the other in a photophil phase, the inhibition caused by the interruption in the photophobe phase can only partially be overcome by a light interruption in a photophil phase either before or after the photophobe perturbation.

Two-hour perturbations may initiate weak rhythms which interact with one another to some extent, however, they are not capable of altering the basic endogenous rhythm which is initiated by the 8-hour photoperiod.

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Regulation of Photosynthesis in Chlamydomonas reinhardi [1, 2]

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Phototrophic growth is possible only in those organisms that possess photoreceptors, photopigments, the photosynthetic electron transport chain, and the reductive pentose-P cycle or other biochemical pathway for carbon reduction. There are many different organisms, however, that are facultative phototrophs, for they can grow as heterotrophs when provided with an appropriate form of reduced carbon as an energy source.

The activity of one enzyme of the reductive pentose-P cycle, ribulose 1,5-diP (RuDP) carboxylase, is a function of growth conditions in several organisms (2, 3, 8). This enzyme catalyzes the reaction of CO_2 with RuDP in photosynthetic organisms (4, 6, 12, 19). Fuller and Gibbs (2) showed that the activity of this enzyme in albino mutants of barley was low compared to that found in green barley. Dark-adapted or streptomycin-bleached *Euglena* were found to contain lower RuDP carboxylase activity than green, light-grown *Euglena*. *Chlorella variegata* contained high levels of RuDP carboxylase activity when grown as a phototroph, but heterotrophic growth, either in the light or in the dark, resulted in both a decreased chlorophyll content and decreased RuDP carboxylase activity. Fuller et al. (3) have also shown that the presence of sodium acetate or malate in the growth medium of *Chromatium* results in a decrease in RuDP carboxylase activity. Lascelles (8) demonstrated that the synthetic of this enzyme occurs at about the same rate as bacteriochlorophyll synthesis in *Rhodopseudomonas spheroides*. Growth conditions which resulted in a cessation of bacteriochlorophyll synthesis also resulted in an immediate cessation of RuDP carboxylase synthesis.

These observations suggest that it should be possible to investigate the cellular regulation of photosynthesis by examining chloroplast formation, pigment synthesis, the level of activity of certain enzymes of photosynthesis, and the activity of the photosynthetic electron transport chain under conditions of phototrophic and heterotrophic growth. To this end, we have initiated an investigation concerned with the regulation of photosynthesis in the unicellular green alga *Chlamydomonas reinhardi*. The purpose of this report is to describe the effect of growth conditions

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on the activities of RuDP carboxylase and photosynthetic pyridine nucleotide reductase (PPNR), and on the rates of O_2 evolution, CO_2 fixation, and TPN photoreduction. It will be demonstrated that the rates of these reactions are correlated with pigment content. Whereas the rates of certain photosynthetic reactions change in a manner which is directly related to chlorophyll content, an indirect and more complex relationship exists for others.

Materials and Methods

Organisms. The wild type strain (137c) of *C. reinhardi* and mutant strains derived from it were used in these studies. The wild type strain is capable of phototrophic growth on a minimal medium composed solely of inorganic salts. It can be cultured as a heterotroph in the dark with sodium acetate as a source of reduced carbon and energy. Chlorophyll and carotenoid synthesis occur under both conditions of growth, but as will be pointed out below, the chlorophyll content of dark-grown cells is about 50 % less than that of light-grown cells. In addition there are minor differences in the carotenoid contents of cells cultured under the 2 conditions (7).

A series of ultra-violet induced mutant strains derived from the wild type strain are unable to reduce CO_2 in the light and must be provided with

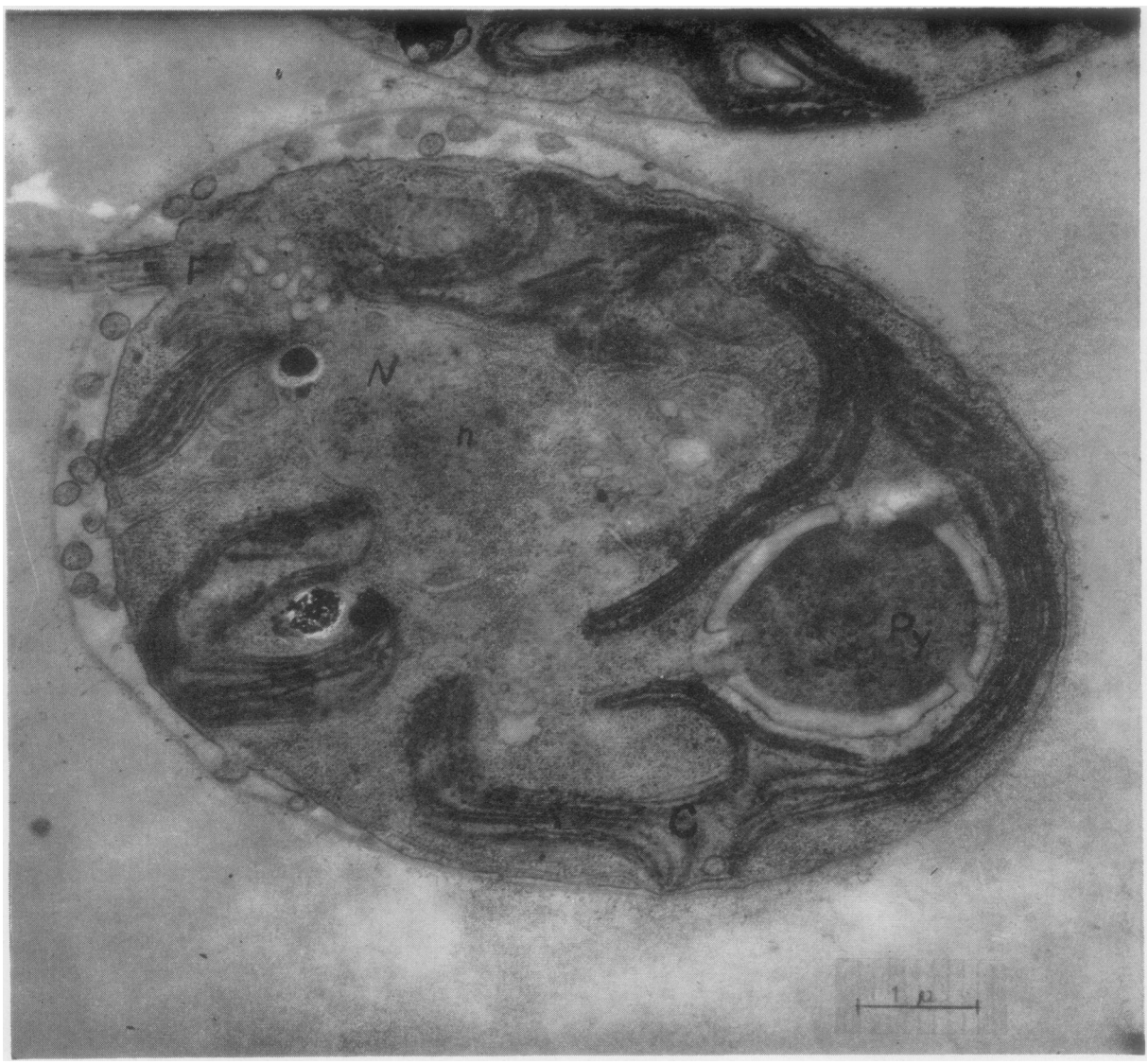
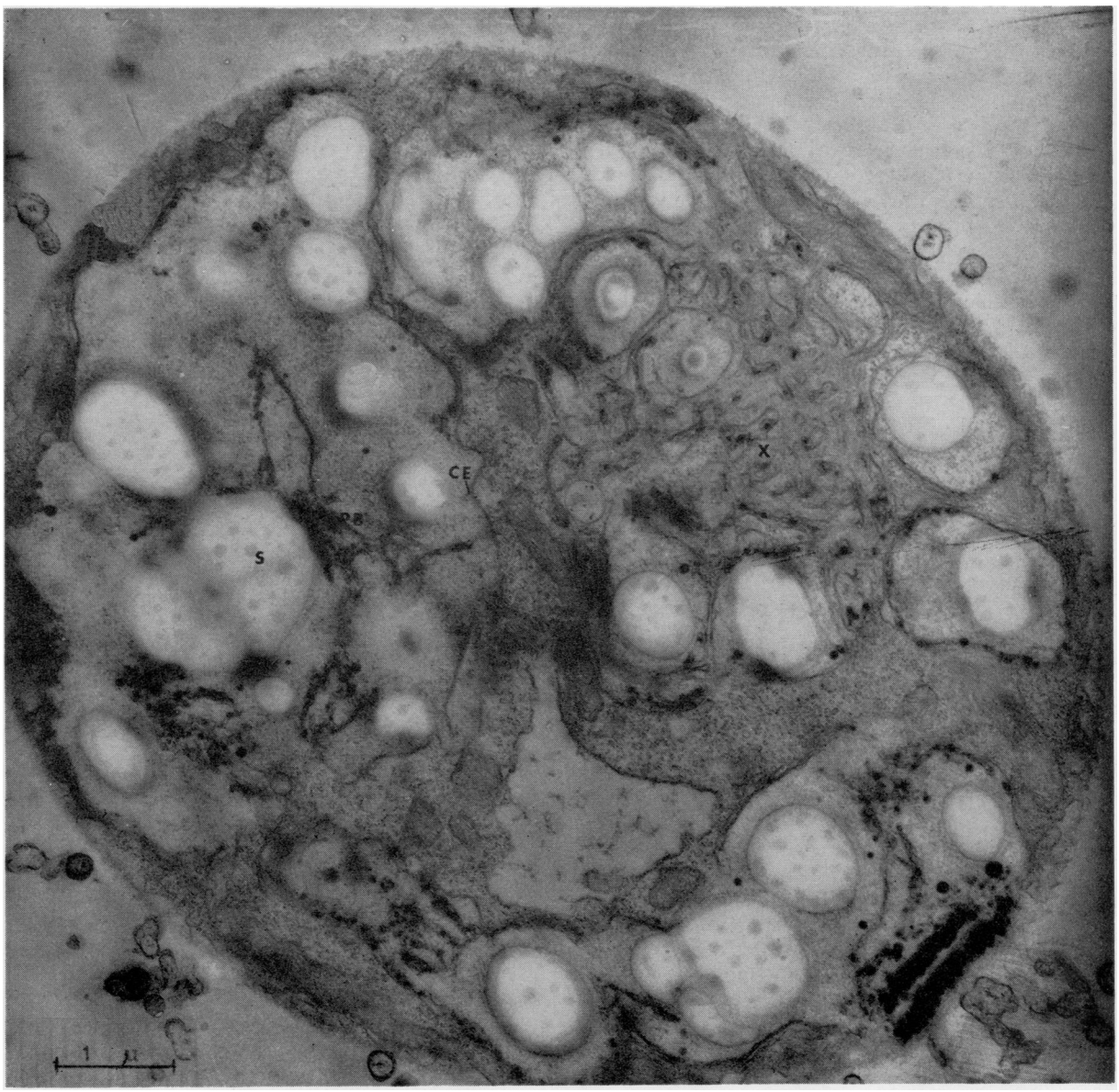


FIG. 1. Electron micrographs of the mutant strain *y-2*. C = chloroplast, CE = chloroplast envelope, F = flagellum, N = nucleus, n = nucleolus, PB = prolamellar body, Py = pyrenoid, S = starch grain. A (*above*), the mutant strain *y-2* during growth in the light. B (*opposite page*), the mutant strain *y-2* following growth in the dark for 96 hours.

sodium acetate either in the light or in the dark. Three such mutant strains, *ac-21, ac-40,* and *ac-115,* have been characterized (10) and were used in this study. Each of these mutant strains is capable of chlorophyll synthesis in the dark.

A fourth mutant strain of *C. reinhardi* used in this study is designated as *y-2*. It differs from wild type in that it can neither form a normal chloroplast (fig 1) nor synthesize chlorophyll (fig 2) in the dark. When dark-grown cells are returned to the light, a process of regreening ensues during which chlorophyll is synthesized (fig 2) and a normal chloroplast is formed. Light-grown cells of *y-2* are indistinguishable from light-grown wild type with respect to both chloroplast structure and chlorophyll content. Thus, this mutant strain is similar to others reported for *C. reinhardi* (15).

Growth Conditions. Cultures of the wild type and *y-2* mutant strains of *C. reinhardi* were grown at 25° in the liquid high salt (HS) medium of Sueoka (18) or in this medium supplemented with 0.20 % sodium acetate (Ac medium). All cultures of the acetate-requiring strains were grown in the acetate-supplemented medium. Cultures were exposed to a light intensity of 8,500 lux from daylight fluorescent lamps or placed in flasks covered with black electrical tape. To establish the effect of CO_2 on the activity of RuDP carboxylase, cultures were aerated with 5 % CO_2 in air or with air from which CO_2 had been removed by passage through 2 N KOH. After



the effect of CO_2 had been established, cultures were aerated by continuous shaking on a gyrotary shaker. Cells from cultures in the exponential phase of growth were harvested by centrifugation (2 min at 4,000 × *g*). Dark-grown cultures were maintained in exponential growth by transfer to fresh medium every 48 hours. When cells of *y-2* were to be transferred from the dark to the light, they were harvested by centrifugation and resuspended in fresh Ac medium.

Chlorophyll Determination. Cells were harvested and washed once in 0.068 M K phosphate buffer at pH 6.8. The cells were resuspended in a small volume of this buffer. Cell density was determined by Hemacytometer count. Following a determination of chlorophyll concentration by Arnon's (1) modification of Mackinney's (11) procedure, chlorophyll content was expressed as $\mu g/10^6$ cells. The concentration of chlorophylls a and b was calculated using the method of Mackinney (11).

Preparation and Assay of RuDP Carboxylase. A concentrated suspension of cells in 0.05 M tris at pH 8.0 was disrupted by sonic oscillation at 0° for 3 minutes with a Mullard 20 KC Ultrasonic Disintegrator. The preparation was then centrifuged for 20 minutes at 27,000 × *g* at 0°. The clear, pale green, supernatant fluid was used as a crude preparation of RuDP carboxylase. A unit of enzyme activity was defined as that amount which fixed 1 μmole of CO_2 per hour. Specific activity is given as units per mg protein.

RuDP carboxylase activity was assayed by a modification of the method of Smillie (17). Reactions were initiated by the addition of $NaHC^{14}O_3$ (specific activity 1.0 $\mu c/\mu$mole) and terminated by the addition of 1.30 ml of a hot ethanol-acetic acid mixture (1.0 ml glacial acetic acid in 50.0 ml 95 % ethanol). It was determined that the rate of reaction was linear under these conditions for at least 15 minutes. Accordingly, reactions were run for a standard period of 10 minutes. A 1.0 ml sample of the reaction mixture was transferred to a planchet and the incorporated radioactivity was determined.

Carbon Dioxide Fixation by Whole Cells. Cultures in exponential growth phase were harvested, washed once in 0.068 M K phosphate buffer at pH 6.8, and resuspended in 5.0 ml of this buffer. Chlorophyll and cell concentrations were determined after which assays were carried out in 25.0 ml Erlenmeyer flasks. Each flask received a volume of cell suspension equivalent to 0.30 mg of chlorophyll or to a known number of cells and HS medium to a final volume of 2.50 ml. The cell suspension was then incubated in saturating light (30,000 lux from tungsten reflector flood lights) for 10 minutes at 25° with constant shaking after which reactions were started by the addition of 0.50 ml of a solution of $NaHC^{14}O_3$ (100 μmoles/ml, 0.20 $\mu c/\mu$mole). The rate of CO_2 fixation was linear for at least 15 minutes under these conditions. Reactions were terminated after 5 minutes by the addition of 0.30 ml of 1.0 N acetic acid. Dark controls were identical except that the flasks were covered with aluminum foil to exclude all light. An aliquot of cell suspension was transferred to a weighed planchet, dried, and the incorporated radioactivity was determined with a gas flow counter. After correction for self-absorption and efficiency of counting, the specific activity was determined as μmoles CO_2 fixed/hour/mg chlorophyll or as μmoles/hour/10^8 cells.

Oxygen Evolution by Whole Cells. O_2 evolution by whole cells was measured manometrically in a solution composed of equal volumes of 0.07 M $KHCO_3$ and 0.13 M $NaHCO_3$ (14). Cells were harvested and washed as above. The chlorophyll concentration and cell density were determined and a volume of cell suspension equivalent to 10^8 cells was placed in a 15.0 ml Warburg vessel. The final volume was brought to 3.20 ml with the bicarbonate solution. Control flasks were identical except that they were covered with aluminum foil. The reaction vessels were flushed with 5 % CO_2 in air for 10 minutes and then allowed to equilibrate in the dark until a constant rate of respiration was obtained. After equilibration, the flasks were illuminated at a light intensity of 30,000 lux from tungsten lamps and the rate of O_2 evolution was measured for at least 1 hour. It was found that chlorophyll formation by *y-2* in bicarbonate buffer was negligible during this period. The rate of O_2 evolution was determined as μmoles O_2 evolved/hour/10^8 cells from the measurements made during the first 20 minutes of exposure to light.

Preparation of Photosynthetic Pyridine Nucleotide Reductase. PPNR activity was measured in soluble protein extracts of algal cultures. Crude PPNR was prepared in a manner identical to RuDP carboxylase except that the cells were suspended in 0.05 M Tris buffer at pH 7.5. Purified PPNR from *C. reinhardi* was used in some experiments. An acetone powder was prepared from light-grown wild type cells. This powder was extracted with 0.10 M K phosphate buffer and dialyzed against this buffer overnight. The extract was then placed on a DEAE column and washed with 0.20 M K phosphate buffer. The PPNR was eluted from the column with 0.40 M K phosphate buffer. All buffers were at pH 7.0. A purification of approximately ten-fold was obtained.

Preparation of Chloroplast Fragments. Chloroplast fragments were prepared by a modification (10) of the method of San Pietro and Lang (16). Cells were harvested and washed in 0.05 M Tris buffer at pH 7.5. The chlorophyll content was determined and the cells were disrupted by sonic oscillation at 0° for 30 seconds. The suspension was centrifuged for 7 minutes at 480 × *g* to remove whole cells. The supernatant fiuid was then decanted and centrifuged for 20 minutes at 20,000 × *g*. The chloroplast fragments were resuspended in 0.05 M Tris buffer at pH 7.5 and flocules were broken up with a Ten Broek homogenizer. All operations were performed at 0° in the dark. The chlorophyll concentration in the preparation was determined. From the chlorophyll content per 10^6 cells and the chlorophyll concentration/ml of the chloroplast preparation, it was possible to determine the number of cells to which a given volume of chloroplast preparation was equivalent.

TPN Photoreduction. The rate of TPN photoreduction was measured in a Cary Model 14 recording spectrophotometer by a modification (9) of the method of San Pietro and Lang. The reaction was measured in 2 different ways: first, with chloroplast fragments from dark-grown or regreening *y-2* and an excess of PPNR purified from light-grown wild type *C. reinhardi;* and, second, with crude PPNR prepared from dark-grown or regreening *y-2* and chloroplast fragments prepared from light-grown *y-2*. In the first instance, the rate of TPN photoreduction was limited by the dark-grown *y-2* chloroplast fragments, whereas, in the second case, the reaction was limited by *y-2* PPNR. When the rate of the reaction was limited by PPNR, specific activity was expressed as units/mg protein. When the rate of the reaction was limited by the chloroplast fragments, specific activity was expressed as units/10^8 cells. A unit of activity will reduce 1 μmole of TPN/hour.

Protein Determination. In all cases, protein concentration was determined by the biuret method (5).

Results

Cultures of wild type were grown for 48 hours under 5 growth conditions and the specific activity of RuDP carboxylase was then determined in extracts of these cultures (table I). The highest specific activity of RuDP carboxylase was obtained in extracts of cells cultured as obligate phototrophs. The presence of sodium acetate in the growth medium, in the light or in the dark, and with or without exogenous CO_2, resulted in a decrease in enzyme activity. In no case, however, was a difference greater than two-fold noted.

Cultures of *ac-21, ac-40, ac-115* and wild type were grown in Ac medium in the light with CO_2 or in the dark in the absence of exogenous carbon dioxide. After 48 hours RuDP carboxylase was assayed in cell-free extracts of these cultures. The chlorophyll content of the cells decreased two-fold during growth in the dark. Whereas there is slight variation in the activity of this enzyme from strain to strain, the activity within a strain was independent of growth conditions studied.

Table I. *Specific Activity of RuDP Carboxylase in Cell-free Extracts of Wild Type C. reinhardi Grown Under Various Conditions*

Reaction mixtures contained in a total volume of 0.70 ml: enzyme preparation (0.10 ml), RuDP (0.10 μmole), $NaHC^{14}O_3$ (20 μmoles, specific activity 1.0 μc/μmole), Tris buffer, pH 8.0 (3.8 μmoles), $MgCl_2$ (1.50 μmole), ethylenediamine tetraacetic acid (0.009 μmole), GSH, pH 8.0 (0.09 μmole), and water to the final volume.

Growth conditions			
Medium	Light	CO_2	Specific activity units/mg protein
High salt	+	+	0.25
Acetate	+	+	0.18
Acetate	—	+	0.14
Acetate	+	—	0.12
Acetate	—	—	0.18

Chlorophyll synthesis and the rates of CO_2 fixation and O_2 evolution by whole cells of *y-2* during growth in the dark and following return to the light are given in figure 2. Under the conditions used, the rates of decrease and increase of photosynthetic oxygen evolution, carbon dioxide fixation, and chlorophyll synthesis were reproducible in over 10 experiments. It can be seen that a decrease in chlorophyll content began simultaneously with growth in the dark and that chlorophyll content decreased two-fold with each cell division in the dark until a relatively constant value was reached. This value is about 4 % of the chlorophyll content of light-grown cells. On return to the light there was a short lag period followed by chlorophyll synthesis at a rapid, exponential rate.

The rate of CO_2 fixation (fig 2) decreased in a manner identical to that of chlorophyll content during growth in the dark. On return to the light, the rate increased following a lag period similar in length to that observed for chlorophyll synthesis. This rapid rise was followed by a steady state level of carbon dioxide fixation comparable to that of light-grown cells.

Only values for O_2 evolution greater than 0.50

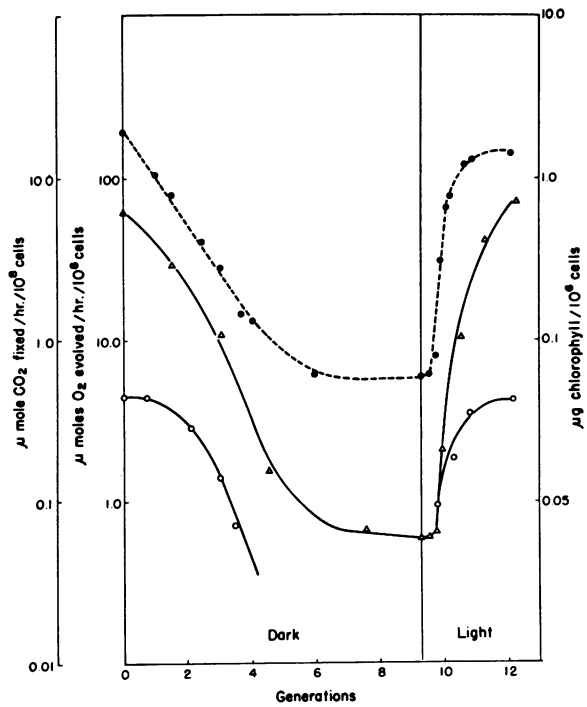


FIG. 2. Oxygen evolution, carbon dioxide fixation, and chlorophyll content of the mutant strain *y-2* during growth in the dark and following return to the light. ○ = μmoles oxygen evolved/hr/10^8 cells; △ = μmoles carbon dioxide fixed/hr/10^8 cells; ● = μg chlorophyll/10^6 cells.

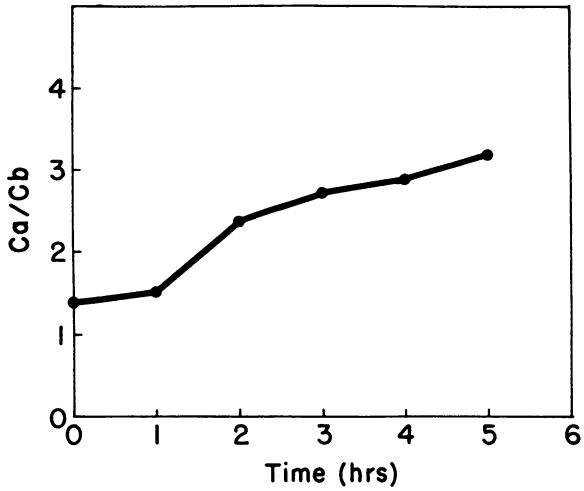


FIG. 3. The ratio of chlorophyll a to chlorophyll b (Ca/Cb) during the first 5 hours of exposure of a culture of *y-2* to the light following growth in the dark for 96 hours.

μmole/hour/10^8 cells could be detected by the method used here and detailed data derived from a more sensitive method will be presented in another report. However, it can be seen from the data given in figure 2 that the rate of O_2 evolution decreased about two-fold with each cell division in the dark following a lag period of about 1 generation. On return to the light, there was a rapid increase in the rate of oxygen evolution until the steady state level was reached.

The ratio (mg/mg) of chlorophyll a to chlorophyll b (Ca/Cb) was found to be 3.0 in light-grown *y-2*. It decreased to 1.2 to 1.4 in 96 hour dark-grown cultures. On return of a dark-grown culture to the light, the ratio increased to approximately 3.0 during the process of regreening (fig 3).

The soluble protein content of *y-2* was determined during growth in the dark and following return to the light, and was found to be relatively constant under both culture conditions.

The specific activity of RuDP carboxylase, measured in cell-free extracts prepared from aliquots of a culture of *y-2* during growth in the dark and following return to the light, did not follow changes in chlorophyll content. These data are given in figure 4. Whereas each cell division in the dark resulted in a two-fold decrease in chlorophyll content, about 2.5 generations were required for a two-fold decrease in enzyme activity. This decrease did not begin until after 2 generations of growth in the dark. On return of the dark-grown cells to the light, increase in the activity of RuDP carboxylase began shortly after the onset of chlorophyll synthesis and the normal level of activity was reached within a period of 2 cell generations.

The rate of TPN photoreduction and the specific activity of PPNR in extracts of *y-2* prepared from cells during growth in the dark and following return to the light were also compared to changes in chlorophyll content (fig 5). When the rate of TPN photoreduction was limited by the *y-2* chloroplasts, it changed at about the same rate as chlorophyll content. On the other hand, changes in the specific activity of PPNR were similar to those observed for the specific activity of RuDP carboxylase. During growth in the dark, about 2.5 generations were required for a two-fold decrease in PPNR activity.

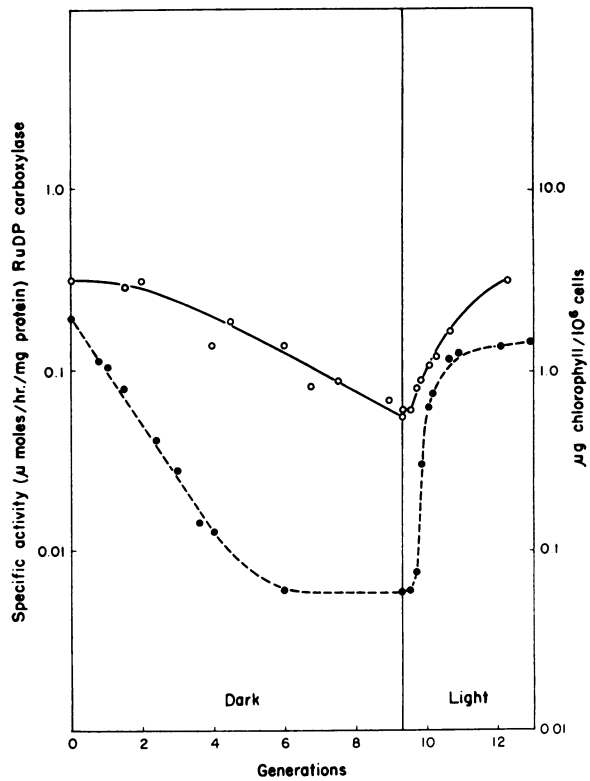


FIG. 4. Chlorophyll content and specific activity of RuDP carboxylase in the mutant strain *y-2* during growth in the dark and following return to the light. ● = μg chlorophyll/10^6 cells; ○ = specific activity of RuDP carboxylase (μmoles/hr/mg protein).

During 96 hours of growth in the dark, the chlorophyll content of *y-2* decreased about ten-fold whereas the specific activity of both PPNR and RuDP carboxylase decreased only three-fold. On return to the light (fig 4, 5) a lag period was observed during which the specific activities of these enzymes remained constant. Following this lag, there was a rapid rate of increase in enzyme activity until the levels found in light-grown cells were approached. A plot of increase in the activities of these enzymes against chlorophyll content is given in figure 6. The most striking feature shown by these data is that the enzyme activities began to increase markedly only after a chlorophyll content of 0.50 to 0.60 μg/10^6 cells was reached.

The chlorophyll content of a cell might be directly correlated with not only reactions such as carbon dioxide fixation, oxygen evolution, and TPN photo-

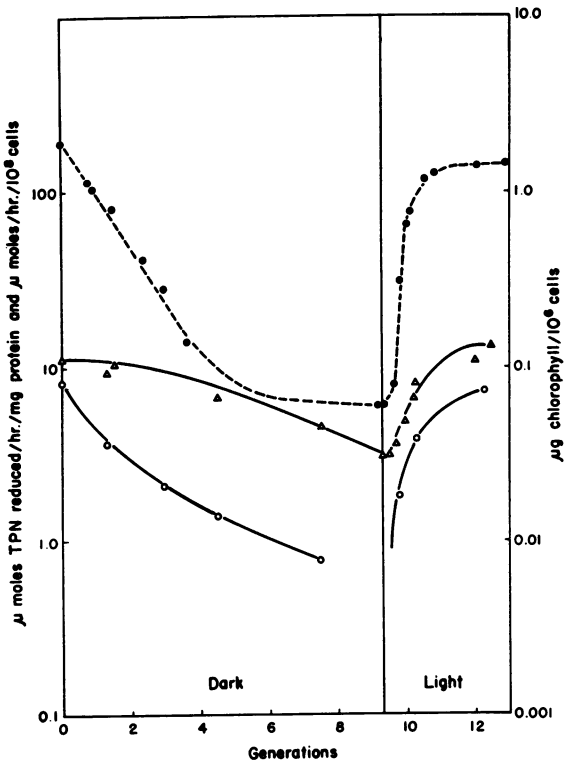


FIG. 5. TPN photoreduction by the mutant strain *y-2* during growth in the dark and following return to the light. Reaction mixtures contained in a total of 0.80 ml: $MgCl_2$ (0.006 μmole), TPN (0.02 μmole), crude or purified PPNR (0.10 ml), a volume of chloroplast fragments equivalent to 10.0 to 15.0 mg of chlorophyll or to 10^8 cells, and 0.05 M Tris buffer at pH 7.5 to the final volume. ○ = μmoles TPN reduced/hr/10^8 cells; △ = μmoles TPN reduced/hr/mg protein; ● = μg chlorophyll/10^6 cells.

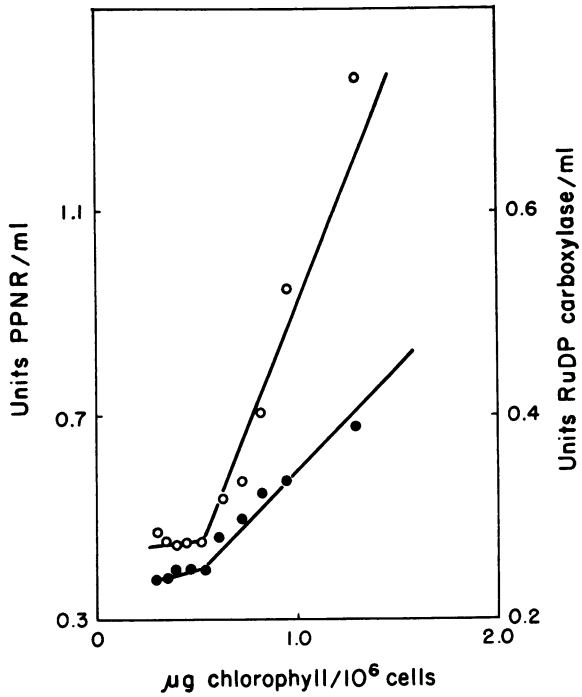


FIG. 6. Units of PPNR/ml and units of RuDP carboxylase/ml plotted as a function of μg chlorophyll/10^6 cells during the first 5 hours of regreening of a 96 hour dark-grown culture of *y-2*. ○ = units PPNR/ml; ● = units RuDP carboxylase/ml.

reduction, but also with the activities of certain enzymes involved in the photosynthetic process. If there is a direct correlation between chlorophyll content and the activities of these enzymes, a differential plot of increase in units of enzyme/ml against increase in chlorophyll/ml in a regreening culture of *y-2* should be linear (13). In order to test this relationship, cultures of *y-2* were grown in the dark for 96 hours and returned to the light at an intensity of 8,500 lux. Aliquots were taken at zero time and at half-hour intervals for 5 hours. The chlorophyll content/ml and the activities of both PPNR and RuDP carboxylase were measured as above. The results obtained were nonlinear and thus they did not indicate a direct or simple correlation between chlorophyll content and enzyme activity.

Discussion

In studies with *Euglena* (2) and *Rhodopseudomonas spheroides* (8), variations in the activity of RuDP carboxylase were seen when the organisms were grown under conditions which resulted in a decrease in chlorophyll or bacteriochlorophyll content. Large variations in chlorophyll content were controlled and separated from other variables in the present study by the use of several mutant strains of *C. reinhardi*. The chlorophyll content of the wild type strain as well as that of the 3 acetate-requiring mutant strains decreased about two-fold during 48 hours of growth in the dark, but no further decrease was seen during growth in the dark for extended periods of time. The presence of sodium acetate in the growth medium of *C. reinhardi* may result in a decrease in RuDP carboxylase activity but no variation of growth conditions produced more than a two-fold change in enzyme activity.

These results show that there are no large variations in the activity of RuDP carboxylase in *C. reinhardi* in the absence of major changes in the chlorophyll content of the cells. Mutant strains that are incapable of photosynthetic growth also showed constant activity comparable to that of wild type.

The *y-2* mutant strain of *C. reinhardi*, which differs from wild type in that it is incapable of chlorophyll synthesis or chloroplast formation in the dark, was used to investigate the loss and redevelopment of photosynthetic activity. Growth of this mutant strain in the dark resulted in a simple dilution of chlorophyll, the chlorophyll content decreasing two-fold with each cell division. On return to the

light, a lag period of 30 to 60 minutes was observed during which no chlorophyll synthesis occurred. Following this lag period, there was a rapid rate of chlorophyll synthesis until the normal chlorophyll content was attained within a period of 8 to 10 hours. During the initial exposure of a dark-grown culture of *y-2* to the light, the doubling time of chlorophyll was 80 to 90 minutes whereas the doubling time of the cells was 10 hours.

The rates of several photosynthetic reactions were studied in cultures of *y-2* during growth in the dark and following return to the light. Two general classes of reactions were found. The rates of reactions in the first class, including carbon dioxide fixation and oxygen evolution by whole cells and TPN photoreduction measured with chloroplast fragments prepared from bleaching and regreening *y-2*, are directly correlated with chlorophyll content (fig 2, 5). The kinetics of decrease of the rates of these reactions in preparations of dark-grown cells and the kinetics of increase upon return of the cells to the light are essentially identical to changes in chlorophyll content. Since these reactions require photosynthetic electron transport, it is not surprising that their rates are directly correlated with chlorophyll content. The decrease in chlorophyll content in a culture of *y-2* during growth in the dark is also accompanied by a loss of lamellar structure in the chloroplast (fig 1). Any loss of organized structure in the chloroplast could affect the electron transport system and thus limit directly the rates of certain photosynthetic reactions.

The rates of reactions in the second class, including the activities of RuDP carboxylase and PPNR, changed in a manner that was different from changes in chlorophyll content. Whereas chlorophyll content decreased two-fold with each cell division in the dark, the activities of these enzymes decreased two-fold only after about 2.5 cell divisions. On return to the light, there was a period of rapid chlorophyll synthesis without an increase in enzyme activity, followed by a period of rapid increase in enzyme activity. Both enzyme activity and chlorophyll content reached the normal value for light-grown cells at approximately the same time. These results differ from those of Lascelles (8) who found that the rates of RuDP carboxylase and bacteriochlorophyll synthesis were parallel in *Rhodopseudomonas spheroides*.

In the *y-2* mutant strain of *C. reinhardi*, chlorophyll synthesis ceases in the dark, but RuDP carboxylase synthesis continues at a decreasing rate. The difference in the regulation of RuDP carboxylase activity in *C. reinhardi* and in *R. spheroides* may be a reflection of different mechanisms controlling chlorophyll synthesis. In both organisms, however, changes in enzyme activity are accompanied by changes in chlorophyll content. While there is no direct relationship between chlorophyll synthesis and enzyme activity in *C. reinhardi* (fig 7), an indirect relationship is apparent.

An explanation of this indirect relationship may be offered on the basis of quantitative data presented. During 96 hours of growth of *y-2* in the dark, the chlorophyll content decreases ten-fold whereas the specific activities of both PPNR and RuDP carboxylase decrease only three-fold. During the first 2.0 to 2.5 hours of regreening, the rate of photosynthesis would be limited by the chlorophyll content of the cells. The activities of PPNR and RuDP carboxylase could remain constant during this time and not limit the rate of photosynthesis. However, when the chlorophyll content increased three-fold, an increase in the enzyme activities would be necessary to maintain an optimum rate of photosynthesis. As reported here, a three-fold increase in chlorophyll content represents 0.50 to 0.60 μg of chlorophyll/10^6 cells. This is precisely the chlorophyll content at which rapid increase in the activities of both PPNR and RuDP carboxylase were seen to occur (fig 6).

Alternative suggestions can be made to explain the indirect relationship between enzyme activity and changes in chlorophyll content. It is possible that the rates of both chlorophyll synthesis and increase in enzyme activity are related to a third factor. This factor could be the total chlorophyll content of a cell. On the other hand, it might be the appearance of a particular species of chlorophyll molecule, a particular structural component or its orientation in the chloroplast, or a change in the ratio of chlorophyll a to chlorophyll b. Although the data presented do not permit a choice among these alternatives, they do suggest a regulatory function for a critical amount of chlorophyll or perhaps a critical structural orientation of chloroplast components.

Summary

The effect of growth conditions on the activity of ribulose 1,5-diphosphate carboxylase was studied in the wild type strain of *Chlamydomonas reinhardi* and in 3 acetate-requiring mutant strains. No variations of growth conditions produced more than a two-fold variation in enzyme activity and little change in chlorophyll content was noted during heterotrophic growth in the dark.

Large decreases in the rates of several photosynthetic reactions were seen in dark-grown cultures of the *y-2* mutant strain of *Chlamydomonas reinhardi* which is unable to synthesize chlorophyll in the dark. Chlorophyll was diluted out by growth in the dark and a ten-fold reduction in chlorophyll content was seen over a period of 96 hours. On return to the light, there was a lag period during which no chlorophyll synthesis occurred followed by chlorophyll synthesis at a rapid rate. Changes in the rates of oxygen evolution and carbon dioxide fixation by whole cells, and of TPN photoreduction by chloroplasts prepared from *y-2* followed the same kinetics as changes in chlorophyll content. The activities of ribulose 1,5-diphosphate carboxylase and photosynthetic pyridine nucleotide reductase, on the other hand, followed a different pattern. Large changes in the

activities of these enzymes were always accompanied by large changes in chlorophyll content, but it was shown that this relationship was not direct.

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