Table 2. The water potential, measured with a thermocouple psychrometer, of five leaves of a pepper plant and of the nutrient solution in which the plant was growing.

Sample	Water potential (bars)
Leaf 1	−4.6
Leaf 2	−4.0
Leaf 3	−4.3
Leaf 4	−4.3
Leaf 5	−4.0
Nutrient solution	−7.2

ured here the gradient in vapor pressure away from the leaves would not affect e_{air} significantly if the length of the equivalent diffusion path caused by atmospheric resistance to vapor diffusion was < 1 cm; undoubtedly, atmospheric resistance in the wind tunnel was not so great. The observed values for k are given in Table 1.

The k for tobacco ranged from an average high of about 3.4×10^{-8}, when 95 percent of the initial leaf weight remained, to about 1.4×10^{-8} when 56 percent of the initial weight remained. The k for laurel also decreased with water loss. So little water was lost from philodendron that no decrease in permeability was detected. In all three species, leaf permeability was sufficiently small to cause considerable error in measurement of water potential in leaves with this psychrometer. For example, if 20 cm^2 of leaf at the greatest hydration shown in Table 1 were placed into the psychrometer for which Fig. 1 is drawn, the relative error of measurement for tobacco would be about 0.14; for laurel, about 0.36; and for philodendron, about 0.85. At the least hydration, the error for tobacco would be about 0.24 and for laurel about 0.66.

To determine how closely Eq. 6 predicts the evaporation rate from a drop in a real psychrometer, a drop was measured onto the wet bulb, θ was measured as time passed, and finally the total loss from the drop was measured. As is seen by integrating Eq. 6, the area under the curve relating observations of θ to t equals the loss in mass from the drop divided by a term which is constant for any psychrometer at constant temperature. An idealized psychrometer with a spherical sample chamber is assumed in Eq. 12 and Fig. 1; different geometry would be expected to change the evaporation rate from the drop. For a cylindrical chamber 1.5 cm in diameter and 5.5 cm long, with other variables the same as for Fig. 1, loss from the drop was within 10 percent of that predicted by Eq. 6 for a spherical chamber with $r_c = 1.0$ cm; the errors predicted above are realistic.

The magnitude of error that can be introduced by the presence of the water drop in the chamber is illustrated in Table 2. The pepper plant that yielded these data grew in nutrient solution, with polyethylene glycol added to decrease the water potential in the root. Prior to sampling, the plant was kept in the dark overnight to slow transpiration. The water potentials of five leaves and of the nutrient solution were determined in psychrometers similar to the one described in the preceding paragraph. The data show the water potential of the leaves to be about 3 bars higher than that of the nutrient solution. Since the water potential gradient must be in the opposite direction for water to move passively from the roots to the leaves, these data indicate that, without a correction for leaf permeability, large errors may be introduced into measurements made with this thermocouple psychrometer of water potential in leaves.

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References and Notes

1. L. A. Richards and G. Ogata, *Science* **128**, 1089 (1958).
2. C. F. Ehlig, *Plant Physiol.* **37**, 288 (1962).
3. W. H. McAdams, *Heat Transmission* (McGraw-Hill, New York, 1954), p. 181.
4. ———, *ibid.*, p. 15.
5. D. N. Moss, *Conn. Agr. Expt. Sta. New Haven Bull. No. 664* (1963).

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Biological Rhythms: A New Type in Strains of a Mutant of Neurospora crassa

Abstract. *The formation of growth bands in* Neurospora, *with periods ranging from 15 to 90 hours, depends strongly on temperature and on composition of the growth medium, but not upon cycles of light and dark. The rhythm is endogenous but not "circadian."*

Insight into cellular processes may be gained by observation of biological rhythms. Goodwin (*1*) explains in his recent book how the observed period of these rhythms can be determined by a statistical treatment of biochemical reactions. Endogenous daily rhythms with a period of approximately 24 hours, which have been termed "circadian" rhythms (*2*), are prominent examples of systems of this kind. However, these rhythms exhibit a number of additional special properties which relate to their inferred role in a biological clock mechanism. First, they may be synchronized, within limits, by appropriate light-dark cycles simulating day-night conditions of illumination. Second, in the absence of light-dark cycling (under constant conditions of light and temperature), the rhythms persist with a well-defined period close to 24 hours. Their deviations from 24 hours are useful diagnostic features since we thereby deduce that no environmental factor is synchronizing the rhythm. Third, the period, when observed under constant conditions, is relatively independent of the physical and chemical aspects of the environment; the so-called temperature-independence of biological clocks is well known.

We have examined mutants of *Neurospora crassa* that possess an endogenous rhythm which is not circadian in character. The role of this rhythm may only be related to a series of repetitive biochemical events rather than serve as a special adaptation to the day-night cycle. The linear growth rate of the several "clock" mutants (*3*) is constant, but their hyphae form a regular series of periodic bands by increasingly dense terminal branching toward the end of a period (Fig. 1). A new growth band is initiated by the relatively small number of surface hyphae advancing beyond the terminal growth edge at random locations. This process is repeated at regular intervals depending on temperature and medium composition. In contrast to previously reported rhythmic *Neurospora* isolates (*4*) Sussman's strains form very distinct bands with no conidia between 16° and 35°C. Formation of bands continues as long as adjacent fresh medium is available for growth. Since there is no overgrowth from one band to another, a permanent record of their growth pattern is left. No bands form on a liquid medium, but they do form on Millipore filters floating on liquid medium.

Although some of the mutants have a period of about 24 hours at room temperatures, the rhythm bears no special relation to the light-dark cycle and it is not possible to demonstrate synchronization. When strain A is main-

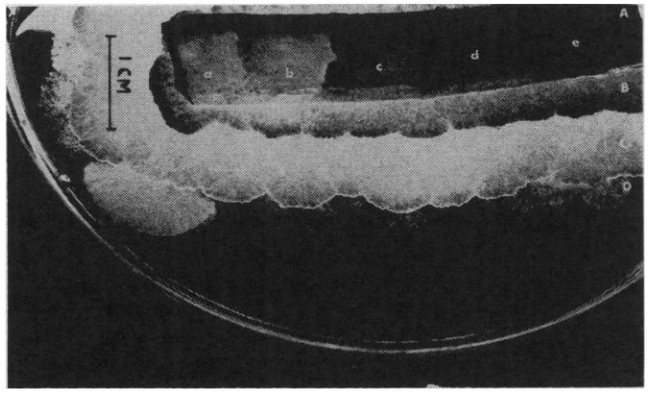

Fig. 1 (above). *Neurospora crassa*, Sussman's "clock" strain, 16g-3c-A/cl-CLlla, A strain. *A*, Inoculum strip consisting of five (*a–e*) growth rings planted on fresh medium. *B*, First growth ring showing independence of new growth from the age of the inoculum. *C*, Second growth ring. *D*, Beginning of a third growth ring at different points on the perimeter of a completed band. Within a few hours the hyphal initials will merge to form a band with a continuous growth front.

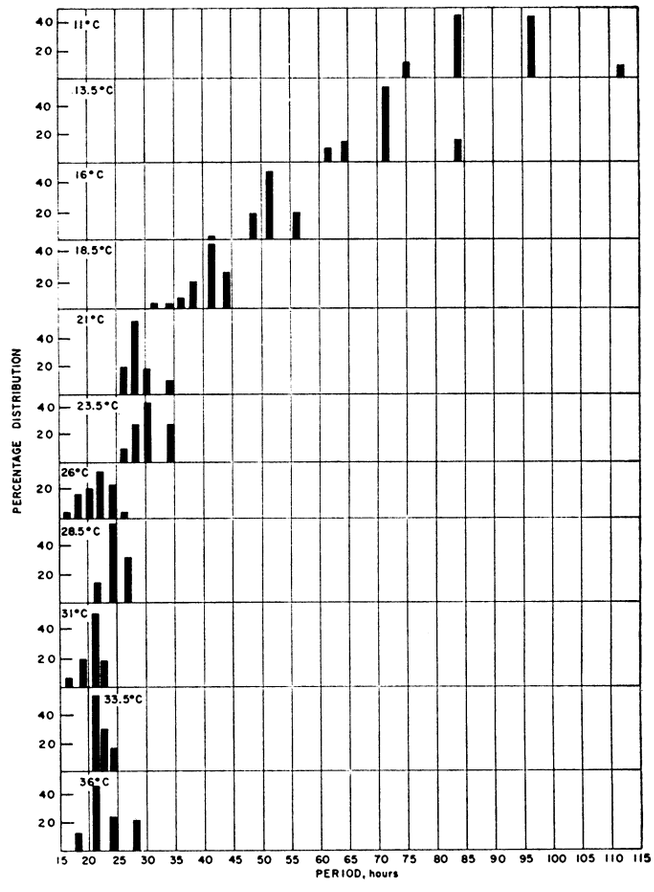

Fig. 2 (right). *Neurospora crassa*, strain A (Sussman's designation: 16g-3c-A/cl-CLlla): Percentage distribution of the periods in total darkness on complete medium. From 11° to 25°C the growth rate increases from 3 to 13 mm/day and then decreases again to 3 mm/day at 36°C.

tained on a regime of 12 hours of light and 12 of darkness, the period at 26°C was 22 hours; at 21°C it was 28 hours. These periods are essentially identical with periods measured at these temperatures in the dark (Fig. 2). There was marked temperature-dependence of this rhythm. Periods much longer than 24 hours occur at lower temperatures.

The period of these *Neurospora* mutants is also dependent upon the composition of the medium. At 26°C in darkness the period of strain C-a15 × T^{5}-CL 199 is approximately 30 hours on Mycophil agar (Baltimore Biological Laboratories) and 50 hours on corn meal agar with dextrose, yeast extract, and malt extract. The growth rate is the same on both.

In the search for a time-phasing or time-setting mechanism for this clock we observed that cutting an agar inoculum block or strip and setting it on fresh medium induced the start of a new band, regardless of whether the inoculum was from the leading hyphae or from anywhere else in a newly completed band, or one that was several days old. Figure 1 shows this strikingly. The time of inoculation determines the starting time for a new series of bands irrespective of time of day or illumination cycle. The cutting in itself is not the operating factor; a cut with a hot needle will not appreciably change the growth pattern of a culture, wherever made.

This unique setting mechanism indicates a biochemical basis of control of the rhythm, which appears to be caused by either the depletion or the accumulation of some chemicals in the growth medium. Additional evidence for this interpretation is given by the experiments showing that, through changes in the chemical composition of the growth medium, the periods of these mutants at a given temperature could be changed by a factor of 2 or more without proportional changes in the linear growth rates.

In circadian rhythms the mechanism of the control exerted by biochemical reactions remains obscure, though Karakashian and Hastings (*5*) showed a blocking effect of actinomycin D and puromycin. This "chemical insensitivity" has been attributed to compensation mechanisms. In the rhythm described here these compensatory mechanisms are absent. In addition, these *Neurospora* mutants are auxotrophic; hence some of the essential intermediates in the loops controlling biochemical oscillations may be supplied by the medium.

For these reasons these organisms may be particularly well suited to elucidate the biochemical nature of cellular oscillatory mechanisms giving rise to periods in excess of 12 hours.

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References and Notes

1. B. C. Goodwin, *Temporal Organization In Cells* (Academic Press, New York, 1963).
2. E. Bünning, *The Physiological Clock* (Academic Press, New York, 1964); C. S. Pittendrigh, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159 (1961); J. W. Hastings, *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1964), vol. 1, p. 333.
3. A. S. Sussman, R. T. Lowry, T. Durkee, *Am. J. Botany* **51**, 243 (1964).
4. C. S. Pittendrigh, V. G. Bruce, N. S. Rosensweig, M. L. Rubin, *Nature* **184**, 169 (1961).
5. M. Karakashian and J. W. Hastings, *Proc. Natl. Acad. Sci. U.S.* **48**, 2130 (1962).
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