

enzyme activity is unaffected by transplantation of the tubules. A second observation made by Friedman and Johnson (1) is that this onset of increased activity coincides with the expected time of eclosion in constant conditions. Neither of these statements, however, warrants the conclusion that there is a timer which assures synchrony of the two developmental events in a normal environment: that is, in a light-dark cycle where emergence is a temporally regulated, "gated" phenomenon. If urate oxidase activity can be shown to be similarly gated in a light-dark cycle, then a hypothesis invoking clock control of enzyme activity might be acceptable.

Friedman and Johnson suggest the possibility that "components of the same clock regulating emergence also function in the Malpighian tubules to control the time of appearance of urate oxidase activity in the tubules." The eclosion clock is an accurate timer, but the "developmental clock" in Malpighian tubules has not been shown to be any more than a sequence of biochemical reactions (developmental events) leading to the expression of urate oxidase activity. If it is an autonomous timer analogous to the better studied clock that regulates activity and eclosion, then it should be possible to demonstrate resetting behavior and temperature compensation in addition to a gated expression of enzyme activity. Until this is achieved, it seems premature to speak of a "clock" that resides in the Malpighian tubules.

F. ROB JACKSON

Department of Biology
University of California,
Los Angeles 90024

References

1. T. B. Friedman and D. H. Johnson, *Science* **197**, 477 (1977).
2. C. S. Pittendrigh and S. D. Skopik, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 500 (1970).
3. J. E. Harker, *The Physiology of Diurnal Rhythms* (Cambridge Univ. Press, London, 1964).
4. F. R. Jackson, unpublished data.
5. C. S. Pittendrigh, in *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974).

7 October 1977

Jackson (1), though he sees no fault with the data in our report (2), objects to the speculative remarks in the last paragraph. In our opinion Jackson's comments regarding our investigation can be condensed into the following two statements: (i) If there is only precedence in the literature for X, then one should not broach the possibility of Y. (ii) There are still important questions to be answered regarding the temporal control of urate oxidase activity in *Drosophila melanogaster*. We agree with the latter but not with the former.

We were aware (2) that Pittendrigh and Skopik (3) demonstrated that head eversion, yellow eye pigmentation, and ocellar bristle pigmentation in *Drosophila pseudoobscura*, *D. victoria*, and *D. melanogaster* are not coupled to the circadian oscillator which gates emergence and determines locomotor activity rhythms (4). Whether the onset of urate oxidase activity in the adult is coupled with emergence is an important question. The hypothesis that the appearance of urate oxidase activity in the adult and emergence behavior are coupled can be tested by phase-resetting experiments and transplant experiments (5). If there is absolute temporal coupling between the appearance of urate oxidase activity and emergence, another important inquiry must focus on the mechanism responsible. The contributions of humoral factors (6), emergence hormones (7), endogenous oscillators (3, 4), and the developmental clock in the tubules (2) will have to be considered.

Jackson stated (1) that the "developmental clock" in Malpighian tubules has not been shown to be any more than a sequence of biochemical reactions (developmental events) leading to the expression of urate oxidase activity." We hasten to add that we have only really studied the behavior of the time-keeping mechanism in the Malpighian tubules and have yet to show directly that the timer in the tubules can be explained on the basis of a sequence of biochemical reactions, although we dogmatically and

faithfully presume that all life forms and behaviors can be reduced to chemical reactions and interactions including temperature-compensated endogenous oscillators.

THOMAS B. FRIEDMAN

Department of Zoology, Michigan
State University, East Lansing 48824

DANIEL H. JOHNSON

Department of Biological Sciences,
Oakland University,
Rochester, Michigan 48063

References and Notes

1. F. R. Jackson, *Science* **200**, 1185 (1978).
2. T. B. Friedman and D. H. Johnson, *ibid.* **197**, 477 (1977).
3. C. S. Pittendrigh and S. D. Skopik, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 500 (1970).
4. R. Konopka and S. Benzer, *ibid.* **68**, 2112 (1971).
5. T. B. Friedman and D. H. Johnson, in preparation.
6. J. D. Gee, D. L. Whitehead, J. Koolman, *Nature (London)* **269**, 238 (1977).
7. J. W. Truman, *Am. Sci.* **61**, 700 (1973); _____, A. M. Fallon, G. R. Wyatt, *Science* **194**, 1432 (1976).
8. We thank J. Asher, M. Balaban, P. Friedman, and L. Robbins for their helpful discussions.

20 March 1978

Electropositive Potential Measured in Plant Protoplast

While being grateful to Racusen *et al.* (1) for citing us on the above subject, we would like to point out that our work referred to as unpublished in their report has appeared already (2).

R. HELLER, J. P. RONA

D. CORNEL, C. GRIGNON

Laboratoire de Physiologie Végétale,
Université de Paris 7,
75221 Paris Cedex 05, France

References

1. R. H. Racusen, A. M. Kinnersley, A. W. Galston, *Science* **198**, 405 (1977).
2. J. P. Rona, C. R. Acad. Sci. Ser. D **227**, 185 (1973); R. Heller, C. Grignon, J. P. Rona, in *Membrane Transport in Plants*, U. Zimmerman and J. Dainty, Eds. (Springer-Verlag, New York, 1974), p. 239; J. P. Rona, D. Cornel, R. Heller, *Colloq. Int. CNRS No. 258* (1976), p. 349; *Biochem. Bioenerg.* **4**, 185 (1977).

30 January 1978