THE EFFECT OF LIGHT QUALITY ON GROWTH, PHOTOSYNTHETIC AND EXCRETION RATES, AND THE CARBON METABOLISM OF *CHLAMYDOMONAS REINHARDTII* DANGEARD

by

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B.Sc., Simon Fraser University, 1969

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department of

Biological Sciences

Simon Fraser University

May, 1972

APPROVAL

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Degree: Master of Science

Title of Thesis: The Effect of Light Quality on Growth, Photosynthetic and Excretion Rates, and the Carbon Metabolism of *Chlamydomonas reinhardtii* Dangeard.

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ABSTRACT

The influence of red, blue, green and white light on growth, excretion and photosynthetic rates and carbon metabolism of the freshwater algae Chlamydomonas reinhardtii Dangeard was examined. Relative growth constants were 0.28, 0.32, 0.40 and 0.41 in green, white, blue and red light respectively. Photosynthetic rates were higher in white, blue or red than in green light of the same intensity.

More than 60% of the $^{14}CO_2$ assimilated by cells grown under blue or green light was incorporated into the ethanol-insoluble fraction compared with about 50% in cells grown under white or red light. Within the ethanol-insoluble fraction the percentage of sugars was significantly higher in cells grown under green or red light than in cells cultured in white or blue light. The percentage in the insoluble protein fraction was greatest in blue light.

The percentage release of dissolved organic carbon into the medium was highest in white light and lowest in blue or red light. The nature and quantity of excreted material varied but not in a predictable manner.

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ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. G. H. Geen for the valuable counsel and critical review of the thesis. I also thank the other members of my supervising committee, Drs. L. Druehl and H. Speer for their advice and review of this thesis.

Thanks are due to John Nelson for his skillful technical assistance and helpful suggestions. This research was supported by a National Research Council grant.

INTRODUCTION

The chemical composition of planktonic algae has received some attention (Parsons et al., 1961; Olive and Morrison, 1967; Wallen and Geen, 1971 a, b, c). Variations in composition are induced by a number of factors such as temperature (Fedorov et al., 1968), pH (Orth et al., 1966) and the inorganic nutrients in the medium (Fogg, 1952; Constantopoulos, 1970). The age of the cultured cells, stage of life cycle and previous culture history also have an effect upon the nature of the photosynthetic products (Fogg, 1952; Stewart, 1963; Ahmed and Ries, 1969). Cellular composition may also depend on the wavelength of the incident light (Hauschild et al., 1962; Voskresenskaya and Nechaeva, 1967; Wallen and Geen, 1971 a). Voskresenskaya and Nechaeva (1967), using barley, reported that blue light stimulated the incorporation of ^{14}C into amino acids especially alanine, whereas red light stimulated ^{14}C incorporation into sugars. Blue and green light stimulated a relative increase in the ethanol-insoluble protein fraction relative to white light in two species of marine phytoplankton (Wallen and Geen, 1971 a). According to Wallen and Geen (1971 a) the increase in some amino and organic acids in cells grown under blue light was independent of light intensity, photosynthetic rate or cell density. Kowallik (1965) also found that blue light increased the proportion of proteins in the products of photosynthesis in Chlorella pyrenoidosa. Hauschild et al. (1962, 1965) and Cayle and Emerson (1957), found that blue light stimulated the accumulation of fixed carbon into amino and organic acids, particularly aspartic and malic acids.

Light quality may also have an effect on rates of photosynthesis. Hauschild et al. (1965) demonstrated that blue light affected the rate of photosynthesis in Chlorella vulgaris, irrespective of whether it was given before or during the actual period of monitored photosynthesis. Wallen and Geen (1971 a) showed that the photosynthetic rates of two species of marine planktonic algae were higher in blue light and lower in green light than in white light of the same intensity.

Light quality and intensity both may have an affect upon the quality and quantity of the dissolved organic compounds excreted by unicellular algae. Both Tolbert and Zill (1956) and Watt and Fogg (1966) showed that excretion of glycolate by Chlorella pyrenoidosa was increased at high light intensities and high CO_2 concentrations. Becker et al. (1968) reported glycolate excretion in C. pyrenoidosa and C. vulgaris exposed to high intensity white or red light. No excretion was observed in cells grown in blue light. Wallen and Geen (1971 c) indicated a smaller percentage excretion by cells grown under blue or green than under white light. They also reported changes in photosynthetic pathways and chemical composition of natural populations of planktonic algae with depth in the photic zone. The shift to blue or green light in the lower part of the photic zone resulted in an increased synthesis of proteins. Wallen and Geen (1971 c) noted that the size of the ethanol-soluble and insoluble fractions in natural phytoplankton populations was related to light quality rather than intensity. The depth-dependent shift of the spectral composition of underwater light probably alters the rate and/or direction of most metabolic

reactions in planktonic algae. This may affect the nature and quantity of excreted products which could, as an example, influence the growth of heterotrophic organisms which utilize dissolved organic compounds, or influence the formation of organic aggregates.

In this work I have examined the nature of the photosynthetic and excretion products of the freshwater alga Chlamydomonas reinhardtii grown under light of the same intensity but different spectral composition. This has enabled me to examine the postulate that there is a direct relationship between the nature of the algal photosynthate and the components excreted.

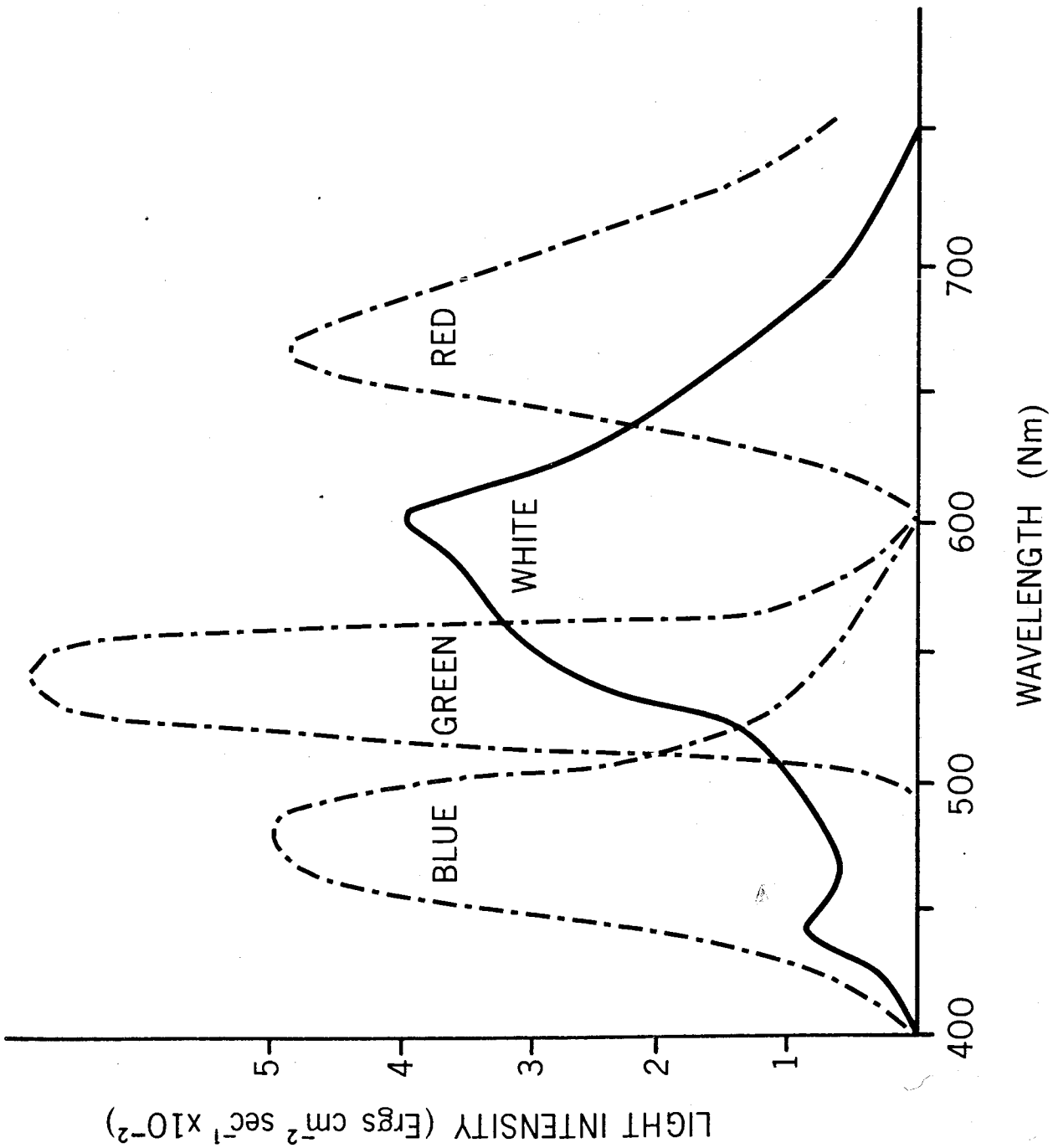
MATERIALS AND METHODS

Culture Techniques

An axenic culture of Chlamydomonas reinhardtii (wild type, mating strain 89, 90) (Starr, 1964) was obtained from the culture collection at Indiana University. The culture was grown in Beijerinck's medium (Stein, 1958) which was previously sterilized by autoclaving for 20 min at 120°C and 15 psi. Stock cultures were maintained with continuous stirring at 18°C ± 0.5°C in cotton-stoppered 2800-ml Fernback flasks. Light was provided by a bank of General Electric cool white fluorescent lamps producing 2.4×10^3 ergs cm^{-2} sec^{-1} at the level of the flask bottoms. Experimental cultures were established with 10^4 cells per ml and placed in incubators operating on a 16:8 hr light-dark regime. The experimental cultures were illuminated from above with General Electric white, blue, or green fluorescent lamps or Sylvania red fluorescent lamps. The band width of the blue, green or red lights reaching the cultures was more narrowly defined by placing plexiglass filters (obtained from Percival Refrigeration and Mfg. Co. Inc. Boone, Iowa 50036) between the lamps and cultures. The maximum energy output from the blue light system at the level of the flasks was 2.4×10^3 ergs cm^{-2} sec^{-1} and was the energy used for culturing cells under each light regime. The spectral distribution of each lamp-filter combination is shown in Fig. 1.

Growth rates were estimated from cell count data obtained with a Coulter Counter equipped with a Model M Volume Converter. Photosynthetic rates and distribution of ^{14}C were determined when the experimental cultures reached the exponential phase of growth ($1\text{-}3 \times 10^5$ cells/ml). Four 160-ml

Fig. 1 The spectral composition of each of the light systems used for culturing Chlamydomonas reinhardtii.


LIGHT INTENSITY (Ergs cm⁻² sec⁻¹ x10⁻²)
1
2
3
4
5
400
500
600
700
WAVELENGTH (Nm)
BLUE
GREEN
WHITE
RED

subcultures were taken from the experimental cultures, inoculated with 400 μ Ci of $H^{14}CO_3^-$ (Sp. activity 76.6%) and incubated under the appropriate experimental light conditions for 30 min prior to the determination of the total ^{14}C uptake and the distribution within the cells.

Separation of the Photosynthetic Products

After incubation with $H^{14}CO_3^-$ the algae were filtered onto Millipore filters (HA 0.45 μ) and placed for 1 hr in boiling ethanol in a reflux apparatus. This separated the photosynthetic products into the hot-ethanol soluble (HES) and insoluble fractions. The HES fraction was adjusted to pH 7.2 (to reduce the expected loss of volatile organic acids under acidic conditions) and dried under vacuum at 35°C. The dried extract was redissolved in 5-ml distilled water and an aliquot was placed in TEG (300-ml ethylene glycol monomethyl ether, 500-ml TOL [42-ml spectrofluor/ℓ toluene]) and the ^{14}C activity measured with a liquid scintillation counter. The HES fraction was then run on ion-exchange resins (Rexyn 101 (H^+) and 201 (OH^-)) to separate the free amino acids, organic acids and sugars. Each fraction was concentrated in a flash evaporator and an aliquote assayed for ^{14}C activity. Each fraction was washed and evaporated three times with distilled water to remove the formic acid and ammonium hydroxide used to elute the organic and amino acids from the ion exchange resins. Failure to remove the formic acid and ammonium hydroxide results in incomplete chromatographic separation of the compounds.

Identification of the Photosynthetic Products

The amino acids were co-chromatogramed with standards in two dimensions using phenol: water: ammonium hydroxide (300: 75: 1) and butanol: acetone: diethylamine: water (120: 120: 24: 60). The organic acids were run in ethanol: water: ammonium hydroxide (280: 104: 16) and ethyl acetate: acetic acid: water: sodium acetate (200: 122: 100: 480). The sugars were run in phenol: water: ammonium hydroxide (300: 40: 1.2) and isopropanol: ethyl acetate: water (315: 45: 90). The sugar chromatograms were dried and rerun in the second direction to achieve better separation.

Chromatograms were exposed to Ilford Ilfex x-ray film for 4-5 weeks to locate the radioactive compounds. All radioactive spots were excised and their radioactivity determined. The efficiency of the spot-counting technique was determined by placing a known quantity of ^{14}C-labelled leucine, serine, glycine and aspartic acids on chromatograms, running in the appropriate solvents, spraying and counting. The spots were identified by their relative positions using spray reagents and known compounds.

The ethanol-insoluble fraction was hydrolyzed in a Soxhlet apparatus. The proteins and polysaccharides were refluxed for 24 hr in 6N HCl and 1N H_2SO_4 respectively. The HCl was removed by three evaporations under vacuum. The H_2SO_4 was removed by precipitation with $BaCO_3$. The hydrolyzates were concentrated under vacuum, redissolved in water, run through ion-exchange resins and separated paper chromatographically as previously described.

The medium in which the cells were grown was examined for organic excretion products after the 30-min period of photosynthesis. The filtrate was brought to pH 2 and bubbled with air for 1 hr to remove the unused

$H^{14}CO_3^-$. Thomas (1971) states that even at a pH of 1-2 and 23 hr of gas washing the removal is not complete (.01% remains after 1 hr of washing). Residual bicarbonate activity was further reduced during a subsequent step in which the filtrate was concentrated to 30 ml at pH 7.2 under vacuum. Only .001% of the $H^{14}CO_3^-$ remained after this evaporation. A 50 λ aliquot was placed in TEG and counted to determine total activity excreted. The filtrate was then run through Rexyn 101 (H^+) and 201 (OH^-) to separate the amino acids, organic acids and sugars prior to chromatographic separation of the components in each fraction.

RESULTS

Growth and Photosynthetic Rates

The exponential growth rate of Chlamydomonas was higher in red and blue light than in green or white light of the same intensity. Relative growth constants (K) and the mean generation times (T) were calculated using the following formulae:

$$K = \frac{\log_e N - \log_e N_o}{t - t_o} = \frac{2.3\,(\log_{10} N - \log_{10} N_o)}{t - t_o}$$

and $$T = \frac{\log e^2}{K} = \frac{0.693}{K}.$$

where N = cell concentration at time t, N_o = cell concentration at time t_o with t and t_o = time in days taken from the linear portion of the graph.

The growth constants for cultures in green, white, blue and red light were 0.28, 0.32, 0.40 and 0.41 respectively. Corresponding mean generation times were 59.4, 52.0, 41.6 and 40.6 hrs respectively. The relationship of cell diameter and cell number is shown in Fig. 3. The average diameter for cells grown in white, blue, green and red light were 5.1, 5.6, 7.2 and 6.0 μ respectively. The average volume of cells grown under green light (198 μ^3) is approximately double that for cells grown in white, blue or red light (70.4, 91.2 and 114 μ^3 respectively).

Cells grown under white, blue or red light had a higher photosynthetic rate in log phase cultures than cells grown under green light of the same intensity. Cells grown under green light had a rate of photosynthesis about one-quarter that of cells grown in white light (Table 1). The log phase

Fig. 2 Growth of Chlamydomonas reinhardtii in red, green, blue and white light of an intensity 2.4×10^3 ergs cm^{-2} sec^{-1}.

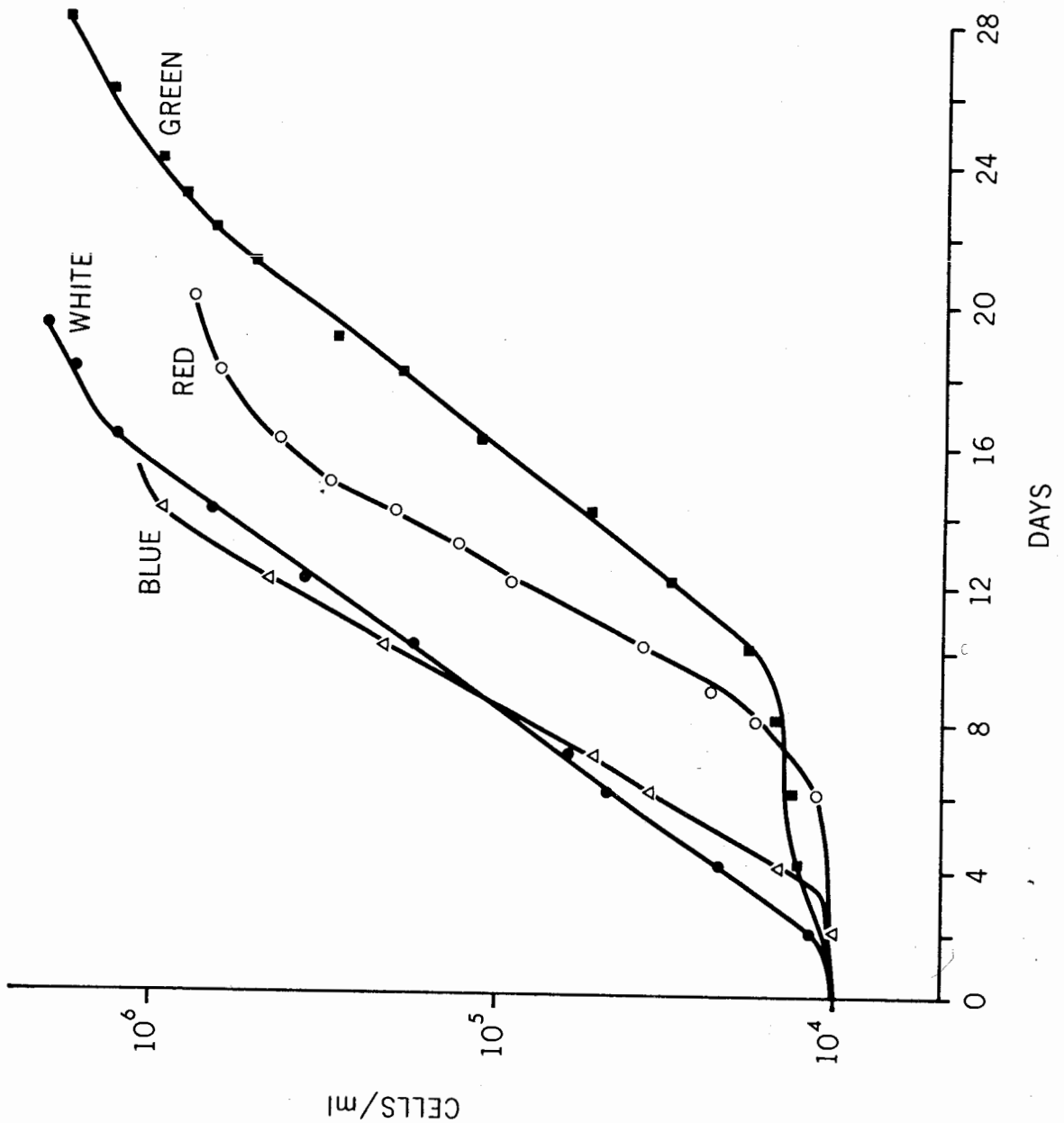

WHITE
GREEN
RED
BLUE
10^6
10^5
10^4
CELLS/ml
0
4
8
12
16
20
24
28
DAYS

Fig. 3 The relationship of cell number and cell diameter in log phase cultures (3×10^5 cells/ml) of Chlamydomonas reinhardtii grown under blue, green, red and white light.

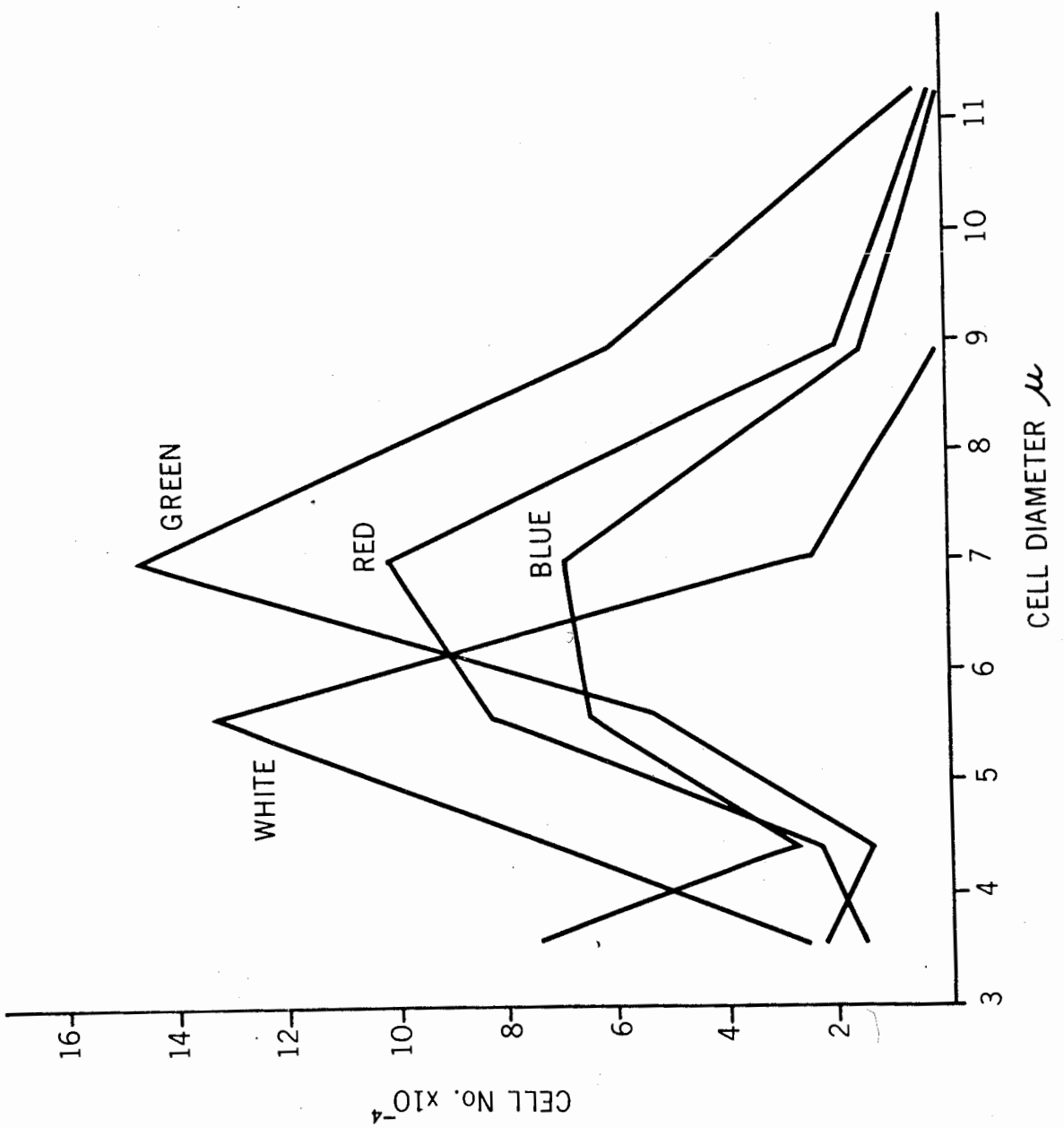

GREEN
RED
BLUE
WHITE
CELL No. x10⁻⁴
CELL DIAMETER μ
16
14
12
10
8
6
4
2
3
4
5
6
7
8
9
10
11

Table 1. Total $^{14}C/10^8$ cells fixed by Chlamydomonas reinhardtii during a 30-min incubation under 2.4×10^3 ergs cm^{-2} sec^{-1} of white, blue, green or red light and the distribution of ^{14}C between the ethanol-soluble and insoluble fractions expressed as a percent of total ^{14}C fixed ± standard error.

Light Quality	Total ^{14}C Fixed x 10^{-6} DPM/10^8 Cells	Ethanol Soluble %	Ethanol Insoluble	Replicates
Blue	66.4 ± 14.7	31.0 ± 3.4	69.0 ± 3.4	4
Green	14.9 ± 5.1	38.5 ± 1.3	61.5 ± 1.3	7
Red	69.1 ± 12.2	47.1 ± 1.6	52.9 ± 1.6	8
White	59.9 ± 13.6	51.8 ± 0.5	48.2 ± 0.5	9

in cultures grown under green or red light was longer than for cells grown under white or blue light (Fig. 2).

^{14}C Distribution in the Photosynthate

Cells grown in blue and green light fixed a greater percentage of the activity in the ethanol-insoluble fraction than cells grown in white or red light (Table 1). Conversely the percentage activity in the ethanol-soluble fraction was higher in cells grown under white and red light.

The distribution of ^{14}C recovered in the ethanol-insoluble fraction is shown in Table 2. The percent activity in the protein amino acids in cells grown under red and green light was significantly lower ($P \leq .01$) than under the other light regimes and highest in cells grown in blue light. Conversely, the percent in the sugar hydrolysis fraction was significantly higher ($P \leq .01$) in cells grown under green or red light than under white or blue light conditions.

The distribution of ^{14}C observed in the various compounds of the ethanol-soluble fraction after 30 min of photosynthesis is shown in Table 3. The highest percent ^{14}C activity in organic acids was recovered in cells grown under white light, whereas cells grown under blue or green light had the highest percent activity in the amino acid fraction. Cells grown in red light had the highest percent activity recovered in the sugar fraction a large percentage of which was incorporated into an unknown sugar. The

Table 2. Distribution of ^{14}C among the major components of the ethanol-insoluble fraction after 30 min of photosynthesis by Chlamydomonas reinhardtii. The results are expressed as a % total activity recovered in the ethanol-insoluble fraction ± standard error.

Light Quality	White	Blue	Green	Red
Protein Hydrolysis				
Amino Acids	82.3 ± 3.3	93.5 ± 0.7	68.5 ± 5.7	59.3 ± 3.3
Sugar Hydrolysis				
Sugars	17.7 ± 3.3	6.5 ± 0.7	31.5 ± 5.7	40.7 ± 3.3

Table 3. Distribution of ^{14}C among compounds of the ethanol-soluble fraction in Chlamydomonas reinhardtii after 30 min of photosynthesis. The results are expressed as a percent of total activity recovered in the ethanol-soluble fraction ± standard error.

	White	Blue	Green	Red
Malic	6.4 ± 0.5	2.0 ± 0.2	3.0 ± 0.4	5.4 ± 0.5
Glycolic	3.0 ± 0.4	2.7 ± 1.0	3.3 ± 0.9	1.8 ± 0.1
Pyruvic	6.9 ± 1.5	0.8 ± 0.4	2.5 ± 2.0	2.3 ± 0.3
Oxalic	3.9 ± 0.7	2.1 ± 0.6	2.9 ± 0.9	1.2 ± 0.3
3-PGA	6.1 ± 1.1	4.7 ± 0.9	5.6 ± 1.2	3.6 ± 0.6
Fumaric	1.1 ± 0.3	- -	- -	- -
Succinic	0.4 ± 0.3	1.7 ± 0.3	2.9 ± 0.5	1.6 ± 0.2
α-Keto Glutaric	- -	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2
Citric	- -	- -	0.4 ± 0.1	0.2 ± 0.2
OA Origin	2.4 ± 0.7	3.7 ± 2.5	1.0 ± 0.3	0.8 ± 0.2
OA Unknown	5.8 ± 1.0	2.6 ± 0.4	7.1 ± 0.9	9.3 ± 1.1
OA Total	36.0	20.6	29.1	26.6
Aspartic	10.1 ± 1.3	9.8 ± 1.1	8.0 ± 2.1	0.2 ± 0.2
Glutamic	10.4 ± 1.8	11.8 ± 1.5	17.4 ± 2.0	0.7 ± 0.5
β-Alanine	0.8 ± 0.8	2.7 ± 1.6	2.6 ± 0.3	2.7 ± 0.3
Serine	- -	- -	1.6 ± 0.2	1.0 ± 0.2
Proline	0.2 ± 0.2	- -	0.6 ± 0.3	1.9 ± 0.3
Leucine	- -	3.3 ± 0.8	1.7 ± 0.4	2.2 ± 0.4
Valine	- -	1.2 ± 1.2	0.8 ± 0.2	1.3 ± 0.2
Arginine	- -	- -	- -	0.9 ± 0.4
Glycine	- -	- -	0.4 ± 0.4	- -
AA Origin	5.0 ± 0.9	5.7 ± 1.4	1.6 ± 0.3	1.4 ± 0.2
AA Unknown	1.0 ± 1.0	- -	4.9 ± 0.7	8.3 ± 1.8
AA Total	27.5	34.5	39.6	20.6
Glucose	- -	- -	- -	1.3 ± 0.8
Fructose	- -	5.9 ± 1.9	- -	- -
Glycerol	- -	- -	- -	0.9 ± 0.6
Sugar Origin	3.7 ± 0.6	3.8 ± 0.3	5.1 ± 0.9	8.1 ± 1.5
Sugar Unknown	32.8 ± 2.8	36.1 ± 6.9	26.8 ± 2.5	42.5 ± 3.9
Sugar Total	36.5	45.8	31.9	52.8

- Below detectable limit

percent activity in the isolated compounds in the HES fraction vary but do not follow a consistent trend. However cells grown in blue light had a low percent activity in the HES organic acid fraction and high percent activities in the HES amino acid and protein fraction. Cells grown in red light also had a low percent activity in the organic acid fraction but had higher percent activities in the sugar and polysaccharide fraction.

Hydrolysis of the ethanol-insoluble fraction yielded the compounds indicated in Tables 4 and 5. There were no significant differences between the protein amino acids ($P \leq .01$) under different light qualities whereas the hydrolysis of the insoluble sugar fraction yielded glucose and fructose in varying amounts. The percent glucose present in cells grown in green and red light was significantly higher than in cells grown in white and blue light. Cells grown in green and red light had a significantly lower percent activity ($P \leq .01$) in the unknown fraction than cells grown in white and blue light. This unidentified sugar appeared in the same area of the chromatograms of the sugars from cells grown under all four light conditions.

Organic Excretion Products

The nature of the compounds excreted by C. reinhardtii under the various light regimes is shown in Table 6. Cells grown under white light excreted a large percent activity in each fraction (i.e. free amino acids, organic acids and sugars). Cells grown under blue and red light excreted mainly sugars and organic acids respectively. The composition of the material excreted by cells grown under green light was not determined because of the low total activity present in the excreted fraction.

Table 4. Distribution of ^{14}C among the protein amino acids after 30 min of photosynthesis by Chlamydomonas reinhardtii. The results are expressed as a percent of the total activity in the protein amino acid fraction ± standard error.

	White	Blue	Green	Red
Aspartic	12.8 ± 2.5	13.4 ± 2.1	14.8 ± 1.7	14.2 ± 0.5
Glutamic	14.7 ± 2.4	9.3 ± 1.1	12.9 ± 0.9	10.8 ± 0.6
Glycine	5.1 ± 1.1	6.4 ± 0.5	3.8 ± 0.8	2.9 ± 0.4
Serine	1.1 ± 0.6	- -	2.9 ± 0.6	1.9 ± 0.2
β-Alanine	15.6 ± 5.0	17.8 ± 2.5	12.2 ± 0.9	12.8 ± 0.6
Leucine	16.8 ± 3.1	29.4 ± 4.0	22.6 ± 0.6	21.6 ± 0.6
Proline	14.3 ± 2.8	6.8 ± 2.5	10.0 ± 1.4	11.4 ± 0.8
Valine	14.7 ± 2.1	12.4 ± 2.1	13.3 ± 0.9	13.1 ± 0.5
Arginine	3.9 ± 1.4	1.3 ± 0.8	- -	0.8 ± 0.5
Phenyl Alanine	- -	- -	3.9 ± 1.3	0.6 ± 0.6
Amino Acid Unknown	- -	3.2 ± 3.2	3.5 ± 2.3	9.5 ± 0.8

- Below detectable limit

Table 5. Distribution of ^{14}C among the sugar hydrolysis fraction after 30 min of photosynthesis by Chlamydomonas reinhardtii. The results are expressed as a percent of total activity in the sugar hydrolysis fraction ± standard error.

Light Quality	White	Blue	Green	Red
Glucose	49.2 ± 12.7	26.9 ± 5.4	83.1 ± 2.3	66.4 ± 4.1
Fructose	- -	- -	5.1 ± 0.1	2.6 ± 0.5
Sugar Origin	8.9 ± 3.8	4.3 ± 0.9	0.2 ± 0.2	0.9 ± 0.6
Sugar Unknown	41.6 ± 9.7	68.8 ± 4.6	11.7 ± 2.1	3.38± 3.9

- Below detectable limit

Table 6. Distribution of ^{14}C in the excreted products after 30 min of photosynthesis by Chlamydomonas reinhardtii expressed as a percent total activity recovered in the excreted fraction ± standard error.

	White	Blue	Red
Glycolate	2.7 ± 0.8	- -	5.1 ± 1.7
3-PGA	2.8 ± 1.1	- -	19.9 ± 3.3
Malic	- -	- -	6.6 ± 0.7
OA Origin	5.0 ± 1.0	- -	11.7 ± 2.2
OA Unknown	3.8 ± 1.3	- -	54.8 ± 9.7
AA Origin	13.8 ± 2.9	- -	- -
AA Unknown	19.5 ± 3.8	- -	- -
Sorbitol	7.6 ± 3.3	14.0 ± 1.3	- -
Sugar Origin	31.6 ± 5.0	81.5 ± 5.7	- -
Sugar Unknown	15.7 ± 4.7	4.5 ± 4.5	- -
Excretion as a % of Total Incorporation	35.6	6.6	4.0
DPM'S (X 10^{-6}) Excreted /10^8 Cells	21.3	4.4	2.8

- Below detectable limit

DISCUSSION

This study has shown that light quality influences photosynthetic rate, growth, as well as the nature of the photosynthetic and excreted products in Chlamydomonas reinhardtii.

C. reinhardtii cultured under blue or white light had a shorter lag phase than cells grown under red or green light of the same intensity (Fig. 2). Wallen and Geen (1971 a), working with two species of marine planktonic algae, found a short lag phase in white light and a slightly longer lag phase in cultures grown under green light. This could be a species difference or a function of the narrower band widths used in my work.

In general, a lag occurs in any system of enzymatic reactions, such as occur in cellular synthesis, when the system is thrown "off balance" by the temporary removal of a critical reactant (Strickland 1960). The precise cause of the slow growth during the lag period may be due to a shortage of a critical growth promoter in the medium produced by the cells themselves (Berglund 1969). This shortage could result from the inoculation of cells into a fresh sterile medium. If this is the case, it appears that the synthesis and release of a specific growth promoter (or complex of promoters) is slower under green or red light than under white or blue light.

A second explanation for the lag phenomenon, put forth by Strickland (1960), is that a very small portion of the cells in the inoculum may have an activity far above the average and multiply in an exponential manner while the majority of the cells are largely static. The lag phase ends

when the number of active cells becomes a significant fraction of the population. The specific differences in the lag phase observed under different light regimes could be due to differential efficiency of absorption of the different wavelengths employed in this study. The reestablishment of the rapid growth by the cells may involve the synthesis of a greater quantity of the same enzymes or the production of different enzymes (isozymes). These are possible explanations for the differences in lag phase of my cultures.

Hess and Tolbert (1967) showed that the growth rates of C. reinhardtii, in blue and red light were initially slow but increased over a period of five days until the growth rates were similar to those of cells grown in white light. In cultures grown in blue light, total chlorophyll per cell increased 20% during the lag period although the chlorophyll a/b ratio decreased (Hess and Tolbert 1967). This is an example of the type of change which might be required during the lag period before rapid growth can take place. This supports Strickland's hypothesis that the cultures grown in new sterilized media actually experience a "period of slow cellular adaptation". The lag phase could result from the slow production of an extracellular growth promoter in combination with pigment or cellular adaptation. The increase in cell size, such as I observed in cultures grown in green light, would decrease the rate of cell division and hence lengthen the lag period. The transfer of cells grown under white light, in the log phase of growth (1-3 x 10^5 cells/ml), to green light produced a 2-day lag period before the green light growth rate was achieved. This suggests that the lag is related to cellular adaptation rather than

the presence of an extracellular growth promoter.

The photosynthetic rates of C. reinhardtii, grown under different light qualities are presented in Table 1. Cells grown in red, blue or white light had a higher photosynthetic rate per cell (as measured by ^{14}C fixed) than those grown in green light. This is in general agreement with the growth constants (Fig. 2). My cultures exhibited low growth rates and long generation times relative to enriched cultures (doubling every 1-2 days). However, these rates and generation times are not unlike those reported for natural populations (Strickland 1960).

Wallen and Geen (1971 a) showed that the photosynthetic rates of two marine planktonic algae were higher in blue and lower in green light than in white light of the same intensity. Voskresenskaya et al. (1971) showed the rate of photosynthesis in bean leaves was similar under red and blue light. These rate differences could be explained by the lower efficiency of energy absorption in green light which may be a function of the pigment composition and concentration.

Light quality has a distinct effect upon the distribution of ^{14}C in the photosynthetic products of C. reinhardtii. The increase I observed in percent ^{14}C fixed in the ethanol-insoluble fraction in green and blue light (Table 1) is similar to that reported by Wallen and Geen (1971 a) who showed that cultures grown in white light, (as at the surface of a lake or ocean) incorporated the greatest proportion of the ^{14}C into the ethanol-soluble fraction, whereas cultures grown in blue or green light (comparable to the lower part of the photic zone) incorporated most of the ^{14}C into ethanol-insoluble compounds, primarily proteins. The relationship they reported was not appreciably affected by light intensity.

The distribution of ^{14}C within the ethanol-insoluble fraction is shown in Table 2. In blue and white light I observed an increase incorporation of ^{14}C in the protein fraction whereas the polysaccharides were highest in green and red light. Kowallik (1965), using Chlorella pyrenoidosa, showed that the enhanced protein production had an action spectrum with a broad maximum of 450-490 nm. Radiant energy above 500 nm resulted in a shift of synthesis to polysaccharides from proteins. This is similar to my results. The results of Wallen and Geen (1971 a) also show a green light enhancement of ^{14}C incorporation into proteins in two species of marine plankton. I did not observe this which could be attributable to the narrower band width used in my work. The addition of only 4% blue light as incident energy to red or green light has essentially the same effect on the distribution of ^{14}C as blue light alone (Hauschild et al. 1965).

Kowallik (1965) found that protein enhancement required the blue part of the spectrum, an equal number of red quanta did not produce an increased protein production. My work showed the insoluble sugar fraction was higher in cells grown in green and red light (31.5, 40.7% respectively) than in white and blue light (17.7, 6.5% respectively) (Table 2). This suggests that short wavelength light (blue) shifts synthesis away from polysaccharides. Blue, green and red light grown cells all show small difference in the insoluble fraction in comparison to white light. The percent in the insoluble fraction is formed at the expense of the total ethanol-soluble fraction.

There were no significant differences ($P \leq .01$) in the percentage composition of the various components of the protein hydrolysis fraction of cells cultured under different light quality. Cells grown under blue light had a higher ^{14}C activity in leucine than cells grown in white light while glycine was significantly higher ($P \leq .05$) in white and blue than in green and red light (Table 4). Serine, on the other hand, was below detectable limits only in cells grown under blue light. The observed patterns of protein amino acids differ from those reported by Wallen and Geen (1971 a) and Voskresenskaya (1956) who noted changes in the rate but not direction of protein synthesis in cells grown under short wavelength light (400-580 nm).

Cells grown under blue and green light had the highest percent activity in free amino acids and a lower activity in organic acids than in cells grown in white light. In general, cells grown in white light contained a larger percent organic acid activity than those grown in blue, green or red light (Table 3). The relative increase in amino acid production in blue and green light and protein synthesis in blue light would drain off the organic acids, precursors of amino acids.

If amino acids are being synthesized from Krebs cycle intermediates, then these intermediates must be replaced to ensure functioning of the cycle. This could be achieved by β-carboxylation of pyruvate or phosphoenol pyruvate (PEP) to give oxoloacetate or malate (Ogasawara and Miyachi 1970). If this pathway were functioning it would be expected that malic or aspartic acid should be high. Since my data do not support this expectation (Table 3) it is doubtful that this mechanism is operative in C. reinhardtii.

Leucine is increased in the ethanol-soluble as well as the protein fraction in cells grown in blue light. Pyruvic acid is very low in cells cultured in blue light relative to white, green and red light grown cells. This could be interpreted as a blue light activation of an enzyme in the leucine synthesis pathway which requires pyruvic acid. Conversely, there was least leucine in the protein and ethanol-soluble fraction of cells grown in white light but the highest relative concentrations of pyruvic acid. This suggests that blue light stimulates production of a specific enzyme in the leucine synthesis pathway while red light inhibits this synthesis. These data do not identify the reactions involved, but do show that short wavelength light alters the direction of algal metabolism in favour of protein synthesis.

Cells grown under red light contained the highest percent activity in the soluble sugars (Table 3) compared to cells grown in white, blue and green light. Glucose was the most significant radioactive sugar recovered on carbohydrate hydrolysis although small quantities of fructose occurred in cells cultured under green and red light. These sugars may have been derived from a reserve polysaccharide or from cell-wall polysaccharides. The cells grown under blue and white light incorporated a smaller percent ^{14}C into glucose and fructose than those grown under green or red light (Table 5). This shows that blue light shifts synthesis away from polysaccharides in comparison to red or green light.

It has been pointed out that red light promotes the synthesis of sugars, whereas, blue and green light enhance the synthesis of amino

acids and proteins (Wallen and Geen 1971 a, Ogasawara and Miyachi 1969, Szász and Barsi 1971). The effect of blue light may be attributed to the activation of PEP carboxylase (Ogasawara and Miyachi 1970) or to the stimulation of respiration which results in a change in the ratio of photosynthesis to respiration (Ries 1970, Kowallik and Gaffron 1967). According to Trukhin (1968), blue light increases the rate of secondary carboxylation of certain organic acids, thereby promoting the accumulation of amino acid precursors.

Trukhin (1968) showed that blue light promotes the formation of reducing agents of the NADP type, even at the expense of ATP formation. Bell and Shuvalova (1971), found that about half the energy stored by Chlorella was fixed as a result of a process not coupled to oxygen evolution. They postulated a pseudocyclic or cyclic photophosphorylation in which the coupling between the cyclic electron flow and ATP formation is improved by blue radiation probably as a result of photostructural changes.

Szász and Barsi (1971) postulated that the stimulatory effect of red light on the polysaccharide accumulation may be explained by an "extra" ATP production in cyclic photophosphorylation, via preferential activation of photosystem I. This is in line with the statement of Metzner (1969) that the effects of red and blue light are probably connected with the changes of the ATP/NADPH ratio.

The nature of the dissolved organic compounds excreted by cells grown in white, blue and red light is shown in Table 6. The rate of excretion by cells grown in green light was not sufficient to permit identification of the constituent compounds. Cells grown in white light excreted amino acids, organic acids and sugars whereas sugars and organic acids were the only products excreted by cells grown in blue and red light respectively. This shows preferential release of material and not excretion due to cell breakage. Allen (1956) showed that 23% of the organic material formed by C. reinhardtii appeared in soluble form in the culture media. She reported that glycolic and oxalic acids and sugars but no amino acids were excreted under white light during an eight day growth period. Maksimova and Pimenova (1966) did not detect any nitrogen-containing compounds in the excreted products of Chlorella. My data do not agree with these findings since amino acid activity was detected in white light although specific compounds were not identified. I did not detect oxalic acid excretion following the short photosynthetic period. Lewin (1956) showed that C. reinhardtii also released soluble polysaccharides into the medium, possibly leached from the mucilaginous sheath. I detected no labelled polysaccharides in the medium after 30 min of photosynthesis although some were observed after 40 min.

Glycolic acid was released into the medium by cultures grown under white and red light but not under blue light. Becker et al. (1968), using Chlorella pyrenoidosa and C. vulgaris observed glycolate excretion by cells grown under high intensity white and red light but not blue light which

agrees with my results. They suggested that the glycolate formed under blue light is further metabolized via glyoxylate to glycine and other amino acids which are utilized during protein synthesis. This pathway was also observed by Lord et al. (1970) who gave evidence for the major involvement of photosystem I (driven by red light) during glycolate biosynthesis in C. pyrenoidosa. The greater utilization of organic acids in blue light could explain their absence in the excretion products of cells grown in blue light. The explanation for the absence of amino acids and sugars in the exudate of cells grown under red light is not at hand.

Wallen and Geen (1971 c) showed the proportion of ^{14}C excreted by marine phytoplankton generally increased as the relative activity in the ethanol-soluble fraction increased. My data do not support this observation. The distribution of ^{14}C between amino acids, organic acids and sugars in the excreted material under different light qualities suggests differential membrane permeability and not passive leaching dependent upon the size of the HES fraction. Vredenberg (1971), using Nitella, showed light-induced changes in membrane potential. These changes in membrane potential were associated with changes in cytoplasmic ion concentrations. The control mechanism probably is powered primarily by electron transport-coupled ion transport at the chloroplast thylakoid membrane, and regulated by changes in ion flux across the cellular and outer chloroplast membranes (Vredenberg 1971). The maintenance of constant intracellular charge in the presence of an ion flux could partially be controlled by the release of small negatively charged organic compounds such as glycolic acid. Tolbert and Zill (1956) suggested that glycolate excretion and absorption represent

a glycolate-bicarbonate anionic exchange across the cell wall without the necessity of a similar cationic shift. The excretion of organic compound could be an active process dependent upon the charge on the cellular membrane and the flux of ions within the cell.

My data (Tables 1-5) show that light quality influences the chemical composition of the planktonic alga Chlamydomonas reinhardtii. Blue light enhanced amino acid and protein production whereas red light enhanced polysaccharide production. A similar situation would be expected in nature due to the differential attenuation of light with depth in the photic zone. These data also show that light quality is associated with differences in the material excreted by C. reinhardtii. The variation in metabolic products within the cell influence the nature and quantity of excreted material.

LITERATURE CITED

Ahmed, A. M. M. and Ries, E. The pattern of $^{14}CO_2$ fixation in different phases of the life cycle and under different wavelengths in Chlorella pyrenoidosa. Progress in Photosynthesis Research Vol. III: 1662-68 (1969).

Allen, M. B. Excretion of organic compounds by Chlamydomonas. Arch für Mikrobiologie 24: 163-168 (1956).

Becker, J. D., Döhler, G. and Egle, K. The effect of monochromatic light on the extracellular excretion of glycolate during photosynthesis of Chlorella. Pflangenphysiol. 58: 212-221 (1968).

Bell, L. N. and Shuvalova, N. P. Blue radiant energy storage uncoupled from oxygen evolution. Photosynthetica 5: 113-123 (1971).

Berglund, H. Stimulation of growth of two marine green algae by organic substances excreted by Enteromorpha linza in unialgal and axenic cultures. Physiol. Plantarum 22: 1069-1073 (1969).

Cayle, T. and Emerson, R. Effect of wavelength on the distribution of carbon -14 in the early products of photosynthesis. Nature 179: 89-90 (1957).

Constantopoulos, G. Lipid metabolism of Mg-deficient algae. Plant Physiol. 45: 76-80 (1970).

Fedorov, V. D., Maksimov, V. N., and Khromov, V. M. Effect of light and temperature on primary production of certain unicellular green algae and diatoms. Soviet Plant Physiol. 15: 635-643 (1968).

Fogg, G. E. The production of extracellular nitrogenous substances by a blue-green algae. Proc. Roy. Soc. B. 139: 372-397 (1952).

Hauschild, A. H. W., Nelson, C. D. and Krotkov, G. The effect of light quality on the products of photosynthesis in Chlorella vulgaris. Can. J. Bot. 40: 179-189 (1962).

Hauschild, A. H. W., Nelson, C. D. and Krotkov, G. On the mode of action of blue light on the products of photosynthesis in Chlorella vulgaris. Naturwissenschaften 52: 435 (1965).

Hess, J. L. and Tolbert, N. E. Changes in chlorophyll a/b ratio and products of $^{14}CO_2$ fixation by algae grown in blue or red light. Plant Physiol. 42: 1123-1130 (1967).

Kowallik, W. Protein production of Chlorella in light of different wavelengths. Planta 64: 191-200 (1965).

Kowallik, W. and Gaffron, H. Enhancement of respiration and fermentation in algae by blue light. Nature 215: 1038-1040 (1967).

Lewin, R. A. Extracellular polysaccharides of green algae. Can. J. Microbiol. 2: 665-672 (1956).

Lord, J. M., Codd, G. A. and Merrett, M. J. The effect of light quality on glycolate formation and excretion in algae. Plant Physiol. 46: 855-856 (1970).

Maksimova, T. V. and Pimenova, M. N. Nature of organic compounds excreted by growing cultures of green algae. Mikrobiologiya 35: 623-732 (1966).

Metzner, H. Comparative biochemistry and photosynthesis. FEBS Letters 5: 93-95 (1969).

Ogasawara, N. and Miyachi, S. Effect of Wavelength on $^{14}CO_2$ fixation in Chlorella cells. Progress in Photosynthesis Research Vol. III, 1653-1661 (1969).

Ogasawara, N. and Miyachi, S. Regulation of CO_2 fixation in Chlorella by light of varied wavelengths and intensities. Plant and Cell Physiol. 11: 1-14 (1970).

Olive, J. H. and Morrison, J. H. Variations in distribution of ^{14}C in cell extracts of phytoplankton living under natural conditions. Limnol. Ocean. 12: 383-391 (1967).

Orth, G. M., Tolbert, N. E. and Jimenez, E. Rate of glycolate formation during photosynthesis at high pH. Plant Physiol. 41: 143-147 (1966).

Parsons, T. R., Stephens, K. and Strickland, J. D. H. On the chemical composition of eleven species of marine phytoplankton. J. Fish. Res. Bd. Canada 18: 1001-1016 (1961).

Ries, E. Effects of light quality on gas exchange and ^{14}C incorporation of Chlorella pyrenoidosa. Photosynthtica 4: 78-80 (1970).

Starr, R. C. The culture collection of algae at Indiana University. Am. J. Bot. 51: 1013-1044 (1964).

Stein, J. R. A morphologic and genetic study of Gonium pectorale. Am. J. Bot 45: 664-672 (1958).

Stewart, W. D. P. Liberation of extracellular nitrogen by two nitrogen fixing blue-green algae. Nature 200: 1020-1021 (1963).

Strickland, J. D. H. Measuring the production of marine phytoplankton. Fisheries Res. Bd. of Can. bull. #122 (1960).

Szász, K. and Barsi, I. S. Stimulatory effect of red light on the polysaccharide accumulation in the leaves. Photosynthetica 5: 71-73 (1971).

Thomas, J. P. Release of dissolved organic matter from natural populations of marine phytoplankton. Marine Biology 11: 311-323 (1971).

Tolbert, N. E. and Zill, L. P. Excretion of glycolate by algae during photosynthesis. J. Biol. Chem. 222: 895-906 (1956).

Trukhin, N. V. Effect of the carbon supply on the response of Chlorella pyrenoidosa to the effect of blue and red light. Soviet Plant Physiol. 15: 544-548 (1968).

Voskresenskaya, N. P. Synthesis of organic and amino acids during photosynthesis under various conditions of illumination. Fiziol. Rast. 3: 49-57 (1956).

Voskresenskaya, N. P. and Nechaeva, I. P. Effect of blue, red and green light on the content of protein, nucleic acids, and chlorophyll in young barley plants. Soviet Plant Physiol. 14: 254-261 (1967).

Voskresenskaya, N. P., Viil'Yu, A., Grishina, G. S. and Pyarnik, T. P. Districution of labelled carbon in the products of photosynthesis of bean leaves under red and blue light at different oxygen content in the medium. Soviet Plant Physiol. 18: 411-415 (1971).

Vredenberg, W. J. Changes in membrane potential associated with cyclic and non-cyclic electron transport in photochemical system I in Nitella translucens. Biochem. Biophys. Res. Comm. 42: 111-118 (1971).

Wallen, D. G. and Geen, G. H. Light quality in relation to growth, photosynthetic rates and carbon metabolism. Marine Biology 10: 34-43 (1971 a).

———— Light quality and concentration of proteins, R.N.A., D.N.A. and photosynthetic pigments in two species of marine plankton algae. Marine Biology 10: 44-51 (1971 b).

———— The nature of the photosynthate in natural phytoplankton populations in relation to light quality. Marine Biology 10: 157-168 (1971 c).

Watt, W. D. and Fogg, G. E. The kinetics of extracellular glycolate production by Chlorella pyrenoidosa. J. of Exp. Bot. 17: 117-134 (1966).

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