A Persistent Daily Rhythm in Photosynthesis

J. WOODLAND HASTINGS, LAZARUS ASTRACHAN, and BEATRICE M. SWEENEY

From the Division of Biochemistry, University of Illinois, Urbana, the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, and the Scripps Institution of Oceanography, La Jolla, California

ABSTRACT The luminescent marine dinoflagellate, *Gonyaulax polyedra*, exhibits a diurnal rhythm in the rate of photosynthesis and photosynthetic capacity measured by incorporation of $C^{14}O_2$ at different times of day. With cultures grown on alternating light and dark periods of 12 hours each, the maximum rate is at the 8th hour of the light period. Cultures transferred from day-night conditions to continuous dim light continue to show the rhythm of photosynthetic capacity (activity measured in bright light) but not of photosynthesis (activity measured in existing dim light). Cultures transferred to continuous bright light, however, do not show any rhythm. Several other properties of the photosynthetic rhythm are similar to those of previously reported rhythms of luminescence and cell division. This similarity suggests that a single mechanism regulates the various rhythms.

INTRODUCTION

Previous studies with the photosynthetic marine dinoflagellate, *Gonyaulax polyedra*, have shown that it possesses two distinct diurnal rhythms. One is a rhythm of bioluminescence. If cells are grown with alternating light and dark periods of 12 hours each, the luminescence of the organism during the dark period is about 60 times greater than it is during the light period (1, 2). Furthermore, the activity of the components of the luminescent reaction (luciferin and luciferase) is greater in cell-free extracts prepared during the dark period (3). Another cell function regulated by a rhythmic mechanism is cell division. At least 85 per cent of all divisions which occur in a 24 hour period take place during a 5 hour interval spanning the end of the dark period and the beginning of the light period (4).

A number of experiments have demonstrated that the rhythmic process is under endogenous control. Cells placed in *constant* dim light (100 to 200 foot-candles) exhibit continued rhythmic patterns of luminescence and cell

division, with maxima spaced at approximately 24 hour intervals. Although the rhythmic fluctuations may be abolished if the cells are kept in constant bright light (1,000 to 1,500 foot-candles), the subsequent resumption of constant dim light results in a return to the endogenous rhythm with its 24 hour period (5, 6).

In order to identify the cellular mechanisms involved in persistent diurnal rhythms, it is imperative that other biochemical processes in the same organism be examined with respect to periodic fluctuations. From information on the number and types of rhythmic processes, and from a comparison of their sensitivity to arbitrary environmental changes, plausible causal relations may be deduced. In the experiments reported in this paper, we have found that the capacity for photosynthesis in *Gonyaulax*, as measured by $C^{14}O_2$ incorporation, also shows a marked diurnal rhythm. This rhythm continues when the cells are transferred to constant dim light and exhibits characteristics which indicate that all three rhythms in *Gonyaulax* (luminescence, photosynthesis, and cell division) have a common controlling mechanism.

MATERIALS AND METHODS

Two liters of modified sea water medium at pH 8.2 (2), contained in Fernbach flasks, were inoculated with sufficient *Gonyaulax polyedra* to yield an initial density of 500 to 1,000 cells/ml. Illumination was scheduled for alternating dark and light (960 foot-candles from "cool white" fluorescent tubes) periods of 12 hours each. The temperature varied from a maximum of 26° when the lights were on to a minimum of 23° in the dark. The doubling time was 2 to 3 days and a cell density of 12,000 to 15,000 cells/ml was attained in the stationary phase.

At a cell density of 8,000 cells/ml, 20 ml portions of the culture were pipetted into a large number of 50 ml Erlenmeyer flasks, and 2 ml portions were distributed to test tubes. The aliquots were returned to the same light-dark schedule for one more day before the experimental manipulations (described in legends to Figs. 1 and 2) were started. The dim light source used for data of Fig. 2 was provided by reducing the light intensity of the fluorescent tubes with translucent tracing paper.

Using the 2 ml aliquots, luminescence of cells stimulated with air bubbled through the suspension was measured (2). Separate samples were used each time.

The 20 ml aliquots were used to compare photosynthetic capacity at designated times. To each flask was added 12.5 microcuries of $NaHC^{14}O_3$ (0.025 ml). The flask was closed with a glass stopper and then incubated for 15 minutes in the light at an intensity of 960 foot-candles. Control flasks were incubated with tracer in the dark. At the end of the incubation period, the reaction was stopped by adding 2 ml of 1 N HCl; the cells were immediately harvested with suction on a 24 mm disk of Whatman No. 54 filter paper, rinsed once with 0.1 N HCl and three times with water, and then air-dried. By proper adjustment of suction, the visible deposit of cells was uniformly distributed on the filter paper. To measure incorporated radioactivity, the filter paper was fixed on a disk with a ring (Tracerlab) and then directly counted with an end-

window Geiger-Mueller tube and scaler. The data recorded were corrected for background and controls. Using this procedure, the incorporation of radioactive carbon in the dark was found to be less than 0.5 per cent of that in the light.

RESULTS

The diurnal rhythm in the rate of $C^{14}O_2$ incorporation is evident in Fig. 1. The curve is representative of photosynthetic *capacity* rather than photosynthetic activity. Samples were taken during the dark period, when no photo-

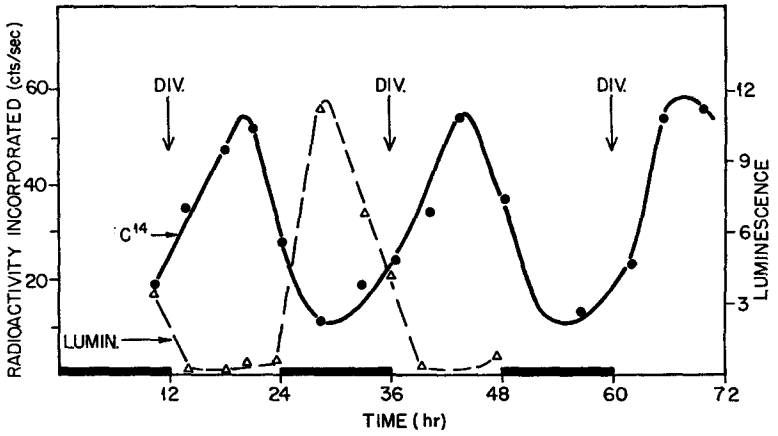

FIGURE 1. Rhythms of photosynthetic capacity, cell division, and luminescence in alternating light and dark periods. Dark periods are indicated by black bars on abscissa. Light intensity in light periods was 960 foot-candles. Curve labeled (C^{14}) refers to the relative amount of $C^{14}O_2$ incorporated when aliquots were incubated with tracer in the light (960 foot-candles) for 15 minutes. Luminescence is in arbitrary units (right ordinate). The times at which cell division occurred were not observed in this experiment but are known to occur at the times indicated by the vertical arrows. Cell density was 11,000 cells/ml.

synthesis was actually occurring, and were incubated with tracer in light at an intensity saturating for photosynthesis in order to determine how much photosynthesis could occur. The figure also shows the phase of the luminescence and cell division rhythms. A difference of only 5-fold between maximum and minimum in photosynthetic capacity was observed, while the luminescence of the organism varied by a factor of about 60.

After the cells were transferred to continuous dim light, the rhythm of photosynthetic capacity continued (Fig. 2 (C^{14})-dim-cap). Again the term photosynthetic capacity is used because the incorporation of tracer was measured at an intensity saturating for photosynthesis rather than in the dim light conditions in which the cells were maintained. Although the difference between the maximum and minimum was less than under the alter-

nating dark and light conditions of Fig. 1, the changes were persistent and the phase relation to the luminescence rhythm was retained. The period of the rhythm was about 26 hours, the same as the period of the luminescence rhythm in dim light at this temperature (7).

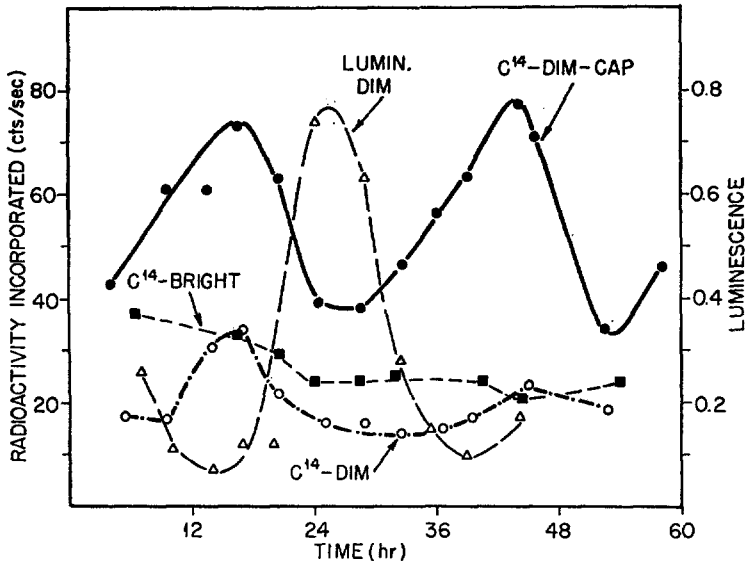

FIGURE 2. Photosynthesis, photosynthetic capacity, and luminescence in continuous light. Cells were transferred from alternating light and dark conditions at the end of a dark period to continuous light 16 hours prior to zero hours on the graph. Dim light was 110 foot-candles and bright light was 960 foot-candles; temperature, 25 ± 0.3°. (C^{14})-dim-cap refers to the photosynthetic *capacity* of cells cultured in constant dim light, and records the relative amounts of $C^{14}O_2$ incorporated when aliquots were incubated with tracer in saturating bright light for 15 minutes. (C^{14})-dim refers to relative rates of photosynthetic *activity* in cells cultured in dim light, and records $C^{14}O_2$ incorporation when aliquots were incubated with tracer for 60 minutes in dim light. (C^{14})-bright refers to relative rates of photosynthesis (or photosynthetic capacity) in cells cultured in bright light, and records the relative amounts of $C^{14}O_2$ incorporated when aliquots were incubated with tracer for 15 minutes in bright light. Luminescence was measured with cells maintained in continuous dim light. The units are arbitrary. Cell density was 11,000 cells/ml.

When the incorporation of tracer was measured in dim light, thereby providing a relative measure of the photosynthetic *activity* actually occurring, no rhythm was observed. With cells maintained in continuous dim light ((C^{14})-dim), the rate of photosynthesis varied slightly during the 1st day but thereafter the changes were damped out and no diurnal rhythm was observed. Cells maintained in continuous bright light ((C^{14})-bright) also showed no

diurnal rhythm of photosynthesis. This result was expected since the rhythms of luminescence and cell division disappear in continuous bright light (6).

Variations in photosynthetic capacity could be caused by changes in a number of different cell constituents such as, for example, photosynthetic pigments. However, as shown in Table I, we have been unable to detect any diurnal variations in either chlorophyll or carotenoid content. On the other hand, there are reports of diurnal fluctuations in the chlorophyll content of

TABLE I

PIGMENT CONTENT OF GONYAULAX CELLS AT DIFFERENT TIMES OF DAY

	In acetone		In methanol	
	Chl. *a*	Chl. *c*	Chl. *a*	Peridinin
Culture				
Dark	0.046	0.021	0.025; 0.039	2.13
Light	0.045	0.021	0.026; 0.038	2.08
Culture II				
Light	0.060	0.028	0.033; 0.043	1.91
Dark	0.055	0.026	0.033; 0.042	1.93

Cultures inoculated in duplicate were grown at 20° with alternating light and dark periods of 12 hours each at an intensity of 500 foot-candles during the light period. A week prior to harvesting, one culture was transferred to an inverted light-and-dark regime without change in light intensity or temperature. Some cells from both cultures were harvested, first at a time corresponding to the middle of the dark period for culture I and the middle of the light period for culture II, and then again 12 hours later when it was the middle of the light period for culture I and dark for culture II. Pigments were extracted with methanol or acetone. The chlorophyll concentrations in acetone were calculated using the equations of Richards and Thompson (20) and are expressed in milligrams per 10^6 cells. The concentration of chlorophyll *a* (chl. *a*) in methanol was calculated using the value 102 as specific absorption coefficient. The concentration of peridinin (the principal carotenoid) was determined by measurements of absorption at 480 mμ.

natural phytoplankton populations (8, 9). Furthermore, Mitrakos *et al.* (10) have reported diurnal variations in the chlorophyll and carotenoid content of the cotyledons of *Perilla ocymoides* maintained under constant conditions. The variations were small (2 to 9.5 per cent) and it is unlikely that they could result in a photosynthetic rhythm of the magnitude reported in this paper.

The photosynthetic capacity might also be altered by changes in the concentration or activity of enzymes involved in dark reactions of photosynthesis. This situation would be similar to the luminescence rhythm in which the activity of the enzyme luciferase shows diurnal fluctuations (3).

DISCUSSION

Persistent diurnal rhythms have been observed in a large variety of animals and plants (11–13). In different plants, for example, many processes such as sporulation, growth rate, leaf movements, and phototaxis, exhibit such rhythms. Diurnal changes in photosynthesis also have been observed with oceanic plankton assayed at different times of day (9, 14). The organisms present were not identified, but presumably the samples contained various species of dinoflagellates. A photosynthetic rhythm also has been reported in single, isolated *Gonyaulax* cells (15). The mechanism of the rhythm thus is not a function of a population of cells but must be considered to reside at the cellular level.

As an approach to the mechanism involved in the rhythm, we considered the possibility that a feedback from photosynthesis could result in rhythmic fluctuations of other functions. This idea was unattractive because it is applicable only to photosynthetic organisms and is difficult to apply to the diverse functions known to exhibit rhythmic behavior. The present experiments do not support this idea; it is photosynthetic capacity rather than photosynthetic activity that is endogenously rhythmic for organisms cultured in dim light. Even by considering that photosynthesis need not be rhythmic to exert a rhythmic feedback control, the hypothesis does not explain the occurrence of rhythmicity in dim light but not in bright light. Other experiments, in which inhibitors of photosynthesis have been used, also have ruled out this feedback possibility (16).

Even though each rhythm studied in *Gonyaulax* has a distinctly different phase, certain similarities in their properties suggest that a common mechanism controls the different rhythms. The rhythms are largely temperature-independent and their phases—but not the relation between the phases—can be changed by changing light conditions. This point may be more definitively established by extending over a longer time in constant conditions the measurements of each of the several rhythms. If any significant change in phase relationship is found, the hypothesis of a common mechanism would not be supported.

Our work did not allow us to identify the mechanism of the rhythm but does extend the range of biochemical and physiological activities known to exhibit rhythmic fluctuations. Furthermore, a restriction on any postulated mechanism is revealed by the observation that each rhythm has a different phase; provisions must exist for qualitative as well as quantitative controls of metabolic processes with respect to time. The observation that the capacities for luminescence and photosynthesis fluctuate with time suggests that the area of nucleoprotein synthesis should be investigated for clues to the mechanism involved in the control of rhythmic processes.

In so far as the question of adaptive significance is concerned, we note that bioluminescence is timed to occur during the night, while maximum photosynthetic capacity is achieved during the day. But whether this regularity has any importance in the economy of the cell is not so clear. For example, the growth rate of *Gonvaulax* is not decreased when cells are grown in light-dark cycles other than 24 hours or in constant bright light. There are cases, however, in which diurnal cycles do seem to be of importance. The alga, *Hydrodictyon*, does not survive when kept in continuous light (17) and the growth of tomatoes is favored by light-dark cycles of 24 hours (18, 19). Perhaps the questionable adaptive significance of rhythmicity in *Gonyaulax* suggests that the mechanism is not sporadically evolved for an adaptive advantage, but may be a general, basic property of this and other organisms. The ability of cells to alter metabolic patterns in a specific, regularly recurring, and controlled fashion is evident in such instances as the mitotic cycle or differentiation of an organism. It is possible that diurnal cycles rely on similar general mechanisms. If so, it is important to note that daily rhythms have a striking additional feature, namely that of the temperature-independent period (6, 7, 12).

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