiment in which the light phase was held constant at 16 hours and was coupled with 8, 20, 32, or 44 hours of darkness (thus creating T's of 24, 36, 48, and 60 hours) (Fig. 1A). Neither the amount of light (constant in each T) nor periods of darkness greater than 8 hours are the measured intervals. Since a peak is not observed at T48, a circadian oscillation is probably not involved in time measurement.

The interpretations based on data derived from the standard T experiment were strengthened when we changed the experimental protocol. Instead of holding L constant, D was held constant at 8 hours, and T was varied by extending L. This experiment gave positive results in all cases (Fig. 1B). The interval that is measured and that results in termination of diapause is 8 hours of darkness. Photoperiodic time measurement occurs irrespective of the absolute value of T. The system thus acts like an hourglass, and the fact that the longer T's (for example, LD 52:8; T60) result in 100 percent pupation after 30 days indicates that the number of 8-hour dark phases required for maximum induction is small.

The only other clear case in insects where circadian oscillations have been ruled out in photoperiodic time measurement is in the aphid *Megoura viciae* (*12*). In this insect the production of sexual and parthenogenetic females is under photoperiodic control. Time measurement is effected by an hourglass mechanism.

M. F. Bowen
S. D. Skopik
Department of Biological Sciences, University of Delaware, Newark 19711

References and Notes

1. P. L. Adkisson, *Science* **154**, 234 (1966).
2. S. D. Beck, *Insect Photoperiodism* (Academic Press, New York, 1968).
3. C. S. Pittendrigh, *Proc. Natl. Acad. Sci. USA* **69**, 2734 (1972).
4. K. K. Nanda and K. C. Hamner, *Bot. Gaz. (Chicago)* **120**, 14 (1958).
5. J. A. Elliott, M. H. Stetson, M. Menaker, *Science* **178**, 771 (1972); M. H. Stetson, J. A. Elliott, M. Menaker, *Biol. Reprod.* **13**, 329 (1975).
6. D. S. Saunders, *J. Insect Physiol.* **20**, 77 (1974).
7. S. D. Beck, *Biol. Bull.* **122**, 1 (1962).
8. C. S. Pittendrigh, *Z. Pflanzenphysiol.* **54**, 275 (1966).
9. D. G. R. McLeod and S. D. Beck, *Biol. Bull.* **124**, 84 (1963).
10. H. H. Shorey, *J. Econ. Entomol.* **56**, 536 (1963).
11. C. S. Pittendrigh and S. D. Skopik, *Proc. Natl. Acad. Sci. USA* **65**, 500 (1970).
12. A. D. Lees, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), pp. 351–356.
13. We thank P. Burbutis and G. Curl for discussions and a continuous supply of eggs, and N. Patel and M. Stetson for discussions, suggestions, and critical reviews of the manuscript. Support was provided by a UNIDEL grant to the Department of Biological Sciences.

19 November 1975; revised 9 January 1976

Tin: A Potent Inducer of Heme Oxygenase in Kidney

Abstract. *Tin greatly enhances heme breakdown in kidney, thus impairing heme-dependent cellular functions, such as cytochrome P-450 mediated drug biotransformation. This novel action of the metal results from a potent induction effect on heme oxygenase, the enzyme that catalyzes heme oxidation in microsomes. The possible toxicological implications of this tin effect in the kidney merit further investigation.*

Heme, derived from hemoglobin and various cellular hemoproteins, is degraded to biliverdin by the microsomal enzyme heme oxygenase (*1*). This enzyme is inducible in liver by certain endogenous compounds (*2*), as well as by certain trace and heavy metals (*3*). These metals are quite potent inducers of hepatic heme oxygenase, cadmium and cobalt, for example, enhancing the rate of heme oxidation from 5 to 15 times the normal rate. Metal induction of heme oxidation is associated with concomitant and marked inhibitory effects on heme-protein mediated cellular functions such as the cytochrome P-450 dependent detoxification of drugs, carcinogens, and environmental chemicals (*3*).

Normally the heme oxygenase activity of tissues other than liver and spleen is not great, nor have such tissues been shown to be particularly responsive to inducers of this enzyme activity. However, during the course of studies of