Circadian Leaf Movements: Persistence in Bean Plants Grown in Continuous High-Intensity Light

Abstract. *A circadian leaf movement has been found in* Phaseolus vulgaris *var. Pinto that will persist under constant temperature and continuous fluorescent light. The period of leaf movement was approximately 26 hours in length, and the amplitude did not diminish for at least 4 weeks. Leaf movements were similar for plants grown in either 1100, 4950, 7700, or 10,450 lumens per square meter. This is the first clearly defined persistent circadian rhythm reported for leaves of higher plants.*

Leaf movements have been used for many years to study circadian rhythms in plants. However, leaf movements that continue to persist for a long period under constant conditions have never, to our knowledge, been reported in the literature. This report presents evidence for a persistent circadian rhythm in leaves under constant conditions and for the long-term existence of a circadian rhythm in higher plants.

Observations and records reported by many investigators generally show that circadian rhythm persists only for a few days, and for the most part these records show a gradual decrease in the amplitude of leaf movements or respiration rates (*1*). In low-intensity light (1100 lu/m^2), plant rhythms have been shown to persist for as long as a week. However, in high-intensity light (4400 lu/m^2 or more), these rhythms are reported to disappear. These results have been interpreted to indicate an attenuation of the rhythm. Some doubts have been expressed concerning the existence of a true circadian rhythm in leaves (*2*). Nowhere in the literature is there a report of a persistent circadian leaf rhythm in higher plants. In this report a persistent rhythm is considered one which continues for 4 weeks or more. Because of the inherent photosynthetic requirements of higher plants, testing for rhythms under continuous dark conditions is difficult. Under continuous light plants may be easily grown, but the high-intensity light required for normal growth has resulted in the cessation of the rhythms (*1*). Thus it is difficult to obtain direct evidence for a persistent circadian rhythm in higher plants. In this report, evidence for the persistence of such a circadian rhythm in the primary leaves of *Phaseolus vulgaris* var. Pinto is presented.

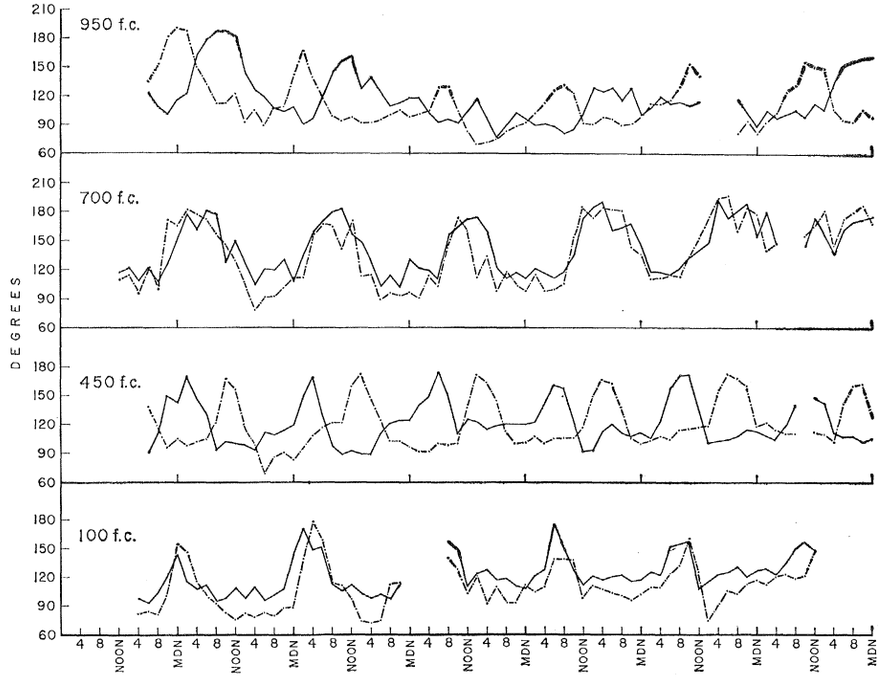

Fig. 1. Circadian rhythm of primary leaves of *Phaseolus vulgaris* var. Pinto plants germinated and grown at 27° ± 0.5°C and under continuous light from slim-line fluorescent tubes. The light intensities were measured at the level of the horizontal primary leaves. For the ordinate, a horizontal leaf was assigned a value of 90 degrees, and a leaf pointing straight down 180 degrees. Time of day is shown along the abscissa. The two curves represent the two primary leaves from one plant. Gaps in curves are due to breakdowns in the recording apparatus.

Bean seeds planted in 10-cm clay pots were placed in constant environmental conditions. The temperature of the room was maintained at 27° ± 0.5°C. The pots containing the seeds were exposed to continuous fluorescent light of given intensities throughout the experiment and were subsequently watered for the first time. When the primary leaves emerged and expanded, they exhibited circadian rhythm movements in the form of sleep movements. After the development of the primary leaves, time-lapse photography records were made. The 16-mm time-lapse movie camera was set to take one frame every 3 minutes. A 24-hour clock was placed near the plants to correlate the time of day. The readings were taken from every 40th frame, that is, with a time lapse of 2 hours between data points. Four plants were used in each treatment, and measurements were taken on the eight primary leaves. The angle of 90 degrees was assigned to horizontally oriented leaves and 180 degrees to leaves pointing straight down. Light intensities of 1100, 4950, 7700, and 10,450 lu/m^2 were used. Each light treatment was repeated at least two times. Figure 1 represents the leaf movements of plants subjected to different light treatments. For each treatment, the two curves shown represent the two primary leaves of the same plant. The leaves are asynchronous but the periods of the rhythm are the same. Asynchrony occurs in these treatments because the leaves were never exposed to a synchronizing dark period. Gaps in the record are due to mechanical failure of the time-lapse camera. The period was about 26 hours for each treatment. The rhythm persisted and the duration remained the same for Pinto beans at all levels of light intensities used. Furthermore, the leaf movement did not decrease in amplitude as the time went on. The movements were similar in amplitude and duration during 4 weeks of observation.

The persistence of the leaf movement in Pinto beans and the uniform

length of the leaf rhythm under different light intensities may just be a unique response of Pinto beans. However, since it does exhibit a persistent rhythm under continuous high-intensity light, the Pinto bean may be useful in further studies on the nature of circadian rhythms in plants.

TAKASHI HOSHIZAKI
Space Biology Laboratory, University of California Medical Center, Los Angeles

K. C. HAMNER
Department of Botany and Plant Biochemistry, University of California, Los Angeles

References and Notes

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Hyperbaric Exposure of Mice to Pressures of 60 to 90 Atmospheres

Abstract. *Albino mice breathing helium-oxygen mixtures remained in good condition for periods of 1 to 13 hours at pressure-equivalent depths of 396 to 914 meters of sea water. They were successfully decompressed to normal atmospheric pressure in less than 5 hours. There were no immediate or delayed adverse effects that could be attributed to the hyperbaric helium-oxygen environment. An occasional death was attributable to hypoxia or the decompression procedure.*

Our objective was to obtain a respirable synthetic atmosphere for mammals which would be adequate for maintaining normal physiological functions at very high ambient pressures.

Marshall (*1*) reported that mice began to show a loss of equilibrium at nitrogen pressures of 10 to 17 atm. Helium, however, at 41 atm caused no signs of equilibrium loss in mice exposed for periods up to 2 hours. When the pressure was raised to 54 atm (helium), mice became stuporous in about 40 minutes, but recovered completely after decompression. From data of Carpenter (*2*) the "isonarcotic" concentrations of nitrogen and helium required to protect mice against electroseizures are 18 and 163 atm, respectively (*3*). Translated into impairment produced by physiologically inert gases at depths tolerated by divers, it appears that helium might have approximately a ninefold advantage over nitrogen. At 10 atm (91.4 m) divers breathing air usually show varying degrees of impairment, and at 15 atm (137.2 m) they show loss of useful function within a matter of minutes. In a helium-oxygen atmosphere, comparable degrees of impairment might not appear until the pressure reaches 60 to 90 atm. Limiting diving depths in pressurized atmospheres are generally estimated to be at a much lower range of about 305 to 427 m. In view of the renewed interest in exploration of the Continental Shelf and problems related to deep submarine submergence, it seemed appropriate to initiate preliminary tests with mice to observe reactions at depths equivalent to 610 to 914 m (60 to 90 atm). A proper decompression procedure is especially important in such experiments.

Helium was chosen as a substitute for nitrogen because of the well-known narcotic properties of nitrogen (*4*) and because of the proven effectiveness of helium-oxygen mixtures at pressures unattainable by a diver breathing air (*3*).

There is evidence that increased oxygen tension rather than barometric pressure itself is responsible for the morbidity and mortality which occurs under conditions of prolonged exposure to air at high-pressures (*5*). Workman *et al.* have shown that rats and monkeys exposed to synthetic atmospheres of helium and oxygen under conditions of pressure equivalent to 61 m of sea water for periods of two weeks tolerated the procedure without deterioration (*6*). The oxygen tension in their experiments was controlled at 3 percent of the total helium-oxygen pressure at 7 atm absolute. This is equal to 0.21 atm and equal to that found in air at 1 atm.

Although oxygen at 0.21 atm is probably adequate even under pressure equivalent to a depth of 1000 m, we attempted to keep the oxygen tension throughout the wide range of pressures within 0.21 and 0.60 atm. The upper limit of oxygen tension used (0.6 atm) is considered to be the upper limit of oxygen tension which is safe for human exposure for prolonged periods of time (*7*).

Mice were considered especially suitable for these experiments because of their small size and consequent rapid uptake of inert gas during compression, and subsequent rapid elimination of the gas during decompression. It has been estimated that mice attain gaseous equilibrium within 1 hour of being placed in the hyperbaric environment, in contrast to man who requires 12 hours to attain equilibrium.

The test compartment was a small pressure chamber hydrostatically tested to 191 atm. The chamber measured 30.48 cm in length and 15.24 cm in diameter and had a volume of 805.18 cm^3. The chamber was provided with internal illumination, and portholes in each side enabled us to observe the mice. A thermocouple was used for measuring temperature. The oxygen content of the chamber was measured when the oxygen tension was high enough to be within range of the instrument used (*8*) (above 1 percent). Baralime was placed in the chamber to aid in the absorption of carbon dioxide. During most of the experiments in which the pressure was equivalent to a depth of 610 m of sea water, a small exercise wheel was placed in the chamber and used actively by several mice.

In our first experiment, two mice were placed in the pressure chamber which was purged with oxygen. The pressure was raised to 1.16 atm (absolute) with a mixture of 25 percent oxygen and 75 percent helium to provide an oxygen tension slightly higher than that found in air at 1 atm. Subsequently, the pressure was raised to 42 atm absolute, or pressure at a depth of 412 m of sea water over a period of 30 minutes, pure helium being used as the additive. The oxygen tension was maintained at approximately 0.3 atm. The mice were kept at this pressure for 13 hours and 40 minutes, then gradually decompressed to normal atmospheric pressure. They tolerated the procedure well and survived with no immediately apparent physiological deterioration. However, 1 week after the experiment one mouse died. Gross examination revealed several milliliters of serosanguinous fluid in the abdominal cavity. Histopathological studies (*9*) showed normal lung tissue. The liver showed definite lymphatic dilatation,