Changes in Chlorophyll a/b Ratio and Products of $^{14}$CO$_{2}$ Fixation by Algae Grown in Blue or Red Light

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Summary. Chlamydomonas and Chlorella were grown for 10 days in white light, 955 μw/cm$^2$ blue light (400-500 μ), or 685 μw/cm$^2$ red light (above 600 μ). Rates of growth in blue or red light were initially slow, but increased over a period of 5 days until normal growth rates were reestablished. During this adaptation period in blue light, total chlorophyll per volume of algae increased 20% while the chlorophyll a/b ratio decreased. In red light no change was observed in the total amount of chlorophyll or in the chlorophyll a/b ratio. After adaptation to growth in blue light and upon exposure to $^{14}$CO$_{2}$ with either blue or white light for 3 to 10 minutes, 30 to 36% of the total soluble fixed $^{14}$C accumulated in glycolate-$^{14}$C which was the major product. However, with 1 minute experiments, it was shown that phosphate esters of the photosynthetic carbon cycle were labeled before the glycolate. Glycolate accumulation by algae grown in blue light occurred even at low light intensity. After growth of the algae in red light, $^{14}$C accumulated in malate, aspartate, glutamate and alanine, whereas glycolate contained less than 3% of the soluble $^{14}$C fraction.

Several groups of investigators have reported an effect of blue light upon the rate of photosynthesis and upon the distribution of $^{14}$C among the products of $^{14}$CO$_{2}$ fixation. Initially, Warburg et al. (24, 25) reported a stimulation of photosynthesis by Chlorella in red light upon addition of blue light. In a recent confirmation of this phenomenon with Acetabularia, Tenborgh (19) provided reasons why this blue light potentiation is different from the Emerson enhancement effect associated with the 2 pigment systems for electron transport. When photosynthesis was restricted to blue light only, Roux et al. (17), Tyszkiezwicz (22) and Voskresenskaya and Grishina (23) all found an increased proportion of amino acids, particularly glycine and serine, and generally less starch synthesis. More recently Zak (28), using Chlorella, and Andreeva and Korzhova (1), using sunflower leaves, have made similar observations. Cayle and Emerson (4) in 1957 using Chlorella reported that glycine was labeled in the C-2 carbon after 5 minutes of $^{14}$CO$_{2}$ fixation in blue light but uniformly labeled from experiments in white light. Thus, the general trend of these investigations has been that blue light affected glycine and serine biosynthesis and perhaps other amino acids and protein. However, Krotkov's group (6, 7, 21) also using Chlorella as well as tobacco leaves, found that blue light, although stimulatory, promoted $^{14}$C labeling of aspartate and glutamate. In some of the above experiments, light intensity may have been a limiting factor. Horvath and Szasz (10) have reported that amino acids were major products of photosynthesis at low light intensity and that sugars were formed at high light intensity.

Since blue light appeared to effect glycine and serine, we reasoned that glycolate formation would also be affected, for in the higher plant glycolate is a precursor for glycine and serine (16). When the work was initiated in 1963, we could confirm with Chlorella the stimulation of glycine and serine formation in blue light, but there was no pronounced effect upon glycolate. Afterwards it was discovered that algae, unlike most higher plants form serine from P-glycerate (9). A C$_2$ pathway may be functioning in algae (26), however, most of the free glycolate is not metabolized to serine but excreted, since the algae lack a normal glycolate oxidase (8, 9). However, the algae, after growth for several days in the blue light, altered their photosynthetic or metabolic process in such a way that glycolate became the single major product of short periods of $^{14}$CO$_{2}$ fixation. Consequently, this report is concerned with algae grown for several days in blue light in contrast to all previous work with algae grown in white light and only exposed to blue light during the test period.

In this investigation, we also examined the algae for any gross spectrophotometric changes during growth in blue light. Fujita and Hattori (5) reported that changes in chlorophyll a and b concentrations in Tetraphix responded to light quality rather...
than light intensity. Jones and Myers (11) reached a similar conclusion from work with *Anacystis*. Light intensity apparently affects only the rate of pigment conversion.

**Materials and Methods**

*Hlyae*. Cultures were obtained from the “Culture Collection of Algae” at Indiana University, Bloomington, Indiana. *Chlorella pyriformis* Chick (Emerson) (No. 395) was cultured in an inorganic salt medium (14) with Hoagland’s micronutrients. *Chlamydomonas reinhardtii* Dangeard (−) (No. 90) was grown on the high phosphate medium described by Orth et al. (15). Cells were cultured in 1.5 L of medium in 2.8 L “Low form” Fernbach flasks fitted with air inlets and agitated on a reciprocating shaker by about 60 excursions per minute. For culturing of algae in white light, the shaker was kept in a controlled environment chamber at 15° which maintained a temperature in the culture medium of 20°. Continuous light at the surface of the culture was 1200 ft-c from Westinghouse cool, white, super-high, fluorescent bulbs (F96T12/CW/5HO). The cultures were gassed with 0.2% CO₂ v/v in air. Algae were also cultured in light passed by a blue or a red filter system which transmitted light as shown in figure 1. The red filter was “Fire Red” No. 110 gelatin (Grand Stage and Lighting Company, Chicago, Illinois), and light was obtained from a bank of 15 watt red fluorescent lights (General Electric F15T12-R). Blue light was obtained from a bank of 15 watt blue fluorescent lights (General Electric F15T12-B or Sylvania F15T8-B) and was filtered through a blue celluloid filter and 5 cm of a CuSO₄ solution containing 30 g/l. (27). The transmission of both the blue and red commercial filters was determined spectrophotometrically by us. The combination of blue celluloid and CuSO₄ solution passed a band of emission which was predominately between 400 μm and 500 μm and contained no light above 570 μm. All of the culturing and photosynthesis experiments in blue or red light were performed in dark rooms. These cultures were grown under continuous light, aerated with 0.2% CO₂ in air, and maintained at 20 to 21° by a water bath.

Cultures grown in continuous blue light received 955 μW/cm² as measured by a recording spectroradiometer, model SPR from Instrumentation Specialties Company, Inc. This intensity of blue light also measured 400 ft-c by a Weston Illumination Meter, model 756 with a quartz filter. Values in the tables are expressed in ft-c to reflect the actual measurements because the spectroradiometer was not yet available when the research was done. Cultures grown in continuous red light received 685 μW/cm² between 610 to 700 μm or 765 μW/cm² between 610 to 720 μm. As measured by our Weston Illumination Meter this intensity was equal to 200 ft-c. The cultures were diluted every second day with fresh nutrient to an absorbance of 0.2 at 680 μm as measured in a 1 cm cuvette with a Beckman DU spectrophotometer. Aliquots were removed at various times after inoculation to determine the rate of growth as expressed in increase absorbance at 680 μm.

14CO₂ Photosynthesis Experiments. Cells were removed in the dark from the growth media by centrifugation, and a 1% (v/v) suspension of cells was prepared in 1 mxt phosphate with a final pH of 6.5. A 15 ml suspension, in a lollipop fitted with a large bore stopcock for rapid removal of aliquots, was maintained at 20° in a water bath. After 5 minutes preillumination in a designated light, a NaH₁⁴CO₃ (2-5 μmole) solution was added and samples were removed at 1, 3, and 10 minutes. Aliquots were dumped directly into warm methanol and further heated. The 14C products in methanol-water extracts were separated by chromatography and radioautography (3).

Extraction and Determination of Chlorophyll. Ten ml of a 0.5% algal suspension were centrifuged in a clinical centrifuge at maximum speed for 3 minutes. The supernatant fraction was discarded and the tube inverted on an absorbent surface to eliminate excess water. After several minutes, the cells were resuspended in 3 ml of absolute methanol and placed in a stoppered centrifuge tube in the dark for at least 2 hours to insure complete extraction of the pigments. After centrifugation, the absorbance of the green supernatant fluid was measured and chlorophyll a and b concentration calculated (12).

Measurement of in vivo Absorption Spectra. The neutral-density, filter technique of Shibata et al. (18) was employed in which a filter paper saturated with mineral oil was placed between the light source and the cuvette. We found that a double thickness of waxed paper (Schleicher and Schuell Co. No. B-2), placed next to the cuvette on the face toward the light source, most effectively elucidated the fine structure of the in vivo chlorophyll spectrum. The ratio of chlorophyll a/b absorption was evaluated from spectra measured on the Cary 15 spectrophotometer.
while using a slit width of approximately 0.7 mm. Under these conditions a 1% algal suspension gave approximately 0.5 absorbancy units at 750 μm.

Results

Growth of Algae. Cultures, when removed from white light and put in either red or blue light (as described in the Methods section) grew more slowly for 4 or 5 days before attaining a relatively constant, rapid growth rate which approximated that of the cultures in white light of similar intensities. Thus, Chlamydomonas and Chlorella in blue light grew slowly at first and then more rapidly, as measured by the culture’s absorbance at 680 μm (fig 2). All measurements were made on non-synchronized or random cultures which initially had similar cell populations as judged from approximately similar absorbance values. Chlamydomonas in red light also grew more slowly at first, but after several days in the red light, they too grew rapidly. We think that the slow recovery of the growth rate in blue or red light was an adaptation rather than a mutation, since all the results presented in this paper were similarly reproducible when starting over again with stock cultures kept in white light.

Rate of $^{14}$CO₂ Fixation. With Chlorella or Chlamydomonas fully adapted after at least 10 days growth in either white, blue, or red light, experiments on the rate of $^{14}$CO₂ fixation were performed with increasing time and intensity of red, blue or white light. The available intensity of the blue light was the limiting factor, but representative data in figure 3 indicated that 400 ft-c was approaching light saturation. In the experiments nearly linear $^{14}$C fixation rates over the 10 minute period were observed, and thus CO₂ availability did not become limiting. The fixation rates with Chlorella grown in blue light were unusual since relatively high levels of fixation occurred in 10 minute periods at low blue light intensities.

Distribution of $^{14}$C Among Soluble Products of Photosynthesis. For the purpose of presentation, the $^{14}$C distribution among the products of CO₂ fixation has been divided into 3 groups of compounds: A) phosphate esters representing components of the photosynthetic carbon cycle, B) malate, aspartate, glutamate, and alanine which are the compounds associated with the citric acid cycle that accumulate $^{14}$C, and C) glycolate and glycine plus serine which are associated with the glycolate pathway (16). The percent distribution of $^{14}$C among each product of $^{14}$CO₂ fixation by paper chromatography is on file along with results from other variations of light intensity and quality (8).

Algae grown in either white, blue or red light

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**Fig. 2.** Rate of growth of *Chlorella pyrenoidosa* and *Chlamydomonas reinhardii* after designate days in blue light. Vertical lines designate the variation of rate of growth from repeated experiments after 10 days culture in blue light.
and allowed to fix $^{14}$CO$_2$ in the same type of light incorporated initially the highest percentage of $^{14}$C into the phosphate esters of the photosynthetic carbon cycle. Typical data for Chlamydomonas are summarized in figure 4. As indicated by the rapid reduction in the percent of the total $^{14}$C fixed which accumulated in these esters, the pool sizes in the blue adapted algae appeared smaller than in the algae grown in red light.

Chlamydomonas grown in blue light rapidly accumulated 31% of the newly fixed $^{14}$C into glycolate during photosynthesis with 350 ft-c of blue light. If the algae were grown in red light, they accumulated only a trace of $^{14}$C labeled glycolate (fig 4). Similar results were obtained with Chlorella except that the total percentage of fixed $^{14}$C in glycolate was only about half that found with Chlamydomonas.

For Chlamydomonas the percent $^{14}$C in glycine and serine was not greatly altered by their growth in either blue or red light. Chlorella, however, showed a 1 to 2 fold increase in the percent of the total $^{14}$C in serine immediately after culture in blue light was initiated. This result was similar to earlier experiments cited in the introduction with Chlorella. Simultaneously with increase serine-$^{14}$C, we observed that the percent of $^{14}$C accumulating in P-glycerate decreased about half.

For both algae the percent of the total $^{14}$C incorporated into malate, aspartate, glutamate, and alanine was somewhat greater with algae grown in red light than white light and much greater than with algae grown in blue light (fig 4). Thus, growth in blue light promoted $^{14}$C accumulation during the initial minutes of $^{14}$CO$_2$ fixation into glycolate while growth in red light resulted in $^{14}$C accumulation in malate, aspartate, glutamate, and alanine.

If blue light were used for $^{14}$CO$_2$ fixation experiments with algae grown in white light or during the first to third day after initiation of their growth in blue light, the large significant changes in the percentage of $^{14}$C incorporated into glycolate were not observed. Since accumulation of a large percentage of the $^{14}$C in glycolate in blue light did not occur with algae grown in white light, glycolate accumulation was probably not related to possible alterations in assimilatory power produced by the blue light.

Algae grown for 10 days in blue or red light produced about the same products regardless of whether $^{14}$CO$_2$ fixation was measured in white, blue or red light. Thus, the same accumulation of $^{14}$C-glycolate by algae grown in blue light occurred when the 10 minute $^{14}$CO$_2$ fixation period was run in either blue or white light. These facts support the hypothesis that the cultures grown in blue light actually

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**Fig. 3.** Rate of total $^{14}$C fixation by algae in increasing intensities of blue light. In part A, Chlorella pyrenoidosa were grown in white light and in part B in blue light for 10 days before the experiment. In part C, Chlamydomonas reinhardtii were adapted for 10 days in blue light. Ordinate values are total $^{14}$C in the soluble fraction from 15 ml of a 1% algal suspension.
experienced a period of slow cellular adaptation which was not rapidly reversed by white light.

The data in figure 5 emphasizes the effectiveness that growth of *Chlamydomonas* in blue light has on glycolate production. It is established that the amount of glycolate production by algae increases at higher light intensities (2,13,20,26). In the present experiments glycolate production at 110 ft-c by *Chlamydomonas* grown in either red or white light was less than 3% after 10 minutes (see fig 5B for algae grown in red light), but increasing the light intensity to 1200 ft-c resulted in considerable formation of glycolate-14C. However, if the *Chlamydomonas* were grown in blue light for 10 days, then they formed in 110 ft-c of blue (fig 5A) or white light (data not shown) nearly the same amount of glycolate-14C which the algae grown in white or red light could only produce at 1200 ft-c. For *Chlamydomonas* grown in blue light 14C in glycolate amounted to 15 to 36% of the total 14C fixed between 3 and 10 minutes, and consequently glycolate-14C was the major soluble product of CO2 fixation. This amount was 3 to 4 times more 14C than in any other single product, yet as seen from figure 4, during the first minute of 14CO2 fixation, there was more 14C in P-glycerate or sugar phosphates than in glycolate. Thus, algae grown in blue light accumulated large amounts of glycolate-14C even at low light intensities and high light intensity further increased the amount of glycolate which was formed. It is also apparent in figure 5, that glycine and serine accumulation was not affected by growing the *Chlamydomonas* in blue light. This is consistent with the fact that serine and glycine synthesis is independent of glycolate formation in algae (9), in contrast to plants in which serine and glycine are formed from glycolate (16).

**Chlorophyll Content.** Using a Cary 15 recording spectrophotometer, absorption spectra were measured on cell suspensions as described in the Methods section. The spectra in figure 6 have equal absorbance values at 550 m\(\mu\) but, for clarity of presentation, the curves have been separated in the figure. The change of the 680 m\(\mu\)/655 m\(\mu\) absorbance ratio, which represents in vivo maxima for chlorophylls a and b respectively, indicated a significant decrease in the chlorophyll a/b ratio as the period of culture in blue light increased. No significant changes in these regions of the spectrum were observed for algae grown in red light. Other portions of the spectrum did reflect absorption changes caused by growing the cells.

![Graph](https://example.com/graph.png)

**Fig. 4.** Percent distribution of 14C among soluble products formed by *Chlamydomonas* after adaptation to blue or red light for 10 days: (0—0) Cells grown in blue light and 14CO2 photosynthesis was performed in 350 ft-c blue light; (●—●) Cells grown in red light and 14CO2 photosynthesis was performed in 200 ft-c of red light (>600 m\(\mu\)).
in red light, but these were not consistent and too complicated for a careful evaluation by this technique.

The above in vivo measurements were verified by results from spectral measurements of chlorophyll in extracts from the algae. The total chlorophyll content on the basis of the cell volume increased about 20\% during the first 6 days of culture in blue light, and a significant decrease appeared in the chlorophyll a/b ratio (fig 7). Although the ratio of chlorophyll a/b varied from 1 to 2.8 for different starting cultures which had been grown in white light, a consistent trend was a decrease in the chlorophyll a/b ratio during culture of the algae in blue light. Similar results were obtained for Chlorella. The data from Chlamydomonas extracts, however, were more consistent than with Chlorella, perhaps, because the pigments from Chlorella were extracted less quantitatively.

Discussion

Three major changes were observed when Chlamydomonas or Chlorella were grown in 955 $\mu$W/cm$^2$ of continuous blue light (425-540 nm) or in 765 $\mu$W/cm$^2$ red light (above 600 nm). A) Growth rate slowed for 3 to 5 days, but returned after 5 to 10 days to rates equal to those with similar amounts of white light. B) After 5 to 10 days adaptation to blue light the algae incorporated about 30\% of the total $^{14}$C fixed in 10 minutes into glycolate at low (110 ft-c) blue or white light intensities and even more at 1200 ft-c light. This phenomena did not occur until

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**Fig. 5.** Total $^{14}$C in glycolate or glycine plus serine formed by 0.5 \% (v/v) suspension of Chlamydomonas reinhardii grown in (A) Blue light or (B) red light; (●–●) after $^{14}$CO$_2$ fixation in 350 ft-c blue light or (■—■) in 1200 ft-c white light.
after the initial period of adaptation. After growth in blue light, \(^{14}\text{CO}_2\) fixation in white light produced the same \(^{14}\text{C}\) distribution among products as in blue light. Algae grown in red light incorporated more \(^{14}\text{C}\) into malate, aspartate, glutamate and alanine and only trace amounts into glycolate. C) During growth in blue light, chlorophyll content increased 20\% while the chlorophyll a/b ratio decreased. The results were reproducible when starting again with fresh cultures which had been grown in white light. The results suggest a slow environmental adaptation over several generations to the specific light quality. The data invite speculation that both \(^{14}\text{CO}_2\) fixation and the pigments for electron transport in photosynthesis are so intimately interdependent that complementary changes in both systems compensate for environmental alterations. These changes could maximize the capacity of an organism to utilize light and \(^{14}\text{CO}_2\).

The accumulation of such large amounts of \(^{14}\text{C}\) in glycolate by algae grown in blue light does not indicate that glycolate was being formed by a separate pathway of \(^{14}\text{CO}_2\) fixation. In all experiments P-glycerate and sugar phosphates contained the most \(^{14}\text{C}\) during the first minute of \(^{14}\text{CO}_2\) fixation, while samples taken at 3 minutes contained much more glycolate-\(^{14}\text{C}\) than P-glycerate-\(^{14}\text{C}\) or any sugar phosphate (fig 4). The results suggest that glycolate-\(^{14}\text{C}\) was accumulating as an end product of photosynthesis, and probably it was being excreted (9, 20).

Our experiments do not prove that the increased chlorophyll b content with respect to chlorophyll a is linked to the altered \(^{14}\text{CO}_2\) fixation. Both changes occurred upon growing algae in blue light, but they need not be associated. The decrease chlorophyll a/b ratio during growth in blue light might be expected from the data of Fujita and Hattori (5). The Soret band absorption for chlorophyll b is approximately 50\% greater than the absorption for chlorophyll a between 400 to 500 \(\text{m}\mu\). Plants grown in shade also seem to have more chlorophyll b.

Unlike glycolate-\(^{14}\text{C}\) formation, the rate of labeling of glycine and serine did not increase when \(Chlamydomonas\) were adapted on blue light. When \(Chlorella\) were placed in blue light \(^{14}\text{C}\)-label in serine increased immediately and before similar changes occurred for glycolate-\(^{14}\text{C}\). With \(Chlorella\), increased serine-\(^{14}\text{C}\) was accompanied by decrease \(^{14}\text{C}\) in P-glycerate. These results appear consistent with the absence of a typical glycolate oxidase in algae and with the formation of serine from P-glycerate rather than from glycolate as occurs in higher plants (16). Movement of \(^{14}\text{C}\) from P-glycerate to serine may occur due to limited availability in low blue light of NADPH and ATP which is needed to convert P-glycerate to triose phosphate. Although not investigated, one might predict with higher plants, which rapidly metabolize glycolate to serine and then to sucrose, that growth in blue light would also increase the pool size of glycine and serine and sugars. Thus glycine and serine accumulation by higher plants in blue light could arise from both P-glycerate and glycolate.

Cayle and Emerson (4) ran \(^{14}\text{CO}_2\) fixation experiments with \(Chlorella\) grown in the presence of 8 microeinstein/cm\(^2\)/min of blue or red light. Their algae had been grown in white light and only the subsequent 5 minute experiment was done in monochromatic light. As we also observed, they found no significant difference in total amount of \(^{14}\text{CO}_2\) fixation or distribution of \(^{14}\text{C}\) between amino acids and phosphate esters. However, they observed that the specific activity of the alanine, glycine and
serine which was produced in 5 minutes of blue light was greater than that from red light. Further, the distribution of C in glycine, but not in alanine, was altered by the blue light. It would appear that metabolic changes in blue light begin immediately. The large change in C distribution which leads to an accumulation of glycate by algae in blue light, as observed by us, seems to occur only after several days of growth in blue light.

In a series of papers from Krotkov’s laboratory, it has been reported that the addition of blue light to red light stimulated C incorporation into aspartate, malate and glutamate during 30 minute experiments and decreased C in glycine and glycate (6, 7, 21). Although these results appear the opposite from ours, the 2 types of experiments are not comparable with respect to growth of the algae or type of monochromatic light employed. Krotkov’s group used algae grown in white light with 5 % CO₂, rather than in the blue or red light with 0.2% CO₂. Further, they used a dark pretreatment period which varied from 1.5 to 20 hours. Their blue light only supplemented red light and even when their blue filter alone was used, it transmitted much light between 500 to 600 μm, some at 680 μm and all light about 710 μm (21). Our use of a CuSO₄ solution, as emphasized by Withrow (27), completely eliminates red light with blue pigment filters.

Literature Cited