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# Studies on the Control of the Rhythm of Photosynthetic Capacity in Synchronized Cultures of *Euglena gracilis* (Z)[1, 2, 3, 4]

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## ABSTRACT

**Synchronous cell division in *Euglena gracilis* (strain Z) was obtained in 24-hour light cycles consisting of 10 hours of light and 14 hours of darkness; cell division was restricted to the dark period. Photosynthetic capacity was found to vary in a cyclic manner during the cell cycle, reaching a peak 2 hours before the onset of darkness. Light reactions were investigated during the cell cycle to determine what role they played in the control of the observed rhythmic changes in capacity. Light-saturation curves showed no major change in the light-limited region. No fluctuations were found in Hill reaction activity or photoreduction of methyl red during the cell cycle. These results imply that the reactions comprising photosystems I and II do not generate the capacity rhythm.**

**Some of the photosynthetic dark reactions were also followed during the cell cycle in an attempt to determine their possible role in the control of the rhythm of photosynthetic capacity. The activity of ribulose-1,5-diphosphate carboxylase showed no correlation with the rhythm. On the other hand, the activity of glyceraldehyde-3-phosphate dehydrogenase was found to parallel the change in photosynthetic rate under various growth conditions. The rhythm in photosynthetic capacity could be effectively divorced from the cell cycle itself by placing cultures in high frequency light cycles (*LD*: 2,4) or in stationary growth-phase conditions. If synchronously dividing cultures previously grown in *LD*: *10*,14 were released into continuous dim illumination and constant temperature, the rhythm of capacity persisted for only one full cycle.**

Recently, several conflicting reports have appeared on the rhythm of photosynthetic capacity during the cell cycle of *Euglena* (9, 11, 32); none of these investigations, however, has examined the role which light reactions may play in its regulation. Because of these differing results from physiological studies on photosynthetic capacity in *Euglena* and the dearth of inquiries into its control, a more thorough study of the generation and mediation of the photosynthetic rhythm in this alga was undertaken. In this paper we characterize the rhythm of capacity in synchronously dividing and in non-dividing cultures and investigate the role which light and dark reactions play in the control of the observed rhythm. Finally, since *Euglena* has been shown to exhibit persisting circadian rhythms of phototaxis (5), dark motility (4), amino acid incorporation (20), cell division (17–19, 27), and cell settling (56), we have also explored the possibility that a circadian biological clock may likewise underlie the overt 24-hr periodicity in photosynthetic capacity by placing synchronous cultures into conditions held constant with respect to light and temperature.

## MATERIALS AND METHODS

**Organism and Culture Conditions.** Cultures of *Euglena gracilis* Klebs (strain Z) maintained in this laboratory for over 7 years were grown axenically and photoautotrophically on a modified Cramer and Myers' (13) inorganic growth medium (pH 6.9) supplemented with vitamins $B_1$ and $B_{12}$ as described by Edmunds and Funch (18). Batch cultures (either 8 or 20 liter capacity) were magnetically stirred and aerated at a bubbling rate of 575 to 625 ml/min. The only source of $CO_2$ supplied was that in the air. The cultures were housed in Hotpack environmental chambers (Hotpack Corporation, Philadelphia, Pa.) and maintained at 24 C. Illumination was supplied by three banks of cool white (40 w) flourescent tubes, each bank containing six bulbs. The incident intensity on the culture vessels from each bank of lights was 12,000 lux. Cells were synchronized by light-dark cycles of either 12 hr of light and 12 hr of dark (*LD*: *12*,12)[7] or 10 hr of light and 14 hr of dark (*LD*: *10*,14). Cell number was monitored every 2 hr across the cell cycle by a miniaturized fraction collector and automatic pipeting device (15) and a Coulter Model B electronic cell counter (Coulter Electronics, Hialeah, Fla.).

**Gross Biochemical Determinations.** The biosynthetic patterns of nucleic acids were followed every 2 hr during the cell cycle. Replicate samples of 300 ml each were siphoned from the master culture and frozen for future analyses. The frozen pellets were extracted four times (2 ml each) with cold acetone,

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[3] The research reported herein is derived from the doctoral thesis of W. Walther accepted by the Division of Biological Sciences of the State University of New York at Stony Brook in partial fulfillment of the requirements for the Ph.D. degree.

[4] Some of these results were reported by L. N. Edmunds, Jr., W. G. Walther, R. Jarrett, and A. Uzzo at the XI International Botanical Congress, August, 1969, Seattle, Washington. (Abstract No. 608).

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[7] Abbreviations: a repetitive cycle of $x$ hr of light and $y$ hr of darkness will be denoted *LD*: $x$,$y$ (where the period of the cycle = $x + y$); RuDP: ribulose-1,5-diphosphate. PGA: phosphoglyceric acid; GPD: glyceraldehyde-3-phosphate dehydrogenase (= triose phosphate dehydrogenase); $LL_d$: continuous dim illumination.

once with 5 ml of cold 0.3 N perchloric acid, and twice with 5 ml of 0.5 N perchloric acid at 70 C for 20 min each. The two 0.5 N perchloric acid extracts were combined, and the Burton (7) modification of the diphenylamine colorimetric test for deoxyribose was used for assaying total cellular DNA. RNA determinations were made on the same 0.5 N perchloric acid extracts using the orcinol colorimetric test for the estimation of ribose as described by Edmunds (16).

Total chlorophyll was assayed according to Arnon (2) and carotenoids were determined by the method of Kirk and Allen (29). Twenty milliliters of culture were centrifuged, and to the pellet were added 3 ml of 80% (v/v) acetone in 0.05 M tris-HCl buffer (pH 7.4). The supernatant was read in a Beckman DU-2 spectrophotometer at 480, 645, 652, and 663 nm.

Protein content was determined by the Folin-Ciocalteau phenol colorimetric test developed by Lowry *et al.* (33). Soluble protein was assayed by taking a 0.6-ml sample from crude extracts and testing directly for protein content. Total cellular protein analyses were performed directly on 0.6-ml aliquots of cell suspension.

**Measurement of Photosynthetic Capacity.** Photosynthetic capacity, arbitrarily defined as the amount of $H^{14}CO_3$ incorporated into cells in 10 min when exposed to bright light (12,000 lux), was assayed during the cell cycle every 2 hr by siphoning off 10-ml aliquots into test tubes to which was added 1 $\mu$c $NaH^{14}CO_3$ (20.5 mc/mmole). The reaction was run for 10 min and terminated with 1 ml of 0.5 N HCl. Dark controls were run to correct for any dark $CO_2$ fixation which occurred. One 10-ml aliquot was put directly onto a Millipore filter, rinsed several times with 0.5 N HCl and distilled water, and counted in toluene. This portion represented the "whole cell" uptake. A second aliquot was centrifuged (after being incubated with isotope); 5 ml of boiling 80% (v/v) ethanol were added to the pellet. This fraction was then centrifuged to remove cell debris, and 0.1 ml of the ethanol supernatant was spotted onto a Millipore filter to be counted. This represented the "soluble supernatant' uptake. The residual pellet was resuspended in distilled water and put onto a Millipore filter, rinsed several times with distilled water, and counted. This constituted the "residual" uptake.

Alternatively, oxygen evolution was measured across the *LD*: *10*,14 cycle and used as an assay of photosynthetic capacity. Cells were grown on inorganic medium (without aeration) supplemented with 20 mM sodium acetate. Aliquots of 100 ml were taken every 2 hr during the cell cycle and exposed for 10 min to saturating light. Oxygen evolution was measured with a YSI Model 51 oxygen analyzer (Yellow Springs Instruments Co., Yellow Springs, Ohio).

**Light Reactions.** A series of light saturation curves of photosynthesis were measured during the cell cycle of *Euglena*. Aliquots of 10 ml were taken from the master culture at different times of the cell cycle and incubated with isotope at various intensities of illumination for 10 min, after which the reaction was terminated.

The Hill reaction was determined by a modification of the method of Russell and Lyman (39). Two liters of culture were centrifuged, and the pellet was resuspended in 5 ml of 0.05 M phosphate buffer (pH 6.8). The suspension was sonicated for 30 sec and centrifuged at 3000 rpm for 5 min to remove cellular debris. The supernatant was respun at 10,000 rpm for 10 min; the pellet from this final centrifugation contained the chloroplast fragments. The reaction was run in 3-ml cuvettes which were exposed to an actinic beam of 20,000 lux. The reaction was read at 600 nm every 30 sec for 5 min in a Beckman DU-2 spectrophotometer. The reaction mixture contained in $\mu$moles: 0.05 M phosphate buffer (pH 6.8), 37.5; KCl, 18.75; dichlorophenol indole phenol, 0.1875; and chloroplast fragments, 0.3 ml.

The photoreduction of methyl red was used as an indicator of the activity of photosystem I. The procedure used was a modification of the method of Hoober *et al.* (25). The chloroplast preparation was identical to that used for the Hill reaction. The reaction was run in 3-ml cuvettes which were exposed to an actinic beam of 20,000 lux. The reaction was read at 430 nm every 30 sec for 3 min in a Beckman DU-2 spectrophotometer. The reaction mixture (3 ml) contained in $\mu$moles: methyl red, 0.4; 0.05 M sodium phosphate buffer (pH 7.2), 150; DCMU, 0.06; dichlorophenol indole phenol, 0.6; ascorbic acid, 3.5; cytochrome 552; and 0.1 to 0.4 ml chloroplast fragments. Nitrogen gas was bubbled through the reaction mixture for 1 to 2 min during the addition of the ingredients.

Cytochrome 552 was prepared according to Perini *et al.* (37). The cytochrome was extracted in a reduced form from 22 liters of a synchronous population of *Euglena* and purified on a diethylaminoethyl cellulose column. In each methyl red determination 0.1 ml of the eluate from this column was used; this amount was determined to be saturating for each time point tested throughout the cell cycle.

**Dark Reactions.** Ribulose-1,5-diphosphate carboxylase activity was assayed by a modification of the method of Fuller and Gibbs (21). Two-liter aliquots were taken every 2 hr across the cell cycle, centrifuged, and frozen for future analysis. It has been shown in spinach chloroplasts that freezing does not inactivate the enzyme; 99% of the activity could be recovered after freezing (31). Likewise, we have found the loss of activity to be minimal in our *Euglena* system. The frozen pellets were thawed and suspended in 5 ml of 0.05 M tris-HCl buffer (pH 7.5). The suspension was sonicated for 30 sec and centrifuged at 17,000 rpm for 30 min. The final concentration of the 2-ml reaction mixture (in $\mu$moles of each component) was as follows: $MgCl_2$, 20; EDTA, 10; GSH, 12; 0.05 M tris-HCl, 47.5; and RuDP, 2. The reaction was started by adding 0.1 ml of $NaH^{14}CO_3$ (100 $\mu$c/ml) and was stopped after 10 min by adding 0.1 ml of the reaction mixture to 0.3 ml of 0.5 N HCl in a scintillation vial. The mixture was air-dried, and 5 ml of scintillation fluid was added to each vial.

Glyceraldehyde-3-P dehydrogenase was assayed according to the method of Russell and Lyman (39). Two-liter aliquots were removed from the master culture every 2 to 3 hr, centrifuged at 10,000 rpm for 10 min, and the pellet resuspended in 4 ml of 0.1 M tris-HCl buffer (pH 8.4). The suspension was sonicated for 30 sec and centrifuged at 17,000 rpm for 30 min. The activity was measured by following the oxidation of NADPH (or NADH) at 340 nm. The reaction mixture (in $\mu$moles of each component) consisted of: tris-HCl buffer, 30; $MgSO_4$, 10; ATP, 2; NADPH, 0.1; neutralized cysteine HCl, 5; and PGA, 3. A 0.2-ml cell extract was used as the source of enzyme. The reaction was started by adding PGA. PGA kinase was not limiting in the reaction mixture since the addition of excess kinase did not alter the activity. Similarly, none of the substances for the reaction were limiting.

## RESULTS

**Cell Division.** The synchronous pattern of cell division obtained in an *LD*: *10*,14 cycle under our experimental conditions is shown in Figure 1. Cell division typically commenced at (or just before) the onset of darkness and was restricted solely to the dark period. A step-size of nearly 2.00 was obtained routinely, indicating an almost exact doubling of cell number. Cells were generally harvested for experiments when the cell concentration at the beginning of the light period reached approximately 20,000 cells/ml, which was the highest

concentration that yielded a step-size close to 2.00. Similar results have been published (12, 15).

**Biosynthetic Patterns.** In order to determine whether balanced growth was occurring, gross biochemical patterns were followed during the cell cycle. Chlorophyll, carotenoids, soluble and total cell protein, and RNA all doubled linearly during the entire light period; DNA synthesis, on the other hand, increased in a step-wise manner beginning at the 6th or 7th hr after the onset of light. All parameters, on a per milliliter of culture basis, remained constant during the dark period. The biosynthetic patterns and absolute amounts (pg/cell) obtained under our growth conditions agree with other published results (10, 14, 16).

**Photosynthetic Capacity during Light-Dark Cycles.** Figure 2 demonstrates a typical pattern of photosynthetic capacity

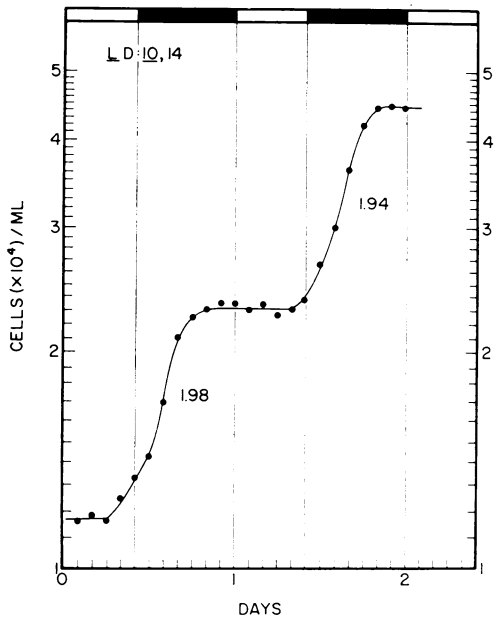


FIG. 1. Synchronous cell division of *Euglena gracilis* (Z) grown photoautotrophically at 25 C in *LD*: *10*,14 cycles. Numbers by division curves indicate step-sizes (factors by which cell concentration increases).

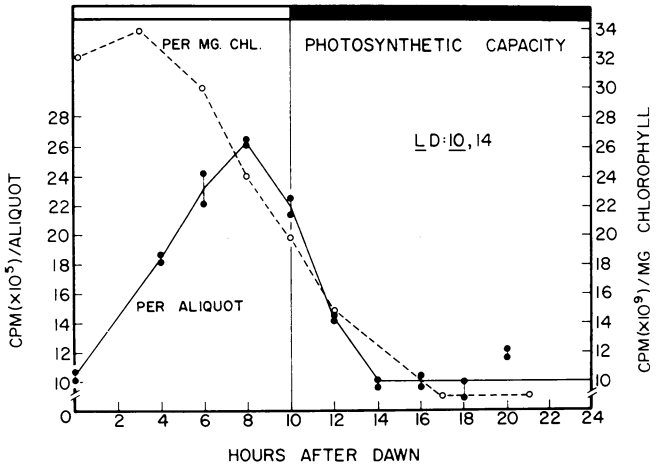


FIG. 2. Photosynthetic capacity during an *LD*: *10*,14 cycle. Aliquots (10 ml) of the master culture were exposed to 1 $\mu$c of $NaH^{14}CO_3$ for 10 min in saturating light. Photosynthetic capacity per aliquot: solid line; per mg chlorophyll: dashed line. Double points indicate duplicate determinations.

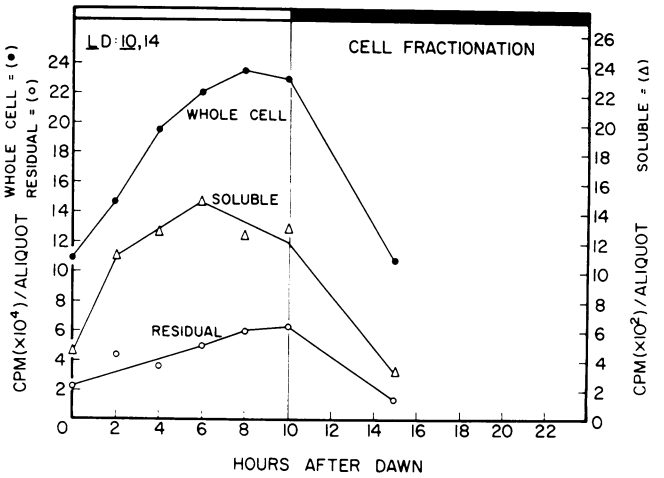


FIG. 3. Hot 80% ethanol fractionation of whole cells after 10 min of incubation with $NaH^{14}CO_3$ under saturating light. Note that residual fraction is more active than soluble fraction.

during the cell cycle of *Euglena* grown in *LD*: *10*,14. The rate of photosynthesis per aliquot increased linearly until it reached its maximum capacity at the 8th hr of the light period, 2 hr before the onset of darkness. The increase in rate of photosynthesis was much more rapid than the rate of chlorophyll production; therefore, when plotted on a per unit chlorophyll basis, photosynthetic capacity reached a maximum about the 3rd hr of the light period and decreased steadily throughout the rest of the cell cycle.

As an alternative method for assaying photosynthetic capacity during an *LD*: *10*,14 cycle, oxygen evolution was measured with a YSI Model 51 oxygen analyzer across the cell cycle. Cell division occurred as usual with step-sizes approximating 2.00. The results obtained by these measurements were similar to the incorporation data.

When *Euglena* was cultured in *LD*: *12*,12 cycle, a similar pattern (not shown) of photosynthetic capacity was observed as in *LD*: *10*, 14 cycles. Photosynthetic capacity reached its maximum 10 hr after the onset of light (2 hr before darkness) and decreased during the last 2 hr of light and the first half of the dark period. Cell division was not restricted completely to the dark period but began about 2 hr before the onset of darkness.

**Fractionation of Cells.** After the whole cell pattern of photosynthetic capacity was obtained the cells were fractionated by adding hot 80% (v/v) ethanol to determine the pattern of alcohol-soluble and insoluble material. The alcohol-soluble fraction consisted of cellular metabolic products which had been labeled during photosynthesis, and the insoluble (residual) portion consisted mainly of pellicular components. The results are shown in Figure 3. The soluble portion increased during the first half of the light period until it reached its peak at the 6th hr. During the last half of the light period and during the dark period it declined to the basal level. On the other hand, the residual fraction paralleled the pattern of whole cell uptake, reaching a peak at the 8th hr and returning to the original value by the end of the dark period. Incorporation into the residual fraction was 100 times higher than into the soluble fraction (Fig. 3). In an *LD*: *12*,12 cycle a similar distribution of label was found after ethanol fractionation.

**Photosynthetic Capacity under Continuous Illumination.** Experiments were undertaken to determine whether the rhythm of photosynthesis found in LD cycles would also persist under conditions of continuous illumination and constant tempera-

ture. Because many circadian rhythms damp out in constant bright light, continuous dim illumination ($LL_d$) was utilized. (The use of constant darkness was necessarily excluded inasmuch as photosynthetic rhythms immediately damp out under these conditions.)

Cultures were placed in $LL_d$ after several synchronous growth and division cycles in *LD*: *10*,14. Dim light was afforded by a bank of six fluorescent bulbs over which six layers of artists' tracing paper had been placed, thereby lowering the intensity from 12,000 lux to about 750 lux. Constant dim light intensities ranging from 750 lux to about 2000 lux were tried in order to find an intensity which would allow the photosynthetic capacity rhythm to be expressed.

Figure 4 illustrates the photosynthetic capacity rhythm when synchronous cells were placed into $LL_d$ of 750 lux. Only the first 24-hr cycle is shown here. Although the capacity rhythm in *LD*: *10*,14 cycles reaches its maximum at the 8th hr of the light period (see Fig. 2), the capacity in dim illumination increased until the 10th hr (the subjective light-to-dark transition) and then decreased during the remainder of the 24-hr time span, even though the cells were now still experiencing continuous light. It should be noted that the amount of radioactive uptake (cpm) was reduced by a factor of 10 when the cells were placed in $LL_d$. This low light intensity completely suppressed cell division: the number of cells remained the same during the entire experiment. When the capacity was measured on succeeding days there was no apparent rhythm; thus, during the second and third 24-hr cycles the rhythm damped out. Intensities higher than 750 lux caused the cell population to undergo logarithmic growth, resulting in a steady increase in capacity; likewise, lower intensities did not allow expression of the rhythm.

**Light Reactions.** To investigate whether light reactions control the capacity rhythm observed in light-dark cycles, light-saturation curves were obtained at 2-hr intervals during the 10-hr photoperiod. The results are presented in Figure 5. The initial slopes of all the curves at the stated time intervals were approximately the same until the intensity reached about 1000 to 2000 lux. At this intensity the organisms started to become saturated with light. In young cells complete saturation was reached at a low level, whereas cells taken from later stages of the cell cycle showed an elevation in saturation. The highest level of saturation was obtained from cells taken at the 8th hr of the cycle, the same point at which photosynthetic capacity reached its maximum on an aliquot basis. Cells at later developmental stages (beyond 8 hr of development) showed a decrease in the level of saturation.

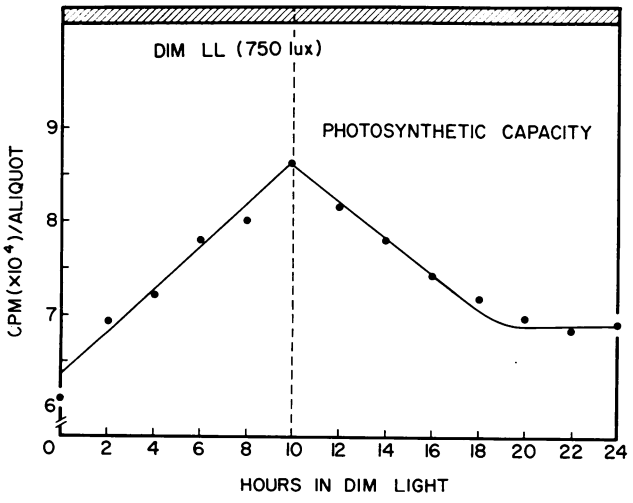


FIG. 4. Rhythm of photosynthetic capacity in synchronous *Euglena* cultures placed in constant dim illumination (750 lux) following entrainment in *LD*: *10*,14. The oscillation did not persist for more than one cycle.

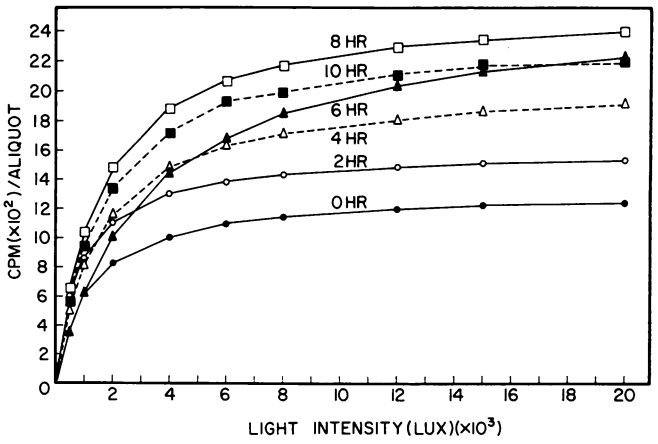


FIG. 5. Light saturation curves during the cell cycle of *Euglena*. Numbers on curves represent hours after onset of light. Complete saturation is reached about 11,000 lux.

Table I. *Hill Reaction Activity and Photoreduction of Methyl Red during the Cell Cycle of Euglena*

| Time After Onset of Light | Hill Activity | Methyl Red Photoreduction |
|---|---|---|
| *hr* | *Δ absorbance/3 min·aliquot* | |
| 0 | 0.060 | 0.070 |
| 3 | 0.075 | 0.045 |
| 6 | 0.080 | 0.050 |
| 8 | 0.065 | 0.030 |
| 9 | 0.090 | 0.050 |

To substantiate further that light reactions were not controlling the rhythm of photosynthetic capacity in *Euglena*, the activities of photosystems I (methyl red reduction) and II (dichlorophenol indole phenol reduction) were assayed throughout the cell cycle (Table I). In relation to other photosynthetic parameters, Hill reaction activity changed only slightly during the cell cycle; furthermore, this change showed no correlation with the observed changes in photosynthetic capacity. Similarly, there was no agreement between the capacity rhythm and the small changes which occurred in the photoreduction of methyl red (Table I).

**Activity of RuDP Carboxylase during the Cell Cycle.** During the light period, the activity of RuDP carboxylase increased linearly until the onset of the dark period, at which time it had more than doubled (Fig. 6). On a per aliquot of culture basis, the activity remained at this elevated level during the dark period, whereas the activity *per cell* decreased to the original level by the end of the darkness since the cell concentration had doubled. If heat-treated supernatants taken at various times during the cycle were mixed, no significant change in the pattern of RuDP carboxylase activity occurred, indicating that the increase in activity during the cell cycle is not due to a heat-labile molecule such as a light-activated factor or a dark inhibitor. This finding does not necessarily imply, however, that the observed change in activity is due solely to *de novo* synthesis since the possibility of activation by heat-stable molecules was not excluded.

**Activity of GPD during the Cell Cycle.** Since the pattern of activity of RuDP carboxylase did not follow the rhythm in

photosynthetic capacity, the activity of GPD was assayed during the cell cycle of *Euglena*. NADP-dependent GPD is associated with the Calvin photosynthetic cycle, whereas the NAD-linked enzyme is thought to be primarily nonphotosynthetic (22). In *Euglena* NADP-activity is catalyzed by a completely NADP-specific enzyme as opposed to an enzyme which has both activities (38); there is no conversion of NAD- to NADP-dependent enzyme (26). The activities of both the NADP- and NAD-dependent enzymes were followed during the synchronous growth cycle. Microscopic examination showed complete cell breakage at all points of the cell cycle. Likewise, the rate of oxidation of NADH and NADPH was proportional to the amount of extract at each cell cycle stage. A typical pattern of activity for each form is shown in Figure 7. At the beginning of the cell cycle, the NADP-dependent activity per aliquot of culture was extremely low but increased rapidly throughout the light period until it reached maximum activity after 8 hr of light, 2 hr before the onset of darkness. The decrease in activity during the last several hours of the light period *before* the onset of darkness was not caused by reduced amounts of PGA, ATP, or NADPH, since the addition of a 3-fold excess of these

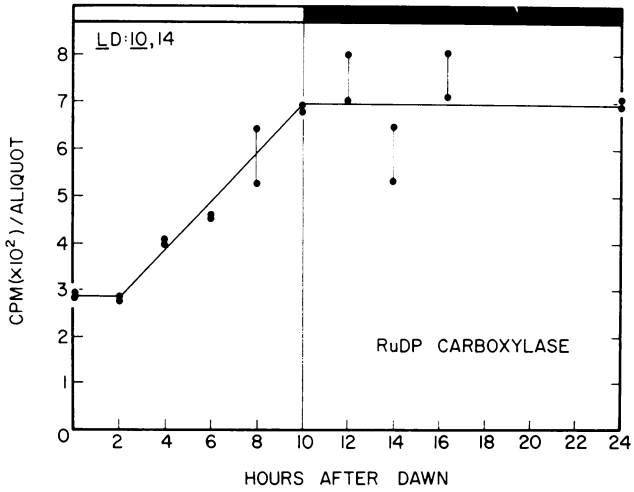


FIG. 6. Activity of RuDP carboxylase during synchronous growth of *Euglena* in *LD*: *10*,14. Two-liter aliquots were removed from the master culture every 2 hr, and the pellets were frozen for later analysis. After sonication the crude supernatant was tested directly for enzyme activity; duplicate determinations are shown.

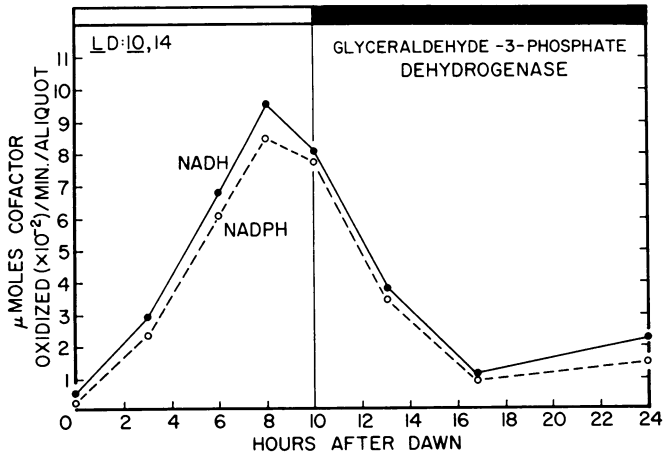


FIG. 7. Activity of GPD over the cell cycle in synchronized *Euglena* cultures. Both the NADPH and the NADH enzymes were assayed.

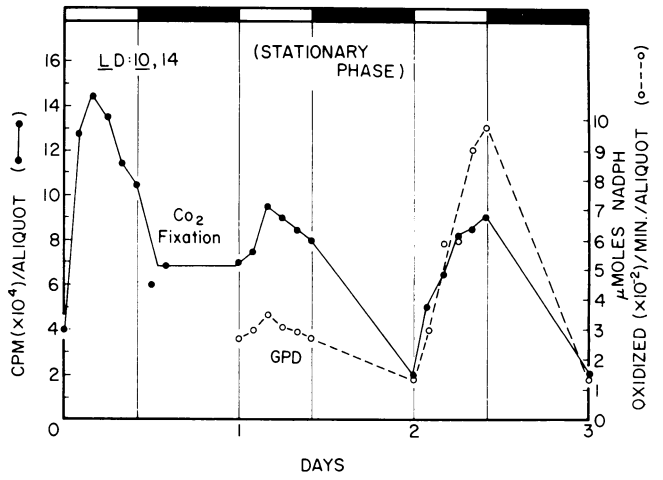


FIG. 8. Photosynthetic capacity and GPD activity in nondividing (stationary-phase) cultures of *Euglena* maintained in *LD*: *10*,14. The culture was kept in the stationary phase for 1 week before assays were performed.

constituents to the reaction mixtures did not alter the phase of the GPD activity rhythm during the cell cycle. Mixing experiments similar to those discussed for RuDP carboxylase also indicated that the change in activity was not due to heat-labile molecules. The activity of the NAD-dependent enzyme closely paralleled the activity of the NADP-dependent enzyme, although the activity of the former was slightly higher.

**Independence of the Photosynthetic Capacity Rhythm from the Cell Cycle.** In order to determine whether the photosynthetic capacity rhythm could be uncoupled or dissociated from the cell division cycle and periodic gene duplication, two types of experimental conditions were employed: stationary-phase cultures and high frequency light-dark cycles. Persisting circadian oscillations in the activities of several enzymes have already been demonstrated in non-dividing cultures of *Euglena* (52).

Cultures of *Euglena* were grown synchronously in *LD*: *10*,14 on inorganic minimal medium until the stationary growth phase was reached. This is a phase in which mitosis, cell division, and cell death are demonstratively negligible (52). These stationary-phase cultures were maintained in *LD*: *10*:14 for 1 week, whereupon photosynthetic capacity and GPD activity were assayed for several days (LD cycles). The results are shown in Figure 8. During the first of the three cycles that were measured, photosynthetic capacity reached a maximum after 4 hr of light. In the second and third cycles, both capacity and GPD activity were assayed. Photosynthetic competence reached a maximum value at hour 4 of the second cycle and at hour 10 during the third cycle. The pattern of activity of GPD consistently paralleled the rhythm of capacity throughout the two successive days shown. There appears to be no precisely fixed phase relation, however, between photosynthetic capacity and GPD activity on the one hand and the light-dark cycle on the other in stationary cultures. A gradual decrease in the absolute level of the oscillation occurred during the three consecutive cycles that were measured.

*Euglena* can be grown synchronously with a period length of approximately 24 hr when high frequency LD cycles (*e.g.*, *LD*: *2*,4 or *LD*: *1*,3) are used as an entraining agent (18). The cells are not considered to be locked on to any particular phase of the high frequency cycles but rather appear to be effectively in a "free-running" state with the timing of division being rated by an endogenous circadian "clock(s)." Although the period length (time between onsets of division) of successive cell cy-

cles is variable, those cells which are prepared to divide (several subpopulations almost certainly exist) do so at the same time, thereby generating the observed fission bursts in the population. Since cell division occurred synchronously and with a circadian period under these high frequency cycles in our present experiments, the question arose as to whether the rhythm of photosynthetic capacity would behave in a similar fashion, resembling the pattern observed in *LD*: *10*,14 cycles.

Interestingly, two types of responses were discovered. One typical pattern of photosynthetic capacity obtained in *LD*: *2*,4 (Fig. 9) indicated that the rhythm of capacity was directly synchronized by the high frequency LD cycle: capacity increased during each 2-hr light period and then decreased during each subsequent 4-hr dark period. GPD activity (not shown) was also measured; it followed *precisely the same pattern as the capacity rhythm*. The 6-hr period of both parameters, therefore, contrasts with the 24-hr period found for the cell division rhythm. On the other hand, an alternative pattern of capacity was sometimes observed in high frequency cycles and is depicted in Figure 10. Although the changes in capacity showed a reduced amplitude, the pattern was similar to the one found in *LD*: *10*,14 cycles, in that the capacity increased for 6 hr (despite the fact that this time span now comprised a 4-hr dark period) before it began to decrease. The activity of GPD closely followed the rhythm of photosynthetic capacity in this case also.

## DISCUSSION

The rhythmic pattern of $CO_2$ fixation in LD cycles presented in this investigation is rather similar to results in several other organisms. When *Gonyaulax polyedra* was grown in *LD*: *12*,12 the maximum in photosynthetic capacity occurred during the mid- to latter part of the light period and then declined to its original level by the middle of the dark period (23). A similar pattern of capacity was observed during the life cycle of *Scenedesmus obliquus* (46). The photosynthetic rhythm in *Acetabularia crenulata* has been measured polarographically in an *LD*: *8*,16 cycle and found to reach a peak during the latter hours of the light period and then to decrease rapidly during the dark period (55).

Our results differ, however, from other observations reported for *Euglena*. Lövlie and Farfaglio (32), using a Cartesian diver, measured photosynthetic activity of single *Euglena* cells taken from a synchronized (*LD*: *17*,7) population. Their results showed that photosynthetic activity increased during the interdivision (light) period and remained constant during division. In contrast to these results, Cook (11) indicated that oxygen evolution increased during the entire cell cycle of *Euglena*, that is, throughout both the light and dark periods. Simultaneous measurements of RNA and protein showed that these two parameters also continued linearly throughout both light and dark periods, which contradicts the results presented here as well as previous work (10, 16). On the other hand, the pattern of chlorophyll and carotenoid syntheses which Cook observed were similar to those presented in our study.

Recently, Codd and Merrett (9) found that the rate of $CO_2$ fixation doubled in a continuous manner throughout the light phase followed by only a slight reduction in the dark phase during synchronous growth (*LD*: *14*,10) of *Euglena*. These workers did not examine whole cell uptake, however, but instead studied incorporation into various fractions. Their ethanol-soluble and cell residue fractions closely paralleled the results presented in this investigation. Furthermore, if one summates their various cellular fractions, the "reconstituted" whole cell uptake thus obtained is similar to the data obtained under our conditions.

When the rhythm of photosynthetic capacity in *Euglena* was examined under constant conditions of illumination and temperature (Fig. 4), the rhythm continued for 1 full day but then did not persist thereafter. Stationary-phase cells placed in constant illumination of various intensities (not shown) also showed an immediate damping of the rhythm. The rhythm of photosynthetic capacity in *Euglena* presented in this discussion, therefore, is unlike the strongly persisting 24-hr rhythms (*i.e.*, circadian rhythm *sensu stricto*) demonstrated in other algae (23, 36, 54, 55, 57), since it does not appear to persist for any length of time under a variety of intensities of dim illumination. Speculations about this lack of persistence appear later in the discussion.

Several workers have reported that the diurnal variation in photosynthesis observed in natural plankton populations is a result of a corresponding change in chlorophyll *a* concentrations (47, 58). However, in the majority of cases there has been no correspondence between photosynthetic capacity and chlorophyll content (23, 28, 36). Chlorophyll is not a causal parameter underlying the observed photosynthetic capacity rhythm in *Euglena*. Chlorophyll increased linearly during the entire light period and, on an aliquot basis, remained at an elevated level during the ensuing darkness. Photosynthetic capacity, in contrast, did not increase linearly during the entire

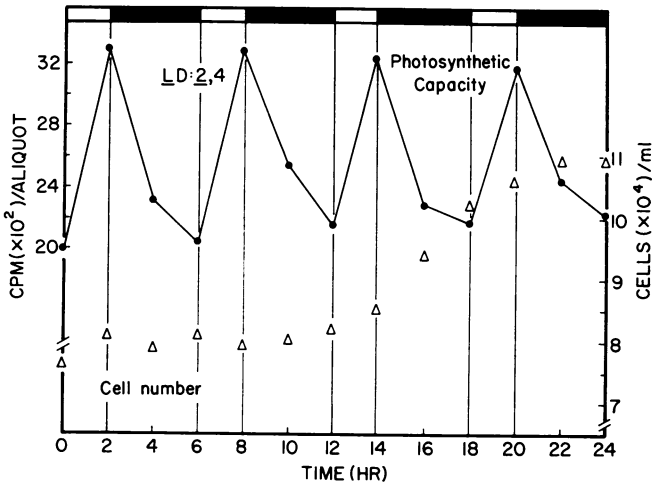


FIG. 9. Entrained rhythm of photosynthetic capacity in *Euglena* exposed to high frequency (*LD*: *2*,4) light-dark cycles. ●: Capacity; △: cell concentration.

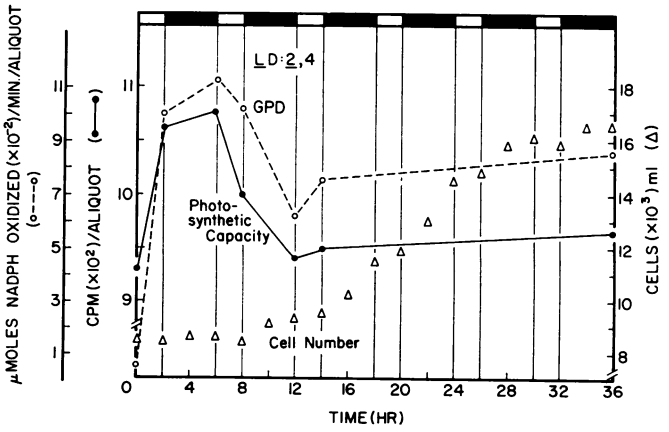


FIG. 10. Nonentrained rhythms of photosynthetic capacity (●), GPD activity (○), and cell division (△) in *Euglena* cultures maintained in high frequency (*LD*: *2*,4) light-dark cycles.

light period but began to decline during the last several hours of light and continued this decrease during the dark period (Fig. 2). Also, when plotted on a per milligram of chlorophyll basis, the capacity increased at a much faster rate than chlorophyll synthesis. Thus, it appears that the capacity of cells (based on chlorophyll content) to perform maximum photosynthesis occurs in the early developmental stages of the cell cycle. This was also found to be the case in the high temperature strain *Chlorella* 7-11-05, where the rate of photosynthesis surpassed that of other cellular constituents during the early developmental stages (48). Sorokin (50) observed in nonsynchronized suspensions of *Chlorella* subjected to fractional centrifugation that the smaller (younger) cells possessed higher photosynthetic activity than the larger (older) cells.

Several studies on photosynthetic capacity during the cell cycle have implicated a control (or partial control) by fluctuations in the light reactions of photosynthesis (24, 41–46, 49). The data reported here collectively indicate, however, that light reactions do not directly underlie the observed rhythm of capacity in *Euglena*. During the 10-hr light period, when photosynthesis was rapidly increasing, there were no significant variations in the light-limited portion of the light-saturation curves, in Hill reaction activity, or in photoreduction of methyl red. Light-saturation curves did show a corresponding change in the light-saturated portion of the curves implying a control by dark reactions.

When the activity of RuDP carboxylase was followed during the cell cycle of *Euglena*, no direct correlation between enzyme activity and $CO_2$ fixation (Fig. 6) was found. These results differ from those of Codd and Merrett (9) who have discussed the function of RuDP carboxylase as a regulatory mechanism in *Euglena*. However, although they found that the activity of this enzyme paralleled changes in photosynthetic rate, the activity was not great enough to satisfy the rates of $CO_2$ fixation at all of the stages investigated. The activity pattern itself of carboxylase that we find (Fig. 6) is similar to that of Codd and Merrett (9). Our data also indicate that rates of $CO_2$ fixation exceed those of enzyme activity. Initial evidence in *Gonyaulax polyedra* showed a direct correlation between the rhythm of capacity and carboxylase (53), although more recent data revealed that this was artifactual (8).

Because the activity of RuDP carboxylase was found not to parallel the pattern of photosynthetic capacity, and since other enzymes have been implicated in the regulation of the Calvin cycle, GPD was investigated. The similarity between both the phase and pattern of GPD activity and the photosynthetic rhythm in dividing cultures during LD cycles (Fig. 7) suggests a possible control of the latter by GPD. The relation between enzyme activity and photosynthetic capacity in cells grown in stationary phase conditions (Fig. 8) and in high frequency cycles (Figs. 9 and 10) offers further support to this hypothesis.

This enzyme has been studied in detail by Melandri *et al.* (34). In greening experiments with pea plants, NADP-dependent GPD activity has been shown to be light-induced and independent from net protein synthesis; activation was found to correspond to a reversible increase of the $V_{max}$ and to involve a stable, reversible arrangement of the enzyme structure rather than a single allosteric effect. The authors conclude that NADPH and ATP are not the true activators. These latter results are similar to those we obtained for *Euglena*. (In contrast, in spinach (35) the activity of GPD was strongly dependent upon the cofactors ATP and NADPH.) Furthermore, the light activation of GPD observed by several investigators (29, 34, 35, 51) indicates that this enzyme could control carbon fixation.

On the other hand, it is quite possible, of course, that capacity and GPD activity are causally unrelated independent variables whose rhythmicity is generated by some other factor(s) such as changes in chloroplast shape (57), structure, membrane permeability, or by other enzymes of the Calvin cycle (1, 6, 24, 30, 51). Indeed, Ben-Amotz and Avron (3) have recently demonstrated that the addition of disalicylidenepropanediamine (an inhibitor of ferredoxin-dependent reactions) to dark-grown *Euglena* cells severely inhibited the light-induced formation of NADP-dependent GPD. However, this inhibitor did *not* prevent the formation of chlorophyll or NAD-dependent GPD or the development of photosynthetic capability (as measured by $O_2$ evolution) in the light.

The experiments under nondividing conditions in LD cycles (Fig. 8) demonstrated that a rhythm of photosynthetic capacity could still continue for at least 10 days in spite of being uncoupled from the cell division cycle and from simple periodic gene duplication. This uncoupling, however, did cause a gradual decrease in the absolute level of the oscillation even though the culture was maintained on a *LD*: *10*,14 regime. Although there was no precisely fixed phase relation between the capacity rhythm and the LD cycles, it is significant that GPD activity nevertheless followed the phase of the capacity rhythm.

The two different patterns of photosynthetic capacity observed in high frequency LD cycles were both unexpected and interesting. In one case (Fig. 9), there was a complete dissociation of the capacity rhythm from the "free-running" circadian cell division pattern manifested by the population—direct entrainment to the 6-hr high frequency cycles occurred. Therefore, although the cell division rhythm either free-ran or perhaps resulted from frequency demultiplication (summation of 4 high-frequency cycles to produce a single circadian output), the capacity rhythm apparently was directly entrained by the LD cycles to a 6-hr period. It is interesting to compare these results to the photosynthetic rhythm in *Hydrodictyon*, a green alga, which will directly entrain to an *LD*: *6*,6 cycle and will persist for at least a few cycles in constant conditions with a period length of 12 hr rather than revert to a 24-hr rhythm, as is typical in most other organisms (40).

The other pattern of photosynthetic capacity in high frequency cycles (Fig. 10) is not uncoupled from the circadian cell division cycle and could also be considered a free-running rhythm (18). In this instance both cell division and photosynthetic capacity displayed a circadian periodicity in spite of the imposed *LD*: *2*,4 cycles. This suggests that the capacity rhythm may be controlled (although somewhat loosely) by the same cellular clock mechanism that controls the free-running rhythm of cell division.

Although the cultures in each type of experiment experienced 2 days of high frequency LD cycles before photosynthetic capacity was assayed, there were noticeable differences between them with respect to the degree of synchronization. In the case where photosynthetic capacity was directly entrained (Fig. 9), the step-size (step-size = factor by which cell concentration increases) during the last two *LD*: *2*,4 cycles, *i.e.*, the last 12 hr of the 24-hr day, was quite reduced (step-size = 1.30), while the period length of the division rhythm was about 24 hr. In contrast, when a free-running rhythm of capacity was obtained (Fig. 10), the step-sizes of the division bursts were much greater (step-size = 1.80) and the period lengths were longer (36 hr in the case illustrated). These differences between the two types of cultures with regard to step-size and period of the cell division rhythm could be responsible for the observed differences in the pattern of photosynthetic capacity.

The lack of any long term persistence of the capacity rhythm in *Euglena*—the classical test for an endogenous circadian

rhythm—could be caused by any one of several factors. The rhythm could be controlled by an oscillator that is coupled to the cell division clock and becomes rapidly disengaged under the imposed dim light conditions when cell division ceases. This seems implausible, however, in view of the finding that the oscillation continued for at least 10 days in *LD*: *10*,14 under nondividing, stationary conditions; it must be noted, however, that the absolute level of the oscillation did gradually decrease over this time span. Alternatively, the capacity rhythm might also be controlled by its own clock mechanism, but the transducing reactions through which the clock operates may not be able to maintain their rhythmicity in $LL_d$. Thirdly, the Calvin cycle enzyme(s) controlling photosynthetic capacity (*e.g.*, glyceraldehyde-3-dehydrogenase) may be dependent on additional cofactors (*e.g.*, NADPH and ATP) which cannot be produced in sufficient quantity in $LL_d$ to support a longer persistence of the rhythm. Still another possibility is that the decay of the rhythm in the population in the absence of a driving *Zeitgeber* reflects the accumulation of spread in period lengths among the individual, still-rhythmic cells comprising it (*i.e.*, secondary asynchrony as opposed to a true lack of primary rhythmicity in the constituent members of the population) (18). If so, such decay would be in contrast to the long term persistence of the rhythms of phototaxis (5), dark motility (4), settling (56), and cell division (17–19, 27) already documented for *Euglena*. The monitoring of photosynthetic capacity in *single* cells in LD and $LL_d$ would be most illuminating (32). Finally, of course, there is the possibility that an endogenous circadian clock does not participate at all in the regulation of the capacity rhythm and that the oscillation observed in $LL_d$ for 1 full day following removal of the *LD*: *10*,14 cycle is merely the reflection of "residual" synchrony.

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## LITERATURE CITED


1. Anderson, L., L. E. Worthen, and R. C. Fuller. 1968. The role of ribose-5P isomerase in regulation of the Calvin cycle in *Rhodospirillum rubrum*. *In:* K. Shibata, A. Takamiya, A. T. Jagendorf, and R. C. Fuller, eds.. Comparative Biochemistry and Biophysics of Photosynthesis. State College, University Park Press, University Park. pp. 379–386.
2. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 25: 1–15.
3. Ben-Amotz, A. and M. Avron. 1972. Is nicotinamide adenine dinucleotide phosphate an obligatory intermediate in photosynthesis? Plant Physiol. 49: 244–248.
4. Brinkmann, K. 1966. Temperatureinflüsse auf die circadiane Rhythmik von *Euglena gracilis* bei Mixotrophie und Autotrophie. Planta 70: 344–389.
5. Bruce, V. G. and C. S. Pittendrigh. 1956. Temperature independence in a unicellular "clock". Proc. Nat. Acad. Sci., U. S. A. 42: 676–682.
6. Buchanan, B. B., P. Schürmann, and P. P. Kalberer. 1971. Ferredoxin-activated fructose diphosphatase of spinach chloroplasts. Resolution of the system, properties of the alkaline fructose diphosphatase component, and physiological significance of the ferredoxin-linked activation. J. Biol. Chem. 246: 5952–5959.
7. Burton, K. 1955. A study of the conditions and mechanisms of the dephenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemistry, 62: 315–322.
8. Bush, K. J. and B. M. Sweeney. 1972. The activity of ribulose diphosphate carboxylase in extracts of *Gonyaulax polyedra* in the day and night phases of the circadian rhythm of photosynthesis. Plant Physiol. 50: 446–451.
9. Codd, G. A. and M. J. Merrett. 1971. Photosynthetic products of division synchronized cultures of *Euglena*. Plant Physiol. 47: 635–639.
10. Cook, J. R. 1961. *Euglena gracilis* in synchronous division. II. Biosynthetic rates over the light cycle. Biol. Bull. 121: 277–289.
11. Cook, J. R. 1966a. Photosynthetic activity during the division cycle in synchronized *Euglena gracilis*. Plant Physiol. 41: 821–825.
12. Cook, J. R. and T. W. James. 1960. Light-induced division synchrony in *Euglena gracilis* var. *bacillaris*. Exp. Cell Res. 21: 583–589.
13. Cramer, M. and J. Myers. 1952. Growth and photosynthetic characteristics of *Euglena gracilis*. Arch. Mikrobiol. 17: 384–402.
14. Edmunds, L. N., Jr. 1964. Replication of DNA and cell division in synchronously dividing cultures of *Euglena gracilis*. Science 145: 266–268.
15. Edmunds, L. N., Jr. 1965. Studies on synchronously dividing cultures of *Euglena gracilis* Klebs (strain Z). I. Attainment and characterization of rhythmic cell division. J. Cell. Comp. Physiol. 66: 147–158.
16. Edmunds, L. N., Jr. 1965. Studies on synchronously dividing cultures of *Euglena gracilis* Klebs (strain Z). II. Patterns of biosynthesis during the cell cycle. J. Cell. Comp. Physiol. 66: 159–182.
17. Edmunds, L. N., Jr. 1966. Studies on synchronously dividing cultures of *Euglena gracilis* Klebs (strain Z). III. Circadian components of cell division. J. Cell. Physiol. 67: 35–44.
18. Edmunds, L. N., Jr., and R. R. Funch. 1969a. Effects of "skeleton" photoperiods and high frequency light-dark cycles on the rhythm of cell division in synchronized cultures of *Euglena*. Planta 87: 134–163.
19. Edmunds, L. N., Jr., and R. R. Funch. 1969b. Circadian rhythm of cell division in *Euglena:* effects of a random illumination regime. Science 165: 500–503.
20. Feldman, J. F. 1968. Circadian rhythmicity in amino acid incorporation in *Euglena gracilis*. Science 160: 1454–1456.
21. Fuller, R. C. and M. Gibbs. 1959. Intercellular and phylogenetic distribution of ribulose-1,5-diphosphate carboxylase and D-glyceraldehyde-3-phosphate dehydrogenases. Plant Physiol. 34: 324–329.
22. Fuller, R. C. and G. A. Hudock. 1967. Triose phosphate dehydrogenase in plant and microbial photosynthesis. *In:* T. W. Goodwin, eds., Biochemistry of Chloroplasts, Vol. II. Academic Press, Inc., New York. pp. 181–190.
23. Hastings, J. W., L. Astrachan, and B. M. Sweeney. 1961. A persistent daily rhythm in photosynthesis. J. Gen. Physiol. 45: 69–76.
24. Hellebust, J. A., J. Terborgh, and G. C. McLeod. 1967. The photosynthetic rhythm of *Acetabularia crenulata*. II. Measurements of photoassimilation of carbon dioxide and the activities of enzymes of the reductive pentose cycle. Biol. Bull. 133: 670–678.
25. Hoober, J. K., P. Siekevitz, and G. E. Palade. 1969. Formation of chloroplast membranes in *Chlamydomonas reinhardi* Y-1: Effects of inhibitors on protein synthesis. J. Biol. Chem. 244: 2621–2631.
26. Hudock, G. A. and R. C. Fuller. 1965. Control of triosephosphate dehydrogenase in photosynthesis. Plant Physiol. 40: 1205–1211.
27. Jarrett, R. M. and L. N. Edmunds, Jr. 1970. Persisting circadian rhythm of cell division in a photosynthetic mutant of *Euglena*. Science 167: 1730–1733.
28. Jorgensen, E. G. 1966. Photosynthetic activity during the life cycle of synchronous *Skeletonema* cells. Physiol. Plant. 19: 789–799.
29. Kirk, J. T. O. and R. L. Allen. 1965. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. Biochem. Biophys. Res. Commun. 21: 523–530.
30. Latzko, E., Garnier, R. V., and M. Gibbs. 1970. Effect of photosynthesis, photosynthetic inhibitors and oxygen on the activity of ribulose 5-phosphate kinase. Biochem. Biophys. Res. Commun. 39: 1140–1144.
31. Latzko, E. and M. Gibbs. 1968. Distribution and activity of enzymes of the reductive pentose phosphate cycle in spinach leaves and in chloroplasts isolated by different methods. Z. Pflanzenphysiol. 59: 184–194.
32. Lövlie, A. and G. Farfaglio. 1965. Increase in photosynthesis during the cell cycle of *Euglena gracilis*. Exp. Cell Res. 39: 418–434.
33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
34. Melandri, B. A., P. Pupillo, and A. Baccarini-Melandri. 1970. D-Glyceraldehyde-3-phosphate dehydrogenase in photosynthetic cells. I. The reversible light-induced activation *in vivo* of NADP-dependent enzyme and its relationship to NAD-dependent activities. Biochim. Biophys. Acta 220: 178–189.
35. Müller, B. 1970. On the mechanism of the light-induced activation of the NADP-dependent glyceraldehyde phosphate dehydrogenase. Biochim. Biophys. Acta. 205: 102–109.
36. Palmer, J. D., L. Livingston, and F. D. Zusy. 1964. A persistent diurnal rhythm in photosynthetic capacity. Nature 203: 1087–1088.
37. Perini, F., M. D. Kamen, and J. A. Schiff. 1964. Iron containing proteins in *Euglena*. I. Detection and characterization. Biochim. Biophys. Acta 88: 74–90.
38. Pupillo, P. 1972. The specificity of glyceraldehyde-3-phosphate dehydrogenases in green plants, *Euglena* and *Ochromonas*. Phytochemistry 11: 153–161.
39. Russell, G. K. and H. Lyman. 1968. Isolation of mutants of *Euglena gracilis* with impaired photosynthesis. Plant Physiol. 43: 1284–1290.
40. Schön, W. J. 1955. Periodische Schwankungen der Photosynthese und Atmung bei *Hydrodictyon*. Flora 142: 347–380.
41. Schor, S., P. Siekevitz, and G. E. Palade. 1970. Cyclic changes in thylakoid membranes of synchronized *Chlamydomonas reinhardi*. Proc. Nat. Acad. Sci., U. S. A., 66: 174–180.
42. Senger, H. 1970. Charakterisierung einer Synchronkultur von *Scenedesmus obliquus* ihrer potentiellen Photosyntheseleistung und des Photosynthese-Quotienten während des Entwichlungscyclus. Planta 90: 243–266.
43. Senger, H. 1970. Quantenausbente und unterschiedliches Verhalten der beiden

Photosysteme des Photosyntheseapparatus während des Entwicklungsablaufes von *Scenedesmus obliquus* in Synchronkulturen. Planta 92: 327-346.

44. SENGER, H. AND N. I. BISHOP. 1967. Quantum yield of photosynthesis in synchronous *Scenedesmus* cultures. Nature 214: 140-142.
45. SENGER, H. AND N. I. BISHOP. 1969. Changes in the photosynthetic apparatus during the synchronous life cycles of *Scenedesmus obliquus*. *In:* H. Metzner, ed.. Progress in Photosynthetic Research, Vol. I. Verlag C. Lichtenstern, München. pp. 425-434.
46. SENGER, H. AND N. I. BISHOP. 1969. Emerson enhancement effect in synchronous *Scenedesmus* culture. Nature 221: 975.
47. SHIMADA, B. M. 1958. Diurnal fluctuations in photosynthetic rate and chlorophyll *a* content of phytoplankton from eastern pacific waters. Limnol. Oceanog. 3: 336-337.
48. SOROKIN, C. 1957. Changes in photosynthetic activity in the course of cell development in *Chlorella*. Physiol. Plant. 10: 659-666.
49. SOROKIN, C. 1960. Photosynthetic activity in synchronized cultures of algae and its dependence on light intensity. Arch. Mikrobiol. 37: 151-160.
50. SOROKIN, C. 1965. Photosynthesis in cell development. Biochim. Biophys. Acta 94: 42-52.
51. STEIGER, E., I. ZIEGLER, AND H. ZIEGLER. 1971. Unterschiede in der Lichtaktivierung der NADP-abhängigen Glycerinaldehyd-3-phosphat-Dehydrogenase und der Ribulose-5-phosphat-Kinase bei Pflanzen des Calvin- und des $C_4$-Dicarbonsäure-Fixierungstypus. Planta 96: 109-118.
52. SULZMAN, F. M. AND L. N. EDMUNDS, JR. 1972. Persisting circadian oscillations in enzyme activity in non-dividing cultures of *Euglena*. Biochem. Biophys. Res. Commun. 47: 1338-1344.
53. SWEENEY, B. M. 1969. Transducing mechanisms between circadian clock and overt rhythms in *Gonyaulax*. Can. J. Bot. 47: 299-308.
54. SWEENEY, B. M. AND F. T. HAXO. 1961. Persistence of a photosynthetic rhythm in enucleated *Acetabularia*. Science 134: 1361-1363.
55. TERBORGH, J. AND G. C. MCLEOD. 1967. The photosynthetic rhythm of *Acetabularia crenulata*. I. Continuous measurements of oxygen exchange in alternating light-dark regimes and in constant light of different intensities. Biol. Bull. 133: 659-669.
56. TERRY, O. W. AND L. N. EDMUNDS, JR. 1970. Rhythmic settling induced by temperature cycles in continuously-stirred autotrophic cultures of *Euglena gracilis* (Z strain). Planta 93: 128-142.
57. VANDEN DRIESSCHE, T. 1966. Circadian rhythms in *Acetabularia:* photosynthetic capacity and chloroplast shape. Exp. Cell Res. 42: 18-30.
58. YENTSCH, C. S. AND J. H. RYTHER. 1957. Short-term variations in phytoplankton chlorophyll and their significance. Limnol. Oceanog. 2: 140-142.