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THE EFFECT OF LIGHT QUALITY ON THE GROWTH, PHOTOSYNTHESIS, EXTRACELLULAR RELEASE AND PHOTORESPIRATION OF FOUR FRESHWATER ALGAE.

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THE EFFECT OF LIGHT QUALITY ON THE GROWTH, PHOTOSYNTHESIS, EXTRACELLULAR RELEASE AND PHOTORESPIRATION OF FOUR FRESHWATER ALGAE

by

Brendan Charles Birmingham

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ABSTRACT

Depth-dependent changes in light quality occur in natural waters. In spite of this only a minor fraction of light quality related studies of algae have been done in this context, mainly using marine algae. This study represents an attempt to analyse the impact of restricted portions of the visible spectrum on the growth, photosynthesis, chemical composition and carbon metabolism of freshwater algae. Four unicellular freshwater algae were grown in equal intensities of white, blue, green and red light. The intensity (550 $\mu W\ cm^{-2}$) and spectral distribution of these light sources simulated conditions in the lower part of the photic zone of natural waters. In the case of Chlamydomonas reinhardtii and Chlorella vulgaris, the complete spectrum of white light was more effective for growth than any part of it. The growth rate of Navicula pelliculosa and Anacystis nidulans was highest in red light.

Photosynthetic rates of the two green algae were highest in blue or red light grown cells and lowest in green light grown cells. A similar pattern of photosynthetic adaptation was observed in Navicula though green light adapted cells had higher photosynthetic rates than similarly adapted green algae. Rates of photosynthesis were highest in red light adapted Anacystis and uniformly low in blue or green light adapted cells. All four algae showed increased photosynthetic efficiency following adaptation when compared to white light adapted cells exposed to the same light source.

This was not reflected in large changes in the total pigment

content of the two green algae. However, blue light grown green algae showed increased light absorption in the blue part of their *in vivo* absorbance spectra. Chlorophyll *a* content was highest in red and green light grown *Navicula*. Blue and white light grown diatom cultures were visibly browner and had a higher total carotenoid: chlorophyll *a* ratio than green or red light grown cells. The phycocyanin content of blue light grown *Anacystis* was 32% of the total cellular protein and resulted in visibly blue cells. The phycocyanin content was 4.5-fold lower in red light grown cells and suggests that some sort of inverse chromatic adaptation had occurred.

No strong effect of light quality on the protein, carbohydrate, nucleic acid or lipid content of these algae was observed. Slightly higher levels of protein in blue light grown cells of the two green algae and *Anacystis* were reflected in lower carbohydrate:protein ratios when compared to cells grown in red light. The minor changes in chemical composition observed may reflect changes in chloroplast structure induced by adaptation to restricted spectral wavebands.

The photosynthetic carbon matabolism of these algae during $^{14}CO_2$-incorporation was followed by partitioning the cells into water-, chloroform- and insoluble fractions following hot ethanol extraction. Most radioactivity was found in the soluble fractions of red light grown cells of *Navicula* and the two green algae. This situation was reversed in blue and white light grown cells where ^{14}C-activity was higher in the insoluble fraction. These results suggest that light quality may account for similar depth-dependent increases in the radioactivity of the insoluble fraction observed during ^{14}C-uptake by marine

and freshwater phytoplankton.

Extracellular release as a percentage of total ^{14}C-fixation was < 5% for all algae. Extracellular release was lowest in red light and highest in white or blue light. No direct relationship between the size of the soluble fraction and extracellular release was observed. The low extracellular release in red light grown cells was striking since soluble ^{14}C-activity was high under these conditions.

Photorespiration was initially estimated from the rate of $^{14}CO_2$ release into CO_2-free air from ^{14}C-labelled cells. In all algae, rates of $^{14}CO_2$ release both in the light and the dark were highest following or during exposure to blue or green light. $^{14}CO_2$ release rates were lowest in red or white light. The $^{14}CO_2$ release method seriously underestimates the magnitude of photorespiration. By measuring the differential influx of $^{12}CO_2$ and $^{14}CO_2$ in a closed system in white light the rate of photorespiration as a percentage of the $^{14}CO_2$-fixation rate was calculated. These calculated values ranged from 17 to 32% for Navicula and the two green algae and from 10 to 26% for Anacystis. The effect of light quality on photorespiration and dark respiration suggests that these processes may be more important than extracellular release for the carbon economy of phytoplankton deep in the photic zone.

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ABBREVIATIONS AND SYMBOLS

ANOVA	:	analysis of variance
C_3 plant	:	primary mechanism of carbon fixation by the photosynthetic carbon reduction pathway (Calvin cycle).
C_4 plant	:	primary mechanism of carbon fixation by the C_4 dicarboxylic acid (Hatch-Slack) pathway
CCA	:	complementary chromatic adaptation
chl	:	total chlorophyll
µCi	:	micro Curie
DCMU	:	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DNA	:	deoxyribonucleic acid
DR	:	dark respiration
dpm	:	disintegrations per minute
EDTA	:	ethylenediaminetetraacetic acid
ft cd	:	foot candle(s)
GP	:	gross photosynthesis
g	:	gram(s)
mg	:	milligram(s)
µg	:	microgram(s)
xg	:	gravity
αHPMS	:	α-hydroxy-2-pyridinemethanesulphonic acid
INH	:	isonicotinyl hydrazide
IRGA	:	infrared gas analyser
klux	:	kilolux
L/D	:	light/dark
λ_{Hmax}	:	peak wavelength of maximum irradiance

l	:	liter(s)
ml	:	milliliter(s)
µl	:	microliter(s)
nl	:	nanoliter(s)
M	:	molar
mM	:	millimolar
µM	:	micromolar
nM	:	nanomolar
nm	:	nanometer
N.P.	:	net photosynthesis
PAR	:	photosynthetically available radiation
PCA	:	perchloric acid
PEP	:	phosphoenol pyruvate
3-PGA	:	3-phosphoglyceric acid
PIB	:	postillumination CO_2 outburst
PR	:	photorespiration
ppm	:	parts-per-million (= µl/l (gas) or mg/l (solution))
psi	:	pounds per square inch
RNA	:	ribosenucleic acid
RuDP	:	ribulose -1,5-diphosphate
s	:	second
S.A.	:	specific activity
TCA	:	trichloracetic acid
TRIS	:	tris(hydroxymethyl) aminomethane
v/v	:	volume/volume
w/v	:	weight/volume
w	:	watt

mW : milliwatt

μW : microwatt

 : light | dark used in $^{14}CO_2$ release figures

INTRODUCTION

1. The Underwater Light Climate

1.1 Introduction

Solar radiation is the starting point for all energy flow through the biosphere (379). About one half of the solar energy influx takes place in the visible portion of the solar radiation spectrum (wavelengths between 400 and 700 nm). This portion of the solar radiation spectrum is photosynthetically available radiation (PAR) and can penetrate almost without loss down to the earth's surface, except where clouds are present (322, 331). PAR controls the primary productivity, the rate at which plants photosynthetically convert carbon dioxide and water into organic matter, of terrestrial and aquatic plants. The absorption of solar energy outside the visible range and its dissipation as heat also has profound effects on the thermal structure, water mass stratification and circulation patterns of lakes and oceans (247, 373).

For the purposes of this review only the influence of the solar radiation spectrum on the aquatic component of the biosphere will be considered.

In the aquatic environment the interception and capture of PAR by aquatic plants occurs in a thin layer called the photic zone. The thickness of this layer is limited by the depth to which incident light can penetrate and maintain net growth of aquatic plants. Freely suspended unicellular algae or phytoplankton are responsible for most primary productivity in the oceans and deep lakes (235). In rivers, shallow bodies of water and around the margins of the oceans and large lakes,

benthic or attached algae or aquatic vascular macrophytes make large and important contributions to primary productivity (215, 373). While primary productivity in natural waters is directly controlled by the intensity and spectral quality of the available light, temperature and the replenishment of the major nutrient ions containing nitrogen and phosphorus can be limiting factors (235, 390, 373).

The PAR incident on the surface of the photic zone varies in intensity, spectral quality and daylength or photoperiod. Apart from being a major factor directly controlling primary production via photosynthesis, this complex underwater light climate also influences the photobiology of algae and aquatic plants. This would include the chemical composition, growth rate and photomorphogenetic processes such as changes in growth form, photomovement and production of reproductive structures of algae and aquatic plants (134, 271).

To better understand and appreciate the underwater light climate controlling aquatic productivity, the physical processes affecting the spectral irradiance of PAR incident on and penetrating the photic zone will be described.

1.2 The solar irradiance spectrum incident on the surface of the photic zone

The amount of direct solar energy per unit time incident on the earth's atmosphere is called the solar constant. Recent evidence suggests that it has a value of about 1.94 cal cm^{-2} min^{-1} (107, 331, 343). Solar radiation penetrating the atmosphere is attenuated by adsorption, reflection and scattering by molecules of oxygen, ozone, carbon dioxide, and larger particles such as water droplets, dust and other particles in the size range of the incoming radiation (161, 211,

331, 373). A major factor controlling both the intensity and spectral distribution of solar energy reaching the earth's surface is the angular height of the sun incident to the earth (107, 331, 373). Solar elevation varies markedly with the time of the day or season, latitude or altitude and is basically an expression of the length of the light path through the atmosphere.

Daylight or the total solar irradiance penetrating the atmosphere has two major components, direct solar radiation or sunlight and indirect solar radiation or skylight. The spectral distribution of sunlight is the dominant component of daylight on clear days and is mainly dependent on solar elevation. The air mass or density of the atmosphere will change the spectral quality of daylight on clear days and has been classified into different air mass types on this basis (Moon (1940) cited in 17, 147). Most spectral shifts in the spectral distribution of solar radiation can be expressed as a change in the ratio of the blue to the red part of the spectrum with a "hinge" point centered near 560 nm (221). The largest changes in the spectral quality of daylight occur at low solar angles i.e. sunrise and sunset, which has been dubbed the "twilight" effect (107, 146, 161, 211).

1.3 The solar irradiance spectrum penetrating the photic zone (the underwater light climate)

Water is a light absorbing and scattering medium. The spectral irradiance distribution of incident daylight is immediately attenuated by true reflection and vertical backscattering by particles just below its surface. The amount of light entering the water (downward irradiance) is strongly influenced by solar elevation as long as sunlight dominates the daylight. This dependence is removed when atmospheric light is mainly diffuse, i.e. overcast conditions or solar angles less

than 10^{o} (147, 159, 290, 331, 373).

The intensity and spectral distribution of light penetrating the water's surface are also further attenuated by absorption and scattering processes. Absorption processes include the absorption properties of water itself, absorption by dissolved or colloidal organic material (gelbstoff or "yellow substance") and absorption by living and non-living particulate matter (turbidity) (147, 159, 290, 331, 373).

Rayleigh scattering causes much of the shortwave attenuation in clear waters. This scattering process increases rapidly at smaller wavelengths. The presence of living and non-living suspended particles also contributes to the scattering properties of natural waters. With increasing depth the irradiance is reduced more or less logarithmically.

The "transparency" or amount of light that can penetrate natural waters is often described by its vertical extinction coefficient (k^{o}, K) which measures the removal of radiation with depth (147, 159, 290, 331, 373). This is analogous to the monochromatic extinction coefficient or absorptivity used in spectrophotometry, however, the vertical extinction coefficient is based on a multichromatic light source and most natural waters do not represent true solutions. This vertical extinction coefficient is strongly influenced by the presence of Gelbstoff (dissolved organic matter which absorbs maximally in the blue part of the spectrum), suspended sediment and phytoplankton.

The various optical types of oceanic and coastal waters have been classified according to their spectral distribution underwater (159). In very transparent, blue oceanic waters, the peak wavelength of maximum irradiance (λ_{Hmax}) tends to be in the range 440 to 475 nm.

The depth at which 0.5% of the surface irradiance is reached tends to be greater than 100 m. In blue-green coastal waters, the presence of Gelbstoff causes both a decrease in the depth of maximum irradiance and a red shift of the λ_{Hmax} into the wavelength range 475 to 515 nm. In nearshore, estuarine and shallow eutrophic situations which can range in colour from very green to reddish brown, the adsorption by dissolved organic matter and turbidity caused by suspended sediment and/or the standing crop of phytoplankton can severely limit the optical depth of the transmitted light and is a major cause of spectral variation resulting in λ_{Hmax} greater than 515 nm. Typically, coastal water type 9 (containing the most Gelbstoff) reached 0.5% surface irradiance at 7 m. In silt-laden estuarine waters, this point is reached less than 1 m from the surface. The spectral distribution of light in freshwaters varies in a similar fashion to that of the marine environment. Early characterization of the spectral transmission properties of natural waters was mainly done with filters placed over submarine photometers (15, 147, 159, 331). With the development of submarine spectroradiometers and quanta meters more detailed measurements of the underwater spectral energy (and quanta) distribution have been reported (123, 160, 211, 221, 300, 314, 317).

Except for the large deep lakes (15, 314) which are characterized by maximum light penetration in the blue and blue-green parts of the spectrum, most freshwater environments are relatively shallow and strongly coloured by dissolved organic matter, particulate matter and phytoplankton (15, 373). In eutrophic waters, light is rapidly attenuated and 1% surface light levels are reached within 2 to 10 meters (15, 160, 300, 317). High spectral variation occurs even in closely

confluent bodies of water. McFarland and Munz (211) cite an example from Florida waters where the rusty red Carrabelle River (maximum transmission from 550 to 700 nm) flows into the green waters of Saint George Sound. The major causes of spectral variation in shallow and highly eutrophic Lough Neagh were dissolved organic matter and the algal standing crop (160). This resulted in rapid attenuation of the blue part of the spectrum and light absorbed by photosynthetic pigments especially the chlorophyll a peak at 680 nm. Maximum light penetration occurred around 575 to 600 nm. An earlier study in Japanese lakes showed the strong influence of a diatom bloom on the vertical transmission of the violet, blue and red parts of the spectrum (293). Surveys of the underwater spectral distributions of 14 English Lakes (317), 8 lakes in the Experimental Lakes Area, N. Ontario (300) and the Great Lakes (15) clearly show a gradient from relatively deep lakes transmitting mainly blue light near the bottom of the photic zone through lakes of intermediate depth transmitting mainly green light to shallow lakes transmitting mainly green to red light.

1.4 Changes in the underwater light field with increasing depth in the photic zone

The physical processes attenuating PAR in natural waters have been described. Consider for the moment the underwater light field of algal cells as depth increases. The spectral distribution of vertically downward irradiance is initially dependent on the spectral distribution of the incident surface light. In the upper meter or so of the photic zone, algal cells are exposed to a downward spectral irradiance only slightly attenuated from that at the surface. Underwater measurements at sunrise show that short-wavelength light becomes progressively more

penetrating than longwave light as the day progresses, i.e. with increasing solar elevation (159). Because of the disperse reflectance of red and blue parts of the spectrum at the water's surface at low solar angles, it seems unlikely that variation in the red part of the spectrum during the "twilight effect" would have much impact on the underwater spectral distribution of water deeper than 1 meter. However, the transient increase in the blue part of the spectrum at sunset is of some significance. During twilight, the underwater spectrum in clear oceanic waters is "blue-shifted" from a broad flat spectrum (450 nm to 600 nm) to a narrow one (450 nm to 500 nm) (211). Details in the daylight spectra caused by the sun's altitude and other factors modifying it on sunny days and during twilight are smoothed out and eliminated by passage through the upper 5 to 10 meters of the water. With increasing depth, phytoplankton and dissolved organic matter cause the distinctive changes in underwater spectral curves which characterize the particular body of water. Thus in a eutrophic "greenish" lake, the underwater "blue shift" during twilight was only apparent in the upper 2 meters. With increasing depth, the characteristic asymptotic radiance distribution of the lake water dominated (211).

In the lower layers of the photic zone, algal cells encounter a highly attenuated, spectrally limited, asymptotic radiance distribution. Thus the underwater climate of algal cells in all but the clearer, shallow water can be considered a shade habitat of limited spectral distribution (317).

1.5 Changes in the quanta versus energy distribution of PAR underwater

The primary act of photosynthesis is photochemical and is

initiated by quanta absorbed by the photosynthetic pigments in plant cells. The flux of quanta in the spectral distribution of PAR reaching algal cells in the photic zone is therefore of greater significance than the energy flux which is normally measured. The development and use of detectors for measuring quanta flux in the last decade or so has led to a re-evaluation of the impact of the spectral distribution of underwater light on the productivity of the various algal and aquatic plant groups (123, 211, 221).

The amount of energy per quantum is wavelength dependent as expressed in the following equation

$$E = h\nu = hc/\lambda \qquad (1)$$

where E = energy content of one quantum at wavelength λ; λ is expressed in nm; h is Planck's universal quantum constant (6.626×10^{-34} Joules s^{-1}); ν = frequency in cycles s^{-1}, and c = the velocity of light (2.9979×10^{17} nm s^{-1}). This relationship may also be expressed as the number of quanta per unit radiant energy i.e. quanta.m^{-2}.s^{-1}/W.m^{-2}

$$\text{or} \qquad \lambda/hc \qquad (2)$$

In nature, relatively broad wavelength bands of PAR occur, therefore, for polychromatic radiant energy, the number of quanta per unit energy must be integrated over the wavelength region being investigated. Within a given wavelength band, a measurement of spectral irradiance $dE(\lambda)/d\lambda$ (W.m^{-2}.nm^{-1}), the total quanta, E_Q(quanta m^{-2}.s^{-1}) or the total energy E_w(W.m^{-2}) can be obtained using the following equations;

$$E_Q(\lambda_1,\lambda_2) = \int_{\lambda_2}^{\lambda_1} \frac{dE(\lambda)}{d\lambda} \cdot \frac{\lambda}{hc} \cdot d\lambda \qquad (3)$$

$$E(\lambda_1,\lambda_2) = \int_{\lambda_2}^{\lambda_1} \frac{dE(\lambda)}{d\lambda} \cdot d\lambda \qquad (4)$$

where λ_1 = 400 nm and λ_2 = 700 nm for PAR. The number of quanta per unit energy is given by the ratio of equation (3) to equation (4). This is called the Q:W ratio (260). The Q:W ratio will vary with changing spectral energy distributions in the atmosphere and with depth in the various optical types of water.

Morel and Smith (221) using a spectroradiometer, confirmed that there was very little variation in the Q:W ratio of atmospheric radiation in the PAR wavelength region and obtained an average value of Q:W = 2.77×10^{18} quanta $s^{-1}.W^{-1}$. In a study of the Q:W ratio in a variety of oceanic and coastal waters, the ratio varied as a function of depth and optical water type. With increasing depth Q:W approached a more or less constant value. For blue waters (λ_{Hmax} = 400 to 475 nm), the Q:W value was low, typically $2.35 \pm 0.13 \times 10^{18}$ quanta $s^{-1}.W^{-1}$. For blue-green waters (λ_{Hmax} = 475 to 515 nm), Q:W was $2.5 \pm 0.13 \times 10^{18}$ quanta $s^{-1}.W^{-1}$. and for green or discoloured waters (λ_{Hmax} = 515 nm). Q:W was $2.65 \pm 0.13 \times 10^{18}$ quanta $s^{-1}.W^{-1}$. While estuaries and other silty waters were not examined, waters with high λ_{Hmax} were expected to have high Q:W ratios. The "red-shift" of λ_{Hmax} is correlated with chlorophyll content of the water. When chlorophyll is added to clear water, λ_{Hmax} approaches asymptotically the wavelength region (560 to 570 nm) where chlorophyll a has minimum absorption and absorption due to water

itself begins to increase rapidly (221). Published spectral extinction curves and spectral photon intensity curves from eutrophic estuarine and lake situations support the idea of high Q:W ratios in highly productive waters (15, 160, 317).

Interestingly, for monochromatic radiant energy, blue light (2.014×10^{18} quanta. $s^{-1}.W^{-1}$ at 400 nm) has about 43% less quanta per unit energy than red light (3.525×10^{18} quanta. $s^{-1}.W^{-1}$ at 700 nm), whereas the range of Q:W for natural waters is much less. Deep blue waters (2.3×10^{18} quanta. $s^{-1}.W^{-1}$) have only 18% less quanta per unit energy than shallower green waters (2.8×10^{18} quanta. $s^{-1}.W^{-1}$). It has been suggested on this basis that using an average Q:W value (2.5×10^{18} quanta $s^{-1}.W^{-1}$) will only introduce an error of 5 to 10% into practical investigations of the relationship of irradiance to photosynthesis in natural waters. However, studies of photosynthetic action spectra (the rate of photosynthesis per unit energy received from different parts of the PAR spectrum) and spectral quantum yields of photosynthesis (the rate of photosynthesis per unit rate of absorption of quanta from different parts of the PAR spectrum) in various algal groups and higher plants suggests that not only the number of quanta but also the wavelength region of the spectrum to which the cells are exposed can be a controlling factor in algal photosynthesis and growth.

2. Light Quality Effects on Growth

2.1 Higher Plants

Early work on light quality effects on plant growth went hand in hand with similar work on photosynthesis. Sachs (1864)(cited in 217) plants in double-walled glass cylinders (senebier jars) containing

coloured solutions. Subsequent workers also used Senebier jars or coloured glasses as filters (reviewed in 33, 34, 217). As pointed out by Popp (268) much of this early work was unreliable due to lack of knowledge of the intensity or spectral composition of the light used. He grew a number of higher plants in greenhouses constructed of Corning short wavelength cut-off glass filters that progressively removed the ultraviolet and blue parts of the daylight spectrum. Plants grown in light lacking the blue end of the spectrum tended to resemble etiolated plants, being taller and having lower fresh weights and dry weights than plants grown in the full daylight spectrum. His data indicated that the violet-blue part of the spectrum (wavelengths < 529 mm) was required for normal vigorous growth of these plants. Similar experiments by Shirley (312) also indicated that this end of the spectrum was more efficient in dry weight production, when light intensities were 10% of the outside sunlight. Lease and Tottingham (194) on the contrary showed that excluding wavelengths < 520 mm increased dry matter production in wheat seedlings grown under tungsten lamps using the same Corning filters as Popp (268).

In more recent work where tomato plants were exposed to equal intensities of violet, blue, green, yellow and red light, the dry weight of the plants increased towards the red end of the spectrum (367). In green, yellow and red light, plants showed marked stem elongation so that increases in dry weight mainly reflect increased stem weight. In experiments where tomato seedlings were grown under a variety of coloured fluorescent lamps at equal but subsaturating light intensities, growth in red fluorescent light was significantly greater than growth under white, blue or green light (17). Similar results were obtained

using pea plants (17). Szasz and Barzi (335) also reported that the dry weight of Vicia faba leaves grown in red light was 10% higher than leaves grown in blue light of equal intensity.

2.2 Green Algae

Artari (1899) (cited in 217) observed that blue-violet light accelerated development of Chlamydomonas ehrenbergii. Nadson (1910) (cited in 217) grew Stichococcus bacillaris Naegeli under Senebier jars and found poor and abnormal growth in yellow-orange light. Cultures grew slowly in blue light but were more normal and similar in colour and morphology to white light cultures. Dangeard (1913) (cited in 217) irradiated Chlorella vulgaris cells on white blotting paper soaked with Knops solution with light from a quartz spectrograph. Maximum growth occurred in the 660 to 680 nm waveband with decreasing growth towards the blue part of the spectrum. This pattern of growth presumably reflects the decreasing energy in this part of the projected spectrum. Klugh (181) used daylight and red, green and blue Wratten filters adjusted to give equal transmittance. Cell multiplication of Volvox aureus and Closterium acerosum was found to be much greater in red than in blue or green light. Meier (217) investigated cell multiplication of Stichococcus bacillaris Naegeli illuminated with tungsten light filtered with Corning short wavelength cut-off filters. While her results were not clearcut due to technical problems, red light was best for cell multiplication whereas blue light was required for maximal chlorophyll formation.

More recently, data of Kowallik (182) clearly suggested that Chlorella pyrenoidosa grew faster in red light of equal intensity to

blue light since red light was reduced by a factor of 1.8 to achieve equal dry weight production rates. By contrast, cell elongation in Acetabularia mediterranea was 4-fold greater in blue light after 31 days compared with equal intensity red light (49). This result was confirmed by Terborgh (342) who showed that Acetabularia crenulata grew more rapidly in blue light than in white light of equal intensity. Growth in red light virtually ceased within 2 weeks. Wallen and Geen (361) found that Dunaliella tertiolecta grew more rapidly in blue light than in white or green light of equal intensity. The exponential growth rate of Chlamydomonas reinhardtii was higher in red light and blue light than in green or white light of equal intensity (29). Jones and Galloway (164) grew Dunaliella tertiolecta under high (3600 $\mu W\ cm^{-2}$) and low (800 $\mu W\ cm^{-2}$) intensities of blue and white light of equal quanta flux. No significant difference in growth rate due to light quality was found at the high light intensity. At the lower light intensity, the growth rate of Dunaliella tertiolecta was significantly higher in blue light.

2.3 Diatoms

While studies on the growth and culture of diatoms go back in time as far as the 1880s (88), reports of light quality effects on diatom growth are sparse and more recent. Baatz (5) found the growth rate of marine diatoms was higher in green light than in blue light of similar energy. The growth of these diatoms was depressed to lower rates following transfer to red light. He also showed that blue and green light stimulated auxospore formation. In contrast to the results of Baatz (5), Wallen and Geen (361) found that Cyclotella nana grew more

rapidly in blue light than in either green or white light. Jeffrey and Vesk (158) found no difference in the growth rate of Stephanopyxis turris exposed to either white light or blue-green light of equal intensity.

2.4 Blue-green Algae

Early work tended to concentrate on light quality effects on pigment variation in blue-green algae (cf. section 3.2.4.3). A red light requirement for photoinduction of development in some nostocacean blue-green algae was first reported by Lazaroff and Schiff (1962) (cited in 193) Subsequent studies showed that this effect could be reversed by green light. Most of this work was related to microscopic studies of colony morphology rather than actual growth rate (reviewed in 193). More recently, Dohler and Przybylla (76) found that Anacystis nidulans cultured in blue and green light only exhibited slight growth. Pulich and van Baalen (273) showed that while a few marine blue-green algae were capable of very slow growth on minimal medium with nitrate as sole nitrogen source in blue light, most species examined were dependent on organic compounds for comparable growth under blue light.

3. The Effects of Light Quality on Chemical Composition

3.1 Carbohydrate, protein, nucleic acid and lipid content

3.1.1 Introduction

Studies of the chemical composition of algae can be traced back to Ketchum (172). He investigated the development of nitrate and phosphate deficiencies in phytoplankton and the recovery of these cells after starvation. Carbon, oxygen, nitrogen and phosphorus ratio determinations were made by Ketchum and Redfield (173) and for protein,

carbohydrate and fat by Parsons et al (254). Variation in chemical composition as a function of nutrient deficiency was first examined by Spoehr and Milner (318) who studied nitrogen-deficiency in Chlorella. Analyses of the chemical composition of algae have been used as an indicator of nutrient deficiency in natural waters (87, 131, 227, 294), to assess the nutrient value of an algal standing crop (332) and to study cellular metabolism during their life cycle (339).

The major chemical components of plant cells may be broadly divided into two main groups:

i) basal materials (nucleic acids, proteins, membrane lipids and photosynthetic pigments)

ii) storage materials (polysaccharides, lipids and storage proteins).

As pointed out by Herbert (136) it is virtually meaningless to speak of the chemical composition of a micro-organism without specifying the environmental conditions that produced it. In the case of algae, chemical composition is influenced by intensity and spectral quality of light, temperature, nutrient supply and in the case of synchronized cultures, the stage of the cell cycle. Many investigations of the chemical composition of algae in response to environmental variation have often been quite selective in their approach. Pigment content or nucleic acid content or protein or carbohydrate content has been measured while the other cellular components were ignored. In considering algae, especially unicellular algae, it should be remembered that their life style is autotrophic and that the chloroplast or photosynthetic lamellae is a dominant organelle or part of the cell structure. In this context the adaptation

of the photosynthetic apparatus to the prevailing light climate and the cellular events required to optimize this process would have an important influence on the chemical composition of the cell.

Apart from studies of photosynthetic pigments, very few analyses of the other cellular components of algae as a function of water depth have been reported.

3.1.2 The carbohydrate and protein content of algae and higher plants

As already pointed out, studies on the effect of light quality on the growth, pigment content and photosynthesis of algae and higher plants go back to the last century. Investigations of the ability of plants exposed to different parts of the spectrum to absorb nitrogen and phosphorus lead to early reports of light quality effects on chemical composition. Lease and Tottingham (194) showed that the crude fiber, available carbohydrate and reducing sugar fractions of wheat seedlings increased when the blue part of the spectrum was removed by shortwave-length cut-off filters during growth. The crude fat and true protein fractions were highest when a complete PAR spectrum was used during growth, however, these fractions decreased when the blue part of the spectrum was removed. In 1952, Voskresenskaya (355) reported that leaves of higher plants contain more protein after growth in blue light than in red light. This result was confirmed for other higher plants (4, 270, 358). Using synchronized high CO_2 (1.5%) grown Chlorella pyrenoidosa 211/8b Pirson and Kowallik (266) reported that cells in blue light contain more protein and less carbohydrate than cells in red light. The light intensity of both light sources had been adjusted to

yield equal rates of dry weight production. The increased protein content of blue light cells was independent of the nitrogen source offered e.g. nitrate, ammonia or urea (182, 267). Kowallik (183) further investigated the effect of blue light on protein production in Chlorella using cells grown on 1.5% CO_2 and 1% glucose. In dark grown cells the ratio of carbohydrate to protein was 2.9. Twenty $\mu W\ cm^{-2}$ of blue light was enough to lower the carbohydrate/protein ratio to ca. 2.0. At saturating light intensities (100 to 500 $\mu W.cm^{-2}$), the action spectrum of the carbohydrate/protein ratio showed a shoulder at 410 to 420 nm (ratio equals 1.7) and a minimum at 480 to 500 nm(ratio equals 1.3), the ratio increased to ca. 2.9 at 550 nm. At longer wavelengths the ratio stayed above this value, reaching a maximum of 3.6 at 680 nm (183).

Pickett (263) starved cells of Chlorella pyrenoidosa 211/8b by growing them in darkness or flashes of red or blue light. Protein content only dropped 2 to 6% on a packed cell volume basis in darkness or blue flashes. On the same basis, protein content dropped 23% in red flashes. Recalculating his data, protein content on a dry weight basis ranged from 39 to 43% for cells grown in the light, darkness or red flashes, whereas blue flash treated cells contained 50% protein. Cells of Acetabularia mediterranea showed a 4-fold increase in protein content after 31 days growth in blue light compared with red light of equal intensity (49). However, protein content of these Acetabularia cells was not different on a dry weight basis and the cells had ceased to grow in red light.

Subsequent studies by Wallen and Geen (360) showed higher protein levels in cells of Dunaliella tertiotecta and Cyclotella nana grown on air in blue light compared to cells grown in green or white

light. The carbohydrate to nitrogen ratio of Chlorogonium elongatum cells grown under high CO_2 in blue or red light was 1.5 and 2.7 resp. (320). The stimulatory effect of red light on polysaccharide accumulation was also re-emphasized by Szasz and Barzi (335) who found 1.5-fold greater levels of sucrose and starch on a dry weight basis in red light grown bean leaves when compared to leaves grown in blue light of equal intensity. Voskresenskaya's group have confirmed their earlier work showing increased soluble protein in the leaves of C_3 plants, e.g. barley (358) beans and peas (270), grown in blue light compared with red light causing equal organic matter accumulation. This effect on soluble leaf protein was also shown to be true for C_4 plants e.g. maize (270).

3.1.3. The nucleic acid content of algae

Synchronized cultures of Chlorella pyrenoidosa (211/8b) with equal dry weight accumulation rates in blue or red light form greater amounts of RNA in blue light (182, 267). DNA production is identical under these conditions, however, the RNA/DNA ratio is higher in blue light cells. Using a chlorophyll-free carotenoid containing mutant of Chlorella pyrenoidosa (C-1.1.10.31), Senger and Bishop (304) showed that a light dependent nucleic acid synthesis could occur in dark-grown non-photosynthetic cells. The action spectrum for this process showed a main peak at 465-480 nm with a shoulder at 430 nm. This was a classical blue light effect since wavelengths > 550 nm were not effective. While there was no pronounced effect of wavelength on DNA synthesis, irradiation with blue light enhanced RNA production in this mutant (20% increase of RNA over dark control at 480 nm).

Wallen and Geen (360) found that the RNA content of blue light

adapted cells of Cyclotella nana and Dunaliella tertiolecta was higher than in cells adapted to white or green light. This corresponded to similar differences observed for protein concentration in these cells. Steup (327) has shown that blue light enhances the synthesis of both cytoplasmic and chloroplastic ribosomal RNA in the same strain of Chlorella pyrenoidosa used by Pirson and Kowallik (266). This effect was apparent at very low intensities of blue light (13 μW cm^{-2}). Cycloheximide severely inhibited this blue light enhancement of ribosomal RNA synthesis.

In a subsequent report Steup et al (328) showed that blue light of low intensity (13 μW cm^{-2}) stimulated incorporation of ^{3}H-guanosine into transfer RNA and 5S RNA, whereas red light of equal quanta flux (9 μW cm^{-2}) had no effect. Red light of higher intensity (90 μW cm^{-2}) stimulated incorporation into both RNA species, but only about 1.3 times the levels incorporated in blue light at this light intensity. This incorporation in blue light was severely inhibited by cycloheximide. DCMU did not inhibit the blue light enhanced incorporation into 5S and transfer RNA. Higher intensities of blue light (130 μW cm^{-2}) also stimulated incorporation into cytoplasmic ribosomal RNA, 5S and transfer RNA in the non-photosynthetic Chlorella mutant (211 - 11h/125), whereas red light of equal quanta flux (90 μW cm^{-2}) was ineffective (328).

Steup et al (328) propose two different mechanisms for the regulation of RNA synthesis, a) a DCMU-sensitive photosynthesis dependent system which requires high light intensities, and b) a low intensity blue light sensitive system which regulates transcription of 5S RNA transfer RNA and cytoplasmic ribosomal RNA in the nucleus. This second system is clearly cytoplasmic in nature since it occurs in the presence of DCMU and in non-photosynthetic Chlorella mutants.

The photoregulation of chloroplastic and cytoplasmic ribosomal RNA synthesis in greening Euglena has also been examined. Cohen and Schiff (52) analysed the ribosomal RNAs during greening of wild type cells and a mutant (W_3BUL) which lacks chloroplastic DNA and contains only cytoplasmic ribosomes. During plastid development the level of cytoplasmic rRNA remains fairly constant, however, ^{32}P-incorporation studies suggest that synthesis or turnover of cytoplasmic rRNA occurs during this period. By contrast, there is a 15-fold increase in the level of plastid rRNAs. After 48 hours this represents 25% of the total cellular rRNA. This increase in plastid RNA is not surprising since the proplastid of dark grown Euglena undergoes a 60-fold increase in volume during chloroplast development (179).

In wild type and mutant Euglena cells, blue and white light were much more effective in stimulating cytoplasmic and plastid ribosomal RNA synthesis than red light. Green light was least effective. Cohen and Schiff (52) suggest that transcription of cytoplasmic RNA is under control of the non-plastid blue light receptor system while Protochlorophyll(ide) may be the receptor controlling plastid RNA synthesis.

3.1.4 The lipid content of algae

The major importance of algal lipids is probably related to their role as membrane components, especially in the thylakoids. References to "fat" or "oil" as reserve materials are common in the earlier algal literature, however, it is not clear whether this was in fact "reserve" or membrane material. The lipid composition of algae has been well studied (65, 108, 171, 248, 254, 378), however, changes in lipid content

in response to changes in light intensity or quality have not been well investigated.

Early studies showed that lipid or fat accumulation takes place in many algae in response to nitrogen deficiency (220, 318). High light intensity stimulated fat accumulation in Chlorella pyrenoidosa under these conditions (318). Fogg (91) also showed that light and nitrogen deficiency increased fat accumulation in Navicula pelliculosa. The most recent work of this nature is that of Opute (248) working with Nitzschia palea. He found that more fat accumulated in nitrogen-deficient cells than in cells with an adequate nitrate supply. He also found that a benzene-soluble fat fraction accumulated more ^{14}C in the presence of red or blue light than in cells exposed to white or green light during 5 min. ^{14}C-incorporation experiments.

3.2 Photosynthetic pigment content and composition

3.2.1 Chromatic adaptation

Early observations on the vertical distribution of benthic marine algae, which is characterized by the presence of green algae in shallow waters and red algae in deep water and brown algae in an intermediate position, led to a controversy as to whether the ambient light intensity or the spectral quality of underwater light lead to this distribution (reviewed in 21, 134, 274). Engelman (1883/4) attributed this distribution to the predominant colour of light. Since sunlight becomes attenuated to the bluish-green part of the spectrum after passage through several meters of sea water, a pigment must have a colour complementary to that of the medium in order to efficiently absorb this transmitted light. Gaidukov (1902) and Engelman (1902) both described this phenomenon

of complementary chromatic adaptation in which the colour taken on by the alga was complementary to the incident illumination. Gaidukov showed that Oscillatoria sancta was red when grown under green light and blue-green in colour when cultured in orange light. Oltmans (1893, 1905) opposed Engelman's theory and suggested that the vertical distribution of intertidal algae was determined by the intensity rather than the colour of the light. This controversy has continued for many years. Early studies of algal pigments in relation to depth did not resolve the question of light quality versus light intensity adaptation (274). Functional differences between green, brown and red algae in terms of their ability to photosynthesize with increasing water depth did suggest that pigment composition imposed limits on depth distribution (196). However, while the limited spectral quality of underwater light obviously can exert strong selective pressures on the type and pigment composition of deep water algae, modern ecological studies have also shown that the vertical zonation of intertidal algae is also controlled by non-photosynthetic parameters such as resistence to dessication, successful establishment of sporelings and grazing pressures of littoral fauna (206, 253).

3.2.2 Pigment changes in response to water depth

Engelman's theory (1883) of "chromatic adaption" to explain the colour stratification of large marine algae has stimulated many studies examining the relationship of photosynthetic pigment composition and concentration as a function of water depth. The pigment composition of algae is genetically determined. Changes in the relative amounts of these pigments therefore reflect the effect of the light environment

on pigment gene expression. In nature, especially in the well mixed upper part of the photic zone, the residence time of cells in this light environment is relatively short term and algae do not have to respond to these changes. Under stratified conditions or deep in the photic zone or in the laboratory where light conditions are more constant and cells may grow for many generations, the possibility that long term adaptation may lead to genetic selection arises. Generally, light adaptation is considered a short term, reversible process and not much attention has been paid to this latter possibility (225). Studies of pigment content of algae in relationship to water depth in the natural environment are complicated by at least two other factors besides the changes in the intensity and quality of light. One is the nutrient status of the water since both nitrogen and iron deficiencies had strong effects on chlorophyll content (1, 106, 310). The other factor is turbulence since pronounced vertical mixing of the water column will cause a relatively homogeneous light climate for phytoplankton. Clearly in a stratified water column sub-thermocline phytoplankton algae will adapt to ambient light conditions.

Actual reports of the in situ cellular chlorophyll concentration with varying depth are few in number. Dutton and Juday (78) investigated the influence of depth on pigment composition of freshwater phytoplankton (primarily green photosynthetic bacteria) in Scaffold Lake, Wisconsin. This organism had a yellow or greenish-yellow colour at the surface but rapidly changed to a bright green colour below 4m. Since the lake was anaerobic below 4m this colour change probably reflected the anaerobic nature of photosynthetic bacteria, i.e., cells in the upper aerobic layer were under O_2 stress. The chlorophyll/carotenoid ratio at various depths in two other lakes (containing mixed populations of blue-greens, greens

and diatoms) showed no significant variation even though one was a highly coloured bog lake.

In a study of pigment concentrations of a natural phytoplankton population, mainly diatoms, in Saanich Inlet, B.C., Fulton et al (1969) (in 360) found that the carotenoid:chlorophyll a ratio decreased with depth. More recently, Kiefer et al (175) have shown that the deep chlorophyll maximum (140-160m) in the central North Pacific is due to increased chlorophyll content per cell rather than increased cell number. In a recent study Ramus et al (278, 279) anchored macroscopic green, red and brown algae at various depths and analysed their pigment content after a 7-day adaptation period. All species showed increased pigment content with depth. In the red and green algae, the ratio of accessory pigments (phycobiliprotein or chlorophyll b) to chlorophyll a increased with depth. In the brown algae the chlorophyll c:a ratio remained relatively constant with increasing depth, however, the fucoxanthin: chlorophyll a ratio decreased with depth. By comparison, changes in pigment content and ratios of seaweeds collected from sun and shade intertidal habitats were not as marked as in the anchored situation.

3.2.3 Pigment changes in response to light intensity

Amongst higher plants, those species which normally grow in the shade usually have higher chlorophyll content than those which normally grow in bright sunlight (20, 177). Shade plants have fewer chloroplasts, but these are larger and contain more chlorophyll than those of sun plants. Shade species also tend to have a higher proportion of their chlorophyll in the b form. Similar changes in chlorophyll content and chlorophyll b:a ratios can be induced by growing plants at high and low

light intensities (18, 336). Reversible changes in chlorophyll concentration and chlorophyll b:a ratios of this nature have been observed in leaves on a seasonal basis (302). At the ultrastructural level chloroplasts from low light grown plants contain numerous well formed grana as opposed to fewer rudimentary, poorly developed grana in chloroplasts in leaves exposed to high light intensities (8, 18, 112).

Amongst the algae it is well established that the chlorophyll content of cells grown at high light intensity is dramatically reduced when compared to cells adapted to low light intensities. This was first shown by Emerson (1935)(cited in 275) who found that following the dilution of Chlorella pyrenoidosa cells from a dense light limited culture in fresh medium, the cellular chlorophyll content decreased by a factor of 3 after 16 hours.

This change in the chlorophyll content of Chlorella pyrenoidosa cells adapted to different light intensities was extensively investigated by Steeman-Nielsen and Jorgensen (236). Similar effects of light intensity on chlorophyll content have been shown in many green algae (165, 177, 284, 291, 306, 313, 352) and blue-green algae (76, 102, 165, 226, 250). By contrast, the chlorophyll content of marine diatoms grown at high and low light intensities appears to be unaffected (165). The green alga Scenedesmus quadricauda also fell into this group.

This finding may not be true for all diatoms since total chlorophyll content in Phaeodactylum tricornutum cells adapted to 15 klux decreased 3- to 5- fold when compared to cells grown at 1 klux (310).

Changes in the chlorophyll b:a or carotenoid:chlorophyll a ratios in response to changes in light intensity have been less well reported. In Chlorella vannielli (284) increasing the growth light

intensity from 300 to 6000 ft cd caused a 5-fold decrease in the total chlorophyll content per cell and a doubling in the chlorophyll a:b ratio.

Senger and Fleischbacker (306) compared cells of Scenedesmus obliquus grown at light intensities of 500 $\mu W\ cm^{-2}$ and 2800 $\mu W\ cm^{-2}$. Total chlorophyll content of high light cells only decreased to 60% of the low light value. The chlorophyll a/b ratio was not significantly higher in cells grown at the high light intensity.

Shimura and Fujita (310) reported that cells of Phaeodactylum tricornutum adapted to various intensities of white light at 10°C or 23°C did show pigment variation. While the concentrations of chlorophyll a and total carotenoids decreased at high light intensity, the ratios of chlorophyll c:a and total carotenoids:chlorophyll a were higher. They also showed that pigment content and pigment ratios could be lowered by decreasing the nitrate concentration of the medium.

Dramatic changes in the phycocyanin/chlorophyll a ratio of blue-green algae can be induced by changes in light intensity. Since phycocyanin content is also affected by temperature, CO_2-concentration and nitrogen content of the medium, cross comparisons between different reports are difficult. However, in studies where other growth conditions were constant, cells grown at high light intensities had lower overall pigment content and lower phycocyanin/chlorophyll ratios (76, 102, 106, 226, 250). Changes in chloroplast ultrastructure similar to those found in terrestrial plants have been found in Chlorella grown at high and low light intensities, (284, 352). Thylakoid lamellae were numerous and densely packed in low light chloroplasts whereas only scattered pairs of thylakoids were found in the chloroplast stroma after growth in high intensity light. Ultra-

structural changes in the chloroplasts of diatoms and blue-green algae in response to light intensity have not been reported.

3.2.4 Light quality effects on pigment content and chloroplast structure

3.2.4.1 Higher plants

Chlorophyll synthesis requires continuous illumination in angiosperms and some algae (177). Wiesner (1877) (217) using Senebier jars filled with either potassium dichromate (transmitting the green to the red part of the spectrum) or ammoniacal copper oxide (transmitting green to violet radiation) observed that plants in weak light greened sooner in the red end of the spectrum but in strong light they greened sooner under blue light.

Other early workers using plants grown under short wavelength cut-off filters, showed that removal of blue light from the daylight spectrum did not prevent good development of chlorophyll, in fact chlorophyll formation (i.e. greening) occurred more rapidly in red light than in blue or green light (194, 268, 297). Later workers found on the contrary that crop plants had a lower chlorophyll content when the blue part of the spectrum was removed (122, 194, 312).

Measurement of the action spectrum of the photoconversion of protochlorophyll(ide) (PChl(ide)) to chlorophyll(ide) (Chl(ide)), the primary event in the greening process of etiolated plants, suggests that both blue and red light are important. The action spectrum has peaks of effectiveness at 645-650 nm and 440 to 445 nm with minor peaks at 545 and 575 to 580 nm and thus parallels the absorption spectrum of PChl(ide) (177, 241, 280).

Recent work using broad waveband red, green or blue light

indicates that chlorophyll formation during greening in barley seedlings is most rapid in blue light (120, 180, 358). Buschman et al (35) have summarized the differences occurring in the barley chloroplasts during this greening process in low intensity red and blue light of equal quanta flux. In red light chloroplasts were more elongated, possessed a higher grana content and contained a higher number of thylakoids per granum than in blue light. Similar results for grana content and number of thylakoids per granum have been reported for pepper plant chloroplasts grown in red and blue light (151). The chlorophyll content and chloroplast ultrastructure of leaves of sun and shade adapted plants species (20), show parallels to those reported for leaves adapted to blue and red light respectively (35, 151).

3.2.4.2 Green algae

While pigment variation due to chromatic adaptation is well investigated in the "coloured" algae, i.e. blue-greens, reds and brown algae, surprisingly few studies of pigment variation in green algae adapted to different light quality regimes have been reported. Meier (217) found that chlorophyll formation in the green alga Stichococcus was best when blue light was included in the incident radiation. Hess and Tolbert (137) grew Chlorella pyrenoidosa and Chlamydomonas reinhardtii on 0.2% CO_2 under white light (approx. 4000 μW cm^{-2}), blue light (955 μW cm^{-2}) and red light (765 μW cm^{-2}). During growth in blue light, the ratio of absorbance at 655 nm and 680 nm (the in vivo maxima for chlorophylls a and b) changed, indicating a significant decrease in the chlorophyll a:b ratio. No significant changes in this ratio were oberved for algae grown in red light. These in vivo measurements were verified by chlorophyll

determinations. Total chlorophyll content increased 20% in blue light accompanied by a significant decrease in the chlorophyll a:b ratio.

Wallen and Geen (360) grew Dunaliella tertiolecta in white, blue and green light of equal intensity (800 μW cm^{-2}). Total chlorophyll, chlorophyll a and chlorophyll b concentrations were highest in blue light and lowest in green light. The lower chlorophyll a:b ratio in blue light was accompanied by decreased total carotenoid content when compared to white or green light cells. Vesk and Jeffrey (354) grew Dunaliella tertiolecta in equal intensities (400 μW cm^{-2}) of white or blue-green light. The total chlorophyll content was 39% higher in cells grown in blue-green light.

Unlike in angiosperms, chlorophyll synthesis in most algae can take place in total darkness (177). The photocontrol of chlorophyll synthesis during greening has been investigated in algae which require continuous light for this process, i.e. Euglena, mutants of Chlorella, Chlamydomonas and Scenedesmus or "glucose bleached" Chlorella. The action spectrum of chlorophyll synthesis in dark grown Euglena has maxima at 430 and 650 nm resembling the absorption spectrum of PChl(ide) (9, 237). The action spectrum of chlorophyll synthesis in dark grown mutants of Scenedesmus only showed peaks at 390, 455 and 465 nm with a shoulder at 480 nm (305). Wavelengths > 550 nm were not effective in stimulating chlorophyll formation. Blue light was also most effective in enhancing chlorophyll formation in "glucose bleached" cells of Chlorella protothecoides (243, 244). Clearly, chlorophyll formation in Chlorococcalean algae such as Chlorella and Scenedesmus responds differently to light quality when compared to Euglena which resembles higher plants in its response.

3.2.4.3 Blue-green algae

Following the studies of Gaidukov and Engelman (1902), Kylin (1912) and Boresch (1919, 1922)(cited in 21, 341) perceived that the colour changes of blue-green algae were due to the presence of different amounts of phycoerythrin and phycocyanin, i.e. the colour of alga was controlled by the phycoerythrin/phycocyanin ratio. The pigment most effective in absorbing the colour of the illumination was produced in greater amount. The complementary chromatic adaptation properties of the red alga, Cyanidum caldarum and two blue-green algae Tolypothrix tenuis and Fremyella diphosiphon have been recently reviewed (21). Cyanidium caldarum, a single-celled acidophilic eukaryote, contains phycocyanin, allophycocyanin and chlorophyll a when grown in the light. When grown in the dark on glucose, phycobiliproteins are not formed. Blue light (420 nm) and to a lesser extend green light (550-595 nm) were most effective in promoting phycocyanin formation in a chlorophyll-less mutant of this alga (231). In the chromatic adaptation system of Tolypothrix tenius, red light (wavelengths >600 nm) promotes phycocyanin and allophycocyanin formation, whereas green light (500-600 nm) promotes phycoerythrin synthesis (70). A similar response to red or green light is found in Fremyella diplosiphon (128). The CCA abilities of a wide variety of blue-green algae (44 strains) was surveyed by Tandeau de Marsac (341). This property of complementary chromatic adaptation is restricted to those algae capable of synthesizing phycoerythrin as well as phycocyanin and even then not all phycoerthrin-containing blue-green algae responded to green and red light treatment. The response of the pigment system of Anacystis nidulans (Synechococcus AN) in response to various external environmental factors has been studied

exhaustively. Earlier studies showed that the phycocyanin, chlorophyll and carotenoid content of Anacystis was affected by light intensity (76, 102, 106, 163, 226, 250), light quality (76, 102, 163, 225) CO_2 concentration (82), temperature (106, 226) and nitrogen content of the medium (1, 191). Examination of whole cell absorption spectra, where the phycocyanin content and chlorophyll content can be crudely estimated from the peak heights at 625 nm and 680 nm respectively, suggest that "normal" cells have approximately equal peak heights at these wavelengths. Cells having this sort of "normal" absorption spectra are obtained under conditions of high temperature (35°C to 40°C) high CO_2 (1%) and low to medium intensities of white light (60 - 700 μW cm^{-2}) (102, 163). Alternatively this "normal" absorption spectrum could be obtained in cells grown on air levels of CO_2 (0.03%) at lower temperatures (25°C - 30°C) and low levels of white light (60 - 130 μW cm^{-2}) (76, 182). These cells have a 10-fold lower growth rate than the high CO_2-high temperature grown cells. Deviations from this "normal" situation were found at higher light intensities (500 - 2100 μW cm^{-2}). High intensities of white and orange light caused a depression of the 625 nm peak with a corresponding decrease in the phycocyanin/chlorophyll ratio (76, 102, 250). This was considered to be a form of inverse chromatic adaptation where the proportion of the pigment that best absorbs the light supplied for growth is reduced in strong light. High intensities of red light (nm > 650 nm) caused an increase of the 625 nm peak relative to the 680 nm peak (76, 102, 163). In this case, chlorophyll content was reduced while phycocyanin content remained at "normal" levels, again this appears to be another form of inverse control with the pigment absorbing light for growth being chlorophyll in this case. Pulich and van Baalen (273) reported that

marine unicellular blue-green algae did not grossly shift pigment ratios during adaptation to blue light i.e. the same chlorophyll/ phycocyanin ratios in blue light as in white light grown cells.

Nitrogen chlorosis in blue-green algae was classically demonstrated by Allen and Smith (1). Since phycocyanin captures much of the light essential for photosynthesis and can constitute up to 24% of the total dry weight of Anacystis (226) there is a selective advantage for Anacystis to control synthesis of this pigment. Lau et al (191) investigated the turnover rate of the phycocyanin apoprotein in Anacystis during nitrate starvation and restoration. During nitrate starvation the disappearance of phycocyanin was correlated with apoprotein degradation suggesting that expression of phycocyanin apoprotein genes was depressed. Resumption of phycocyanin production in the light following nitrate restoration was inhibited by darkness, chloramphenicol and DCMU suggesting that repression and derepression of de novo apoprotein synthesis in light was induced by nitrogen content of the medium.

3.2.4.4 Diatoms

Mothes and Sagromsky (223) observed that Chaetoceros was dark brown when grown under green light but was golden when grown under red light. This was attributed to a shift in the carotenoid/chlorophyll ratio. Mann and Myers (213) compared the pigment composition of Phaeodactylum tricornutum grown in unspecified intensities of long wavelength red light (wavelength > 660 nm) with cells grown in white light. Concentrations of chlorophyll a were equal under both light regimes but chlorophyll c concentration was decreased by 40 to 60% in long wavelength red light grown cells. The relative concentrations of fucoxanthin or the other carotenoids under both light treatments were not reported.

Jupin and Giraud (167) observed changes in the far red portion of the *in vivo* absorption spectrum of *Detonula* sp. when comparing white light grown cells (1500 μW cm^{-2}) with cells cultured in dim red fluorescent light (150 μW cm^{-2}, peak emission 650 nm). A distinct peak at 707 nm was observed in room temperature *in vivo* absorption spectra of red light cells which was not present in white light cells. While total chlorophyll pigments on a dry weight basis were lower in red light grown cells, due to a 6-fold decrease in chlorophyll *c* content, the chlorophyll *a* concentration was higher than in white light cells (166). Total carotenoid pigments were 25% lower in red light compared with white light and this was mainly due to decreased fucoxanthin content. These changes in the gross pigment composition of the cells was accompanied by a change in plastid structure. The plastids of white light grown cells contained about 20 thylakoids disposed in groups of 4, within an abundant stroma. Those of cells grown in low intensity red light contained up to 40 thylakoids evenly distributed throughout the plastid.

Wallen and Geen (360) investigated the pigment composition of *Cyclotella nana* grown in white, green and blue light of equal intensities. Total chlorophyll concentrations were highest in blue light grown cells and lowest in those from green light. Total carotenoid concentrations were highest in green light. Thus in terms of complementary chromatic adaptation, cells grown in blue light, i.e. strongly absorbed by chlorophyll, had a higher chlorophyll and lower carotenoid content than cells grown in green light. Conversely cells grown in light strongly absorbed by carotenoids, e.g. green light, had higher concentrations of carotenoids and a lower concentration of chlorophylls. Shimura and Fujita (310) could not observe any marked changes in pigment composition of *Phaedactylum tricornutum*

grown under green or red light (unspecified intensity or spectral quality). Based on their inability to observe pigment variation during growth of Phaeodactylum tricornutum under red or green light and the pigment changes observed in nitrogen-deficient cultures, they suggested that the pigment variations reported by Wallen and Geen (360) for Cyclotella nana grown in blue or green light were not due to chromatic adaptation. Instead they suggested that the pigment variation in Cyclotella nana occurred as a result of nitrogen deficiency. This point is unfounded since Wallen and Geen (360) used 1g KNO_3/ℓ in their culture medium. Jeffrey and Vesk (158) investigated pigment content and chloroplast structure in Stephanopyxis turris grown in low intensity (400 μW cm^{-2}) white and blue-green light (peak emission at 480 nm). Total chlorophyll per cell almost doubled in blue-green light grown cultures but no major change in the chlorophyll c:a or carotenoid:chlorophyll a ratios occurred. Chloroplasts in white light grown cells were elongated and contained several well-placed thylakoids running parallel to the major axis of the plastid. Cells grown in blue-green light contained large numbers of spherical chloroplasts with increased stacking of the thylakoids. The increased number of chloroplasts per cell resulted in deep chocolate coloured cultures in blue-green light as opposed to the golden orange colour of white light cultures. In an accompanying paper Vesk and Jeffrey (354) examined the photosynthetic pigments and chloroplast structure in 10 diatoms grown under the same light regime as the previous paper (158). Increases in total chlorophylls of 0% to 146% occurred in blue-green light grown cells. Phaeodactylum tricornutum showed a 2.4-fold increase in total chlorophylls in blue-green light compared with white light of equal intensity. This was accompanied by a slight increase in the ratios of chlorophylls $c_1 + c_2$/chl a and

fucoxanthin/chl a. More substantial increases in chl $c_1 + c_2$/chl a ratios were observed in three other diatoms concurrent with increased total chlorophyll concentrations following growth in blue-green light. However, since blue-green light only caused slight increases in the fucoxanthin/chl a ratio in these cells, it was concluded that complementary chromatic adaptation (blue-green light increasing the concentration of blue-green light absorbing carotenoids) had not occurred. They suggest that their work with S. turris and that of Shimura and Fujita (310) with P. tricornutum support the suggestion of Halldal (123) that complementary chromatic adaptation probably does not occur in planktonic algae which use carotenoids and chlorophyll c as accessory pigments. However, the effect of blue-green light on chloroplast structure was independent of light intensity over the range 100 - 400 μW cm^{-2}, and the increases in total chlorophyll concentration and chlorophyll $c_1 + c_2$/chlorophyll a ratio suggest that some form of chromatic adaptation had occurred.

4. The Effects of Light Quality on Photosynthesis

4.1 The effect of light intensity on photosynthesis

Two kinds of processes are involved in photosynthesis; a) photochemical processes which are dependent on pigment concentration and the intensity of illumination, and b) enzymatic processes which are dependent on the relative amounts of photosynthetic enzymes in the plant cell, the relative importance of both kinds of processes is demonstrated when the rate of photosynthesis is plotted as a function of light intensity. The shape of the light curve so produced being hyperbolic in nature.

Blackman (1905)(cited in 275) proposed a law of "limiting factors" to explain this phenomenon of light saturation of photosynthesis with

increasing light intensity. He called the limiting reactions involved "dark" or thermal reactions since temperature is an important factor regulating enzymatic reactions. Comparison of the shape of light curves of various plants soon revealed the different photosynthetic responses of "sun" or "shade" adapted plant species to light intensity (reviewed in 275).

The photosynthetic adaptation of algae to light intensity has been well studied (165, 236, 275, 284, 292, 306). Typically the major difference between high and low light adapted cells is the light intensity at which photosynthesis is saturated. Photosynthesis in low light adapted cells usually saturates at lower light intensities. Depending on whether photosynthetic rate is expressed on cell number, dry weight or unit chlorophyll basis the slope of the linear part of the light curve may vary. Generally low light adapted cells have a steeper slope over the linear response range than high light adapted cells. This sort of relationship is found in the light curves of cells taken from increasing depths of water (292, 311, 337, 381).

The light intensity at the point of intersection of the initial slope of the linear part of the light curve with a line extrapolated back from the horizontal saturating part of the curve is called I_k (337). This point describes to a certain degree the ratio between the light and dark reactions and has been widely used to compare the physiological adjustments of algae grown in or sampled from different light habitats (165, 236, 292, 311, 337, 381).

4.2 The effect of water depth on photosynthesis

The shape of the light curves of algae has been well investigated

in the primary production literature, particularly in relation to the photosynthesis of algae at different depths (292, 311, 337, 381). The concept of sun and shade adapted species has been well known since algae must adapt to the low light intensities prevailing deep in the photic zone.

The existence of deep chlorophyll maxima or deep living algal populations was first reported by Kemmerer et al (1924) (cited in 174) who reported a maximum concentration of Mougeotia at 90 m in Lake Tahoe. The vertical distribution of chlorophyll in natural waters generally shows a maximum which may sometimes be found near or at the surface, and at other times, at or below the apparent euphotic depth (256, 344). Deep chlorophyll or subthermocline phytoplankton maxima have been reported for both marine (2, 175) and freshwater environments (27, 89, 174, 290, 344). Deep chlorophyll maxima appear to be a seasonal feature of summer vertical profiles, usually occuring below the thermocline or associated with pynoclines in the marine situation (141). Deep is a relative word since subthermocline maxima occur in shallow stratified freshwater lakes (89), where light is rapidly attenuated, as well as in the large lakes and oceans. Light levels in the chlorophyll maxima zones were at or below the 0.1 - 1% surface illumination range. Clearly the spectral distribution of light is highly restricted at these depths.

Early studies of the vertical profile of in situ algal photosynthesis were limited by the sensitivity of the methods available (mainly the Winkler O_2 method) and suggested that production maxima occurred within 10 - 15 m from the surface (see refs cited in 332). More detailed investigations of the vertical distribution of photosynthesis, especially deep maxima have been done in recent years using the ^{14}C-

technique (89, 109, 190, 296, 311, 337). These studies confirmed the fact that most photosynthesis occurs in the upper part of the euphotic zone close to the surface and decreases rapidly with depth. Lesser photosynthetic maxima have been detected in association with deep chlorophyll maxima even though the light level was exceedingly low (2, 109, 175, 190). In other cases, cells from these deep chlorophyll maxima have been shown to have high photosynthetic potential (98, 174, 344). Much of the early work on the photosynthetic response of benthic algae from different depths to light intensity was reviewed by Rabinowitch (275). Later studies with benthic algae (196, 277, 279) have shown that photosynthesis decreases with depth parallel to the decrease in light intensity. Deep chlorophyll maxima phenomena were not observed, though depth greatly affects the pigment content and composition and photosynthetic light curves of benthic algae.

4.3 The effect of light quality on photosynthesis

4.3.1 Introduction

The first act of photosynthesis is light absorption. Only visible light (380 - 720 nm) is absorbed and used for photosynthesis in eukaryotic and blue-green algae. This information was obtained by measurements of the absorption spectra (absorbance as a function of wavelength) and the action spectrum of photosynthesis (O_2-evolution or CO_2-assimilation per incident energy or quanta as a function of wavelength). With the increasing sophistication of techniques for making precise measurements of absorption, light intensity or quanta flux and rates of photosynthesis, much has been learned since Senebier (1788)(cited in 275) conducted experiments on CO_2 assimilation using double-walled bell jars

filling the space between the walls with coloured solutions.

Much of the earlier work has been reviewed by Rabinowitch (275). In 1871 Lommel pointed out that the basic principle of photochemistry, known as Herschel's law, states that there is no photochemical action without light absorption (275). This requires that the spectral maximum of photosynthetic efficiency coincide with the absorption maximum of the photosynthetic pigments. Subsequently several workers showed that the photosynthetic efficiency of green plants decreases steadily from red through yellow to green, parallel to the decline in absorbing capacity of chlorophyll. Engelman (1882) noticed that in addition to the main maximum in the red, there was a second maximum in the blue-violet which he associated with the strong absorption band of chlorophyll in this region.

Early work was hampered by a lack of understanding of what an "action spectrum" was, certainly light cast by a prism would contain decreasing amounts of energy in the blue because of the declining energy of the light source and energy in the red would decline due to dispersion. Use of light fluxes of equal light intensity confirmed Engelman's finding of the second maximum in the blue. "Isoenergetic" action spectra can be highly variable due to the varying compositions of pigment systems. This variability involves a number of factors a) the O.D. or absorbance of the sample - in thick suspensions or tissues all light is absorbed blurring the action spectrum; b) light intensity - at saturating light intensity, photosynthesis is limited by dark reactions, not light and the rate of photosynthesis at saturating light intensity is the same for all wavelengths of light (265, 275). For accurate action spectra, photosynthesis measurements have to be made in a low intensity

range where the response of photosynthesis of all wavelengths investigated is linear; c) cell density - this is related to a) because at low light intensities where the light intensity/photosynthesis response is linear, the cell density of the sample can substantially reduce the net light intensity "seen" by the cells, consequently the photosynthetic rate would be lowered. Hence the cell density should be such that photosynthetic rate is proportional to light intensity and the same for all wavelengths investigated; d) quantum correction - according to Einstein's law of photochemical equivalency, equal numbers of absorbed quanta would be expected to produce the same effect on photosynthesis at different wavelengths rather than equal quantities of absorbed energy. If the maximum quantum yield is the same for all wavelengths, then the "quantized" action spectrum would be expected to parallel exactly the absorption spectrum. The "isoenergetic" action spectrum, on the other hand would always be askew with red light always appearing more efficient than blue because of the higher number of quanta in red light.

If a "quantized" action spectrum differs markedly from the absorbance spectrum this would indicate that quanta of different wavelengths have different photochemical effects on photosynthesis or that the light is absorbed by photosynthetically inactive pigments or other structures in the cell or tissue.

Monochromatic quantum yields (the rate of O_2-evolution per absorbed quantum as a function of wavelength) were first measured by Warburg and Negelein (1923)(cited in 275) using Chlorella. They found highest quantum yields in red and yellow light with a dip in the green and if the absorption due to all pigments in the blue was included then blue yields were slightly lower than the green. This lower yield in the blue was

corroborated in experiments by Warburg (1946-48)(cited in 275) twenty-five years later.

Briggs (1929)(cited in 275) made yield determinations on higher plants in "Isoenergetic" light of three colours which showed the expected decline in yield with decreasing wavelength. Gabrielsen (1935)(cited in 275) using Sinapis alba and three colours of isoenergetic light again found a maximum quantum yield in the red with 20% less in yellow-green and 30% less in blue-violet. Hoover (1937)(cited in 275) using wheat leaves obtained an action spectrum (isoenergetic) with a high red peak, a green "dip" and a blue peak slightly lower than the red. When this curve was "quantized" the red and blue peaks were comparable (31).

Since this time the action spectra of many green crop plants (31,210) and trees (47) have been measured. Since many of these action spectra were measured using equal light intensities, they display the typical high broad maximum in the red part of the spectrum with a "dip" in photosynthetic efficiency somewhere between 550 and 450 nm and a second maximum in the blue part of the spectrum, usually lower than the red maximum. When these spectra are quantized (31) the rates of photosynthesis in the blue and red parts of the spectrum are generally equalized. McCree (210) in his survey of 22 crop plants found that the action spectrum and quantum yield of photosynthesis in the blue part of the spectrum was consistently lower than the red maximum, this may in part be due to the absorbance of these leaves which was very high in the blue part of the spectrum and presumably was caused by non-photosynthetic absorption processes. Clark and Lister (47) also found a considerably reduced blue shoulder in the normalized isoenergetic action spectra of

trees when comparing a broadleafed deciduous species with leaves of increasing "blueness" in conifers. They discussed the masking effect of wavelength-specific photosynthetic screening processes. Three basic photosynthetic mechanisms were proposed:- a) metabolic screening, i.e. via some metabolic process that reduces net photosynthesis such as photorespiration; b) absorption screening by inactive pigments; and c) physical screening by selective light filtering at the leaf surface. Since the blue colouration of the conifer leaves was due to a glaucous bloom of waxy cuticular projections, with high blue reflectance, an inverse relationship between decreasing blue light utilization and increasing blue light reflectance was proposed (48).

4.3.2 Green algae

Emerson and Lewis (86) extensively reinvestigated the spectral quantum yield experiments of Warburg and Negelein using Chlorella pyrenoidosa. The yield was constant between 580 and 685 nm, below 580 nm the yield declined reaching a minimum at 490 nm, then rose again to a smaller maximum at about 412 nm. The average quantum yield deficiency between 400 and 580 nm was 15% suggesting that the light energy absorbed by the carotenoids in this region was not transferred efficiently to the chlorophyll. Thus in the "action spectrum" of photosynthesis for Chlorella we have red and blue peaks of approximately similar height with a large dip at about 550 nm.

Since the experiments of Emerson and Lewis (86) the action spectrum of photosynthesis in various spectral wavebands of Chlorella has been extensively investigated (23, 69, 224, 239, 265, 289). The action spectra in all these studies exhibit the typical red and blue maxima with

a minimum in the green part of the spectrum. In terms of light saturated photosynthesis when the dark reactions of photosynthesis are limiting (i.e. 15-20 mW cm^{-2}) it would appear that light quality has no effect (69, 265) at the level of primary photochemical reactions, e.g. quantum yield, O_2-evolution, photophosphorylation and cyclic electron flow.

This is in contrast to the spectral behaviour of photosynthesis at very low levels of light (80 - 200 μW cm^{-2}) when the drop in efficiency of photosynthesis in green light becomes highly exaggerated and the blue photosynthesis maxima is attenuated to 50% or less of the red maximum (239, 249, 289). This change in behaviour of photosynthesis rate in the red and blue parts of the spectrum can be clearly demonstrated by inspection of the monochromatic light intensity curves in Fig. 4 of Pickett and Myers (265). This drop in the blue maximum relative to the red maximum in low light of equal intensity is not completely explained by unequal quantum distribution and even appears in "quantized" action spectra (249). This may be due to non-photosynthetic respiratory processes in the cell stimulated by blue light which may become more apparent at very low light intensities (357).

The effect of light quality on the photosynthesis of other green algae has also been investigated (29, 137, 281, 361). These other green algae fall into the same spectral "mold" for photosynthesis as Chlorella and terrestrial green plants.

4.3.3 Diatoms

Early work by Gabrielsen and Steeman Nielsen (1938) (cited in 275) showed that for equal incident light intensity, the rate of O_2 production by diatoms was consistently greater in the blue than in the red. The

difference was particularly strong in relatively low light intensities, the ratio of photosynthesis in blue light to photosynthesis in red light being about 1.8. At high light intensities this ratio decreased to about one (275). Mothes and Sagromsky (223) also measured the photosynthetic rate (green light)/photosynthetic rate (red light) ratio for Chaetoceros grown in red, green and blue light. This ratio was 1.12 for red adapted cells, 1.26 for green adapted cells and 1.21 for blue light grown cells. This suggests that diatoms photosynthesize more actively in green light and cells adapted to green light can use it optimally. Dutton and Manning (79) measured the quantum yield of Nitzschia closterium in narrow bands of monochromatic red (665 nm) green (546 nm), blue-green (496 nm), blue (436 nm) and violet (405 nm) light. Because they used the dropping mercury O_2 electrode and the diatom was poisoned by the mercury, their data varied widely, however, by using ratios they concluded that the quantum yields in the violet, blue and green were practically equal to that in the red. Tanada (340) made the first detailed investigation of quantum yield as a function of wavelength in diatoms using the freshwater diatom Navicula minima. He measured the quantum yield in narrow spectral bands from 400 to 700 nm. The quantum yield was constant between 520 and 680 nm dropping sharply to zero above 710 nm. The yield dipped about 20% between 520 and 475 nm, rose to a blue maximum at about 430 nm and dropped off again in the violet. This lowered quantum yield in the blue-green to violet part of the spectrum (520-400 nm) is also evident in the action spectrum of Phaeodactylum tricornutum (fig. 3, curve (a) Mann and Myers (214), where action is less than but parallel to absorption in this part of the spectrum. However, even under these conditions the blue maximum is

not less than the red.

Studies on the ^{14}C-fixation rates of the marine diatom Cyclotella nana (361) grown in white, blue or green light, show that photosynthesis in blue light adapted cells was 74 - 80% higher than white adapted cells. Green light adapted cells had photosynthetic rates 32 - 39% lower than cells adapted to white light of equal light intensity.

Jeffrey and Vesk (158) compared the photosynthetic carbon fixation rates of the diatom Stephanopyxis turris grown in white or blue-green light (400 $\mu W\ cm^{-2}$). Cells grown in white light fixed more carbon in white light than cells grown in blue-green light. However, blue-green light adapted cells fixed 42% more carbon in low intensity blue-green light than did cells adapted to white light. Shimura and Ichimura (311) examined apparent photosynthetic efficiencies of marine phytoplankton (predominantly diatoms) sampled from various depths in the northwestern North Pacific. Light intensity curves were measured using blue, green and red fluorescent lamps. The order of apparent photosynthetic efficiency was blue >red > green light irrespective of depth. However, green light was utilized as efficiently as red light by cells from the lower part of the photic zone.

In summary, diatoms differ from green plants in that the blue maximum of photosynthesis is usually equal to or greater than the rate in red light. They also utilize light in the yellow to green part of the spectrum more efficiently.

4.3.4 Blue-green algae

At about the same time that Emerson and Lewis were investigating

the spectral quantum yield of Chlorella, they also examined the blue-green alga Chroococcus using the same methods (85). Quantum yield was approximately constant between 570 and 690 nm, there was a large drop in quantum yield between 550 and 450 nm with a minimum at about 480 nm and the yield rose again in the violet to a value less than the red maximum. These changes in quantum yield are reflected in the photosynthesis action spectrum, which closely parallels the absorption spectrum in the region above 570 nm, showing the high efficiency with which light captured by phycocyanin is transferred to chlorophyll for photosynthesis. The photosynthetic action spectrum dipped dramatically in the region 550 - 420 nm, then rose in the violet to a low maximum, suggesting that carotenoids were inefficient in transferring light energy to chlorophyll.

Jones and Myers (162) showed that the action spectrum of O_2-evolution in Anacystis nidulans closely follows the absorption curve of phycocyanin with a single peak maximum about 630 nm which drops steeply from about 640 to 690 nm. From 570 nm to 400 nm the photosynthesis rate drops to a very low rate with a minor peak at 420 nm.

The quantized action spectrum of CO_2-uptake in Anacystis was investigated by Dohler and Przybylla (76) using IRGA. Cells grown in low intensity white light (60 μW cm^{-2}) exhibited a broad peak overlying the absorption curve of phycocyanin with a maximum at 625 nm, a large dip in photosynthesis at 475 nm and a minor peak in the violet-blue part of the spectrum.

Stevens and van Baalen (329) measured the O_2-evolution rate of Agnemellum quadriplicatum at 681, 620, 550 and 430 nm using a light intensity of 680 μW cm^{-2}. Their data points overly the action spectrum

of Jones and Myers (162). The quantized action spectrum of photosynthetic ^{14}C-fixation in Anacystis nidulans was recently reported by Sorensen and Halldal (316). Again a broad peak centered around 600 nm with photosynthesis dropping off towards 700 and 500 nm was observed. A very low minimum at 480 nm was accompanied by an increase to a maximum in the violet part of the spectrum of about 1/2 the value at 600 nm.

Evidently for blue-green algae containing the C-phycocyanin-chlorophyll a pigment system, normal photosynthesis shows an action spectrum which closely parallels the in vivo absorption spectrum of C-phycocyanin. Light at wavelengths less than 550 nm is absorbed and utilized very inefficiently by these algae (363).

5. The Effects of Light Quality on Photosynthetic Carbon Metabolism

5.1 The Calvin cycle and the glycolate pathway

Plants obtain carbon for growth and for energy storage through photosynthesis by incorporating CO_2 from the external medium. The major metabolic pathway by which CO_2 is fixed and reduced to organic compounds in autotrophic organisms is the photosynthetic reductive pentose phosphate pathway, or Calvin cycle. Research leading to the elucidation of this pathway using ^{14}C started in 1947 (38).

Due to the convenience with which they could be killed and extracted, the main experimental organisms used were the unicellular green algae Chlorella pyrenoidosa and Scenedesmus obliquus. The first stable product of CO_2 assimilation is 3-phosphoglyceric acid (3-PGA) (38) hence the term C_3-pathway. Glycine and glycolic acid were also rapidly labelled with $^{14}CO_2$. The absence of CO_2 or presence of O_2 during illumination enhanced formation of labelled glycine and glycolic acid (16).

Figure 1. Proposed pathway and spatial localization of the glycolate pathway in a green algal cell.

The specific reactions are:-

1. RuDP carboxylase;
2. RuDP oxygenase;
3. P-glycolate phosphatase;
4. Glycolate dehydrogenase (site of action of αHPMS):
5. Glutamate-glyoxylate aminotransferase (site of action of INH);
6. Serine hydroxymethyltransferase (major source of photorespiratory CO_2; energy may be partially conserved as ATP);
7. An unidentified aminotransferase;
8. NADH - hydroxypyruvate reductase;
9. Glycerate kinase.

Organelles involved are:-

A. Chloroplast
B. Microbody
C. Mitochondrion.

Based on figures in references 30 and 45.

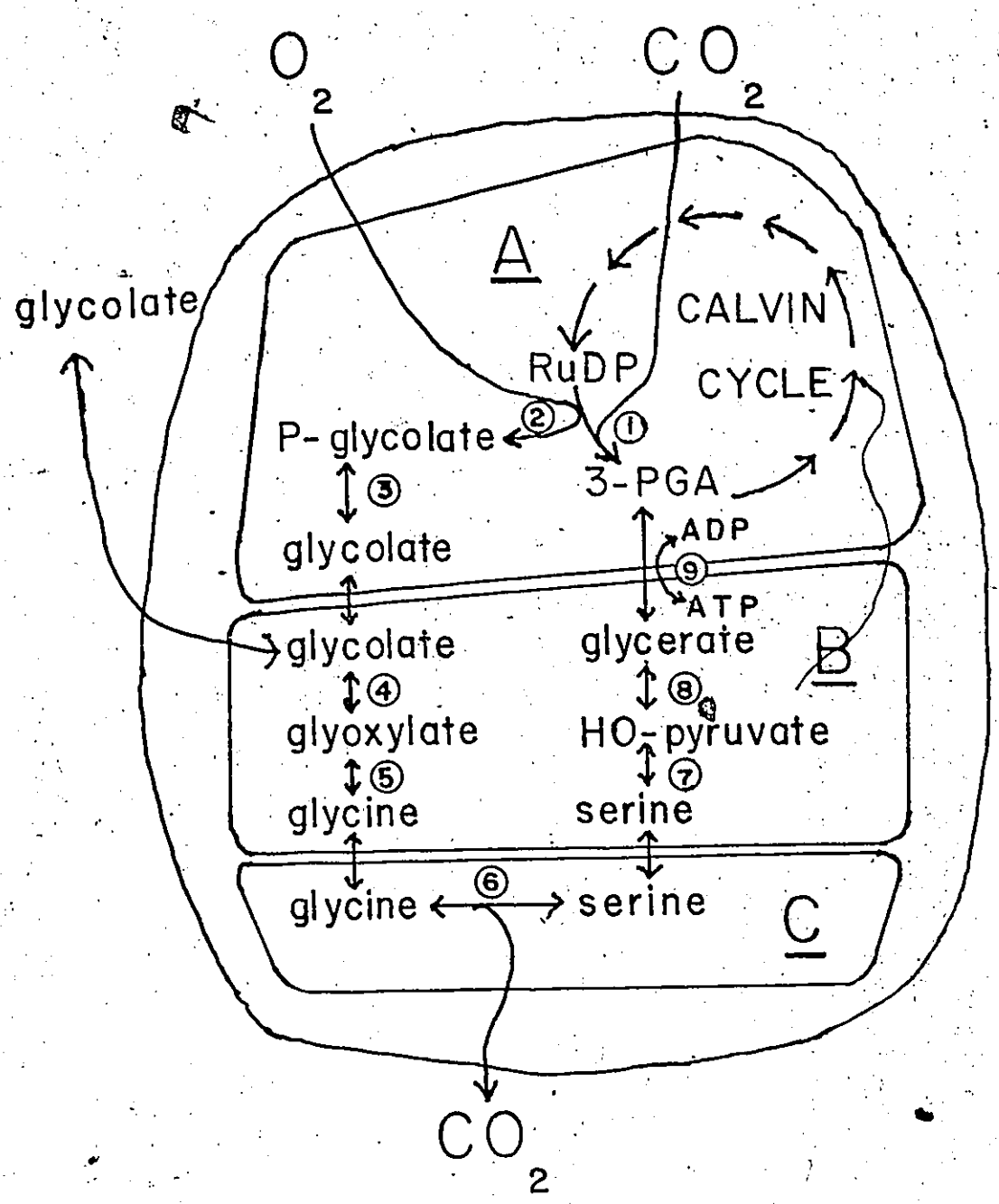

O2
CO2
A
glycolate
CALVIN
CYCLE
RuDP
P-glycolate
2
1
3-PGA
3
glycolate
ADP
9
ATP
glycolate
glycerate
B
4
8
glyoxylate
HO-pyruvate
5
7
glycine
serine
6
glycine
serine
C
CO2

Figure 1.

Wilson and Calvin (376) studying CO_2 concentration dependent changes in the radioactivity of photosynthetic intermediates, showed that the level of radioactivity in glycolic acid was very low in 1% CO_2 but rose rapidly, mainly at the expense of 3-PGA when the CO_2 concentration was dropped to 0.003% CO_2. They also showed that RuDP was the actual CO_2-acceptor in photosynthesis and gives rise to 3-PGA. Bassham and Kirk (10) showed that a very large stimulation of glycolic acid production occurred in an O_2 atmosphere. This was accompanied by a dramatic loss of radioactivity in RuDP. The details of glycolate metabolism in plant leaves were worked out by Tolbert's group (276, 346) and designated the glycolate pathway. This is a sequence of reactions whereby glycolate is converted via glyoxylate, glycine and serine into glycerate and ultimately into sucrose (Fig. 1). In higher plants the key enzyme in the pathway is glycolate oxidase. This enzyme catalyses the oxidation of glycolic acid to glyoxylic acid. Oxygen acts as a hydrogen acceptor and hydrogen peroxide is formed. This H_2O_2 can then react non-enzymatically with glyoxylic acid to produce formic acid and CO_2, however, in higher plants this reaction is normally prevented by catalase. Glycolate oxidase is inhibited by α-hydroxysulfonates (382). Treatment of Chlorella and Chlamydomonas with α-hydroxysulfonates did not result in the accumulation of glycolate-^{14}C as in treated leaves of higher plants (348). On this basis it was proposed that glycolate oxidase was not present in these algae. Further failures to detect glycolate oxidase in five strains of unicellular green algae (138) and the dilemma of an incomplete glycolate pathway in algae was resolved by the finding that algae contain glycolate dehydrogenase which does not link to oxygen and which is repressed when the cells are grown on 1% CO_2 (230). A glycolate pathway similar to

terrestrial plants but using glycolate dehydrogenase was shown to be present in unicellular green algae (30). The mechanism of glycolate synthesis has been a major problem of photosynthetic carbon metabolism. Glycolate could be produced *in vitro* from fructose-6-P via the enzyme-bound thiamine pyrophosphate glycoaldehyde intermediate in the reaction mediated by the chloroplast enzyme transketolase (22, 307). This was assumed to be the major pathway of glycolate formation. Bassham and Kirk (10) suggested that glycolate may be derived from phosphoglycolate formed by oxidation of RuDP. The finding that molecular O_2 competitively inhibits RuDP carboxylase, the carboxylation enzyme of the Calvin cycle, led to the proposal that O_2 substitutes for CO_2 in the carboxylase reaction, so that RuDP is oxidised to 3-phosphoglyceric acid and phosphoglycolate (242). Preparations of RuDP carboxylase from higher plant leaves were subsequently shown to catalyse the formation of phosphoglycolate and 3-PGA from RuDP in the presence of O_2 *in vitro* (3). The enhanced production of glycolate during photosynthesis at high O_2, high light intensity and low CO_2 was related to the O_2-inhibition of photosynthesis. Ellyard and Gibbs (83) proposed that this depressing effect of O_2 on net photosynthesis, first described by Warburg (365), was the result of an increased proportion of the total carbon fixed being channelled into the glycolate pathway with subsequent loss of CO_2 through photorespiration.

The isolation of microbodies or peroxisomes from the leaves of higher plants and the characterization of their enzyme content by Tolbert *et al* (349) has led to the knowledge that the glycolate pathway is partitioned into 3 cell organelles in leaves of higher plants (347). Glycolate is formed in the chloroplast converted to glycine in the

peroxisome and two glycines are condensed to form one serine plus CO_2 in the mitochondrion. The serine formed can be converted to glycerate in the peroxisome and fed back to the chloroplast re-entering the Calvin cycle. A recent review (205) represents the carbon metabolism of C_3 plants as two interlocking cycles, the photosynthetic carbon reduction cycle (Calvin cycle) and the photorespiratory carbon oxidation cycle (Glycolate pathway). From this viewpoint the glycolate pathway is an integral part of photosynthetic carbon matabolism in C_3 plants, the flux of carbon through both pathways being determined by the prevailing CO_2 and O_2 concentrations.

The peroxisomes of higher plants have a distinct physiological role in the glycolate pathway during photosynthesis in air. They contain catalase which presumably acts to prevent H_2O_2 build-up resulting from the aerobic oxidation of glycolate. The algal glycolate dehydrogenase differs in that O_2 is not the immediate acceptor for glycolate electrons so that the algal enzyme does not have to be associated with catalase in an organelle of the peroxisome type. Microbodies have been detected in electron micrographs of Chlorella (99) Chlamydomonas (104) Euglena (116) and Chlorogonium (100). Catalase has been detected cytochemically by DAB staining in situ in Chlorogonium (100) and Chlamydomonas (104). Experiments to determine the intracellular localization of enzymes concerned with glycolate metabolism have been carried out with Chlorogonium (321), Euglena (53) and Chlamydomonas (259). Broken cell suspensions were separated on sucrose gradients. Catalase or hydroxypyruvate reductase were used as peroxisomal markers and major peaks of activity of these enzymes were associated with a microbody fraction banding from 1.20 to 1.25 g/cm^2. Unlike higher plants most of the glycolate

dehydrogenase activity was found to coincide with malate dehydrogenase activity in a mitochondrial fraction banding from 1.19 to 1.22 g/cm^2.

Even though the fragility of microbodies often results in loss of their matrix enzymes during isolation, the association of glycolate dehydrogenase with the mitochondrial fraction is striking. It remains to be seen whether peroxisomes of the terrestrial plant type occur in green algae such as Nitella and Spirogyra which have relatively high levels of catalase activity and contain glycolate oxidase rather than glycolate dehydrogenase (97).

5.2 Glycolate excretion

In 1956, Tolbert and Zill (350) showed that 3 to 12% of the total $^{14}CO_2$ fixed during short term photosynthesis experiments (2 to 30 min.) with Chlorella was excreted into the medium as glycolate. Pritchard et al (272) showed that glycolate excretion by Chlorella was maximal between 0.03 and 0.2% CO_2 and that isonicotinyl hydrazide (INH) stimulated glycolate excretion over this range of CO_2 concentration. Nalewajko et al (228) examined glycolate excretion in a planktonic strain of Chlorella pyrenoidosa grown in stationary culture under air. They found that the radioactivity of extracellular products, which was primarily glycolic acid, rarely exceeded about 1% of the total ^{14}C-uptake by the cells. This low percentage activity of excreted glycolate was in marked contrast to earlier reports with Chlorella pyrenoidosa grown on 4% CO_2 (155). Nalewajko et al (228) also found that glycolate excretion was enhanced at high pH.

Watt and Fogg (370) showed that maximum glycolate release by Chlorella pyrenoidosa occurred when cells grown on 3% CO_2 were transferred to the low CO_2 conditions of air. Air-grown cells did not excrete

glycolate under these conditions. Glycolate excretion was also not observed in a strain of Chlorella pyrenoidosa 211/8p (114). The failure to excrete glycolate was not the result of an inability to synthesize this compound because addition of α-HPMS caused glycolate excretion by this alga (204). The experiments of Nelson and Tolbert (230) showing that high CO_2-grown, but not air-grown, Chlamydomonas reinhardtii cells excreted glycolate, suggested that the ability to excrete glycolate was related to low levels of glycolate oxidizing enzymes in high CO_2-grown cells. The high levels of glycolate oxidizing enzymes in high CO_2 or air-grown cells of Chlorella pyrenoidosa 211/8p was suggested as a major reason why glycolate was not excreted by these cells.

Colman et al (56) re-examined the kinetics of glycolate excretion and the content of glycolate dehydrogenase in high CO_2 and air-grown cells of Chlorella pyrenoidosa. CO_2-grown cells of Chlorella lost the ability to excrete glycolate after 7 hrs of growth on air. α-HPMS and INH stimulated glycolate excretion in both types of cells. Their results supported the finding of Nelson and Tolbert (230) and Lord and Merrett (204) that air-grown cells produce glycolate without excreting it. Adaptation of CO_2-grown Chlorella to growth on air did not affect the levels of glycolate dehydrogenase in the cells or affect the levels of glycolate dehydrogenase in the cells or affect the rate of dark oxidation of exogeneous ^{14}C-glycolate. Thus while the level of glycolate dehydrogenase may limit further metabolism of glycolate in the cell it is not the cause of massive glycolate excretion. These results also indicate that the lack of glycolate excretion by air-grown or air-adapted cells cannot be explained by changes in the level of

glycolate dehydrogenase.

The fact that the rate of glycolate formation exceeds the rate of its utilization resulting in the transient phase of glycolate excretion observed when high CO_2-grown cells are transferred to air, can be related to transient changes in the ability of the cells to photosynthesize under these conditions. Briggs and Whittingham (25) showed that after Chlorella grown with high levels of CO_2(5%) was transferred to low CO_2 conditions, the rate of photosynthesis was initially very low. Then over a 2 hr period the rate of photosynthesis rose about 5-fold as the cells "adapted" to the low CO_2 concentration. At about the same time Osterlind (251) and Steeman-Nielsen (233) suggested that the inhibition of photosynthesis of high CO_2 adapted cells following transfer to low CO_2 conditions was related to an inability to utilize bicarbonate ions. Thus the increase in photosynthesis rate during the "adaptation" to low CO_2 was related to a slow increase in the ability to utilise bicarbonate.

The fact that lack of available CO_2 was regulating the rate of photosynthesis and glycolate excretion during adaptation to low CO_2 was high-lighted by the work of Nelson et al (229) with Chlamydomonas and Graham and Reed (115) with Chlorella showing that carbonic anhydrase formation was suppressed in these algae during growth on high CO_2. A large increase in the level of carbonic anhydrase activity occurred within 2 hrs following transfer to low CO_2. At high external pH, many submerged plants and algae use external HCO_3^- besides CO_2 for photosynthesis (6, 90). Carbonic anhydrase catalyses the inter-conversion of HCO_3^- and CO_2 and presumably increases the supply of CO_2, which is the species fixed by RuDP carboxylase (59). Findenegg (90) showed

that high CO_2-adapted Scenedesmus obliquus could not use HCO_3^- for photosynthesis at alkaline pH. After 2 hrs aeration with normal air, photosynthesis proceeded rapidly even at pH 11. These changes in photosynthesis were correlated with an increase in the carbonic anhydrase activity of the cells. Reed and Graham (283) also showed a relationship between the level of carbonic anhydrase activity and photosynthesis in Chlorella during adaptation to low CO_2 concentrations.

It would thus appear that glycolate excretion is primarily regulated by the supply of available CO_2 to RuDP carboxylase-oxygenase. Under conditions where the supply of CO_2 inside the cell is very low (during growth on high CO_2, the CO_2 readily diffuses into the cell), the oxygenase reaction will predominate increasing the supply of glycolate to the glycolate pathway and glycolate produced in excess of the cells' ability to metabolize it will therefore be excreted.

5.3 Photosynthetic CO_2-fixation and glycolate metabolism in blue-green algae and diatoms

While most of our current knowledge of photosynthetic carbon metabolism and the glycolate pathway has been derived from extensive studies of the leaves of higher plants and unicellular green algae, investigations of these processes in other photosynthetic organisms such as the blue-green algae and the diatoms has been less extensive.

5.3.1 Photosynthetic CO_2-fixation in blue-green algae

These organisms are prokaryotic in nature and do not possess separate chloroplasts, microbodies or mitochondria as in eukaryotic plants. Early studies of the pattern of ^{14}C incorporation into photosynthetic intermediates in Anacystis nidulans showed a high proportion of the radioactivity in 3-PGA and hexose monophosphates after 5 secs of

photosynthesis and it was concluded that the Calvin cycle operated exclusively (170, 176). However, Richter (285) was unable to detect fructose 1,6-diphosphate aldolase activity in Anacystis nidulans. He also found aspartic acid, glutamic acid, 3-PGA and PEP were more highly labelled with $^{14}CO_2$ than hexose monophosphates during short term $^{14}CO_2$-incorporation experiments (5 - 30 secs). These results raised the suggestion that blue-green algae may also fix CO_2 via the dicarboxylic acid (C_4) pathway described by Hatch and Slack (126). Subsequent attempts to clarify this situation have been somewhat equivocal. Evidence that 3-PGA was the first detectable product of $^{14}CO_2$-fixation and that the subsequent distribution of radioactivity could be accounted for by the Calvin cycle was obtained by Pelroy and Bassham (262) and Ihlenfedlt and Gibson (153). Fixation primarily into aspartate was found occasionally but not reproducibly, depending on experimental conditions in Anacystis nidulans (157).

Dohler (72, 73) looked at the activities of carbonic anhydrase, photosynthetic enzymes and early products of $^{14}CO_2$-fixation in Anacystis nidulans grown on air or 3% CO_2. CO_2 concentration during growth had little effect on the activity of RuDP carboxylase and PEP carboxylase, however, the level of carbonic anhydrase activity was 3-fold lower in CO_2-grown cells (72). Over 50% of the incorporated radioactivity was found in aspartate during the first minute of $^{14}CO_2$-uptake (73). 3-PGA and PEP were also labelled preferentially during this period. CO_2-fixation via the C_4 pathway requires PEP carboxylase levels higher than that of RuDP carboxylase (127). The activity of PEP carboxylase reported by Dohler (73) was 4-fold lower than that of RuDP carboxylase. However, the activity of PEP carboxylase in lysed spheroplast preparations

of 3 species of blue-green algae (including Anacystis nidulans) was found to be 1.5 to 5-fold higher than that of RuDP carboxylase (55). A subsequent study (54) showed that photosynthetic CO_2 fixation of 4 species of blue-green algae was inhibited 82% to 97% by malonic acid, an inhibitor of PEP carboxylase which also inhibits CO_2-fixation in sugarcane, a C_4 plant (7). While these more recent reports are inconsistent with earlier reports that 3-PGA was the first detectable product of CO_2-fixation in blue-green algae (153, 157, 170, 262) it has been pointed out (54) that these earlier reports were obtained with cells grown on high CO_2. These conditions repress carbonic anhydrase (72, 155) and HCO_3^- transport capacity (6) of blue-green algae. Under these conditions CO_2-grown blue-green algae would only utilize CO_2 diffusing into the cells which would be preferentially used by RuDP carboxylase. Thus while blue-green algae clearly have the ability to fix CO_2 via the Calvin cycle, β-carboxylation reactions may also account for a large proportion of the total CO_2 fixed in the light under low CO_2 conditions.

5.3.2 Glycolate metabolism in blue-green algae

Norris et al (238) demonstrated that phosphoglycolic acid was a product of photosynthesis in blue-green algae. Hellebust (133) showed that a species of Coccochloris excreted 2.7% of the carbon fixed in photosynthesis and only 3% of this was glycolic acid. Two species of blue-green algae were shown to assimilate glycolic acid (218). An enzyme catalysing the oxidation of glycolate to glyoxylate was detected in cell-free extracts of both these algae (117). The metabolism of exogenous glycolate was inhibited by α-hydroxysulfonates indicating that part of the glycolate pathway operates in these algae in vivo. However,

glycolate metabolism was not inhibited by INH, suggesting that the further metabolism of glycolate to serine via glycine was not a major pathway in these algae. Dohler and Braun (74) reported a transient "pulse" of glycolate excretion at 20°C lasting 10 min. in Anacystis nidulans cultured on air at 32°C. No glycolate excretion was observed at 35°C. Cheng et al (44) examined glycolate excretion in 2 species of blue-green algae grown on high CO_2 or air. The amount of glycolate excreted was < 1% of the ^{14}C fixed by the algae during photosynthesis and > 25% of the total ^{14}C excreted. Transfer from high CO_2 to air, α-hydroxysulfonates and INH failed to stimulate massive excretion of glycolate.

Han and Eley (124) showed that high CO_2-grown Anacystis nidulans exhibited a light dependent glycolate excretion which occurred in the absence of HCO_3^- and was rapidly inhibited by addition of HCO_3^-. Air levels of O_2 (21%) and INH did not stimulate glycolate excretion in cells without HCO_3^-. Ingle and Colman (156) also showed that glycolate excretion occurred as a "pulse" of about 20 min. duration when high CO_2-grown cells of Coccochloris peniocystis were incubated in low bicarbonate at high light intensities. HPMS stimulated glycolate excretion but INH had no effect on glycolate release.

Carbonic anhydrase activity of blue-green algae had previously been shown to be repressed by growth of high CO_2 (155). When CO_2-grown Coccochloris cells were transferred to air, carbonic anhydrase activity increased while a corresponding decrease in the cells ability to excrete glycolate occurred. This effect was reversible. Diamox, an inhibitor of carbonic anhydrase stimulated glycolate excretion in air grown cells. Their results suggest that glycolate excretion is stimulated by a

decreased internal supply of CO_2 resulting in increased oxidation of RuDP by molecular O_2 to glycolate via the oxygenase activity of RuDP carboxylase. Sorensen and Halldal (316) examined glycolate excretion in high CO_2-grown Anacystis nidulans. Very low amounts of glycolate were excreted (0.2%) of total ^{14}C fixed, confirming earlier reports that glycolate excretion is low in blue-green algae.

Codd and Steward (51) re-examined oxidation of exogeneous glycolate in Anabaena cylindrica. Assimilation of glycolate was inhibited by HPMS but not by INH. Short term labelling experiments with ^{14}C-glycolate did not show significant metabolism of ^{14}C via glycine and serine. Cell extracts catalysed the enzymic condensation of glyoxylate to tartronic semialdehyde with decarboxylative release of CO_2 and also the enzymic reduction of tartronic semialdehyde to glycerate. On the basis of their results, they proposed that in the light glycolate is metabolized mainly via glyoxylate→tartronic semialdehyde → glycerate → 3-PGA.

Grodzinski and Colman (390) found most of the glycolate dehydrogenase activity of a lysed spheroplast preparation of Oscillatoria sp. localized in a particulate photosynthetic lamellae fraction after centrifugation in a discontinuous sucrose density gradient. Codd and Sallal (389) also found about half of the glycolate dehydrogenase activity from the crude extract attached to a photosynthetic chlorophyll-containing cell-free pellet in 3 blue-green algae. Their data and that of Grodzinski and Colman (390) indicates that most of the glycolate dehydrogenase in blue-green algae is associated with the thylakoids. This indicates that blue-green algae metabolize glycolate differently to green algae or higher plants.

5.3.3 Photosynthetic CO_2-fixation in diatoms

Coombs and Volcani (58) reported that most of the ^{14}C-label in the freshwater diatom Navicula pelliculosa was in 3-PGA and sugar phosphates after 10 secs of $^{14}CO_2$-incorporation, suggesting that the Calvin cycle was operating in this alga. Wallen and Geen (361) found that almost half of the radioactivity incorporated in the ethanol soluble fraction of the marine diatom Cyclotella nana during a 30 min. incubation with $Na_2{}^{14}CO_3$ was in amino acids, amides and TCA cycle intermediates. Beardall et al (12) found that cultures of the marine diatom Phaeodactylum tricornutum incorporated 50% or more of the total fixed ^{14}C into amino acids and amides after 30 secs of photosynthesis. Holdsworth and Colbeck (145) using the same diatom found 35% of the total $^{14}CO_2$ fixed in amino acids after 10 secs. In this short time 90% of the ^{14}C in aminoacids was in aspartate. 30% of this ^{14}C fixed into aspartate was located in the β-carbon atom. Thus PEP, the substrate for β-carboxylation must arise from 3-PGA formed via the Calvin cycle. Beardall et al (13) also found significant labelling of C_4 acids and Krebs cycle intermediates in short term (10 sec.) $^{14}CO_2$-assimilation experiments with another marine diatom Skeletonema costatum. The enzyme responsible for β-carboxylation was studied in 3 marine diatoms and reported as PEP carboxykinase (144). Since the pattern of ^{14}C-fixation in Phaeodactylum tricornutum can largely be explained by the action of RuDP carboxylase, the early appearance of ^{14}C in aspartate suggests that the role of β-carboxylation in diatoms may be restricted to providing an anaplerotic sequence to the Krebs cycle (39, 345).

5.3.4 Glycolate metabolism in diatoms

Hellebust (133) found glycolate in the filtrates of 8 marine

diatoms. Glycolate constituted from 1.5 to 38.5% of the carbon excreted by these cells. Watt (369) found that glycolate was the major extracellular compound released by the freshwater diatom Stephanodiscus hautschii. In young, rapidly growing cultures, glycolate formed 36% to 69% of the total extracellular compounds released. Another freshwater diatom Synedra acus did not release glycolate when grown under the same conditions.

Paul and Volcani (258, 260, 261) have examined glycolate metabolism in three marine diatoms. Glycolate dehydrogenase activity was found in all three diatoms and appears to differ from the enzymes found in other algae in that it is CN^- insensitive and prefers the L-isomer of lactic acid. In two of the diatoms the enzyme appears to be localized in the mitochondrion (261). Paul and Volcani (260) detected activity of glyoxylate carboligase and tartronic semialdehyde reductase in Cylindrotheca fusiformis. This diatom can therefore convert glycolate to glycerate via tartronic semialdehyde as well as the more common route via glycine.

Coughlan (60) examined glycolate metabolism in Thalassiosira pseudonana. Uptake of glycolate was slow, presumably via passive diffusion, being about 1% of photosynthetic CO_2-fixation. Over 50% of the label entered the protein fraction during a 2 hr incubation period. INH halved total fixation and the amount of label entering the protein fraction. α-HPMS decreased uptake substantially and diverted label into the lipid and water soluble fraction. In short term ^{14}C-glycolate feeding experiments, label was detected in glycine after 30 secs reaching a maximum after 2 min. From 2 to 4 min, lable accumulated maximally in serine. The type and level of glycolate dehydrogenase

activity was similar to that reported for the same species of diatom by Paul and Volcani (258).

5.4 Photorespiration

5.4.1 Introduction

"Usually, respiration is mentioned in the investigation of photosynthesis only as a bothersome source of uncertainty." This quote from Rabinovitch (274) underlines a problem that has plagued measurements of photosynthesis. The problem was, and still is to some extent, that of measuring the rate of respiration occurring during photosynthesis. The photosynthetic assimilation of CO_2 is partly counterbalanced by the loss of CO_2 by respiration. Net (or "apparent") CO_2 assimilation is equal to the gross (or "true") photosynthesis minus the loss resulting from respiration. "Photorespiration is defined as the respiration (especially the CO_2 evolution) that differs biochemically from normal dark respiration and is specifically associated with substrates produced during photosynthesis" (384). Usually photorespiration is taken to mean the total CO_2 evolved in the light, which, of course, includes dark respiration also occurring in the light. Much of our understanding of photorespiration has been obtained from a number of indirect and direct methods of measuring this process.

5.4.2 Post-illumination CO_2 outburst

Early workers had noticed that the rate of respiration in the dark immediately following illumination could be accelerated. This light-stimulated respiration was shown to be wavelength dependent, blue light stimulating the subsequent rate of dark respiration of Chlorella (86), whereas wavelengths > 500 nm did not. van der Veen (353) used

a thermal conductivity detector to measure changes in the CO_2 content of air passing over plants. In the light CO_2 was removed from the air, however a post illumination peak of CO_2 release was observed prior to the steady increase on CO_2 content of the air in the dark. He postulated that a "light dependent CO_2 absorbing factor released this CO_2 in the dark".

Decker (67) using an infrared CO_2 analyser (IRGA) observed similar post-illumination CO_2 outbursts (PIBs) in leaves of higher plants. The magnitude of PIB increased with light intensity and temperature but was not affected by CO_2 concentration. The PIB was explained as an overshoot of photorespiration into the dark period as the small pool of photorespiratory substrate synthesized in the light was metabolized. It was shown by Tregunna et al (351) that the rate of dark respiration was unaffected by O_2 concentrations between 2 and 47%. Also no outburst occurred in soybean leaves kept in 1% O_2, but it occurred in 21% O_2 and was 3 times as large in 100% O_2 (95). The lack of a CO_2 outburst in maize leaves was confirmed by Forrester et al (96) even when 100% O_2 was present in the atmosphere. These results suggested that photorespiration was low or absent in these plants. Some confusion has arisen since the relationship between the initial rate of CO_2 release during the PIB and the magnitude of the PIB is not clear. The PIB appears to give some estimate of the rate of photorespiration but the complexity of the CO_2 concentration transients make it difficult to use the PIB for routine estimates of the rate of photorespiration (32).

5.4.3 The CO_2 compensation point

The light compensation point is the light intensity at which

photosynthesis and respiration rates compensate each other, resulting in a zero net CO_2 exchange. At constant light intensities above the light compensation point CO_2 is removed from the environment until a CO_2 level is reached at which there is no net CO_2 exchange, the CO_2 compensation point. At both these compensation points there is a steady state between CO_2 uptake and CO_2 release, this is a dynamic equilibrium though net changes are not observed. This CO_2 C.P. was first seriously studied by Miller and Burr (219) using whole plants. They found that the plants could only reduce the CO_2 content of the air to about 100 ppm (µl/l). Similar results for detached leaves were obtained by Gabrielsen using IRGA (98). The CO_2 compensation point in bright light was shown to increase with temperature (132) and O_2 concentration (95) and to differ greatly among various plant species. Moss (222) was the first to report that plants such as maize or sugarcane had CO_2 compensation points < 10 ppm whereas other species such as tobacco, tomato and orchard grass had CO_2 compensation points of 60 ppm or greater. This was confirmed for maize by Forrester et al (96) who also showed that this very low CO_2 compensation point was O_2-insensitive over the range 1% - 100% O_2. This has been shown to be a characteristic of plants using the C_4 pathway of carbon fixation. With the discovery of the C_4 pathway of photosynthesis in sugarcane (126) higher plants have been divided into 2 main groups according to their mode of C-fixation. C_3 or Calvin cycle plants photorespire and have O_2-sensitive CO_2 compensation points of 30 ppm or more whereas C_4 plants have very low rates of photorespiration with O_2-insensitive CO_2 compensation points of less than 10 ppm (45).

Two main approaches have been used to measure CO_2 compensation

points of algae. The first approach involves a chamber for gassing algal suspensions in acid buffers (pH 4-5) linked to an IRGA which measures the CO_2 content of gas bubbling through the algae. This method was first used by Whittingham (374) to measure a CO_2 compensation point in air of about 10 ppm with Chlorella. Brown and Tregunna (28) used the same method to look at the effect of O_2 concentration of the CO_2 compensation point of three species of freshwater unicellular algae, the macroscopic freshwater alga, Nitella and seven species of marine macroscopic algae. Chlorella, Scenedesmus and Gonium all exhibited CO_2 compensation points of 3 ppm or less at air levels of O_2. Lowering the O_2 level to 1 - 2% did not greatly reduce these values, which, however, were at the limit of detection for the IRGA system used. Nitella, and three of the marine macro-algae had air CO_2 compensation points of 25 to 75 ppm which dropped to less than 3 ppm at 1 - 2% O_2. The other marine macro-algae had similar CO_2 compensation points to the three unicellular algae.

The other approach was to suspend algae in thin layers on wet membrane filters in the leaf cuvet of the IRGA system. This was first done by Egle and Schenk (81) using Chlorella and they obtained a CO_2 compensation point of about 10 ppm. Schaub and Egle (299) repeated this work and obtained CO_2 compensation points very close to zero for Chlorella. Lloyd et al (201) extended this wet membrane approach to seven unicellular freshwater algae and three marine unicellular algae. CO_2 compensation points in air for all algae were less than 10 ppm (1 - 8 ppm) and insensitive to O_2 concentration and temperature.

5.4.4 Release of $^{14}CO_2$ in the light

The two methods of detecting and estimating photorespiration

described above are basically indirect methods and techniques to measure CO_2 release in the light directly, were developed. During a study of the isotope discrimination factor for ^{14}C uptake, Steeman Nielsen (234) measured the release of previously assimilated $^{14}CO_2$ in the light and dark. Chlorella pyrenoidosa cells were exposed to $^{14}CO_2$ for 2 hrs, then centrifuged in the dark and washed twice with bicarbonate buffer. After 1 1/2 hrs dark incubation, the cells were centrifuged, resuspended in growth medium and distributed in a light and dark bottle. At various intervals the cells were sampled and filtered, the activity of the $^{14}CO_2$ in the filtrate was determined after being precipitated as $Ba^{14}CO_3$. From 1.8 to 3.3% of the ^{14}C taken up by the cells was released in the light. The rate of $^{14}CO_2$ release in the light was 33-40% the rate of dark release. While this experiment is complicated by the fact that organic ^{14}C released by the cells may have co-precipitated with the $Ba^{14}CO_3$, it is one of the first direct measurements of $^{14}CO_2$ release in the light by algae.

Using IRGA El-Sharkawy and Hesketh (1965)(cited in 384) first described CO_2 efflux into CO_2-free air in the light in higher plants. They found photorespiration in cotton leaves was greater than dark respiration while CO_2 efflux from maize leaves in the light could not be detected. This technique was used to separate species into high or low photo-respiration groups (384). This method was rather insensitive since the sensitivity of IRGA was not much better than 5 - 10 ppm. However, Goldworthy (110) labelled tobacco leaf segments with $^{14}CO_2$ and then measured the $^{14}CO_2$ released into CO_2-free air in the light and the dark. Zelitch (383) independently developed a similar method to Goldsworthy's. Using this ^{14}C-assay with cells of Chlorella and Chlamydomonas, Zelitch

and Day (387) showed that photorespiration exceeded dark respiration. However, it should be pointed out that Zelitch flushed CO_2-free gas over a thin dense suspension of algae cells in rotating Warburg flasks, and it is not clear how efficient the gas exchange between the aqueous and gaseous phases was. Cheng and Colman (43) modified Zelitch's ^{14}C-assay by bubbling algal suspensions in a pear-shaped flask. In all cases except one, the rate of $^{14}CO_2$-release in the light was less than the dark rate. Two blue-green algae were shown to have extremely low rates of photorespiration, i.e. less than 1/10 dark rate.

Considerable doubt has been placed on the value of measuring only the radioactivity of $^{14}CO_2$ released in the light (45). It has been shown that the specific radioactivity of $^{14}CO_2$ released by leaves subsequent to labelling with $^{14}CO_2$ decreases fairly rapidly with time (208). While the technique is very sensitive and clearly shows CO_2 release in the light, the relationship between the radioactivity of the $^{14}CO_2$ released and its mass is unclear.

5.4.5 Changes in the specific radioactivity of $^{14}CO_2$ during photosynthesis

Weigl et al (372) studied changes in the specific radioactivity of $^{14}CO_2$ in a closed recirculating system containing barley leaves. Changes in CO_2 concentration were measured by IRGA and radioactivity in the gas phase decreased rapidly, the specific radioactivity of $^{14}CO_2$ in the gas phase initially rose about 10%, which they ascribed to isotope discrimination, then "the continuous respiratory evolution of inactive CO_2 surpassed the photosynthetic isotope concentration and quickly reduced the specific activity to a very low value", (372). In subsequent light periods, very little $^{14}CO_2$ was released, which may

be related to low CO_2 concentration effects on the stomata of the leaves. They attempted to evaluate the rate of "light respiration" based on the rate of change of the specific radioactivity and came up with a value of about 50% of the dark respiration rate. Krotkov et al (189) placed detached leaves of plants in water in a sealed glass chamber with known amounts of $^{14}CO_2$ and $^{12}CO_2$. After 1 hr in the light or dark, the residual $^{14}CO_2$ and $^{12}CO_2$ was removed and quantified. The difference between the calculated uptake of $^{14}CO_2$ and the actual amount of CO_2 taken up by the plants gave a value for CO_2 output in the light. This CO_2 output in the light was found to be less than, equal to, or greater than the CO_2 output in the dark for pea, kalanchoe and wheat leaves respectively. Krotkov (188) used the closed system of Lister et al (200) to measure changes in specific radioactivity of $^{14}CO_2$ around tobacco leaves. Though the specific radioactivity of $^{14}CO_2$ dropped rapidly in the light, no attempt was made to estimate photorespiration using this data. Hew et al (139) measured changes in the specific radioactivity of $^{14}CO_2$ around leaves of sunflower or maize in a closed system. The CO_2 concentration was rapidly reduced to the compensation point within 5 min. in the light. In sunflower, the specific radioactivity of $^{14}CO_2$ dropped rapidly, indicating that $^{12}CO_2$ was being released by the leaf. In maize, the specific radioactivity of $^{14}CO_2$ remained constant during CO_2 depletion, indicating that no photorespiration of $^{12}CO_2$ could be detected. The rate of CO_2 evolution in the light was calculated from the difference in the rates of $^{14}CO_2$ and $^{12}CO_2$ uptake. Due to the rapid evolution of $^{14}CO_2$ following incorporation into photosynthetic intermediates, i.e. within 1 min. this method can only be applied if accurate measurements can be obtained before $^{14}CO_2$ is

evolved from the leaf, otherwise the rate of CO_2 evolution is underestimated.

To overcome problems associated with closed IRGA systems, mainly related to leaks and the inability to maintain steady state conditions in a closed system due to changing CO_2 concentration, Ludwig and Canvin (209) developed an open gas-exchange system for the simultaneous measurement of CO_2 and $^{14}CO_2$ fluxes around leaves. This method enables steady state rates of true and apparent photosynthesis and CO_2 evolution in the light to be determined. Lloyd et al (201) used this open gas exhcange system to measure the rate of photorespiration in ten species of unicellular algae suspended on wet membrane filters. Most of the algae had rates of true photosynthesis approximately equal to apparent photosynthesis and therefore very little or no photorespiration was observed in either 1% or 21% O_2.

5.4.6 The source of photorespiratory CO_2

In higher plants possessing the glycolate pathway, glycine decarboxylation in the mitochondrion is considered the major source of photorespiratory CO_2 in terrestrial C_3 plants (178, 385). In most green algae where a functional glycolate pathway including the glycine → serine step has been demonstrated (30), this is also probably true. (Fig.1). The fact that INH which inhibits this reaction also causes glycolate excretion in green algae, clearly suggests that this step is important in the metabolism of glycolate by these algae.

As discussed earlier, blue-green algae can form glycolate during photosynthesis but excretion is minimal. The assimilation and oxidation of exogenous ^{14}C-glycolate is inhibited by HPMS but not by INH and label from ^{14}C-glycolate does not accumulate significantly in

glycine or serine. Cell extracts of Anabaena cylindrica catalysed the decarboxylation of glyoxylate under anaerobic conditions. The reaction product appeared to be tartronic semialdehyde, suggesting the presence of glyoxylate carboligase (51).

A second mechanism which may contribute to CO_2 release from glycolate is the direct decarboxylation of glyoxylate to formate in leaf peroxisomes by a non-enzymic reaction with H_2O_2. This can occur because peroxisomal catalase is unable to break down all the H_2O_2 produced during glycolate oxidation. The rates of this decarboxylation at 25°C could not account for more than 25% of the maximum rate of CO_2 release during photorespiration in intact leaves (118). At 35°C ^{14}C-glycolate was oxidized to $^{14}CO_2$ by peroxisomes isolated from leaves of spinach beet about 3 times more rapidly than at 25°C. This increase was mainly due to the increased H_2O_2 available to oxidize glyoxylate non-enzymatically (119).

A third reaction which may convert glycolate to CO_2 is the non-enzymic decarboxylation of glyoxylate by H_2O_2 or O_2^- in illuminated chloroplasts (84, 386). In contrast to green algae such as Chlamydomonas which have high catalase activity, blue-green algae have low or no catalase activity (46). Although the blue-green alga Anacystis has catalase activity, Patterson and Myers (257) observed bursts of H_2O_2 production in this alga during illumination. These experiments strongly suggest that CO_2 release in the light may also occur via the non-enzymic decarboxylation of glyoxylate by H_2O_2 in blue-green algae.

The work of Paul and Volcani (260) showing glyoxylate carboligase activity in Cylindrotheca and the work of Coughlan (60) with Thalassiosira, showing evidence for the conventional glycine → serine

step during glycolate metabolism, suggests that photorespiratory CO_2 may arise from at least two sources in diatoms.

5.5 The effect of light quality on respiration, photosynthetic carbon metabolism and extracellular release in algae

5.5.1 Endogenous oxidative respiration

As previously mentioned, Emerson and Lewis (86) noticed large increases in the rate of O_2 consumption in the dark period following exposure of Chlorella pyrenoidosa to low intensity (330 μW cm^{-2}) light in the 460 to 480 nm range. Since the effect was limited to a wavelength range where most of the light was absorbed by carotenoids, they suggested that a photochemical acceleration of respiration by the yellow pigments was involved. Reid (286) reported that Chlorella pyrenoidosa kept in the dark for several hours and then exposed to flashes of blue light (λ < 540 nm) gave increased rates of O_2-uptake. This increase was not inhibited by 5 x 10^{-6} M DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea). Kowallik and Gaffron (184) found blue light stimulation of O_2-uptake in a yellow, chlorophyll-free, mutant of Chlorella vulgaris (211-11 h/20) after it had been starved for several hours on glucose-free medium in the dark.

The action spectrum for blue light-stimulated O_2-uptake in Chlorella was reported independently by Kowallik (185) using the glucose-starved yellow Chlorella vulgaris mutant and by Pickett and French (264) using Chlorella pyrenoidosa exposed to 5 second flashes of light in the dark. The rate of O_2-uptake caused by the flash of light was not inhibited by 10^{-5} M DCMU which inhibited O_2-evolution (94%) under the same conditions. These action spectra showed a prominent peak at about 480 nm, a characteristic dip at 400 nm and another peak in the near

UV (380 nm). No effect on respiration was noticed at $\lambda > 550$ nm. This blue light effect on respiration saturated at very low light intensities (50 $\mu W\ cm^{-2}$ (450 nm)) in the chlorophyll-free Chlorella mutant (185). Further studies (186, 263) showed that the enhanced respiratory activity has a respiratory quotient (RQ) of 1, and the same temperature dependence as dark respiration. Pickett (263) showed that the cyanide sensitivity of the blue light effect was identical to that part of dark respiration which is inhibited by cyanide. Laudenbach and Pirson (192) investigated the effect of red and blue light on the carbohydrate content of DCMU- poisoned Chlorella and suggested that blue light may have a direct effect on the permeability of the chloroplast membrane.

The blue light effect on oxygen-uptake was attributed on the other hand to an FMN-dependent amino acid oxidase by Schmid and Schwarze (301). They showed that blue light caused enhanced oxygen-uptake on a crude extract of this enzyme from a colourless Chlorella mutant. Sargent and Taylor (295) compared DCMU-poisoned normal Chlorella pyrenoidosa with a colourless mutant possessing this FMN-dependent amino-acid oxidase activity, and found that the classical blue light effect on oxygen-uptake was inhibited by 10^{-4} M cyanide in the normal Chlorella but not in the mutant. They dubbed the cyanide sensitive effect "type I" and the cyanide insensitive effect "type II".

Steup et al (328) showed that blue light stimulated O_2-uptake in DCMU-poisoned Chlorella cells, was unaffected by both rifampicin or lincomycin but was abolished by cycloheximide. Kowallik and Ruyters (187) have also shown that cycloheximide blocks O_2-uptake in a yellow mutant of Chlorella (211-11h/20).

5.5.2 Primary products of $^{14}CO_2$-fixation

Following her early report that blue light increased the soluble protein content of higher plant leaves, Voskresenskaya (1953) (356), reported that the ^{14}C-activity of leaf protein was enhanced in blue light. In 1956, using bean and tobacco leaf discs, she found that blue light increased ^{14}C-incorporation into the amino acids aspartate and alanine and the organic acids malate and citrate (356). Cayle and Emerson (42) exposed Chlorella pyrenoidosa cells to high light intensities (3×10^4 μW cm^{-2}) of blue light and red light and found that after 30 secs. of $^{14}CO_2$-incorporation, the specific radioactivity of the amino acids alanine, glycine and serine, was 2- to 3- fold greater in blue light. Hauschild et al (129) used Chlorella vulgaris grown on high CO_2 (5%) in white light. They found that the addition of blue to red light enhanced ^{14}C-incorporation into aspartic acid compared to red light alone, after 5 min. exposure to $^{14}CO_2$. Increasing the period that the cells were kept in the dark (dark pretreatment) prior to illumination from 60 min. to 3 hrs, further stimulated this blue light effect. After 30 min. exposure to $^{14}CO_2$, addition of blue to red light or blue light alone, increased the ^{14}C incorporated into aspartic acid, glutamic acid, fumaric acid and malic acid. They extended their investigations to Chlorella pyrenoidosa and Scenedesmus acuminatus, also grown on 5% CO_2, Microcystis aeruginosa grown on air and Chromatium grown in N_2 (130). In Chlorella pyrenoidosa with no dark pretreatment, addition of blue to red light did not alter the ^{14}C distribution in the products of photosynthesis after 30 min. of illumination. However, after 3.4 and 21 hrs. of dark pretreatment, ^{14}C-activity rose in aspartic acid, glutamic acid, serine, alanine, fumaric acid and malic acid, in red and blue light and blue light treatments. Similar results were obtained for Scenedesmus.

After 3.4 hr. and 24 hr. dark pretreatments, the ^{14}C-activity of aspartic acid, glutamic acid, alanine and malic acid rose in red and blue light in Microcystis aeruginosa compared with red light alone. In Chromatium, the quality of light had no effect on the distribution of ^{14}C.

Hess and Tolbert (137) studied the effect of light quality on Chlorella pyrenoidosa and Chlamydomonas reinhardtii grown on 0.2% CO_2. Cells were grown under either blue, red or white light. The products of ^{14}C-incorporation following 3 and 10 min. exposures to the light used during growth were examined. Both algae initially incorporated the highest percentage of ^{14}C into phosphate esters of the Calvin cycle. In blue light adapted Chlamydomonas, more than 70% of the ^{14}C was incorporated into phosphate esters after 1 min. exposure to blue light. The percentage of ^{14}C in phosphate esters dropped to about 20% of the total soluble ^{14}C incorporated after 10 min. Concurrently ^{14}C-activity in glycolate rose to about 50% of the total ^{14}C in the soluble fraction. Less spectacular increases in ^{14}C-activity of malate, aspartate, glutamate and alanine occurred over the same period. The high level of ^{14}C-incorporation into glycolate was not observed in red light adapted cells.

Ries (287) and Ries and Metzner (289) studied the action spectrum of ^{14}C-fixation (20 min. incubation) in Chlorella pyrenoidosa using light of equal quantum flux or equal energy. The curves of CO_2-fixation rate as a function of wavelength, showed an increasing suppression of the blue maximum with decreasing light intensity, i.e. light intensity $< 180\ \mu W\ cm^{-2}$ (287). Under these conditions, after 20 min. exposure to light in the blue part of the spectrum > 40% of ^{14}C-incorporated

into the soluble fraction was in aspartic acid, 15-20% was in malate. By contrast > 60% of total soluble ^{14}C-activity was in PGA, sugar-phosphates and sucrose in the red part of the spectrum. Increasing the light intensity 5-fold increased the rate of photosynthesis in the blue part of the spectrum (blue maximum not suppressed) and the amount of ^{14}C in aspartate as a percentage of total soluble counts dropped to about 25%, however, aspartate was still labelled preferentially under blue light. They suggested that at low intensities of blue light, respiration of the cells was enhanced almost compensating photosynthesis. This would have the effect of raising the light compensation point in blue light. Thus under low light conditions the differences in blue and red light CO_2-fixation patterns were dependent on the ratio between respiration and photosynthesis.

At about the same time Ogasawara and Miyachi (239) were studying wavelength dependent effects on CO_2-fixation in Chlorella ellipsoidea. They grew this Chlorella on high CO_2 (1 - 3%) at low intensities (100 - 800 $\mu W\ cm^{-2}$) of white light. The action spectrum of $^{14}CO_2$-fixation (3 min. incubation) at low light intensity (80 $\mu W\ cm^{-2}$) showed a large red maximum and a smaller peak of CO_2-fixation in the blue part of the spectrum. Virtually no ^{14}C-fixation occurred in green light (ca. 540 nm). The distribution of ^{14}C in soluble photosynthesis products in cells exposed to red (40 $\mu W\ cm^{-2}$) and blue light (53 $\mu W\ cm^{-2}$) adjusted to give equal rates of CO_2-fixation was compared to dark $^{14}CO_2$-fixation under the same conditions. The ^{14}C activity of aspartate and glutamate increased in blue light compared to red light or darkness over the initial 10 min. period. A greater than 10-fold increase in light intensity further increased ^{14}C-incorporation into aspartate and malate in blue

light. However, the rate of incorporation in phosphate-esters and the insoluble fraction increased more than the incorporation into aspartate. At high light intensities > 70% of the total soluble ^{14}C was in phosphate-esters after 2 min. of photosynthesis as compared with 10 - 20% in the low light intensity experiments. This clearly shows the reduced operation of the Calvin cycle at very low light intensities and indicates that the blue light effect on ^{14}C incorporation into aspartate and malate is saturated at low light intensities.

When the action spectrum of 3 min. $^{14}CO_2$-fixation at low light (80 μW cm^{-2}) was repeated in the presence of 5 x 10^{-5} M CMU (3-(p-chlorophenyl)-1,1-dimethylurea) the normal action spectrum of photosynthesis was suppressed at wavelengths > 500 nm. A small but reproducible peak of $^{14}CO_2$-fixation was observed in the blue part of the spectrum. This blue light effect on CO_2-fixation in the presence of CMU was saturated at about 30 μW cm^{-2}. Blue light effects on the distribution of ^{14}C in the presence of CMU were similar to those in the absence of CMU though the magnitude of cpm fixed was much lower. Again blue light enhanced ^{14}C-incorporation into aspartate and glutamate compared to ^{14}C-incorporation in the dark or under red light and CMU. They concluded that blue light enhanced ^{14}C incorporation into aspartate, glutamate and malate, was mediated by a mechanism independent of normal photosynthesis. In a subsequent paper Ogasawara and Miyachi (240) found that increasing the dark pretreatment period of the cells by up to 24 hrs. increased the blue light effect on ^{14}C-incorporation into aspartate compared with red light by 2.3-fold. This confirms the results of Hauschild et al (129, 130) on this point. Ogasawara and Miyachi (240) also found that the blue light effect on the distribution

of ^{14}C in photosynthetic products was inhibited by pre-incubating the cells with chloramphenicol (3.2 mg/ml) or cycloheximide (15 µg/ml). This indicated that synthesis of cytoplasmic proteins was involved in the mechanism of the blue light effect.

Kamiya and Miyachi (168) showed that the action spectra of blue light enhanced respiration and $^{14}CO_2$-fixation were essentially the same in the colourless mutant of Chlorella vulgaris (211-11h/125). Both effects were saturated at 30 µW cm^{-2} light intensity. Comparison of the ^{14}C-incorporation into products during $^{14}CO_2$-fixation in the dark or under blue light in this Chlorella mutant revealed enhanced ^{14}C-incorporation into aspartate, glutamate, alanine, malate and fumarate, compared with cells incubated in the dark.

Wallen and Geen (361) investigated the pattern of ^{14}C distribution in the soluble fraction of Cyclotella nana and Dunaliella tertiolecta. The cells had been grown on air in blue, green or white light and were incubated with ^{14}C for 30 min. in the light of adaptation. In blue light, there was an increase in ^{14}C-activity of aspartic acid, glutamic acid, asparagine glutamine, serine, alanine, malic acid and fumaric acid relative to that observed in white light. This effect was found in both algae examined. The blue light effect was not light intensity dependent, similar results being obtained at 80 and 800 µW cm^{-2}.

Brown and Geen (29) used Chlamydomonas reinhardtii grown on air in red, blue, green and white light. The distribution of ^{14}C in the soluble fraction of cells following 30 min. exposure was examined. The highest percentage ^{14}C activity in the amino acid fraction of the total soluble counts was found in cells grown under blue or green light. The percentage ^{14}C activity in the amino acid fraction was lowest in red

light grown cells. Much of this difference in the percentage ^{14}C activity of the amino acid fraction could be attributed to differences in percentage incorporated into aspartate and glutamate. The combined percentage ^{14}C activity of these two compounds being 24 to 28-fold higher in blue and green light. The ^{14}C level of glycolate in air-grown blue light adapted Chlamydomonas in this study (29) was quite low (2% total soluble fraction) suggesting that the high level of glycolate labelled in the experiments of Hess and Tolbert (137) using the same alga adapted to blue light, may reflect an effect of the CO_2 level (0.2%) during growth.

The results of this section and the preceding one suggest a fairly direct relationship between blue light-enhanced respiration and CO_2 fixation into amino acids and C_4-dicarboxylic acids, especially under conditions where only respiration is occurring i.e. in the dark, in chlorophyll-free or photosystem II-deficient algal mutants or in the presence of photosynthesis inhibitors, such as CMU. The implication that blue light affects PEP carboxylase activity stimulating the β-carboxylation of PEP receives some support in the work of Kamiya and Miyachi (169). They showed that low intensity blue light (80 μW cm^{-2}) stimulated PEP carboxylase activity in cells of the colourless Chlorella vulgaris mutant (211/11h/125) during glucose starvation in the dark. Cycloheximide (5 μg/ml) completely suppressed this blue light effect, suggesting the cytoplasmic nature of the effect.

Recently Ries and Gauss (288) have attempted to answer the question of whether the two blue light effects, i.e. the enhanced respiration and the increased synthesis of dicarboxylic acids, result from a change induced in a step common to both responses. They examined the

specific radioactivity of $^{14}CO_2$ released from DCMU-poisoned Chlorella fusca fed with ^{14}C-glucose in red and blue light. In blue light there was an immediate increase in the rate of release of both $^{12}CO_2$ and $^{14}CO_2$ over and above the $^{12}CO_2$ release in response to feeding with unlabelled glucose in the dark. This effect was not observed in red light. In blue light, the incorporation of radioactivity into glutamate, aspartate, alanine and malate was higher than in red light. On the other hand, the amount of ^{14}C-incorporation from ^{14}C-glucose into sucrose and the insoluble cell components in red light was considerably higher than under blue light. These results confirm the earlier work of Laudenbach and Pirson (192) on carbohydrate turnover in blue light and red light in the same strain of Chlorella poisoned with DCMU.

Ries and Gauss (288) point out that the cyanide-sensitive "type I" blue light effect is the one most commonly encountered in Chlorella. They suggest that an increased turnover rate of the Krebs cycle is involved in this type "I" effect. It has been suggested (240, 288) that the decisive step enhanced by blue light is the carboxylation of PEP. This is a classical anaplerotic reaction (39) for replenishing Krebs cycle intermediates. Thus at normal levels of blue light, PGA from the Calvin cycle is diverted to form PEP and ultimately amino-acid carbon skeletons via the Krebs cycle. PGA is thus diverted away from formation of sugar phosphates, i.e. carbohydrates. Under non-photosynthetic conditions, i.e. + CMU, very low light intensities, darkness or non-photosynthesising mutants, PEP is supplied through glycolysis of carbohydrate reserves or exogenous glucose and blue light will accelerate the rate of respiration via increased Krebs cycle activity. The effect of cycloheximide on both blue light enhanced O_2-uptake and CO_2-fixation

patterns (169, 187, 240, 328) emphasizes the cytoplasmic origin of the blue light effect. The increased production of amino-acid carbon skeletons in blue light clearly must have some relationship to the higher levels of protein and RNA in these algae.

5.5.3 Extracellular release (i.e. glycolate excretion)

5.5.3.1 Green algae

Becker et al (14) examined the effect of red light (662, 672 nm) and blue light (452 nm) on glycolate excretion by Chlorella vulgaris. The cells were grown on 3% CO_2 in white light but flushed with air in the dark for 30 min. prior to exposure to white, red or blue light. The release of glycolate in white and red light was rapid and linear, reaching a maximum 5 min. after the start of the light period. The level of glycolate in the external medium then decreased over the next 2 hrs. No glycolate was excreted in blue light. Lord et al (203) reinvestigated the work of Becker et al (14) using Chlorella pyrenoidosa and Euglena gracilis grown on 5% CO_2 in white light. When Euglena cells were illuminated by blue light under conditions favouring glycolate formation, no excretion occurred. The addition of αHPMS did not force excretion, indicating that glycolate biosynthesis was not occurring. In red light, Euglena formed and excreted glycolate, and the rate of excretion was not significantly increased in the presence of αHPMS.

The strain of Chlorella pyrenoidosa used does not excrete glycolate in white light when grown on 5% CO_2. This alga did not excrete glycolate in red or blue light, but addition of αHPMS forced excretion in red but not in blue light. By contrast, Stabenau (319) found that glycolate was excreted at approximately the same rate in red and blue

light of equal quanta flux by the unicellular green alga Chlorogonium elongatum. These cells had been grown on 2% CO_2 in white light.

5.5.3.2 Blue-green algae

Dohler and Koch (75) attempted to measure an action spectrum of glycolate excretion and photorespiration in Anacystis nidulans. These cells were grown on air at 32°C. Glycolate excretion was only observed at lower temperatures (20°C). At this temperature glycolate excretion was minimal in blue or far red light, but showed a broad peak of activity between 550 and 670 nm. Glycolate excretion was uniform and unaffected by the spectral quality of light in the presence of αHPMS. At 35°C, glycolate excretion was only observed in the presence of αHPMS and was relatively uniform across the spectrum. This suggests that glycolate synthesis is unaffected by light quality. Photorespiration was assumed from the presence of CO_2 outbursts following illumination. CO_2 production following illumination was lowest in blue and far red light with a broad maximum occurring between 550 and 670 nm. This action spectrum of "photorespiration" at 35°C is identical to the action spectrum of photosynthesis.

Sorensen and Halldal (316) recently re-examined the action spectrum of glycolate excretion and photosynthesis in Anacystis nidulans. Glycolate excretion was very low, only about 0.2% of the total ^{14}C fixed was found in the medium as glycolate. The cells had been grown at 32°C on 4% CO_2. The action spectrum of glycolate excretion paralleled that of $^{14}CO_2$-fixation with a minor peak around 400 nm, a dip between 440 and 520 nm and a broad peak centered around 600 nm, reflecting the phycocyanin-sensitized photosynthesis of this alga. This confirms the

action spectrum of glycolate excretion observed by Dohler and Koch (75) at 20°C. Clearly the magnitude of glycolate excretion is related to the magnitude of its production during photosynthesis and the cell's ability to metabolize this compound. In red-orange light where the rate of photosynthesis in blue-green algae is maximal, the production of glycolate appears to exceed the cells capacity to utilize it and it is excreted maximally. In the case of green algae (see preceding section) there are usually two photosynthetic maxima, one in the blue and one in the red part of the spectrum. In some cases glycolate synthesis and/or excretion was not observed in white light adapted cells exposed to blue light (14, 203). This lack of glycolate metabolism by these cells may reflect the lower maximum of photosynthesis in blue light or even the absence of photosynthesis in blue light (287, 289).

5.6 The photosynthetic carbon metabolism of natural phytoplankton as a function of depth

While there are many studies of chlorophyll and ^{14}C-fixation as a function of depth, very few studies have examined the partitioning of ^{14}C within phytoplankton or extracellular release under natural conditions.

Fogg (92) showed that appreciable fixation of $^{14}CO_2$ into organic compounds in the filtrate occurs during phytoplankton ^{14}C-productivity measurements. Hellebust (133) and Fogg et al (94) presented evidence that glycolate is a major extracellular product of natural phytoplankton populations. Watt (368) examined extracellular release as a function of depth during ^{14}C-productivity experiments in freshwater reservoirs. The rate of extracellular release generally parallelled that of

CO_2-fixation with depth. When expressed as a percentage of total CO_2-fixation increases near the surface were seen on sunny days, and there was a general trend for extracellular release to increase towards the bottom of the photic zone. Chromatographic analyses of the ^{14}C-labelled extracellular products of natural phytoplankton populations suggested that aminoacids and polysaccharides were being released as well as glycolate (368).

Olive and Morrison (245) examined the partitioning of ^{14}C into various extracts of phytoplankton taken from different depths in an Ohio lake of glacial origin. Following ^{14}C-uptake the filtered phytoplankton were extracted with boiling 80% ethanol. More than 80% of the total ^{14}C uptake was usually in the ethanol and insoluble fractions. As the season progressed from March to June (thermal stratification prevailed from May) up to 50% of the ^{14}C-fixed by the cells in the upper photic zone (0.5m) was found in the ethanol soluble fraction. Up to 60% of the ^{14}C-fixed was found in the insoluble fraction in the middle photic zone (3m) and up to 80% of the ^{14}C-fixed was found in the insoluble fraction in the lower part of the photic zone (6m). Olive et al (246) also showed an increase with depth of ^{14}C-labelling in the insoluble fraction of phytoplankton at a station in western Lake Erie.

Wallen and Geen (362) examined the distribution of newly incorporated ^{14}C in marine phytoplankton from various depths near the coast of British Columbia. In general the ^{14}C in the ethanol soluble fraction decreased with depth. From 59.8 to 78.1% of the ^{14}C fixed by phytoplankton at the 1% surface illumination level (10 or 12 m) was recovered in the insoluble fraction. Excreted organic carbon as a percentage of ^{14}C-fixed generally decreased with depth, reflecting the decrease in the ethanol-

soluble fraction. Excretion ranged from 7.3 to 12.3% of the total ^{14}C-fixed at the 1% surface illumination level whereas 13.5 to 34.2% of the total ^{14}C fixed was released in the upper and middle parts of the photic zone.

STATEMENT OF THE PROBLEM AND RESEARCH OBJECTIVES

The classical experiments of Emerson and Lewis (86) detailing the wavelength dependence of photosynthesis in Chlorella were the culmination of many earlier investigations of the influence of light quality on the growth and photosynthesis of algae and higher plants. Subsequent studies revealed the wavelength dependence of photosynthesis in blue-green algae (85, 316), diatoms, (79, 213, 340) and terrestrial plants (31, 47, 210). Directly or indirectly, this early work has stimulated investigations of the influence of light quality in algae on:-

(a) The products of CO_2-fixation and their subsequent metabolism (section 5.5), including photorespiration and extracellular release,

(b) Endogenous oxidative respiration (section 5.5),

(c) Photosynthetic pigments and chloroplast structure (section 3.2.4),

(d) Cellular constituents such as protein, carbohydrate and nucleic acid (section 3.1),

(e) Cell division and other morphogenetic events related to growth (sections 2.1 to 2.4).

Some of these laboratory studies may be criticized for technical reasons, for example the use of high CO_2 during growth, or the use of white-light-grown cells not adapted to restricted portions of the PAR spectrum (section 3.2).

Depth-dependent changes in light quality occur in natural waters (sections 1.3 and 1.4), but few studies have addressed the possible significance of light quality to to phytoplankton population maxima which often develop in thermocline regions in lakes and oceans (sections 3.22 and 4.2). Clearly, algae remaining for any period at these depths

will have to adapt to the restricted spectrum of light if photosynthesis and growth are to take place. However, adaptation to decreased light intensity has been proposed as the major factor controlling the changes in photosynthetic response and pigment content observed with depth in the water column (235) and changes in light quality have often been discounted or ignored.

Most studies of photosynthesis in relation to underwater illumination have been considered mainly in a marine context (158, 223, 277, 279, 311, 360, 361). However, there has been a tendency to emphasize the blue-to-green part of the PAR spectrum which favours the growth of marine phytoplankton and macroalgae and relatively little work has been reported on the role of red light in the marine environment.

Algal growth is an expression of the carbon economy of the cells and reflects the balance between carbon influx due to photosynthesis and carbon efflux via dark respiration, photorespiration and extracellular release of organic compounds. Studies of the effect of light quality on these processes has not been reported in a manner integrating them with growth. Light quality may affect algal growth by changes in one or more of these processes controlling carbon balance. In particular, photorespiration which can account for the efflux of 30 to 60% of recently fixed CO_2 in terrestrial C_3 plants (45), is a poorly understood process in algae. Photorespiration may be a significant component of algal carbon metabolism and sensitive to regulation by light quality.

The experiments described in this thesis represent an attempt to analyse the impact of restricted portions of PAR similar in spectral distribution and light intensity to those found in the lower part of the photic zone on the growth, photosynthesis, chemical composition and

carbon metabolism of four unicellular freshwater algae. The major emphasis of this study was to study the uptake rates and partitioning of ^{14}C into various cellular fractions and metabolic processes, such as photorespiration and extracellular release and to relate these to measurements of growth, photosynthesis and chemical composition. The responses of two green algae were to be compared with those of a diatom and a blue-green alga following adaptation to the light source.

MATERIALS AND METHODS

1. Growth conditions

Axenic cultures of Chlamydomonas reinhardtii Dangeard (IUCC 89), Chlorella vulgaris Beijerinck (IUCC 259) and Navicula pelliculosa (Breb.) Hilse (IUCC 668) were obtained from the culture collection at Indiana University, Bloomington, IN47401, U.S.A. (now at the University of Texas) (325). Anacystis nidulans, strain 6301, now designated as the cyanobacterium Synechococcus AN (323) was a gift of Dr. H.D. McCurdy, University of Windsor, Windsor, Ontario, Canada.

Stock cultures were maintained on soil extract slants (325) with the Bristol's solution component modified as follows: KH_2PO_4 was omitted and the K_2HPO_4 reduced to 80 mg/l; $Na_2SiO_3.10H_2O$ titrated with 0.1N HCl to neutrality was added to yield a concentration of 40 mg/l and the medium was buffered with 400 mg/l TRIS-HCl (pH 7.8); the trace element solution of the modified FWT medium was used. Anacystis nidulans was grown on BG-11 slants (324).

Slant cultures were grown under the same lighting conditions as the experimental white light cells. Anacystis nidulans slants were grown at 30°C until growth was well established on the slants and then maintained at 20°C. Slant cultures were periodically checked for contamination using standard sterility test media (152).

Experimental cultures were grown in the freshwater Tryptone (FWT) medium of Darley and Volcani (66) modified as follows: bactotryptone was omitted and the medium was buffered with 600 mg/TRIS-HCl (pH 7.8); a trace element solution containing Na_2EDTA, 4 mg; $FeCl_3.6H_2O$, 2.5 mg; $ZnCl_2.7H_2O$, 0.92 mg; $MnCl_2.7H_2O$, 0.65 mg; $Na_2B_4O_7.10H_2O$, 0.47 mg;

$CoCl_2.6H_2O$, 0.42 mg; $CuCl_2.2H_2O$, 0.25 mg; $Na_2MoO_4.2H_2O$, 0.20 mg; $LiCl_2$, 0.10 mg and a vitamin solution containing thiamin-HCl, 0.05 mg; biotin, 0.01 mg, B_{12}, 0.02 mg were added per liter of medium. In the case of Navicula pelliculosa the medium was supplemented with 2.4 g/l sodium lactate (4 ml/l of 60% sodium lactate). The medium was sterilized by autoclaving 20 min. at 120°C and 15 p.s.i.

Experimental cultures were grown in controlled environment cabinets at 20°C and exposed to a photoperiod of 16h light and 8h darkness. Anacystis nidulans was grown at 30°C. One liter experimental cultures were continuously stirred magnetically in 2.8l Fernbach flasks. The cells were illuminated from above by a bank of 6 Canadian General Electric cool white, blue, green or red fluorescent lamps. Blue 380 and green 480 plexiglas filters obtained from Percival Manufacturing Co., Boone, Iowa 50036, U.S.A., were used in conjunction with the blue and green fluorescent lamps, respectively. An ISCO model SR spectroradiometer, calibrated against spectral intensity standard lamp No. 361 in an ISCO spectroradiometer calibrator model SRC, kindly provided by Dr. Buttery, Agriculture Canada Research Station, Harrow, Ontario, Canada, was used to measure the spectral energy distribution, which was then integrated to determine the total incident energy. The maximum output of the red fluorescent lamps at flask level, approximately 26 cm from lamps, was 540 $\mu W\ cm^{-2}$. Since energy output at wavelengths less than 550 nm was unmeasurable at this level these lamps were used unfiltered. The light intensities of the other light sources were adjusted down to approximately 540 $\mu W\ cm^{-2}$ using layers of white gauze. The spectral energy distribution from each of the light sources is shown in Fig. 2.

Figure 2. Spectral energy distribution of light sources used for growth.

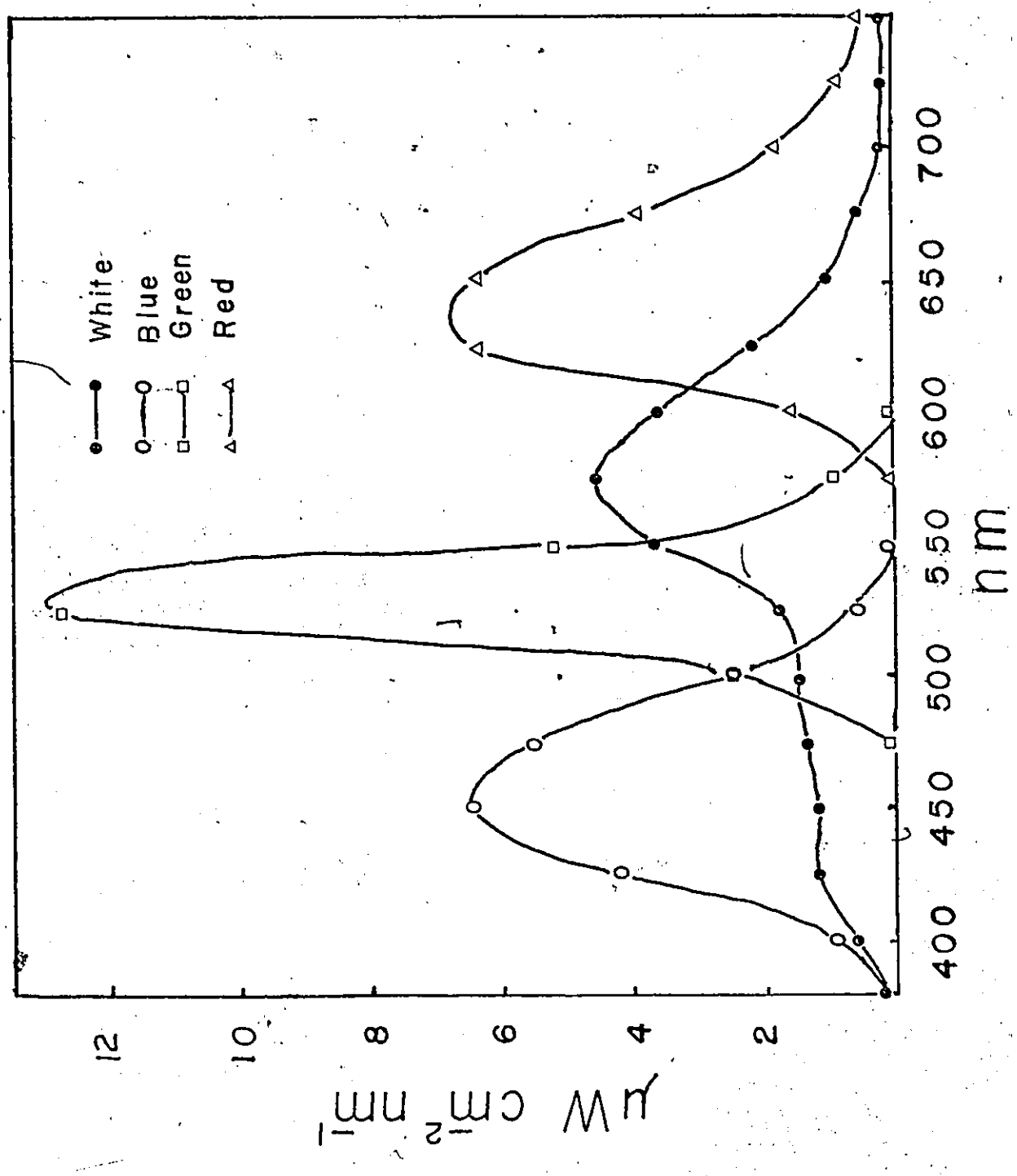

White
Blue
Green
Red
400
450
500
550
600
650
700
nm
2
4
6
8
10
12
μW cm⁻² nm⁻¹

Figure 2.

For growth rate studies, cells were grown with continuous shaking in 125 ml erlenmeyer flasks containing 50 ml medium. Growth rate experiments were performed in quadruplicate. Growth rates were estimated turbidometrically from the increase in absorbance at 540 nm. The following equations were used:

$$k(\text{doublings/day}) = \log_2(A_t/A_i)(1/t)$$
$$= 3.322 \log_{10}(A_t/A_i)(1/t).$$
$$T(\text{mean generation time}) = 1/k$$

where A_i = absorbance at beginning of time interval t, A_t = absorbance at end of time interval t, and t = time interval (in days) of exponential growth. Growth was monitored throughout the exponential growth phase, which was usually from 4- to 10- days duration. To overcome problems of clumping during growth, aliquots of cells used for absorbance measurements were sonicated for 5 to 10 secs. Calibration curves for converting A_{540} to cell number were initially obtained from haemocytometer counts. In later work, calibration curves were obtained using a Coulter Counter, model ZB1 with a 50 µm aperture.

2. Chemical Composition

2.1 Nucleic acid content

2.1.1 Estimation of DNA content

DNA concentration was determined using a modified method of Holm-Hansen et al (148). Ten ml of culture was centrifuged (1000 x g, 10 min.) and the supernatant discarded. Five ml ice-cold 10% trichloroacetic acid (TCA) was added with mixing and the cells let stand on ice for at least 30 min. The TCA was removed by centrifugation and 5 ml ice

cold 95% ethanol saturated with sodium acetate (to remove residual TCA, (143)) was added with mixing. After 15 min. on ice, the 95% ethanol was removed by centrifugation and the rest of the procedure performed at room temperature. Five ml chloroform:methanol (1:2) was added with mixing and removed 15 min. later by centrifugation. The remaining pellet was dried with a gentle stream of air. 100 µl freshly prepared 40% diaminobenzoic acid dihydrochloride in water was added to all test tubes, which were then sealed with Parafilm and heated at 60°C for one hour. 5 ml ice cold 0.5 N perchloric acid (PCA) was added with mixing and let stand for 5 min. followed by centrifugation. Fluorescence of the supernatant was measured using a Turner 430 spectrofluorometer set at 420 nm for excitation and 520 nm for emission. Slit widths were set to 15 nm for excitation and emission. Standard blanks and DNA standards were made by pipetting 100 µl N NH_4OH (blank) and 100 µl N NH_4OH containing 10 and 20 µg calf thymus DNA in test tubes and removing NH_3 with a gentle stream of air before adding diaminobenzoic acid and treating as above. As an independent test of the spectrofluorometer response, the fluorescence of a quinine sulfate standard (0.1 mg/l) was measured using 350 nm for excitation and 450 nm for emission.

2.1.2 Estimation of RNA content

RNA concentration was estimated using the copper-catalysed orcinol reaction of Lin and Schjeide (199). Twenty ml of culture was centrifuged (1000 x g, 10 min.) and the supernatant discarded. Cells were precipitated with 10% TCA, defatted and the resulting pellet dried as described in the DNA determination. One ml N KOH was added with mixing and the cells extracted for 18 to 24 hrs at room temperature. The extraction was

terminated by addition of 3 ml ice cold 0.5 PCA. Cells were allowed to precipitate in the cold for at least 15 min. Following centrifugation, half of the untreated supernatant was removed and transferred to a fresh test tube. The remaining supernatant was treated with 0.5 ml of 30 mg acid activated charcoal/ml for 15 min. Following centrifugation, the treated supernatant was transferred to another fresh test tube. Two ml copper-orcinol reagent (1 ml 50% orcinol in 95% ethanol plus 50 ml 0.15% copper chloride in conc. HCl) was added to treated and untreated supernatants. Samples were boiled for 30 min. cooled on ice and the absorbance at 660 nm read against a PCA precipitated KOH blank. Fifteen µg ribose in PCA precipitated KOH was used as a standard. Subtraction of the absorbance of the charcoal treated portion of supernatant from the absorbance of the untreated portion of the supernatant yielded the absorbance due to the ribonucleotides adsorbed to the charcoal. This value was doubled to account for the initial halving of the supernatant before charcoal treatment. To convert ribose to RNA a factor of 4.5 was used (154).

The use of activated charcoal to discriminate between pentoses and pentose nucleotides (143) was tested by treating mixtures of ribose (3 - 30 µg) and KOH digested yeast tRNA (6 - 60 µg) with activated charcoal. After treatment with charcoal only the added ribose could be detected using the copper-orcinol test. Up to 600 µg KOH digested RNA could be absorbed by 15 mg activated charcoal, the maximum amount of RNA per sample encountered during the study was 99.6 µg.

2.2 Estimation of protein content

Protein concentration was estimated using the Lowry test of

Schacterle and Pollack (298). One or 2 ml of culture was centrifuged (1000 x g, 10 min.) and the supernatant discarded. The TRIS buffer used in the culture medium was found to interfere with this protein test. Therefore cells were resuspended in 1 ml water. One ml of the alkaline copper reagent (containing 2g NaOH, 10g Na_2CO_3, 0.1g potassium tartrate and 0.05g cupric sulphate per 100 ml H_2O) was added and the cells boiled for 5 min., then cooled on ice. Four ml of dilute Folin-phenol reagent (1 ml Folin-phenol reagent (2N) plus 16 ml H_2O) was blown forcibly into the test tube and immediately mixed vigorously. The mixture was heated at 60°C for 5 min., cooled on ice and the absorbance at 750 nm read against a H_2O blank. Eighty and 160 µg bovine serum albumin standards were treated in the same fashion. To avoid frothing, bovine serum albumin was allowed to dissolve in a small amount of distilled H_2O and then gently made up to volume.

2.3 Estimation of carbohydrate content

Carbohydrate content was estimated using the phenol sulfuric acid method of Dubois et al (77) following the precedure of Gershakov and Hatcher (101). One to 2 ml culture was centrifuged (1000 x g, 10 min.) and resuspended in 1 ml H_2O. Replicates were set up in the following fashion:

A. 1 ml sample + 1 ml 10% phenol + 5 ml conc. H_2SO_4.
B. 1 ml sample + 1 ml H_2O + 5 ml conc. H_2SO_4.
C. 1 ml sample + 1 ml 10% phenol + 5 ml H_2O.
D. 1 ml H_2O + 1 ml 10% phenol + 5 ml H_2O.

All test tubes were mixed immediately and the test tubes containing conc. H_2SO_4 cooled on ice. The absorbance at 485 nm was measured against H_2O. Fifty µg glucose standards were treated in a similar fashion. The absorbance of test tubes B to D was subtracted from test tube A to

yield absorbance due to carbohydrate.

2.4 Estimation of lipid content

Lipids were extracted using the method of Bligh and Dyer (19). Lipid content of the chloroform extracts was determined using the phosphovanillin test described by Barnes and Blackstock (9). Ten to 20 ml of culture was centrifuged and the supernatant discarded. The pellet was resuspended in 0.8 ml H_2O, 1 ml chloroform and 2 ml methanol to form a monophasic solution and allowed to extract in the dark at room temperature overnight. The test tubes were centrifuged to remove cells and denatured cellular "scum" and the supernatant transferred to a fresh test tube. Water-soluble impurities were removed by washing with 1 ml 0.1 M KCl and 1 ml chloroform was added with mixing to form a biphasic solution and centrifuged. The final volume of the chloroform extract was measured and the upper aqueous methanol and any remaining interfacial "scum" was discarded. A 0.5 ml aliquot of the chloroform extract was transferred to a fresh test tube and the chloroform evaporated with a stream of nitrogen. A 0.5 ml chloroform blank and 50 and 100 µg lipid standards were evaporated in a similar fashion. The evaporated residue was boiled for 10 min. with 0.5 ml conc. H_2SO_4. Three ml of phosphovanillin reagent (1 g vanillin in 500 ml 11.8 M orthophosphoric acid) was added to 0.2 ml of the H_2SO_4 digest in a fresh test tube with immediate mixing. After 20 to 30 min., the absorbance at 520 nm was measured.

This method, originally devised for total blood lipids, does not seem to have been used for plant or algal extracts. It has considerable potential as a relatively simple method for use in ecological studies.

It depends on the reaction of lipids with H_2SO_4, phosphoric acid and vanillin. The degree of colour development is dependent on the degree of saturation. Zollner and Kirsh (388) showed that although the maximum absorption of the coloured product with various lipids was the same, the relative extinction values were not. On an equal weight basis, linoleic acid was 84.9%; phosphatides 58.9% and stearic acid, 20.8% of the extinction value of cholesterol (388). In the present study the extinction value of stearic acid was 7.4% the value for cholesterol. Cholesterol is generally used as a lipid standard in animal studies, however, cholesterol is not widespread in plant tissues (133), therefore it was decided to use olive oil (12% saturated fatty acids, 80% oleic acid and 8% linoleic acid (113) as an easily obtainable and reasonably typical mixture of plant saturated and unsaturated fatty acids. Therefore 50 and 100 µg olive oil standards dissolved in chloroform:methanol (1:2) were used in this assay and µg lipid/10^6 cells is reported on this basis. Chlorophylls and carotenoids with their - C = C - bonds will react with the colour reagent, however, the reported lipid values are not corrected for this, since the degree of colour formation by these pigments is unknown.

2.5 Photosynthetic pigment analysis

Cells from 10 or 20 ml aliquots of culture were extracted overnight in 90% acetone containing 1 or 2 drops of 1% $MgCO_3$ in darkness at 4°C. Initially Chlorella vulgaris cells in 2 ml 90% acetone containing glass powder (ca. 35 µ diameter) were sonicated for up to 3 min. in 30 sec. bursts alternating with 30 sec. of cooling using a Branson sonifier (model 140) with microtip attachment. In later experiments, cells were sonicated for 30 sec. (no glass powder) in 2 ml methanol. Following

sonication, cells were made up to volume with solvent and extracted overnight as above. Phycocyanin was extracted from Anacystis in dilute phosphate buffer (M/30, pH 7) by sonication for 30 secs and overnight extraction in the cold. Following pigment extraction, cells were removed by centrifugation and the absorbance of the supernatant at the appropriate wavelength read against a solvent blank. Readings were corrected for absorbance at 750 nm and pigment concentrations calculated using the following equations:-

A. Chlorella and Chlamydomonas (90% acetone)

Chlorophyll a = $11.6\ A_{664} - 1.35\ A_{645}$

Chlorophyll b = $19.1\ A_{645} - 4.65\ A_{664}$

Total carotenoids = $4\ A_{480}$

B. Chlorella (Methanol)

Chlorophyll a = $16.5\ A_{665} - 8.3\ A_{650}$

Chlorophyll b = $33.8\ A_{650} - 12.5\ A_{665}$

Total carotenoids = $4\ A_{480}$

C. Navicula (90% acetone)

Chlorophyll a = $10.9\ A_{664} - 0.454\ A_{631}$

Chlorophyll c = $26.8\ A_{631} - 4.07\ A_{664}$

Total carotenoids = $10\ A_{480}$

D. Anacystis (90% acetone)

Chlorophyll a = $11.4\ A_{664}$

Total carotenoids = $4\ A_{480}$

E. Anacystis (M/30 phosphate)

Phycocyanin = $111.7\ A_{620} - 28.7\ A_{680}$

The following equation,

F. $C_{abc} \times \nu(ml)/l(cm) \times V(l) =$ ng pigment/ml

was used to correct for sample volume (V), solvent volume (ν) and cuvet path length (cm). C_{abc} is the value obtained from equations A to E. Equations in A were derived from the absorption coefficients for chlorophyll-a and b in 90% acetone given in Parsons and Strickland (255). Equations in B are those of Holden (142) originally derived from the extinction coefficients for chlorophylls a and b in methanol published by Mackinney (212). Equations in C are those of Wasley et al (366). Equations in D are derived from Parsons and Strickland (255). The equation in E is based on the following rationale. The standard method for phycocyanin estimation is to calculate the absorbance due to PC at 620 nm in aqueous extracts by correcting for dissolved chlorophyll (absorption maximum at 680 nm) using equations similar to those published by Myers and Kratz (226). This corrected absorbance at 620 nm is then multiplied by a factor based on published extinction coefficients for phycocyanin (26, 61, 333). Recent studies on the structure of Anacystis nidulans phycocyanin suggest that it exists as the hexamer in aqueous solutions of circumneutral pH. (105). Each monomer consists of an α and β sub unit, there being 3 phycobilin chromatophores per monomer. Taking the molar absorption coefficient of the hexameric form calculated by Myers et al (225) converted to ml mg^{-1} cm^{-1} and multiplying the equation of Myers and Kratz (226) by this value yields equation E.

3. Measurement of Light Absorption

3.1 In vivo absorption spectra measurement

In vivo absorption spectra were obtained by vacuum filtering enough cells onto 25 mm membrane filters (Sartorius SM 11304) to yield

approximately one unit of absorbance at 540 nm per cm^2. The filters were cleared with glycerol (99%) and sandwiched between glass slides (380). The cells-filter-glass slide sandwich was scanned in the normal sample position of a Bausch and Lomb spectronic 505 spectrophotometer with a piece of opalescent polyethylene (1.5 mm thick) on the side of the sandwich facing the detector (309). A similar glycerol cleared filter-glass slide - polyethylene sandwich without cells was placed in the reference position. Spectra from 380 to 600 nm were obtained using a UV-visible phototube and from 600 to 720 nm with a red-sensitive photomultiplier tube. Wavelength and absorbance accuracy were checked using the built-in mercury lamp and standard solutions of $CuSO_4$ or $K_2Cr_2O_4$ respectively. In vivo absorbance spectra in Figures 4 to 7 have been vertically separated to avoid overlap.

3.2 Calculation of quanta flux of each growth light source

The energy readings ($\mu W\ cm^{-2}.nm^{-1}$) (Fig. 2) for each light source obtained at 25 nm interval using the ISCO spectroradiometer were converted to $quanta.cm^{-2}.nm^{-1}$ using the following formula derived from (221).

$$\frac{\text{Quanta } m^{-2}.s^{-1}}{\mu W\ m^{-2}} = \frac{\lambda}{hc} = \lambda(nm) \times 0.5035 \times 10^{10}$$

where $h = 6.6255 \times 10^{-34}$ Joules s^{-1} and $c = 2.9979 \times 10^{17}$ nm s^{-1}.

The average quanta/μW for each 25 nm waveband was estimated by calculating the quanta flux per μW at the wavelength on either side of the waveband, taking the mean of both values and multiplying it by the integrated energy reading for the waveband.

3.3 Calculation of light absorption factors

The amount of light absorbed by the cells used for *in vivo* absorbance measurements was calculated by converting the absorbance, (A)

$$A = \log \frac{I_o}{I}$$

where I_o = incident light and I = transmitted light, at the same wavelengths used for energy measurements to absorptance (α), the fraction of incident light absorbed,

$$\alpha = 1 - \frac{I}{I_o}$$

The absorptance was multiplied by the incident energy of the light source measured at that wavelength to obtain the quantity of light absorbed by the cells. The amount of light absorbed by the cells was integrated for the waveband of the light source under consideration.

4. Measurement of Photosynthesis

4.1 $^{14}CO_2$ uptake

To measure CO_2-fixation rates, cells were resuspended in growth medium with the TRIS-HCl omitted (pH was adjusted to pH 7.8 with dilute HCl). The TRIS-HCl was omitted to facilitate the estimation of total inorganic carbon. One to four µCi of $Na_2\ ^{14}CO_3$ was added to 50 ml aliquots of cells in three 125 ml erlenmeyer flasks. One flask was wrapped in aluminum foil as the dark control. An aliquot of the same activity was placed in 10 ml scintillation fluid. Flasks were incubated for 1 hr in the growth cabinet under the same light and temperature conditions as for growth. The experiment was terminated by filtering the cells through a Sartorius SM 11304 filter (47 mm, pore diameter 0.8 µ)

using a vacuum pressure of 25 cm Hg. The filters were rinsed with 2 vols of incubation medium and placed in 10 ml TEG scintillation cocktail (359). The scintillation vials were shaken by hand until the membrane filters dissolved and counted in a Nuclear Chicago Mark II scintillation spectrometer. Counting efficiency was calculated by the channels ratio method (36) and counts converted to dpm.

CO_2 fixation rates were calculated as follows:

nmoles CO_2 incorporated/10^9 cells/hr

$$= 1.05 \frac{(\text{dpm incorporated/50 ml})(\text{nmoles } CO_2\text{/50 ml})}{(\text{dpm added/50 ml})(\text{time in hours})(10^9 \text{ cells/50 ml})}$$

µmoles CO_2 incorporated/mg Chl/hr

$$= 1.05 \frac{(\text{dpm incorporated/50 ml})(\text{µmoles } CO_2\text{/50 ml})}{(\text{dpm added/50 ml})(\text{time in hrs})(\text{mg chl/50 ml})}$$

Total inorganic carbon (µmoles CO_2) was estimated by titrating 100 ml of the incubation medium with dilute HCl to the total alkalinity endpoint using a mixed methyl red/bromocresol green indicator as outlined in Golterman (111).

4.2 O_2 - evolution

Cells were harvested by centrifugation at 1000xg for 10 min. and resuspended in 50 mM K_2HPO_4 (pH 7.9). O_2-evolution was measured using a thermostatted Clark-type O_2-electrode (Hansatech Ltd., Kings Lynn, Norfolk, U.K.) (68). Electrode output was recorded on the 1 mV range of a Metrohm chart recorder (Laborgraph E 478). The O_2-electrode was calibrated by injecting µl amounts of 0.12 M H_2O_2 into freshly prepared 20% catalase solutions. The cells were illuminated with light from a cool white fluorescent lamp (GE F15.T12.GW) or blue, green or red light isolated

Figure 3. Spectral transmittance of filter combinations used to isolate blue (peak wavelength 465 nm), green (peak wavelength 535 nm) or red (peak wavelength 638 nm) light for O_2-evolution measurements.

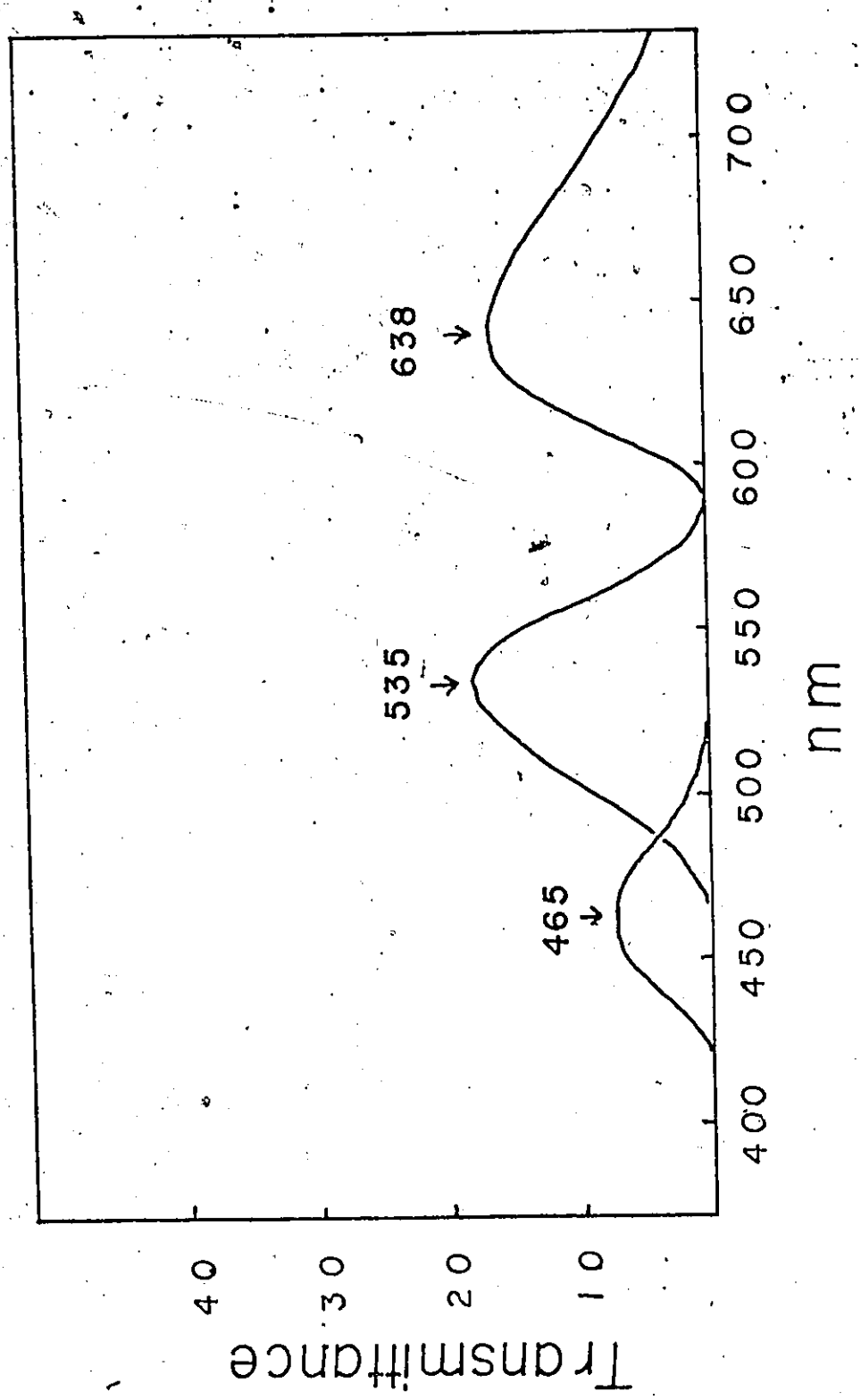

Transmittance
40
30
20
10
400
450
500
550
600
650
700
nm
465
535
638

Figure 3.

from a 150-W tungsten lamp (GE 150 PAR/FL). Red light was obtained using one layer of Cinemoid No. 6 (primary red) (Strand Century Ltd., 6334 Viscount Rd., Malton, Ont.) plus 10 cm of ferrous ammonium sulfate solution (300 g/l containing 1% H_2SO_4) (377). Blue and green light was obtained using one layer of Cinemoid No. 20 (Deep Blue) or Cinemoid No. 39 (Primary green) plus 6 cm of ferrous ammonium sulfate (300 g/l containing 1% H_2SO_4) plus 6 cm tapwater respectively. The spectral transmittance properties of these filter combinations is shown in Figure 3. The rate of O_2-evolution was compared at 600 μW cm^{-2} for each of the light qualities to simulate growth light intensities. The maximum light intensity of blue light that could be obtained was about 800 μW cm^{-2}. Consequently, light saturation curves were determined using cool white fluorescent light only.

The light intensities of the various light sources used in the O_2-evolution rate determinations were measured with an 1L 500 radiometer (International Light Inc., Newbury port, Mass., 01950, U.S.A.) equipped with an SEA 010 photodetector. The radiometer was calibrated against a standard lamp kindly supplied by Dr. R.W. Nicholls, CRESS, York University, Downsview, Ont.).

5. Measurement of photosynthetic carbon metabolism

5.1 ^{14}C-incorporation and extraction procedures

One liter algal cultures were harvested by centrifugation and resuspended in 200 ml growth medium with the TRIS-HCl omitted (pH was adjusted to pH 7.8 with dilute HCl). Thirty ml of this suspension was removed to provide aliquots for measurement of A_{540} and pigment concentration. The remaining cells were returned to the appropriate light

quality in the growth chamber and allowed to equilibrate for 1 hr.

One hundred µl (200 µCi) $Na_2{}^{14}CO_3$ was added to each flask and the flasks swirled for ca. 0.5 min. A 40 ml aliquot was removed immediately and filtered through a bed of Supercel (Johns Mannville) (1-2 g) overlaying a Whatman GF/C glass fiber filter (47 mm diameter) using a vacuum pressure of 25 cm Hg. The cells were rinsed with 50 ml Tris-free growth medium.

The resulting Supercel "cake" and the glass fiber filter was transferred to 50 ml boiling 80% ethanol in a 125 ml erlenmeyer flask and boiled for 10 min. An inverted glass funnel in the neck of the flask was used to prevent excessive loss of volatile compounds. From removal of the 40 ml aliquot of cells to transfer of the Supercel "cake" and filter to boiling 80% ethanol usually took 4 to 5 min. Forty ml aliquots were also sampled at 20 and 40 min. after addition of the $Na_2{}^{14}CO_3$.

The initial filtrate and TRIS-free growth medium rinse of the 20 and 40 min. samples was collected to investigate extracellular release of photosynthate. To monitor ^{14}C-uptake into whole cells, 2 to 5 ml aliquots were filtered on membrane filters as described in the ^{14}C-uptake section.

The hot ethanol soluble fraction was obtained by transferring the resuspended Supercel "cake" to another Supercel/glass fiber filter combination and rinsing with 50 ml cold 80% ethanol and 50 ml boiling H_2O. The remaining "cake" with insoluble materials was stored at $-20^{o}C$ for later analysis. The pH and volume of the hot ethanol soluble fraction was measured. A 200 µl aliquot was added to 10 ml scintillation fluid and counted. The pH of the hot ethanol soluble fraction was generally close to neutrality. The hot ethanol soluble fraction was then

rotary evaporated to a small volume at 20°C using the vacuum produced by an aspirator (ca. 1 atm). Preliminary tests using the Calkins method (37) of glycolate determination showed that under these conditions, the loss of glycolate from an 80% ethanol solution was 13 to 14%. The use of higher temperatures (35°C) and/or acidic conditions led to larger losses (> 85%) of this volatile compound. This resulting ethanol soluble fraction was extracted at room temperature with an equal volume of chloroform in a separating funnel to remove extracted pigments and lipids and also to precipitate soluble proteins. The volume of the chloroform soluble fraction (usually pale green in colour) was measured, 200 µl transferred to 10 ml scintillation fluid for counting and the rest of the extract discarded. The aqueous phase was refiltered on a super-cel/glass fiber filter combination with some dist. H_2O rinsing. The volume of the water soluble filtrate was measured and 200 µl used for liquid scintillation counting. The insoluble residue on the supercel/glass fiber filter combination was pooled with the previous insoluble fraction and stored at -20°C.

5.2 Determination of radioactivity in the extracellular release fraction

The initial filtrate and medium rinse of the 20 and 40 min. samples was acidified (final pH 2) with 2 ml N HCl and aerated vigorously for 30 min. with CO_2-free air to remove $^{14}CO_2$. Following degassing 2 ml N NaOH was added (final pH close to neutrality), the final volume of the fraction noted and 200 µl used for liquid scintillation counting.

5.3 Determination of radioactivity in the insoluble fraction

Radioactivity in the insoluble residue was determined following

wet oxidation using the Mahin-Lofberg perchloric acid-peroxide method (202). Fractions with large amounts of Supercel were incubated at 70 - 80°C in 50 ml erlenmayer flasks with 4 ml 60% perchloric acid and 8 ml 30% hydrogen peroxide for 30 min. 300 µl aliquots of the supernatant were added to 5 ml 2-methoxyethanol and 10 ml toluene containing 6 g 2,5-diphenyloxazole per liter for LSC. 1,4-bis [2(5-phenyloxazolyl)] benzene was omitted since it forms a highly coloured compound with the wet oxidation reagents. Other fractions with smaller amounts of Supercel were placed in scintillation vials with 2 ml 60% perchloric acid and 4 ml 30% hydrogen peroxide and incubated as described above. 400 µl aliquots of the resulting supernatants were counted in the 2-methoxyethanol- 2,5-diphenyloxazole-toluene scintillation fluid.

5.4 Fractionation of H_2O-soluble and extracellular release fractions on ion exchange resins

The H_2O-soluble and the degassed extracellular release fractions were separated into neutral, basic and acidic components by passage through the strong cation exchange resin Rexyn 101(H^+) (Fisher R-203, 16-50 mesh) as supplied and the strong base anion exchange resin Rexyn 201 (OH^-) (Fisher R-205, 16-50 mesh) in the formate form. Resin columns were prepared as follows: a) cation resin - this was usually used as supplied following washing with dist. H_2O. A 5 ml wet bed volume in a 5 ml plastic disposable syringe was used; b) anion resin - this is usually supplied in ammonium hydroxide which was washed off with distilled water, the resin was then boiled in 4 M formic acid and then washed with distilled H_2O until the effluent pH > 4.5. A 10 ml wet bed volume in a 10 ml disposable plastic syringe was used. The syringes were set up so that the effluent of the cation resin drained into the anion resin. In a typical

fractionation, the resins were first rinsed with 60 ml dist. H_2O, then the sample was run through both columns, followed by another 60 ml dist. H_2O rinse. The fraction emerging from both columns and the dist. H_2O wash was termed the neutral fraction and was assumed to contain free sugars, disaccharides and polyols. Sixty ml of 2 N NH_4OH was used to elute the basic fraction from the cation resin. This fraction was assumed to contain mainly amino acids. Sixty ml of 4 M formic acid: 4M ammonium formate (4:1) was used to elute the acidic fraction from the anion resin (11). This fraction was assumed to contain organic acids, their phosphates and sugar mono-and diphosphates. The volume of each fraction was noted and 200 µl used for liquid scintillation counting. 67.4 $\pm$ 14.5%, n = 32, of the total radioactivity present in the H_2O-soluble extract was recovered in these 3 fractions.

5.5 Measurement of $^{14}CO_2$ release in the light and dark

Measurement of $^{14}CO_2$ release was based on the method of Zelitch (383) as modified by Cheng and Colman (43). About 1 hr after the addition of $Na_2{}^{14}CO_3$ to the cells, a 40 ml aliquot of the remaining culture was transferred to a modified 200 ml pipet and bubbled with CO_2-free air (Canadian Liquid Air - < 5 ppm CO_2) in the light for 30 min. to remove residual $^{14}CO_2$. The 200 ml pipet was modified by bending the long tip so that it was parallel to and above the liquid level in the bulb of the pipet and thus acted as a gas inlet. The portion of the pipet above the bulb was cut off and flared out to facilitate addition and removal of the cell suspension. This exit port was fitted with a one hole rubber stopper attached to tygon tubing. During the 30 min. flush with CO_2-free air the gas exiting from the 200 ml pipet was trapped in a large

excess of NaOH solution to prevent contamination of the atmosphere with $^{14}CO_2$.

Following this flushing period, the exit port of the 200 ml pipet was attached to a CO_2-trapping device consisting of a gas dispersion tube in a 100 ml graduate cylinder containing 60 ml monoethanolamine:2-methoxyethanol (2:1). The pipet was positioned under the growth chamber lights in the same position as the culture and 1 ml aliquots of the CO_2-trapping solution were transferred to 10 ml scintillation fluid every 3 min. After 15 min. the pipet was covered with aluminium foil and the lights were turned off to measure dark $^{14}CO_2$ release. The flow rate of the CO_2-free air was 500 - 600 ml per min. as measured with a Labcrest flowmeter (Fisher Scientific Co.).

5.6 Measurement of $^{12}CO_2$ and $^{14}CO_2$ release in the light and dark

A sensitive gas chromatrographic technique in which CO_2 is converted to methane and detected by flame ionization was adapted to measure the differential influx and efflux of ^{14}C and ^{12}C into algae in a sealed aqueous system.

Algae were grown in white light as described above, harvested by centrifugation and resuspended in 100 ml 0.05 M K_2HPO_4 (pH 7.8). Cells were incubated with magnetic stirring at growth temperature in a water jacketed glass cylinder (internal diameter 35 mm) sealed at the top with a 1 - 2 cm layer of parafin oil. This acted as an effective liquid seal to keep atmospheric CO_2 out of the cylinder. Cells were illuminated with a coolwhite fluorescent lamp. The light intensity at the center of the cylinder was 600 $\mu W\ cm^{-2}$. A 10 µl aliquot of $Na_2{}^{14}CO_3$ (1 - 2 µCi) was added and a 5 ml sample immediately withdrawn with a

Figure 4. Schematic diagram showing modifications to the gas chromatograph for measuring dissolved CO_2 as methane.
A: gas stripping column;
B: 4-way valve;
C: oven containing a 6 mm o.d. x 1.4 m coiled glass column;
D: oven containing nickel catalyst in a 6 mm o.d. x 10 cm stainless steel tube;
E: FID;
F: electrometer;
G: Varian A-25 recorder (2 mv full scale, 25 inch hr^{-1});
H: Varian Aerograph 2740 gas chromatograph.
For further explanation see text.

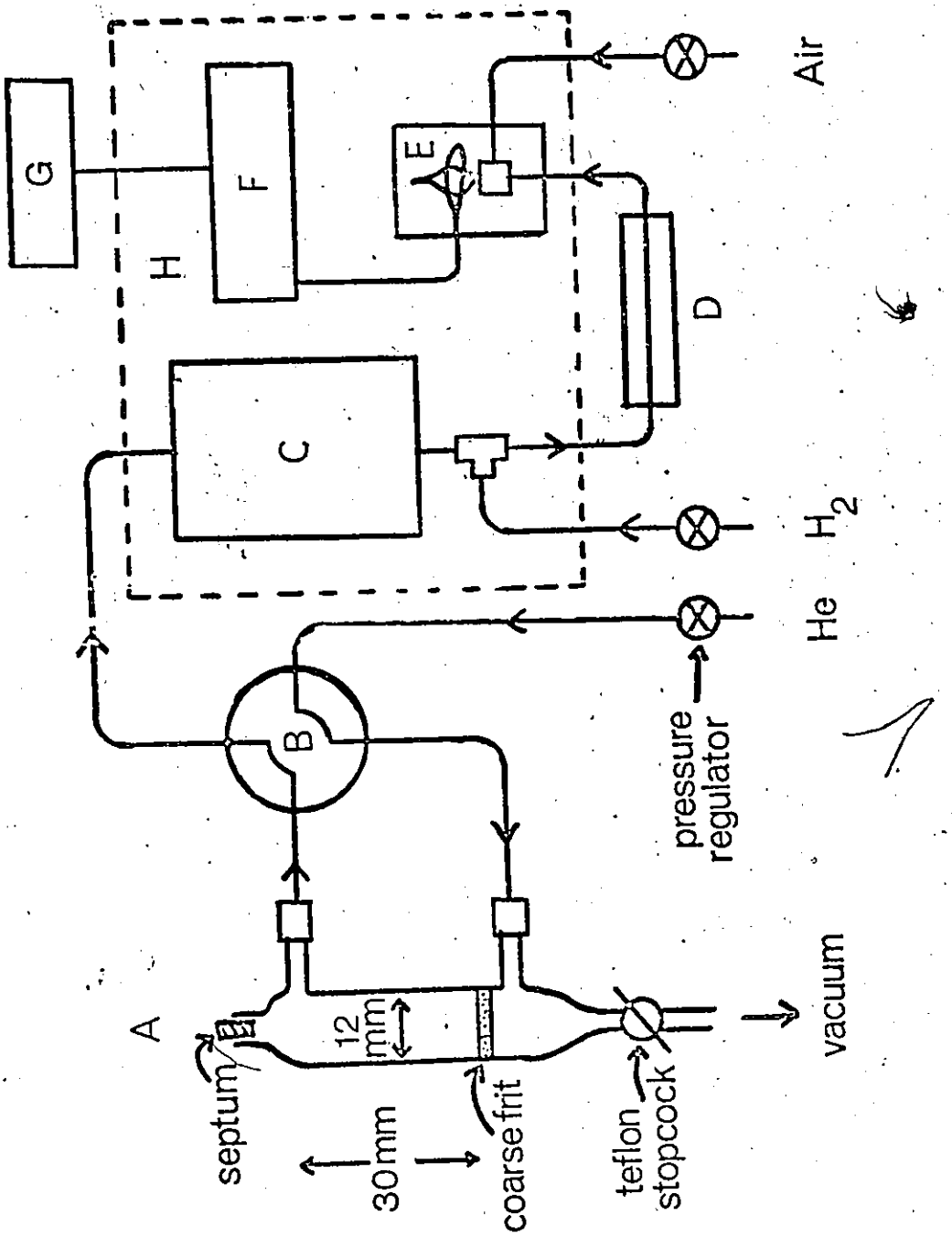

G
F
H
E
D
C
B
A
Air
H_2
He
pressure regulator
vacuum
septum
12 mm
30 mm
coarse frit
teflon stopcock

Figure 4.

syringe via a septum stoppered port at the side of the cylinder. The contents of the syringe were passed through an AP20 or AP25 prefilter and a 0.8 µm Sartorius membrane filter held in a 13 mm diameter Swinnex membrane filter holder (Millipore Corp.) attached to the syringe. One ml of the cell-free medium produced in this way was added to 10 ml of Bray's scintillation cocktail (24), and 1 ml of the same filtrate was injected directly into the gas-stripping column of the gas chromatograph through a hypodermic needle to minimize contamination with atmospheric CO_2.

The flow scheme of the modified gas chromatograph used in this study is shown in Figure 4. The incoming cell-free sample was mixed in the gas stripping column with 0.2 ml previously injected 50% H_3PO_3, and the dissolved gases were stripped from solution by the carrier gas onto the column (334). The detector hydrogen supply was moved upstream so that the CO_2 peak emerging from the column was mixed with H_2 prior to the catalyst oven. The CO_2 is converted to methane by the hydrogenation process of Sabatier and Senderens (1902)(cited in 375), which requires elevated temperatures and a nickel catalyst as follows:

$$CO_2 + 4H_2 \xrightarrow[Ni]{300\text{-}400^{\circ}C} CH_4 + 2H_2O$$

The nickel catalyst packing was prepared using Celite (Johns-Manville) instead of brick dust (269) and was heated externally to the gas chromatograph with Electrothermal heated tape (Canadian Laboratory Supplies). Optimum catalyst temperature was between 350°C and 400°C (326, 375).

Operating conditions for the gas chromatograph were as follows: helium carrier gas flow 25 ml/min.; hydrogen gas flow 25 ml/min.; air

flow 275 ml/min.; injection port temperature 110°C; column temperature 60°C; detector temperature 125°C. The response of the gas chromatograph to CO_2 was calibrated by injecting µl amounts of Na_2CO_3 solutions prepared with boiled distilled H_2O.

The change in the concentrations of $^{14}CO_2$ and CO_2 in the medium was measured at regular intervals until the external inorganic carbon reached a limiting value. The efflux of both of these carbon isotopes was then measured at regular intervals in the dark.

6. Statistical treatment of data

A one-way analysis of variance to determine the presence of significance between the light quality treatments was performed using the sub-program ONEWAY of the Statistical Package for the Social Sciences (SPSS) (232). The results in Tables 1 to 11 and 20 represent mean values from three to six experiments for each light treatment. The underlines connect homogeneous subsets whose highest and lowest means do not differ by more than the shortest significant range. A posteriori multiple range tests following the LSD, Student-Newman-Keuls and Scheffes procedures were applied at the $\rho < 0.05$ level. The data points in other Tables and Figures represent the mean of at least two replicates.

RESULTS

1. The Effect of Light Quality on Growth

The effect of light quality on the growth constant (k) and generation time (T) of the four algae used in this study is shown in Tables 1 and 2. The growth rate of Navicula pelliculosa was significantly higher in red light than in any of the other light treatments ($P < .05$). Growth of this alga in green and white light was higher than in blue light. Growth of Chlamydomonas reinhardtii in white light was significantly higher than in red light ($P < .05$). In both blue and green light, growth rates were significantly lower than in either white or red light treatments. Growth rates of Chlorella vulgaris in blue, green and red light were not significantly different, however, growth was significantly more rapid in white light ($P < .05$). The highest growth rate of Anacystis nidulans was in red light while the growth rates in white, blue and green light do not appear to be significantly different. However, the turnover times (T) in blue and green light were significantly longer than in either white or red light ($P < .05$).

2. The Effect of Light Quality on Chemical Composition

2.1 Nucleic acid content

The DNA content of Anacystis nidulans cells grown in green and red light was about twice that found in cells grown in white and blue light (Table 3). A partial explanation for the significantly higher DNA content ($P < .05$) of red grown cells when compared to white and blue grown cells is the finding of Mann and Carr (216) that the cell volume and hence DNA and RNA content of Anacystis nidulans increases as a

Table 1. The effect of light quality on the growth constant (k)(doublings/day) of four freshwater algae.

	Spectral Quality of Light (550 μW cm^{-2})			
	White	Blue	Green	Red
Navicula pelliculosa	0.352 ± 0.032	0.290 ± 0.045	0.416 ± 0.033	0.722 ± 0.037
Chlamydomonas reinhardtii	0.433 ± 0.026	0.135 ± 0.043	0.152 ± 0.010	0.280 ± 0.033
Chlorella vulgaris	0.598 ± 0.146	0.361 ± 0.064	0.331 ± 0.035	0.245 ± 0.039
Anacystis nidulans	0.358 ± 0.024	0.194 ± 0.022	0.223 ± 0.074	0.932 ± 0.112

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 2. The effect of light quality on the generation time (T) (days) of four freshwater algae.

	Spectral Quality of Light (550 μW cm^{-2})			
	White	Blue	Green	Red
Navicula pelliculosa	2.86 ± 0.27	3.52 ± 0.62	2.42 ± 0.19	1.39 ± 0.07
Chlamydomonas reinhardtii	2.32 ± 0.14	7.95 ± 2.27	6.60 ± 0.41	3.61 ± 0.39
Chlorella vulgaris	1.73 ± 0.34	2.84 ± 0.51	3.04 ± 0.31	4.15 ± 0.63
Anacystis nidulans	2.81 ± 0.2	5.19 ± 0.59	4.76 ± 1.38	1.09 ± 0.14

Numbers connected by a common underline are not significantly different ($P < 0.05$).

function of growth rate. The growth rate of red light grown Anacystis cells was significantly higher than all other light treatments. The DNA content of Anacystis nidulans with a growth rate of 0.04 hr^{-1} i.e. similar to red light cells, was reported by Mann and Carr (216) to be 0.06 µg DNA/10^6 cells and shows remarkable agreement with my results. The high DNA content of green light grown Anacystis cannot be explained on this basis. RNA content of Anacystis nidulans at this growth rate was reported as 0.3 µg RNA/10^6 cells (216). My result is about one half this value, however, my method of RNA estimation is more conservative, not being based on total orcinol positive material in the extract. While RNA content was highest in red light, there was no significant difference in the RNA content of cells grown under any of the light sources.

While the DNA and RNA content of Navicula pelliculosa was highest in blue light grown cells and lowest in red light cells, these differences were not significant (Table 4). The DNA content of Navicula pelliculosa was previously reported to range from 0.08 to 0.11 µg per 10^6 cells (57). RNA content was reported to range from 0.38 to 0.46 µg per 10^6 cells (57). My results show excellent agreement with these published values.

When compared to white, blue and red light grown Chlorella vulgaris cells, green light cells had a significantly lower DNA content (Table 5). Red light grown cells had a significantly higher DNA content ($P < 0.05$) than cells grown in the other three light regimes. The DNA content of Chlamydomonas reinhardtii cells grown in green and red light was higher than in blue or white light grown cells, however, these differences in DNA content were not significantly different (Table 6). No significant differences in the RNA content of Chlorella or Chlamydomonas

cells grown under the various light qualities were found.

DNA values of 0.082 to 0.186 µg per 10^6 cells have been reported for the thermophilic strain of Chlorella pyrenoidosa 7-11-05 (= C. sorokiniana) (41,150) and 0.099 to 0.12 µg per 10^6 cells for Chlorella pyrenoidosa 211/8b (364). The RNA content of Chlorella pyrenoidosa 211/8b has been reported to range from 1.00 to 1.51 µg per 10^6 cells (364). The DNA content of Chlamydomonas reinhardtii has been reported as 0.124 to 0.20 µg per 10^6 cells (41, 198). Chlamydomonas meowusii was reported to contain 0.055 to 0.095 µg DNA per 10^6 cells and 0.34 to 1.2 µg RNA per 10^6 cells (50). My results show reasonable agreement with these reports given the diversity of techniques used for measuring nucleic acid content.

2.2 Carbohydrate content

The carbohydrate content of Anacystis nidulans showed a high degree of variation and while the carbohydrate level was highest in green light grown cells and lowest in red light cells these differences are not significant (Table 3).

Blue-green algae form a storage carbohydrate which is a polymer of glucose with a degree of branching between glycogen and amylopectin. The occurrence of a glycogen-type α-glucan has been demonstrated in Anacystis nidulans (371). The glycogen content of Anacystis nidulans cells rises sharply during exponential growth and reaches a constant level during stationary growth (195). This very large change in glycogen content of Anacystis cells during exponential growth may explain the large variation in carbohydrate content found in the present study. Large variation in the carbohydrate content of planktonic blue-green algae in nature in relation to depth and nutrient deficiency have been reported (103).

The carbohydrate content of Navicula pelliculosa grown in blue light was significantly higher when compared to cells grown in white, green and red light (Table 4). The lowest level of carbohydrate was found in red light grown cells and this was significant when compared to white, blue and green light grown cells ($P < 0.05$). Coombs et al (57) reported carbohydrate levels ranging from 4.4 to 5.3 µg per 10^6 cells for this diatom.

Carbohydrates in diatoms are present in the cells as reserve polysaccharides and as cell wall constituents. The principal reserve polysaccharides in freshwater diatoms is chrysolaminarin, a water soluble β-1, 3 glucan (65). The capsule produced by stationary phase cells of Navicula pelliculosa is a polyuronide composed of glucuronic acid residues (197).

No significant difference in the carbohydrate content of Chlorella vulgaris and Chlamydomonas reinhardtii cells grown in white, blue and red light was found (Tables 5 and 6). Carbohydrate content was lowest in green light grown cells, significantly so in Chlamydomonas but not in Chlorella. Hase et al (125) reported that the carbohydrate level of Chlorella ellipsoidea ranged from 2 to 9 µg per 10^6 cells in synchronized cultures. Duynstee and Schmidt (80) reported that the starch content of synchronized Chlorella pyrenoidosa 7-11-05 rose from 1 µg per 10^6 cells to 5 µg per 10^6 cells over the course of the cell cycle. Guerin-Dumartrait et al (121) found that the carbohydrate content of Chlorella pyrenoidosa ranged from 2.8 to 7.5 µg per 10^6 cells during a study of nitrate deficiency. The starch content of Chlamydomonas reinhardtii has been reported as 3.3 µg per 10^6 cells (198). These cells had a dry weight of 28.0 µg per 10^6 cells.

2.3 Protein content

No significant difference was found in the protein content of Anacystis nidulans cells grown under the various light sources (Table 3). The highest protein level was found in blue light cells and the lowest level was found in red light cells, which would appear to reflect the differences in phycocyanin content. No published reports appear to be available giving protein content per cell for blue-green algae. Reported protein or organic nitrogen values range from 44 to 56% (93, 254). Total organic nitrogen content (% dry weight), of Anabaena cylindrica was reported to rise with increasing growth rate (93), presumably in an analogous fashion to nucleic acid content and cell volume (216). Daley and Brown (64) showed that the organic nitrogen content per unit carbon of Anacystis nidulans rose two-fold during exponential growth and then dropped sharply when nitrate became limiting in the medium.

The protein content of Navicula pelliculosa cells grown under white, blue, green or red light was not significantly different (Table 4). Coombs et al (57) reported protein levels of 10 to 13 µg per 10^6 cells for synchronized cultures of Navicula pelliculosa.

The protein content of green light grown cells of Chlamydomonas reinhardtii was significantly lower when compared to white, blue or red light grown cells (Table 6). The protein content of white or blue light grown cells was significantly higher than green or red light grown cells ($P < 0.05$). No significant changes in protein content of Chlorella cells grown in various light qualities was observed (Table 5). A similar trend in protein content to Chlamydomonas, in that white or blue light grown cells had higher levels than green or red light cells was observed. Hase et al (125) reported that Chlorella ellipsoidea contained 10 to 20 µg

protein per 10^6 cells. Hopkins et al (150) reported a protein content of 1.33 g per 10^6 cells for Chlorella pyrenoidosa 7-11-05. Guerin-Dumartrait et al (121), found 1.52 to 4.2 µg protein per 10^6 cells in Chlorella pyrenoidosa 211/8b. Wanka et al (364) reported values of 4.9 to 6.3 µg protein per 10^6 cells for the same strain of Chlorella pyrenoidosa. Lien and Knutsen (198) found 14.7 to 14.9 µg protein per 10^6 cells in Chlamydomonas reinhardtii. My protein values for Chlorella vulgaris show good agreement with these published reports.

2.4 Carbohydrate:Protein Ratio

The carbohydrate:protein ratio was lowest in red light grown Anacystis nidulans cells and highest in blue and green light grown cells, these differences were not significant (Table 7). The carbohydrate:protein ratio was significantly higher ($P < 0.05$) in white and blue light grown cells of Navicula pelliculosa when compared to green and red light grown cells. This reflects the lower carbohydrate content of green and red light grown cells. The carbohydrate:protein was significantly higher in red light grown Chlamydomonas reinhardtii when compared to white, blue or green light grown cells ($P < 0.05$). This ratio was lowest in white light grown cells when compared to the three other light treatments. A similar situation was found in cells of Chlorella vulgaris, however, the carbohydrate:protein ratios were not significantly different in this case.

2.5 Lipid Content

Significantly higher levels of lipid were found in cells of Anacystis nidulans grown in green light when compared to cells grown in

white, blue and red light (Table 3). When compared to blue, green or red light grown cells the lipid content was significantly lower in white light grown cells ($P < 0.05$).

While the lipid composition of blue-green algae has been exhaustively analysed (170) very few reports of total lipid content in terms of dry weight or cell number exist. The fatty acid content of Anacystis nidulans has been reported as 11% dry weight (140).

Large variation in the lipid content of Navicula pelliculosa was observed and while lipid content was highest in blue and red light grown cells and lowest in green light cells, these differences were not significant (Table 4). The accumulation of fat by diatoms in response to nutrient deficiency or unfavourable growth conditions is well documented (57, 65, 248). In synchronized cultures of Navicula pelliculosa the lipid content of cells rose almost 2-fold during the transition from mid-exponential to early stationary phase of growth (57). The fact that cells were usually harvested during mid to late exponential phase in the present study appears to be reflected in the large variation in lipid content observed. The lipid content of Navicula pelliculosa was reported to range from 4.5 to 9.7 µg per 10^6 cells (57). My data shows excellent agreement with this report.

No significant effect of light quality on the lipid content of Chlorella vulgaris and Chlamydomonas reinhardtii was observed (Table 5 and 6). Hase et al (125) reported that synchronous cultures of Chlorella ellipsoidea contained 5 to 10 µg lipid per 10^6 cells. Guerin-Dumartrait et al (121) reported lipid levels of 3.04 to 5.64 µg per 10^6 cells in Chlorella pyrenoidosa 211/8b. Recalculating µ moles total fatty acids of

Table 3. Concentrations of DNA, RNA, carbohydrate, lipid and protein in Anacystis nidulans grown in white, blue, green or red light (550 μW cm^{-2})

	White	Blue	Green	Red
μg DNA/10^6 cells	0.034 ± 0.008	0.029 ± 0.004	0.065 ± 0.010	0.055 ± 0.004
μg RNA/10^6 cells	0.14 ± 0.03	0.13 ± 0.02	0.14 ± 0.01	0.16 ± 0.01
μg carbohydrate 10^6 cells	0.25 ± 0.18	0.19 ± 0.03	0.33 ± 0.17	0.09 ± 0.03
μg lipid/10^6 cells	0.48 ± 0.07	0.59 ± 0.03	0.74 ± 0.11	0.68 ± 0.11
μg protein/10^6 cells	3.92 ± 0.84	4.24 ± 0.30	3.54 ± 0.17	3.40 ± 0.04

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 4. Concentration of DNA, RNA, carbohydrate, lipid and protein in Navicula pelliculosa grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg DNA/10^6 cells	0.16 ± 0.10	0.19 ± 0.09	0.16 ± 0.01	0.15 ± 0.01
μg RNA/10^6 cells	0.60 ± 0.31	0.64 ± 0.61	0.60 ± 0.15	0.50 ± 0.06
μg carbohydrate/10^6 cells	7.2 ± 2.8	8.6 ± 3.4	4.0 ± 0.6	3.3 ± 0.6
μg lipid/10^6 cells	5.4 ± 3.0	7.2 ± 2.4	3.5 ± 0.01	8.1 ± 1.2
μg protein/10^6 cells	13.3 ± 2.5	14.0 ± 2.3	13.7 ± 0.07	14.9 ± 0.9

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 5. Concentrations of DNA, RNA, carbohydrate, lipid and protein in Chlorella vulgaris grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg DNA/10^6 cells	0.040 ± 0.007	0.045 ± 0.006	0.033 ± 0.006	0.053 ± 0.006
μg RNA/10^6 cells	0.14 ± 0.07	0.15 ± 0.09	0.17 ± 0.02	0.14 ± 0.01
μg carbohydrate/10^6 cells	1.75 ± 0.24	2.05 ± 0.25	1.60 ± 0.10	2.07 ± 0.21
μg lipid/10^6 cells	0.83 ± 0.15	0.87 ± 0.12	0.97 ± 0.06	0.90 ± 0.17
μg protein 10^6 cells	4.12 ± 1.22	4.40 ± 1.01	3.43 ± 0.27	3.50 ± 0.21

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 6. Concentrations of DNA, RNA, carbohydrate, lipid and protein in *Chlamydomonas reinhardtii* grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg DNA/10^6 cells	0.35 ± 0.19	0.35 ± 0.12	0.52 ± 0.10	0.51 ± 0.2
μg RNA/10^6 cells	2.28 ± 1.80	2.00 ± 1.30	2.93 ± 0.29	1.66 ± 0.80
μg carbohydrate/10^6 cells	31.90 ± 2.80	38.10 ± 5.80	14.20 ± 1.30	33.20 ± 3.90
μg lipid/10^6 cells	19.00 ± 4.60	18.30 ± 4.70	14.30 ± 1.80	15.0 ± 0.70
μg protein/10^6 cells	79.70 ± 25.10	81.80 ± 24.50	25.30 ± 5.10	45.20 ± 4.60

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 7. Ratios of carbohydrate:protein for cells grown in white, blue, green or red light.

	µg carbohydrate/µg protein			
	White	Blue	Green	Red
Anacystis nidulans	0.06 ± 0.04	0.09 ± 0.04	0.09 ± 0.04	0.03 ± 0.01
Navicula pelliculosa	0.68 ± 0.18	0.80 ± 0.26	0.32 ± 0.01	0.27 ± 0.02
Chlorella vulgaris	0.41 ± 0.20	0.49 ± 0.14	0.47 ± 0.03	0.59 ± 0.06
Chlamydomonas reinhardtii	0.44 ± 0.16	0.55 ± 0.21	0.58 ± 0.17	0.74 ± 0.13

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Chlamydomonas reinhardtii y-1 to µg yields 13.4 µg total fatty acid per 10^6 cells (108). While my lipid values for Chlorella vulgaris seem low, the values for Chlamydomonas reinhardtii show good agreement with these reported values.

2.6 Pigment content and composition

Total chlorophyll pigment concentrations in cells of Chlorella vulgaris and Chlamydomonas reinhardtii showed large variation (up to 25% of the mean) and were not significantly different ($P < 0.05$) (Tables 10 and 11). This result was also generally true for the concentrations of chlorophylls a and b and total carotenoids. The ANOVA treatment broke chlorophyll b concentrations of Chlorella cells into two homogeneous subsets. In one, chlorophyll b concentrations in white, green and red light were not significantly different ($P < 0.05$) (Table 10). In the other subset, the chlorophyll b concentrations in white, blue and green light were not significantly different ($P < 0.05$). The ratio of concentrations of chlorophylls b:a in all light quality treatments was not significantly different in cells of Chlamydomonas (Table 11). The chlorophyll b:a ratio in Chlorella was broken into two homogeneous subsets. In one subset, the ratios in white, blue and red light were not significantly ($P < 0.05$) different. In the other subset, the values in blue and green light were not significantly different ($P < 0.05$). Changes in the ratio of total carotenoids to chlorophyll a in Chlorella and Chlamydomonas were not significantly different in any light treatment.

The chlorophyll a content of Anacystis cells was not significantly affected by light quality (Table 8). The phycocyanin content was highest in blue light (representing 32% of the total cellular protein) and lowest

in red light ($P < 0.05$). Phycocyanin content of white and green light cells was not significantly different. The concentration of total carotenoids was not significantly different in any of the light treatments. The ratio of phycocyanin:chlorophyll *a* was highest in blue light cells and lowest in red light cells, however, this ratio was not significantly different in white, green and red light. The total carotenoid:chlorophyll *a* ratio was not significantly different in white, green or red cells but was higher in the blue light cells ($P < 0.05$). It is of interest to note that the total pigment content (chlorophyll *a* + carotenoids + phycocyanin) of blue light grown *Anacystis* cells was three times the total pigment content of red light grown cells.

Most of the pigment concentration and pigment ratio parameters of *Navicula pelliculosa* fell into two homogeneous subsets, white and blue light and green and red light respectively, using ANOVA (Table 9). Chlorophyll *c* concentration was not significantly different in any light treatment. Chlorophyll *a* concentrations in the green and red light subset were not significantly different but were higher than the white or blue light subset. This trend is reflected in the values for total chlorophyll content and in the chlorophyll *c*:*a* ratios. Chlorophyll *c*:*a* ratios were lower in red and green light. The higher chlorophyll *a* content of red and green light cells is also reflected in a lower total carotenoid:chlorophyll *a* ratio in these cells. The total carotenoid concentration in white, blue and green light cells is not significantly different in one homogeneous subset of the ANOVA treatment. In the other subset, the total carotenoid content is not significantly different in blue, green or red light.

Table 8. Concentrations of chlorophyll a, phycocyanin and total carotenoids and ratios of chlorophyll a, phycocyanin and total carotenoids in Anacystis nidulans grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg chlorophyll a/10^6 cells	0.098 ± 0.023	0.090 ± 0.013	0.114 ± 0.026	0.109 ± 0.018
μg phycocyanin/10^6 cells	0.565 ± 0.166	1.346 ± 0.225	0.538 ± 0.238	0.297 ± 0.040
μg total carotenoids/10^6 cells	0.057 ± 0.014	0.060 ± 0.007	0.061 ± 0.012	0.060 ± 0.011
μg phycocyanin/μg chlorophyll a	4.98 ± 2.06	15.38 ± 2.91	4.54 ± 2.67	2.80 ± 0.41
μg total carotenoids/μg chlorophyll a	0.55 ± 0.12	0.70 ± 0.12	0.54 ± 0.10	0.58 ± 0.07

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 9. Concentrations of total chlorophylls, chlorophyll *a*, chlorophyll *c* and total carotenoids and ratios of chlorophyll *a* and *c* and total carotenoids in *Navicula pelliculosa* grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg total chlorophyll/10^6 cells	0.55 ± 0.28	0.56 ± 0.26	0.96 ± 0.06	1.04 ± 0.04
μg chlorophyll *a*/10^6 cells	0.45 ± 0.25	0.48 ± 0.23	0.88 ± 0.06	0.97 ± 0.04
μg chlorophyll *c*/10^6 cells	0.07 ± 0.03	0.08 ± 0.03	0.09 ± 0.02	0.07 ± 0.01
μg total carotenoids/10^6 cells	0.37 ± 0.17	0.43 ± 0.19	0.58 ± 0.06	0.61 ± 0.05
chlorophyll *c*/chlorophyll *a*(μg/μg)	0.16 ± 0.06	0.18 ± 0.02	0.10 ± 0.02	0.07 ± 0.01
total carotenoids/chlorophyll *a*(μg/μg)	0.88 ± 0.18	0.89 ± 0.13	0.66 ± 0.06	0.62 ± 0.04

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 10. Concentrations of total chlorophylls, chlorophyll a, chlorophyll b, total carotenoids and ratios of chlorophylls a and b and total carotenoids in Chlorella vulgaris grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg total chlorophyll/10^6 cells	0.154 ± 0.032	0.153 ± 0.041	0.107 ± 0.019	0.117 ± 0.047
μg chlorophyll a/10^6 cells	0.124 ± 0.028	0.123 ± 0.039	0.080 ± 0.016	0.094 ± 0.042
μg chlorophyll b/10^6 cells	0.030 ± 0.006	0.034 ± 0.008	0.029 ± 0.007	0.023 ± 0.005
μg total carotenoids/10^6 cells	0.034 ± 0.008	0.034 ± 0.013	0.029 ± 0.007	0.029 ± 0.009
chlorophyll b/chlorophyll a(μg/μg)	0.25 ± 0.03	0.28 ± 0.08	0.36 ± 0.10	0.25 ± 0.05
total carotenoids/chlorophyll a(μg/μg)	0.27 ± 0.06	0.29 ± 0.09	0.37 ± 0.06	0.34 ± 0.08

Numbers connected by a common underline are not significantly different ($P < 0.05$).

Table 11. Concentrations of total chlorophylls, chlorophyll a, chlorophyll b and total carotenoids and ratios of chlorophyll a and b and total carotenoids in Chlamydomonas reinhardtii grown in white, blue, green or red light (550 μW cm^{-2}).

	White	Blue	Green	Red
μg total chlorophyll/10^6 cells	4.98 ± 1.22	4.63 ± 0.92	4.40 ± 0.76	4.34 ± 0.76
μg chlorophyll a/10^6 cells	3.83 ± 1.02	3.44 ± 0.75	3.14 ± 0.80	3.14 ± 0.80
μg chlorophyll b/10^6 cells	1.17 ± 0.30	1.17 ± 0.17	1.26 ± 0.11	1.22 ± 0.05
μg total carotenoids/10^6 cells	1.18 ± 0.30	1.09 ± 0.23	0.98 ± 0.21	1.08 ± 0.16
chlorophyll b/chlorophyll a (μg/μg)	0.31 ± 0.04	0.35 ± 0.04	0.31 ± 0.02	0.35 ± 0.04
total carotenoids/chlorophyll a (μg/μg)	0.31 ± 0.05	0.32 ± 0.03	0.31 ± 0.02	0.35 ± 0.04

Numbers connected by a common underline are not significantly different ($P < 0.05$).

2.6.1 Colour of cultures

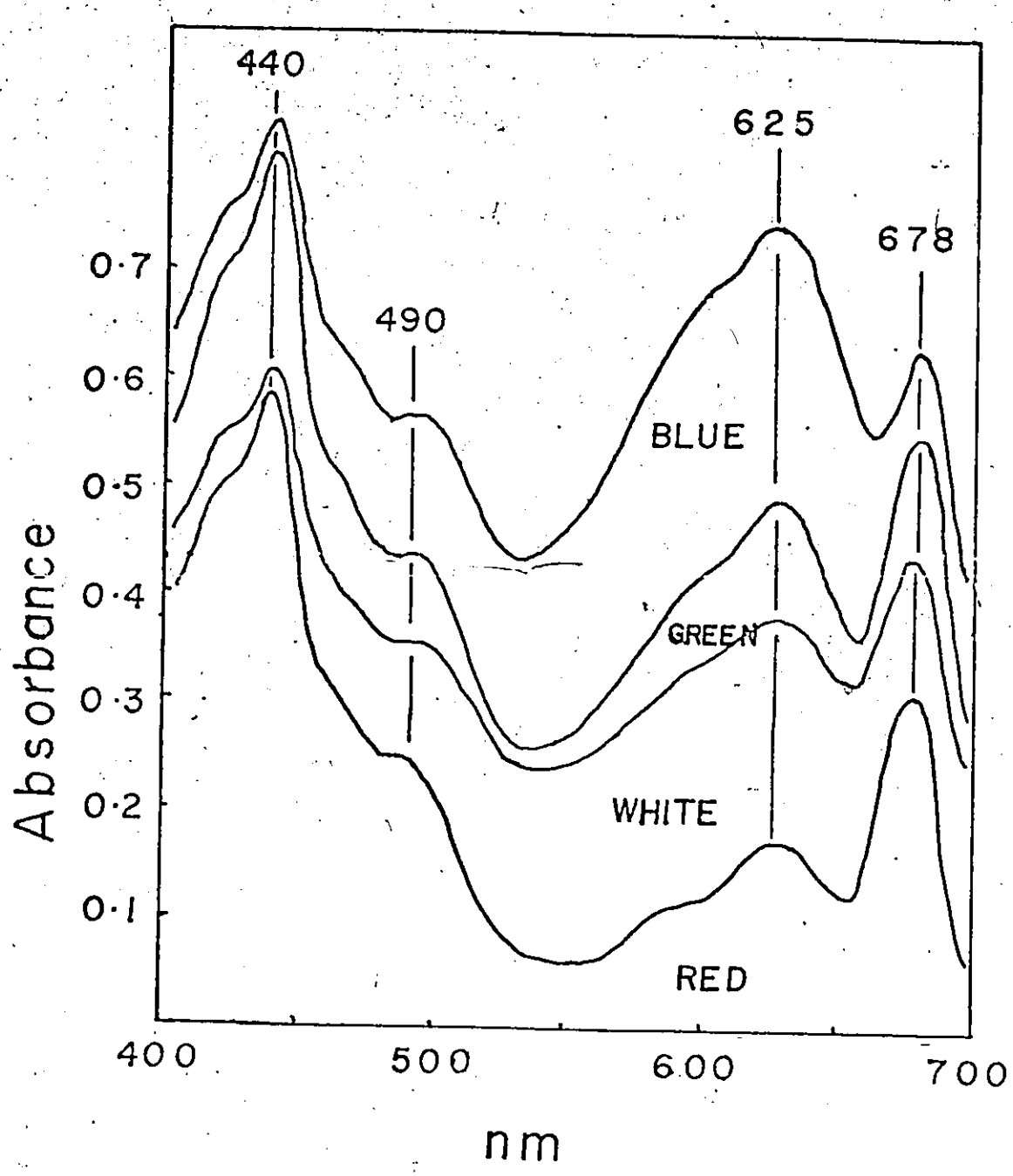
While this is a fairly subjective estimate, light quality did affect the colour of the cultures. Anacystis cultures grown in red light were bright green, whereas cells grown in blue light were blue. Cells grown in white or green light had an intermediate blue-green colour. Red light-grown cultures of Navicula were greenish-brown whereas blue light cultures were a dark chocolate brown. White and green light cultures were an intermediate golden-brown colour. No obvious colour changes were observed in Chlorella or Chlamydomonas cultures.

3. The Effect of Light Quality on Light Absorption

3.1 In vivo absorption spectra

The in vivo absorption spectra of Chlorella and Chlamydomonas show considerable variation in overall absorbance profiles (Figures 7 & 8). This, in part, is due to differing amounts of pigment per unit area of membrane and also variation in the technique used. However, changes in chloroplast structure and arrangement may have altered the apparent absorbance of the photosynthetic pigments in vivo (252). Self shading of chlorophyll within chloroplasts could result in decreased absorbance per unit pigment. A comparison of the absorbance peaks at 680 nm and 440 nm (chl a) suggests that blue light grown cells have greater absorption in this part of the spectrum than other light treatments. This is especially pronounced for Chlamydomonas (Figure 8). Increased absorbance at 440 nm is found in both red and blue light cells of Chlorella (Figure 7). Absorbance primarily due to carotenoids at 480 nm is manifested by a single broad peak at this wavelength and does not show much variation with light treatment. In Chlorella carotenoid absorption is reflected in a series

Figure 5. In vivo absorbance spectra of Anacystis nidulans grown in white, blue, green or red light (550 μW cm^{-2}). Spectra have been vertically separated to avoid overlap.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0·4
0·3
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Absorbance
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400
500
600
700
nm

Figure 5.

Figure 6. *In vivo* absorbance spectra of *Navicula pelliculosa* grown in white, blue, green or red light (550 μW cm^{-2}). Spectra have been vertically separated to avoid overlap.

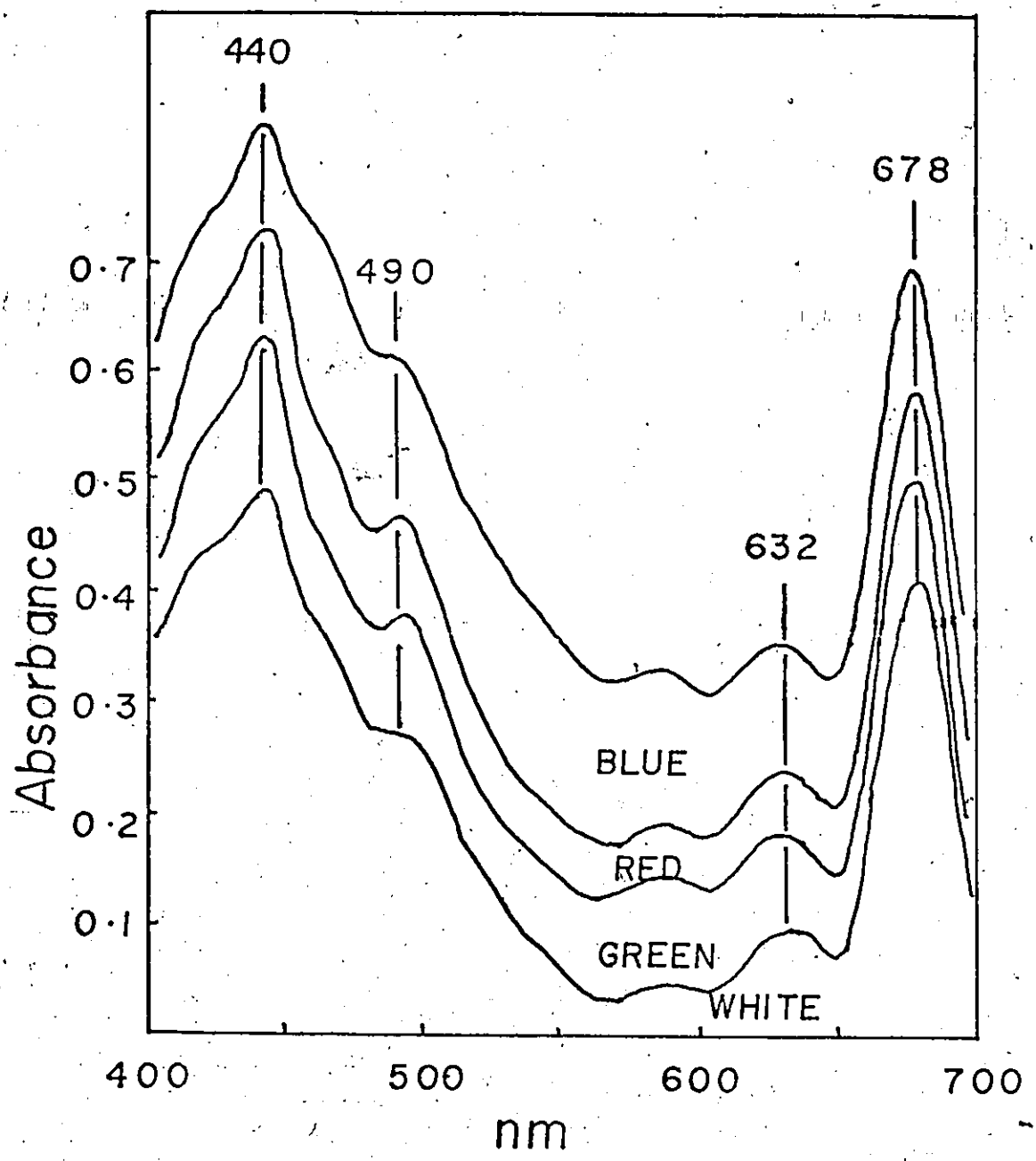
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Figure 6.

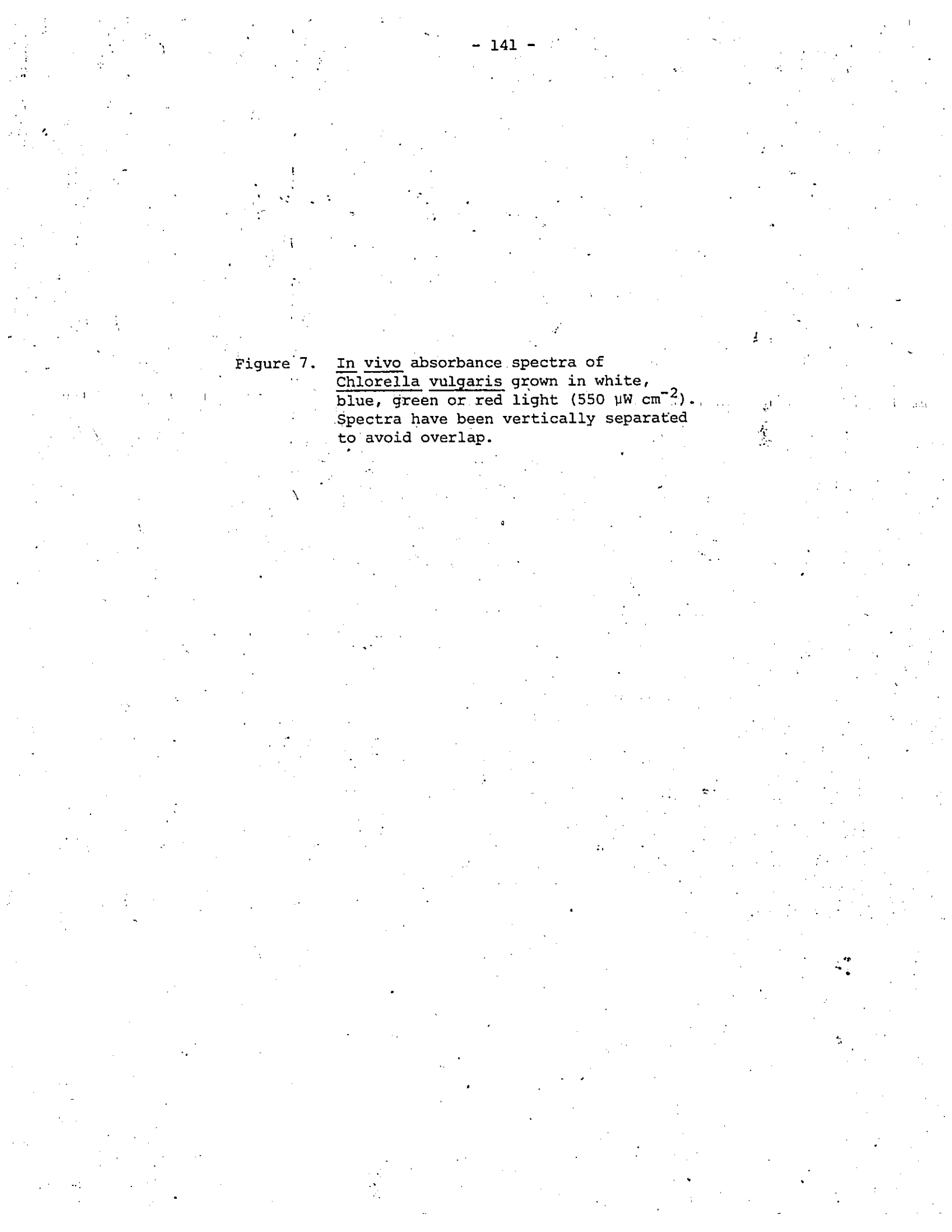
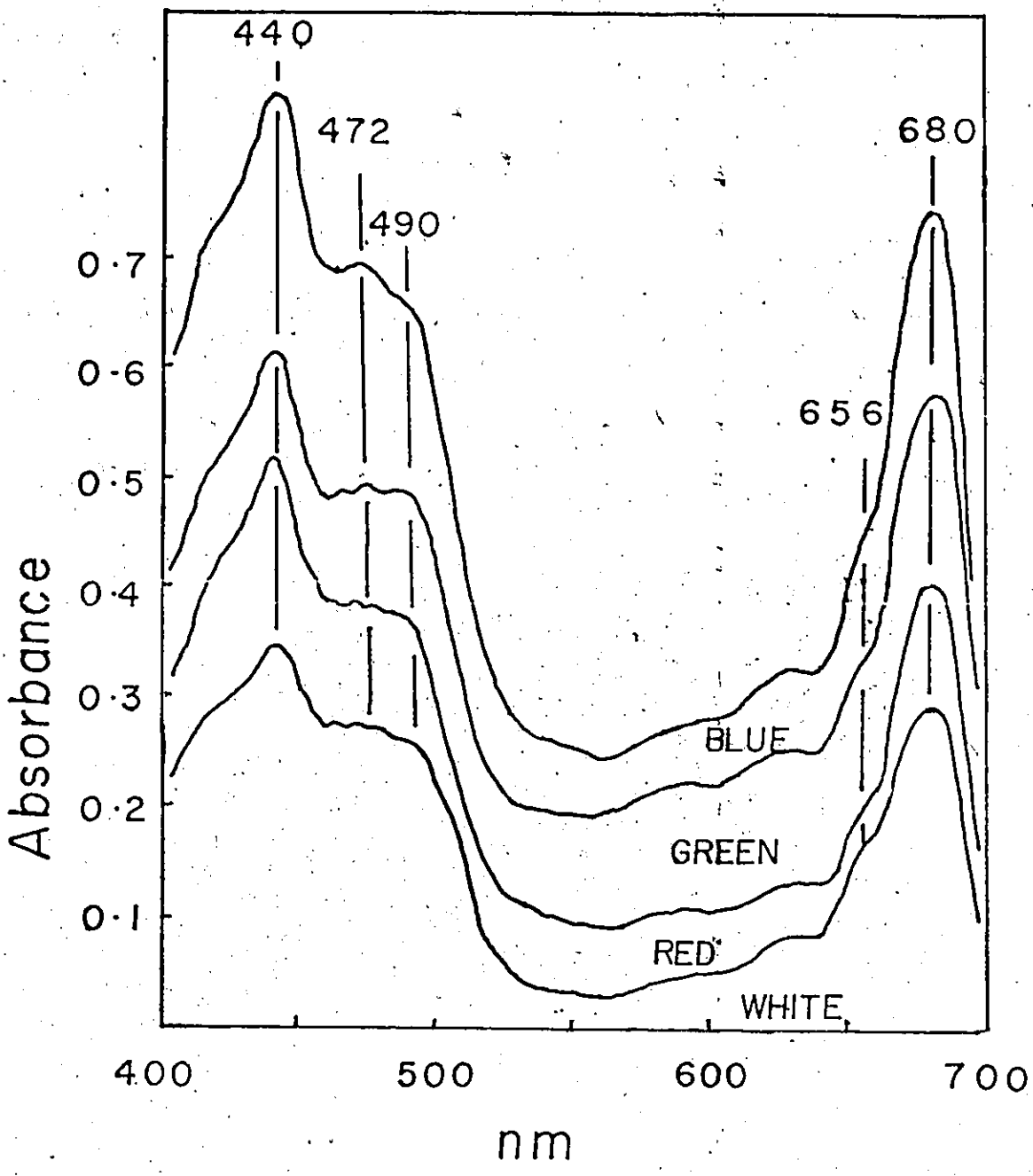


Figure 7. *In vivo* absorbance spectra of *Chlorella vulgaris* grown in white, blue, green or red light (550 $\mu W\ cm^{-2}$). Spectra have been vertically separated to avoid overlap.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Absorbance
BLUE
GREEN
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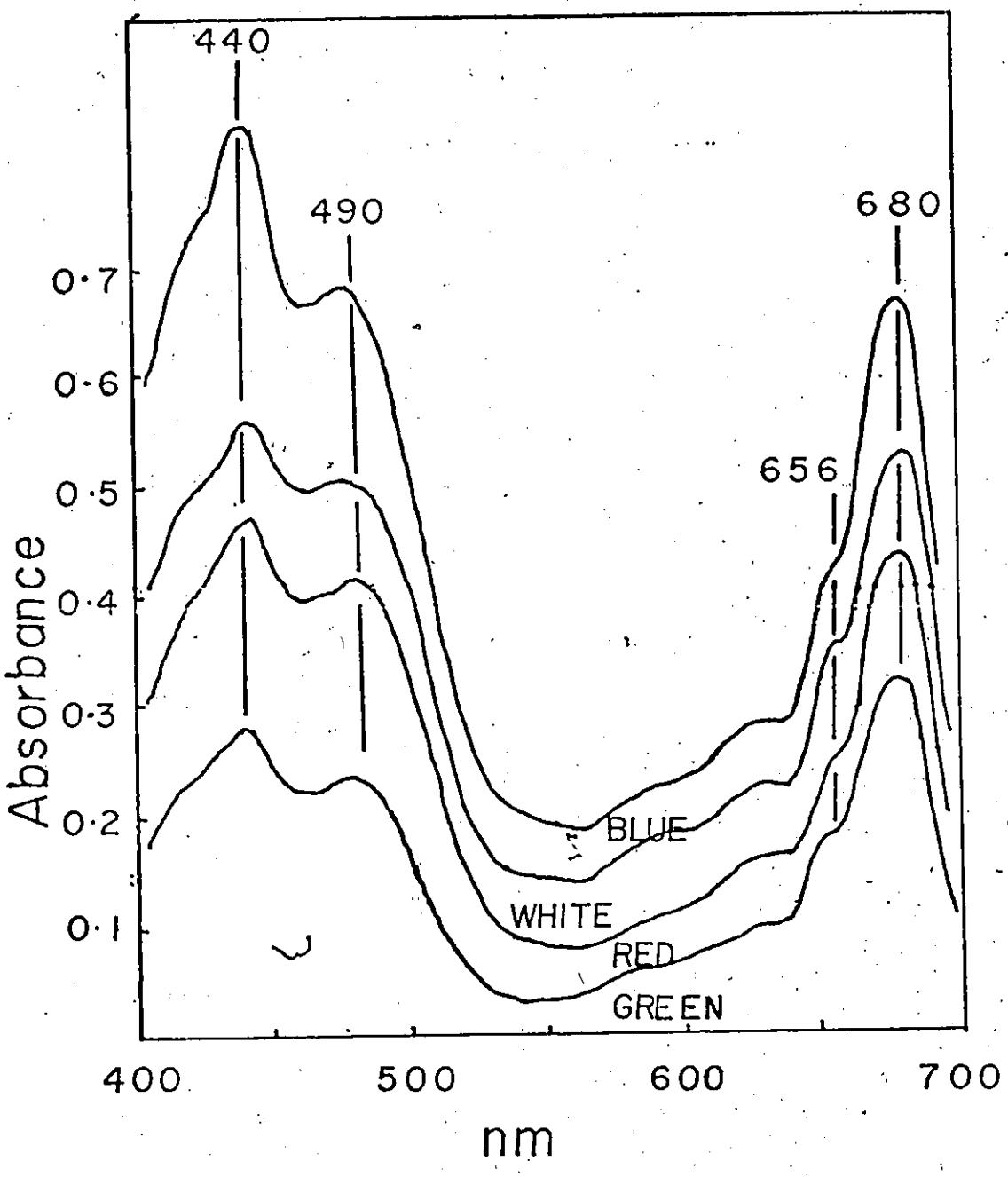
400
500
600
700
nm

Figure 7.

Figure 8. *In vivo* absorbance spectra of *Chlamydomonas reinhardtii* grown in white, blue, green or red light (550 $\mu W\ cm^{-2}$). Spectra have been vertically separated to avoid overlap.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Absorbance
BLUE
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Figure 8.

of minor peaks between the 440 nm peak and a shoulder at 490 nm. A peak at 472 nm is especially pronounced in blue light cells.

The outstanding feature of the Anacystis in vivo spectra is the variation of the phycocyanin peak at 625 nm (Figure 5). The peak height ratio at 680 nm and 625 nm is similar in white and green light cells, somewhat lower in red light and dramatically higher in blue light grown cells. This clearly reflects the importance of phycocyanin in this alga and suggests that a form of inverse chromatic adaptation has occurred. The absorption profile in the blue part of the spectrum reveals no major changes in carotenoid composition in response to light quality.

Two shoulders are observed in in vivo spectra of white and blue light grown Navicula pelliculosa cells at 460 and 490 nm (Figure 6). In red and green light grown cells the 460 nm shoulder is no longer apparent and a minor peak is observed at 490 nm. The higher carotenoid content of red and green light grown cells and the changes in in vivo absorbance at 460 and 490 nm suggest that changes in the carotenoid composition had occurred. No major differences in absorbance were observed in the red part of the in vivo spectrum.

3.2 Changes in light absorption

Changes in the amount of light absorbed in the waveband of the light source used for growth (light absorption factor) are reported in Tables 12 to 15. The calculated quanta flux absorbed under the same conditions is reported in Tables 16 to 19. Blue light adapted cells of the two green algae used in this study have increased in vivo absorption in the blue part of the spectrum (Figures 7 & 8). Blue light adapted Chlamydomonas and Chlorella cells also showed 18% and 37% increases in

Table 12. Integrated light absorption of Anacystis nidulans grown in white, blue, green and red light based on in vivo absorbance spectra (ca. 55.6 x 10^6 cells cm^{-2}).

Light quality for growth	Light source (nm)	Incident light ($\mu W\ cm^{-2}$)	Absorbed light ($\mu W\ cm^{-2}$)	Light absorption factor
White	White (400-700)	527.3	200.8	0.381
	Blue (400-575)	536.3	263.4	0.491
	Green (475-600)	538.0	118.5	0.220
	Red (575-700)	496.4	184.6	0.372
Blue	White (400-700)	527.3	223.1	0.423
	Blue (400-575)	536.3	273.7	0.510
Green	White (400-700)	527.3	208.1	0.395
	Green (475-600)	538.0	126.0	0.220
Red	White (400-700)	527.3	175.5	0.333
	Red (575-700)	496.4	151.8	0.306

Table 13. Integrated light absorption of Navicula pelliculosa grown in white, blue, green and red light based on in vivo absorbance spectra (ca.15.6 x 10^6cells cm^{-2}).

Light quality for growth	Light source (nm)	Incident light ($\mu W\ cm^{-2}$)	Absorbed light ($\mu W\ cm^{-2}$)	Light absorption factor
White	White (400-700)	527.3	170.4	0.323
	Blue (400-575)	536.3	323.4	0.603
	Green (475-600)	538.0	166.0	0.309
	Red (575-600)	496.4	159.6	0.322
Blue	White (400-700)	527.3	166.3	0.315
	Blue (400-575)	536.3	338.6	0.631
Green	White (400-700)	527.3	164.6	0.312
	Green (475-600)	538.0	152.1	0.283
Red	White (400-700)	527.3	173.0	0.328
	Red (575-700)	496.4	159.1	0.321

Table 14. Integrated light absorption of *Chlorella vulgaris* grown in white, blue, green and red light based on *in vivo* absorbance spectra (*ca.* 43.5 x 10^6 cells cm^{-2}).

Light quality for growth	Light source (nm)	Incident light ($\mu W\ cm^{-2}$)	Absorbed light ($\mu W\ cm^{-2}$)	Light absorption factor
White	White (400-700)	527.3	136.4	0.259
	Blue (400-575)	536.3	269.0	0.502
	Green (475-600)	538.0	101.8	0.189
	Red (575-700)	496.4	152.0	0.306
Blue	White (400-700)	527.3	171.1	0.325
	Blue (400-575)	536.3	367.7	0.686
Green	White (400-700)	527.3	148.7	0.282
	Green (475-600)	538.0	94.3	0.175
Red	White (400-700)	527.3	142.2	0.270
	Red (575-700)	496.4	141.6	0.285

Table 15. Integrated light absorption of *Chlamydomonas reinhardtii* grown in white, blue, green and red light based on *in vivo* absorbance spectra (*ca.* 4.35×10^6 cells cm^{-2}).

Light quality for growth	Light source (nm)	Incident light ($\mu W\ cm^{-2}$)	Absorbed light ($\mu W\ cm^{-2}$)	Light absorption factor
White	White (400-700)	527.3	160.9	0.305
	Blue (400-575)	536.3	319.0	0.595
	Green (475-600)	538.0	108.6	0.202
	Red (575-700)	496.4	191.0	0.385
Blue	White (400-700)	527.3	179.4	0.340
	Blue (400-575)	536.3	375.3	0.700
Green	White (400-700)	527.3	128.0	0.243
	Green (475-600)	538.0	84.0	0.156
Red	White (400-700)	527.3	153.4	0.291
	Red (575-700)	496.4	178.1	0.359

Table 16. Quanta flux absorption of Anacystis nidulans based on the calculated quanta flux of the light source and the mean light absorption factor.

Light quality for growth	Incident light (quanta x $10^{15} s^{-1} cm^{-2}$)	Mean light absorption factor	Absorbed light (quanta x $10^{15} s^{-1} cm^{-2}$)
White	1.67	0.383	0.64
Blue	1.28	0.500	0.64
Green	1.47	0.220	0.32
Red	1.80	0.339	0.61

Table 17. Quanta flux absorption of Navicula pelliculosa based on the calculated quanta flux of the light source and the mean light absorption factor.

Light quality for growth	Incident light (quanta x 10^{15} s^{-1} cm^{-2})	Mean light absorption factor	Absorbed light (quanta x 10^{15} s^{-1} cm^{-2})
White	1.67	0.320	0.53
Blue	1.28	0.617	0.79
Green	1.47	0.296	0.44
Red	1.80	0.322	0.58

Table 18. Quanta flux absorption of Chlorella vulgaris based on the calculated quanta flux of the light source and the mean light absorption factor.

Light quality for growth	Incident light (quanta x $10^{15} s^{-1} cm^{-2}$)	Mean light Absorption factor	Absorbed light (quanta x $10^{15} s^{-1} cm^{-2}$)
White	1.67	0.284	0.47
Blue	1.28	0.594	0.76
Green	1.47	0.182	0.27
Red	1.80	0.296	0.53

Table 19. Quanta flux absorption of Chlamydomonas reinhardtii based on the calculated quanta flux of the light source and the mean light absorption factor.

Light quality for growth	Incident light (quanta x 10^{15} s^{-1} cm^{-2})	Mean light absorption factor	Absorbed light (quanta x 10^{15} s^{-1} cm^{-2})
White	1.67	0.295	0.49
Blue	1.28	0.648	0.83
Green	1.47	0.179	0.26
Red	1.80	0.372	0.67

blue light absorption, respectively, when compared with white light adapted cells (Tables 14 & 15). No increase in green or red light absorption was observed in cells of these green algae adapted to green or red light. Cells of Navicula pelliculosa and Anacystis nidulans showed little or no increase in light absorption following adaptation to blue, green or red light (Tables 12 & 13). However, increases in the ability to absorb white light were noticed in cells of Anacystis nidulans following adaptation to blue or green light.

4. The Effect of Light Quality on Photosynthesis

4.1 $^{14}CO_2$-fixation

Table 20 reports the rates of $^{14}CO_2$-fixation of the four freshwater algae following growth in white, blue, green and red light. CO_2-fixation is expressed both per cell number and per unit chlorophyll. On a cell number basis, photosynthetic rates of the diatom Navicula pelliculosa were highest in red light and lowest in blue light. The CO_2-fixation rate in blue light was significantly lower ($P < 0.05$), when compared to rates in white, green and red light. The rates of CO_2-fixation in blue and green light were not significantly different when compared to the rates in white and red light. On a chlorophyll basis the rate of photosynthesis in blue light was greater than rates in white and green light. However, the rates of CO_2-fixation of this diatom were not significantly different under any of the light qualities when expressed on a chlorophyll basis.

The green alga Chlamydomonas reinhardtii had highest rates of photosynthesis in red and blue light on both cell number and unit chlorophyll bases. The rates of photosynthesis in red and blue light were not significantly different. The rate of CO_2-fixation was lowest in green

Table 20. Rates of ^{14}C-fixation of four freshwater algae in white, blue, green and red light (550 µW cm^{-2})

	White	Blue	Green	Red
Navicula pelliculosa				
nmoles CO_2 10^9cells^{-1}hr^{-1}	21.5 ± 8.8	9.4 ± 2.1	15.2 ± 1.0	25.3 ± 4.2
µmoles CO_2 mg chl^{-1}hr^{-1}	18.4 ± 7.4	20.0 ± 6.1	15.8 ± 1.4	24.8 ± 4.1
Chlamydomonas reinhardtii				
nmoles CO_2 10^9cells^{-1}hr^{-1}	75.0 ± 26.0	135.8 ± 24.8	21.8 ± 5.6	118.8 ± 15.8
µmoles CO_2 mg chl^{-1}hr^{-1}	17.7 ± 3.4	35.0 ± 12.9	5.7 ± 1.3	31.5 ± 5.3
Chlorella vulgaris				
nmoles CO_2 10^9cells^{-1}hr^{-1}	9.9 ± 1.3	12.1 ± 1.1	3.3 ± 0.6	8.2 ± 0.5
µmoles CO_2 mg chl^{-1}hr^{-1}	59.0 ± 7.7	92.5 ± 22.4	34.8 ± 8.8	90.0 ± 8.2
Anacystis nidulans				
nmoles CO_2 10^9cells^{-1}hr^{-1}	6.7 ± 2,6	2.1 ± 0.2	2.4 ± 1.3	12.2 ± 0.7
µmoles CO_2 mg chl^{-1}hr^{-1}	100.0 ± 63.8	27.8 ± 2.9	42.5 ± 18.3	230.8 ± 13.5

Numbers connected by a common underline are not significantly different ($P < 0.05$).

light and intermediate in white, either per cell number or per unit chlorophyll. CO_2-fixation rates in green or white light were significantly different ($P < 0.05$).

A similar trend in photosynthetic response to light quality was found in the other green alga Chlorella vulgaris. On a cell number basis, however, the CO_2-fixation rate in white light was significantly higher than the rate in red light.

In the blue-green alga Anacystis nidulans CO_2-fixation rates in red light were significantly higher than in any other light quality on either a cell number or unit chlorophyll basis ($P < 0.05$). The lowest photosynthetic rates were found in blue and green light. The CO_2-fixation rates in blue and green light were not significantly different. On a cell number basis, photosynthesis in white light was intermediate between red and blue and green light and was significantly different. When expressed on a unit chlorophyll basis, rates of CO_2-fixation in white and green light were not significantly different.

4.2 O_2-evolution

The results of the O_2-evolution rate experiments fall into two main categories. In one group of experiments, cells grown in white, blue, green and red light were exposed to equal intensities (600 $\mu W\ cm^{-2}$) of white light or the light they were grown in and the O_2-evolution rate was measured. In the case of white light grown cells, equal intensities of all light qualities were used. The results of these experiments are reported in Tables 21 to 24. In the other group of experiments the photosynthetic response of cells grown in the various light qualities to increasing intensities of white light was measured. The results of these

Table 21. Rate of O_2-evolution of Anacystis nidulans grown in white, blue, green and red light (550 μW cm^{-2})

Light quality for growth	Light quality for O_2-evolution measurement (600 μW cm^{-2})			
	White	Blue	Green	Red
	(μmoles O_2 mg chl^{-1} hr^{-1})			
White	38.7	-18.3	0	53.6
	45.0	-23.0	0	65.6
Blue	81.3	8.1	-	-
	100.3	14.3	-	-
Green	120.0	-	39.3	-
	142.0	-	40.7	-
Red	54.0	-	-	90.6
	58.3	-	-	83.1

Table 22. Rate of O_2-evolution of Navicula pelliculosa grown in white, blue, green and red light (550 µW cm^{-2})

Light quality for growth	Light quality for O_2-evolution measurement (600 µW cm^{-2})			
	White	Blue	Green	Red
	(µmoles O_2 mg chl^{-1} hr^{-1})			
White	25.7	10.2	23.8	7.1
	28.0	11.9	26.1	8.6
Blue	34.7	6.1	-	-
	53.3	9.3	-	-
Green	33.6	-	29.5	-
	42.6	-	34.9	-
Red	27.0	-	-	26.7
	22.5	-	-	19.7

Table 23. Rate of O_2-evolution of Chlorella vulgaris grown in white, blue, green and red light (550 μW cm^{-2})

Light quality for growth	Light quality for O_2-evolution measurement (600 μW cm^{-2})			
	White	Blue	Green	Red
	(μmoles O_2 mg chl^{-1} hr^{-1})			
White	21.3	12.3	5.4	34.0
	23.0	11.1	6.2	32.5
Blue	39.0	32.8	-	-
	41.3	32.5	-	-
Green	52.3	-	19.4	-
	46.5	-	18.4	-
Red	40.6	-	-	50.2
	47.0	-	-	47.5

Table 24. Rate of O_2-evolution of Chlamydomonas reinhardtii grown in white, blue, green and red light (550 μW cm^{-2})

Light quality for growth	Light quality for O_2-evolution measurement (600 μW cm^{-2})			
	White	Blue	Green	Red
	(μmoles O_2 mg chl^{-1} hr^{-1})			
White	19.0	1.5	16.4	35.9
	27.7	2.0	14.5	39.3
Blue	27.7	2.2	-	-
	37.3	3.5	-	-
Green	33.3	-	5.7	-
	39.7	-	10.2	-
Red	27.7	-	-	42.5
	43.6	-	-	58.1

experiments are shown in Figures 9 to 12.

The O_2-evolution rate per unit chlorophyll of white light grown cells of Navicula in response to equal intensities of white, blue, green and red light is shown in Table 22. Photosynthetic rates were highest and similar in white and green light. Low rates of photosynthesis were found in blue and red light. Red light grown cells had similar rates of O_2-evolution to white light cells in white light but showed enhanced photosynthetic rates in red light. Blue light grown cells had greatly enhanced O_2-evolution rates in white light, however, the O_2-evolution rate in blue light was not improved. A partial explanation for this anomalous result may lie in the spectral energy distribution of the blue light used in the O_2-evolution experiments (Fig. 3). While this blue light source has a similar peak emission at 460 nm to the light source used for growth, its's waveband is much more restricted i.e. 420 to 515 nm against 380 to 575 nm for growth. The in vivo absorbance spectra of Navicula (Fig. 6) by comparison to those of Chlorella and Chlamydomonas (Figs. 7 and 8) show greater absorption in the range 520 to 560 nm presumably due to the in vivo absorption of fucoxanthin. The absence of these wavelengths in the blue light used for O_2-evolution measurements may explain the low O_2-evolution rates of Navicula in blue light. Green light grown cells showed enhanced photosynthesis both in white and green light. The O_2-evolution rates of Navicula cells grown in white, green and red light show substantial agreement with the earlier $^{14}CO_2$ fixation experiments on a unit chlorophyll basis (Table 20).

The rates of O_2-evolution of white light grown Chlamydomonas cells in equal intensities of white, blue, green or red light are shown in Table 24. The highest rate of photosynthesis is found in red light.

Lower rates of photosynthesis are found in white and green light. Very low rates of O_2-evolution are found in blue light. Again, blue light grown cells show enhanced photosynthetic rates in white light but no improvement in blue light. This suggests that the restricted waveband of the blue light used for O_2-evolution measurements prevented photosynthetically active pigments absorbing just outside its waveband from participating in photosynthesis. Even higher rates of O_2-evolution were found in red light grown Chlamydomonas cells in both white and red light. While green light grown cells showed enhanced photosynthesis in white light, the rate of photosynthesis in green light was not improved. Again, substantial agreement between the rates of O_2-evolution and $^{14}CO_2$-fixation of white, green and red light grown cells in white, green or red light was found (Table 20).

White light grown Chlorella cells showed highest O_2-evolution rates in red light, followed by white light, blue light and green light in decreasing order of magnitude (Table 23). Blue light grown Chlorella showed increased photosynthetic rate in both white light and blue light, suggesting that its pigment composition was well suited to the restricted waveband of the blue light used. Green light grown cells also showed higher rates of O_2-evolution in white and green light. The highest O_2-evolution rate was found in red light for red light grown cells. The rate of photosynthesis of red light cells in white light was also increased by comparison with white light grown cells. Again, good agreement between the O_2-evolution and CO_2-fixation experiments is observed for all light qualities (Table 20).

White light grown cells of Anacystis showed high rates of O_2-evolution in red and white light (Table 21). Equal intensities of green

Figure 9. The rate of O_2-evolution of Anacystis nidulans grown in white, blue, green or red light (550 μW cm^{-2}) as a function of white light intensity.

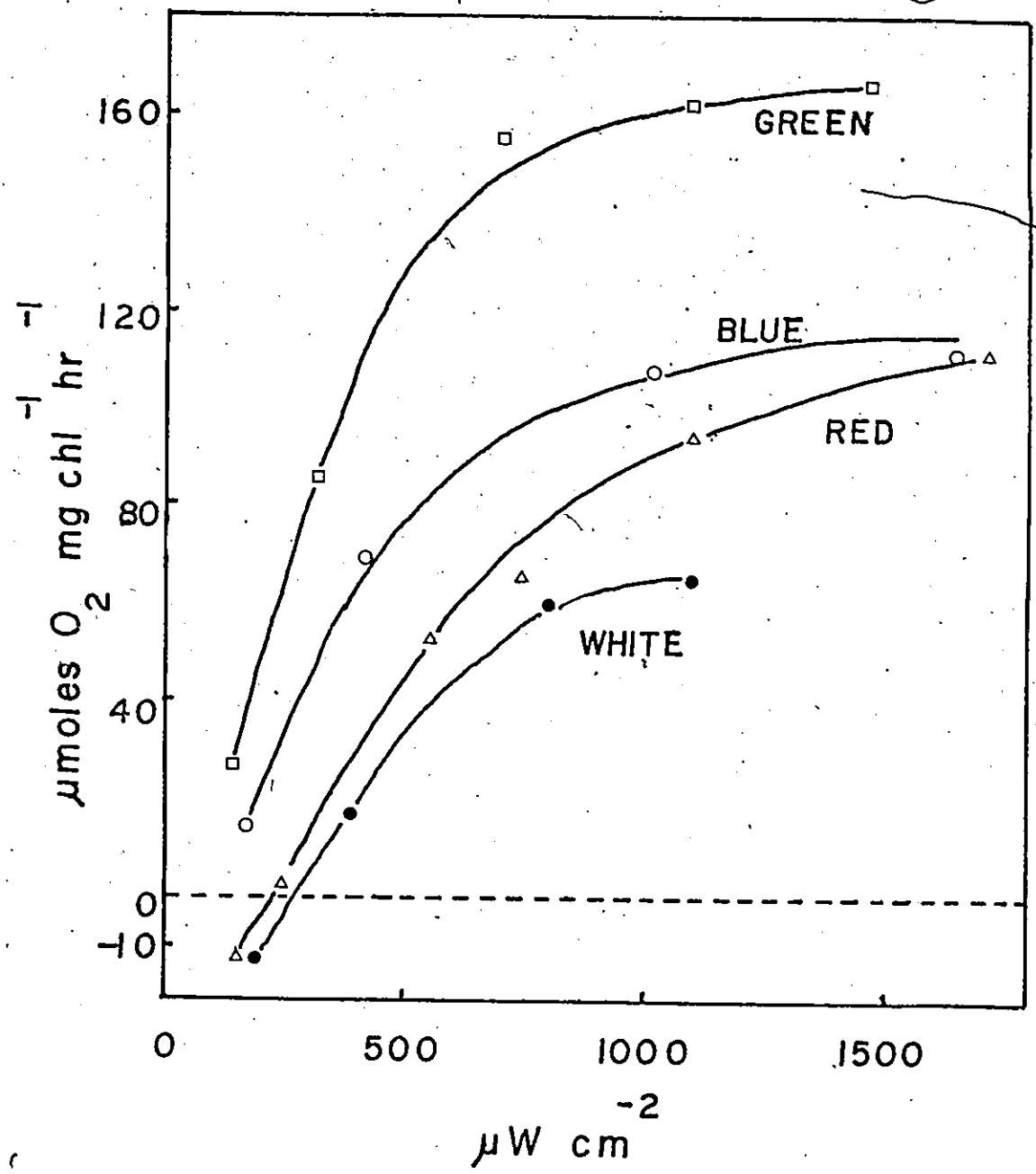
160
120
80
40
0
-10
GREEN
BLUE
RED
WHITE
0
500
1000
1500
μmoles O2 mg chl-1 hr-1
μW cm-2

Figure 9.

Figure 10. The rate of O_2-evolution of Navicula pelliculosa grown in white, blue, green or red light (550 μW cm^{-2}) as a function of white light intensity.

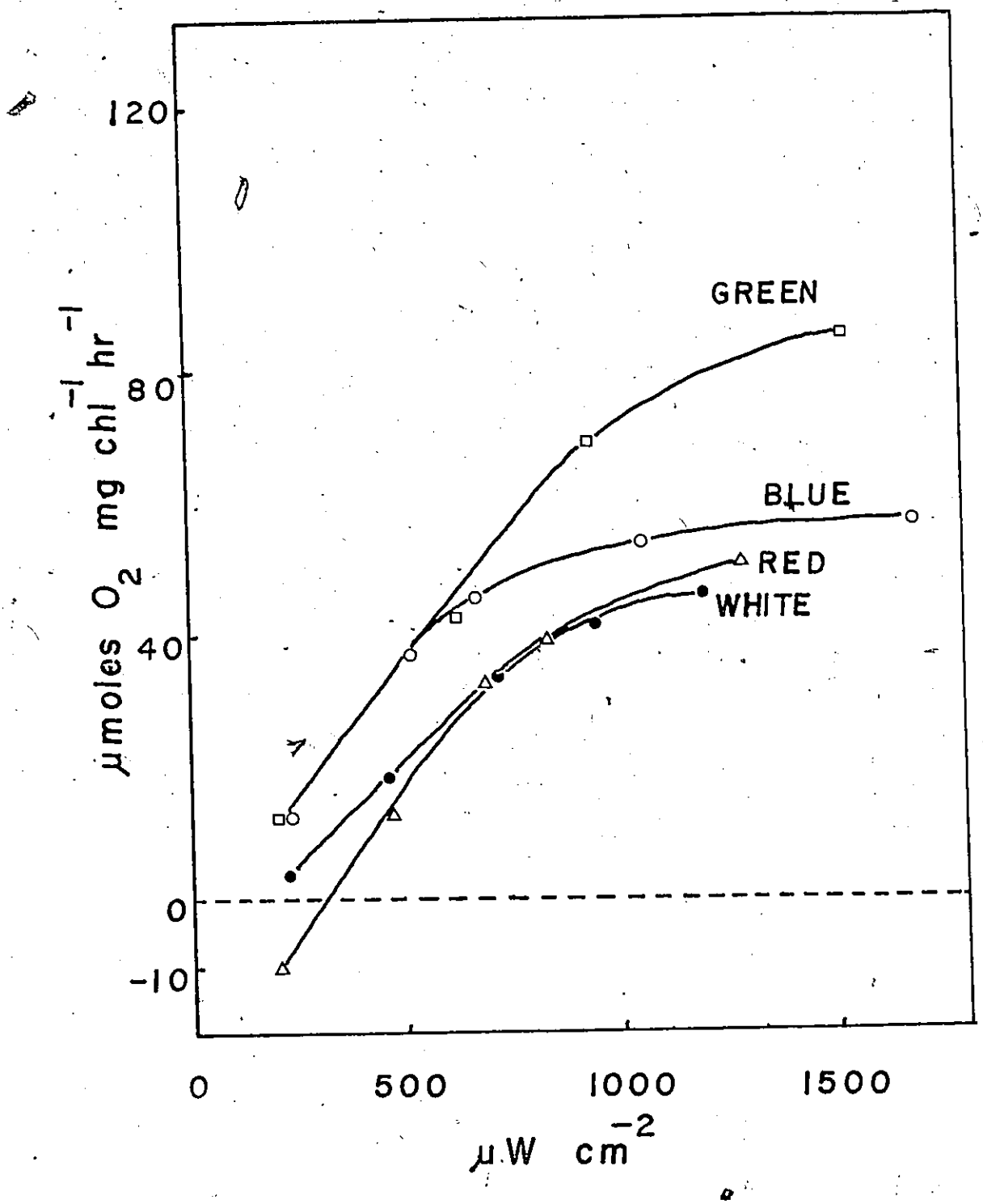
120
80
40
0
-10
μmoles O2 mg chl-1 hr-1
GREEN
BLUE
RED
WHITE
0
500
1000
1500
μW cm-2

Figure 10.

Figure 11. The rate of O_2-evolution of *Chlorella vulgaris* grown in white, blue, green or red light (550 $\mu W\ cm^{-2}$) as a function of white light intensity.

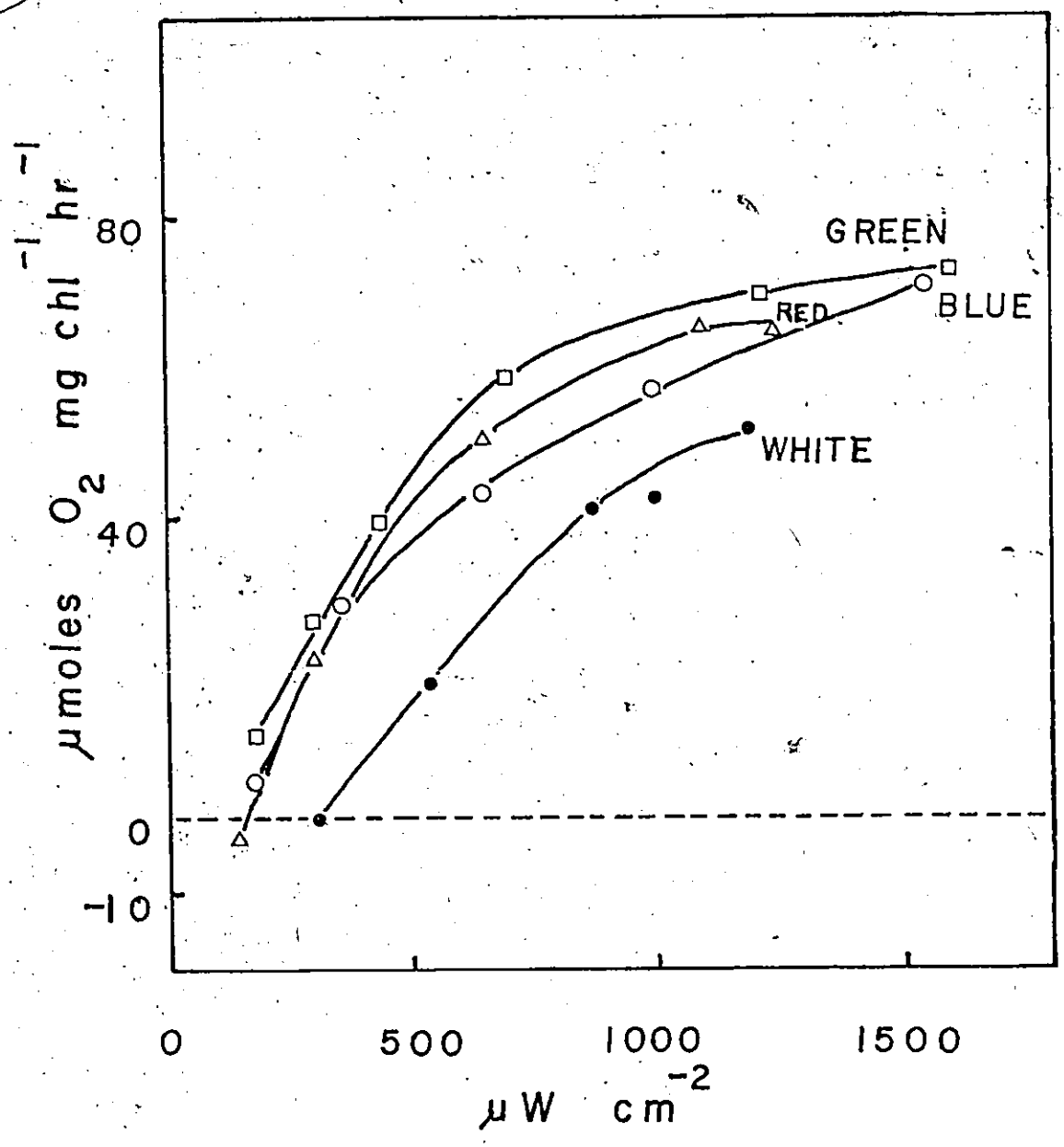
μmoles O_2 mg chl^{-1} hr^{-1}
80
40
0
-10
GREEN
BLUE
RED
WHITE
0
500
1000
1500
μW cm^{-2}

Figure 11.

Figure 12. The rate of O_2-evolution of Chlamydomonas reinhardtii grown in white, blue, green or red light (550 $\mu W\ cm^{-2}$) as a function of white light intensity.

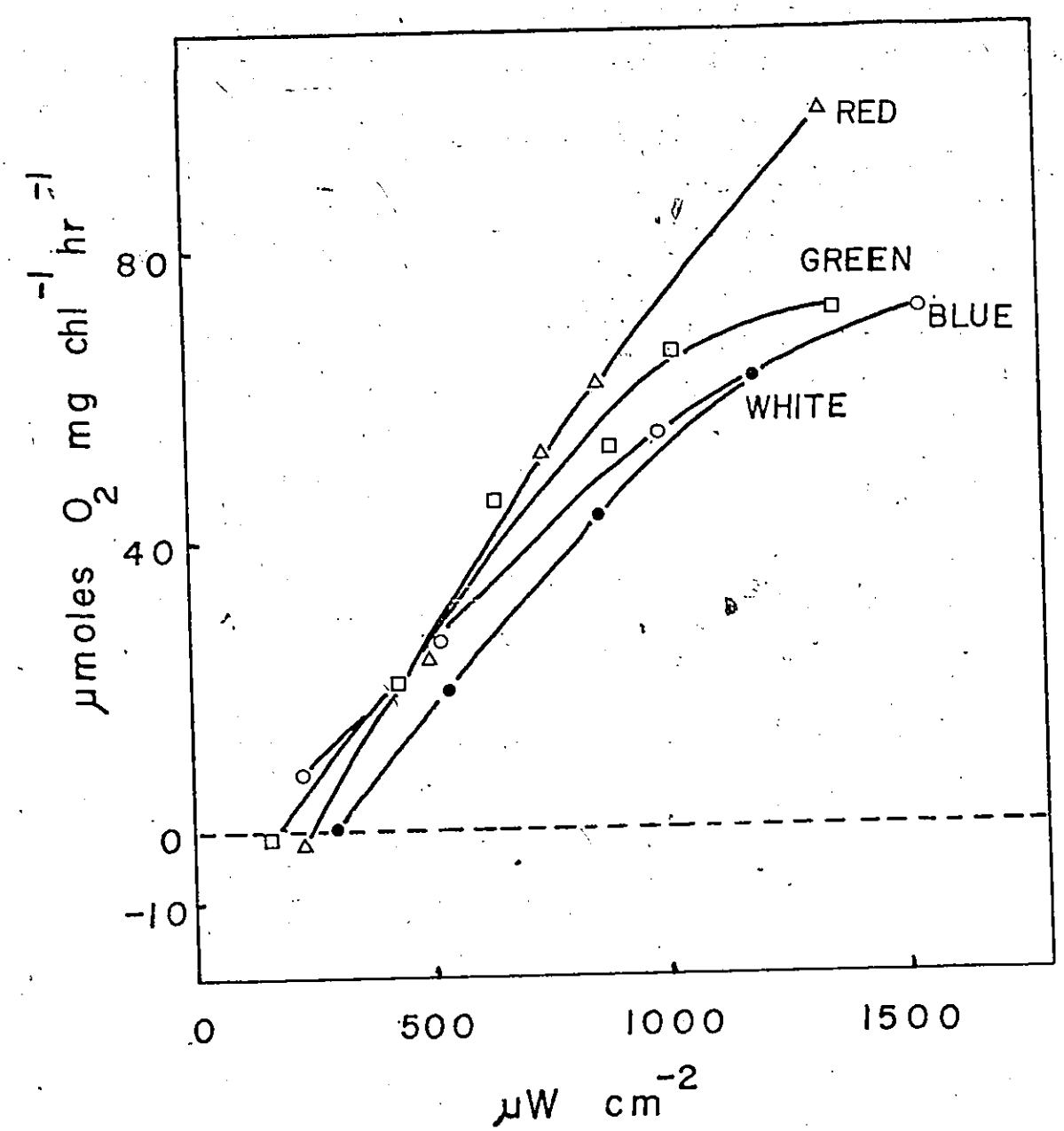
RED
GREEN
BLUE
WHITE
80
40
0
-10
0
500
1000
1500
μmoles O2 mg chl-1 hr-1
μW cm-2

Figure 12.

light produced a light compensation point situation in white light adapted cells and showed a rate of O_2-evolution equal to the rate of O_2-consumption. In blue light, white light adapted cells showed net O_2 consumption. Blue light adapted cells showed higher rates of O_2-evolution in both white and blue light compared with white light adapted cells. Green light grown Anacystis cells showed dramatically higher O_2-evolution rates in white and green light when compared to cells grown in white light. Anacystis cells adapted to red light showed slightly increased photosynthetic rates in white light and much higher rates in red light. The O_2-evolution results parallel those found in the CO_2-fixation experiments (Table 20).

4.3 The effect of white light intensity on O_2-evolution

The results of the white light intensity-photosynthesis curves showed several effects (Figures 9 to 12). Firstly, the magnitude of the photosynthetic response to white light of cells adapted to restricted parts of the spectrum was greater than the response of white light adapted cells in all cases. Secondly, most of the curves show saturation at light intensities > 1000 $\mu W\ cm^{-2}$. This suggests that these cells were all shade adapted. And thirdly, visual inspection of the curves suggests that I_k was lowest for green light grown cells of Anacystis, Chlorella and Chlamydomonas and blue light grown cells of Navicula, suggesting that these are the most shade adapted cells.

5. The Effect of Light Quality on Photosynthetic Carbon Metabolism

5.1 ^{14}C-incorporation into the water soluble, chloroform-soluble and insoluble fractions

When the total radioactivity recovered in the water soluble,

Table 25. Comparison of total radioactivity recovered during the extraction procedure with $^{14}CO_2$-uptake of whole cells

		Total extracts	Whole cells	% Recovery
		(dpm 10^6 cells^{-1} 40 min^{-1})		
CHLORELLA				
White	1	43198	70899	60.9
	2	42215	68815	61.3
Blue	1	43294	59639	72.6
	2	47694	63222	75.4
Green	1	20320	26880	75.6
	2	19154	26257	73.0
Red	1	39031	52895	73.8
	2	31732	38223	83.0
CHLAMYDOMONAS				
White	1	359724	852265	42.2
	2	347645	711826	48.8
Blue	1	280728	584222	48.1
	2	264513	603121	43.9
Green	1	33586	83360	40.3
	2	81691	207905	39.4
Red	1	351476	875882	40.1
	2	325929	872643	37.3
NAVICULA				
White	1	148768	182402	81.6
	2	145961	187347	77.9
Blue	1	136457	192453	70.9
	2	120883	187279	64.5
Green	1	76392	97488	78.4
	2	78644	104222	75.5
Red	1	156343	200440	78.0
	2	163983	210242	78.0
ANACYSTIS				
White	1	17711	64910	27.3
	2	16303	57648	28.3
Blue	1	6126	9958	61.5
	2	6179	10655	58.0
Green	1	6188	15557	39.8
	2	5666	14135	40.1
Red	1	89331	222313	40.2
	2	87495	218153	40.1

Table 26. Comparison of the mean radioactivity found in the water-soluble, chloroform-soluble and insoluble fractions after 40 mins. $^{14}CO_2$ incorporation

	Water soluble	Chloroform soluble	Insoluble
	(% combined activity recovered in fractions)		
CHLORELLA			
White	82.4	3.6	14.0
Blue	86.3	2.8	10.9
Green	95.6	1.4	3.0
Red	89.7	6.0	4.3
CHLAMYDOMONAS			
White	42.8	4.7	52.5
Blue	37.9	7.2	54.9
Green	47.0	5.8	47.2
Red	53.0	18.8	28.2
NAVICULA			
White	64.4	18.8	16.8
Blue	80.3	3.6	15.6
Green	84.0	5.6	10.4
Red	87.3	3.8	8.9
ANACYSTIS			
White	38.8	2.1	59.1
Blue	87.2	1.0	11.8
Green	74.2	2.0	23.4
Red	43.5	5.4	51.1

insoluble and chloroform soluble extracts is compared with the ^{14}C-uptake of whole cells, variable percentage recoveries of radioactivity are seen (Table 25). For Chlorella vulgaris percentage recovery ranged from 60.9 to 83%. For Chlamydomonas reinhardtii 37.3 to 48.8% of the incorporated radioactivity was recovered in the various extracts. 64.5 to 81.6% percentage recovery was found in Navicula pelliculosa. In Anacystis nidulans percentage recovery varied widely from 27.3% to 61.5%.

Examination of the relative distribution of radioactivity in the various cellular fractions reveals wide differences in the incorporation strategies of the various algae (Table 26). In Chlorella vulgaris, most of the incorporated radioactivity was found in the water soluble fraction. If we consider the distribution of label in the 40 min. samples, the amount of ^{14}C in the water soluble fraction ranged from 82.4% in white light to 95.6% in green light. Corresponding changes in the insoluble fraction were noted, incorporation being highest in white and blue light and lowest in green and red light. The amount of radioactivity incorporated into the chloroform soluble fraction in red light was at least twice that found in any of the other light treatments.

By contrast, in the other green alga, Chlamydomonas reinhardtii, amounts of radioactivity found in the insoluble fraction were greater than or equal to those found in the water soluble fraction in white, blue and green light. In red light, insoluble radioactivity was decreased. The percentage of radioactivity of the chloroform soluble fraction in red light was almost three times that found in the other light treatments.

In the diatom Navicula pelliculosa the highest percentage of radioactivity in the insoluble and chloroform soluble fractions was found in white light. In comparing the blue through green to red light

experiments, an increasing percentage of radioactivity in the water soluble and decreasing percentage of radioactivity in the insoluble fractions was observed. Percentage incorporation of radioactivity into the chloroform soluble fraction in blue, green and red light was 1/4 to 1/3 the percentage found in white light.

In Anacystis nidulans, a strong influence of light quality on the percentage of radioactivity in the water soluble and insoluble fractions was observed. In white and red light, more than half the radioactivity was found in the insoluble fraction. In blue and green light 87.2 and 74.6% resp. of the radioactivity was found in the water soluble fraction. The percentage radioactivity of the red light chloroform soluble fraction was at least two times the amount found in the other light qualities.

5.2 Extracellular release

Light quality had variable effects on the extracellular release fraction. Radioactivity in this fraction will be considered as a percentage of the activity of the water soluble fraction, since it is presumably derived from this fraction (Tables 27 to 30).

In Chlorella, the amount of radioactivity released by the cells ranged from 0.6 to 2.1% of the water-soluble fraction. This is a very low value since, in this alga, more than 80% of the incorporated ^{14}C was found in the water soluble fraction. Light quality does not seem to greatly affect this fraction and in all cases the amount released did not increase appreciably with time (Tables 29 and 31).

In the case of Chlamydomonas, larger and more variable amounts of radioactivity were released. This fraction was highest in white light,

Table 27. Radioactivity of the water-soluble, chloroform-soluble, insoluble and extracellular release fractions of Anacystis nidulans

		Water soluble	Chloroform soluble	Insoluble	Excreted
		(dpm 10^6 cells^{-1})			
White					
2.5 min.	1	800	48	-	-
	2	596	41	-	-
20 min.	1	5761	162	4036	635
	2	5283	165	3595	717
40 min.	1	6908	364	10439	1180
	2	6290	363	9650	1238
Blue					
2.5 min.	1	145	41	-	-
	2	189	26	-	-
20 min.	1	3012	62	271	196
	2	3306	57	281	209
40 min.	1	5321	59	746	320
	2	5404	66	709	349
Green					
2.5 min.	1	189	25	-	-
	2	238	59	-	-
20 min.	1	2752	91	672	322
	2	2689	74	431	301
40 min.	1	4563	110	1515	481
	2	4284	121	1261	487
Red					
2.5 min.	1	857	56	-	-
	2	739	59	-	-
20 min.	1	28764	2996	36833	740
	2	32279	3544	33696	806
40 min.	1	41344	4202	43785	1021
	2	35646	5329	46520	1108

Table 28. Radioactivity of the water-soluble, chloroform-soluble, insoluble and extracellular release fractions of Navicula pelliculosa

		Water soluble	Chloroform soluble	Insoluble	Excreted
		(dpm 10^6 cells^{-1})			
White					
2.5 min.	1	1397	418	-	-
	2	1383	725	-	-
20 min.	1	17679	10011	13014	7465
	2	17581	10607	10938	5086
40 min.	1	93508	27609	27651	7440
	2	96260	27840	21861	6642
Blue					
2.5 min.	1	2360	353	-	-
	2	1398	105	-	-
20 min.	1	64112	2942	8985	1160
	2	58414	1784	7852	598
40 min.	1	109155	4972	22330	3021
	2	98651	4361	17871	3496
Green					
2.5 min.	1	2037	404	-	-
	2	2088	347	-	-
20 min.	1	34725	2145	5030	562
	2	38197	4144	4694	500
40 min.	1	65354	2899	8139	617
	2	64920	5769	7955	714
Red					
2.5 min.	1	1560	143	-	-
	2	1837	90	-	-
20 min.	1	64162	3167	5747	428
	2	73930	4195	7560	503
40 min.	1	137342	6442	12559	743
	2	142459	5588	15936	828

Table 29. Radioactivity of the water-soluble, chloroform-soluble, insoluble and extracellular release fractions of Chlorella vulgaris.

		Water soluble	Chloroform soluble	Insoluble	Excreted
		(dpm 10^6 cells^{-1})			
White					
2.5 min.	1	782	45	-	-
	2	1094	29	-	-
20 min.	1	23018	314	3170	601
	2	22687	590	2521	379
40 min.	1	36004	1525	5669	545
	2	34427	1542	6246	381
Blue					
2.5 min.	1	580	46	-	-
	2	625	64	-	-
20 min.	1	10987	254	1610	104
	2	25591	388	1909	276
40 min.	1	35925	1670	5699	115
	2	42586	838	4274	393
Green					
2.5 min.	1	816	53	-	-
	2	680	54	-	-
20 min.	1	8849	112	198	149
	2	13348	204	316	98
40 min.	1	19253	334	733	111
	2	18493	208	453	98
Red					
2.5 min.	1	1261	18	-	-
	2	1210	32	-	-
20 min.	1	15796	727	701	307
	2	14564	539	626	207
40 min.	1	35235	2441	1355	163
	2	28253	1778	1701	190

Table 30. Radioactivity of the water-soluble, chloroform-soluble, insoluble and extracellular release fractions of Chlamydomonas reinhardtii.

		Water soluble	Chloroform soluble	Insoluble	Excreted
		(dpm 10^6 cells^{-1})			
White					
2.5 min.	1	10522	1079	-	-
	2	10106	990	-	-
20 min.	1	119063	10568	110657	16890
	2	88944	10986	100571	15949
40 min.	1	148219	14524	196981	20767
	2	154593	18983	174069	15231
Blue					
2.5 min.	1	6380	580	-	-
	2	5295	839	-	-
20 min.	1	79540	5019	69337	7528
	2	83367	5642	85089	6199
40 min.	1	113740	27155	139833	9616
	2	92662	12498	159353	7566
Green					
2.5 min.	1	2554	549	-	-
	2	7748	657	-	-
20 min.	1	10146	1066	6765	1086
	2	24632	5100	19588	1841
40 min.	1	16846	1530	15210	1798
	2	37324	5203	39164	2773
Red					
2.5 min.	1	8873	420	-	-
	2	9176	367	-	-
20 min.	1	109015	38104	78968	2644
	2	112625	29919	53650	2953
40 min.	1	172984	72591	105901	4460
	2	185819	55276	84834	3455

Table 31. Comparison of the radioactivity found in the water soluble and extracellular release fractions.

		Water soluble	Excreted	(% Water soluble)
		(dpm 10^6 cells^{-1} 40 min.$^{-1}$)		
CHLORELLA				
White	1	36004	545	1.5
	2	34427	381	1.1
Blue	1	35925	115	0.3
	2	42586	393	0.9
Green	1	19253	111	0.6
	2	18493	98	0.5
Red	1	35235	163	0.5
	2	28253	190	0.7
CHLAMYDOMONAS				
White	1	148219	20767	14.0
	2	154593	15231	9.9
Blue	1	113740	9616	8.5
	2	92662	7566	8.2
Green	1	16846	1798	10.7
	2	37324	2773	7.4
Red	1	172984	4460	2.6
	2	185819	3455	1.9
NAVICULA				
White	1	93508	7440	8.0
	2	96260	6642	6.9
Blue	1	109155	3021	2.8
	2	98651	3496	3.5
Green	1	65354	617	0.9
	2	64920	714	1.1
Red	1	137342	743	0.5
	2	142459	828	0.6
ANACYSTIS				
White	1	6808	1180	17.3
	2	6290	1238	19.7
Blue	1	5321	320	6.0
	2	5404	349	6.5
Green	1	4563	481	10.5
	2	4284	487	11.4
Red	1	41344	1021	2.5
	2	35646	1108	3.1

Table 32. Ion exchange separation of the water-soluble and extra-cellular release fractions of Anacystis nidulans.

		Neutral	Basic	Acidic
		(dpm 10^6 cells^{-1})		
White				
Water soluble	1	1101	591	3087
	2	1274	683	2475
Excreted	1	362	309	250
	2	697	302	295
Blue				
Water soluble	1	1198	1619	1112
	2	1022	1334	2461
Excreted	1	527	235	198
	2	476	181	170
Green				
Water soluble	1	920	369	2216
	2	926	488	2474
Excreted	1	433	144	165
	2	426	135	193
Red				
Water soluble	1	7981	8540	4403
	2	8106	7831	4189
Excreted	1	948	332	411
	2	787	278	414

Table 33. Ion exchange separation of the water soluble and extra-cellular release fractions of Navicula pelliculosa.

		Neutral	Basic	Acidic
		(dpm 10^6 cells^{-1})		
White				
Water soluble	1	25757	8754	7216
	2	22506	8479	9095
Excreted	1	1253	1247	4161
	2	647	511	2009
Blue				
Water soluble	1	56041	6845	10612
	2	57085	9384	6802
Excreted	1	620	828	427
	2	674	677	624
Green				
Water soluble	1	24551	12441	4925
	2	22965	7069	6179
Excreted	1	556	155	308
	2	420	250	221
Red				
Water soluble	1	52475	6250	11107
	2	53497	13680	15987
Excreted	1	445	303	377
	2	438	226	348

Table 34. Ion exchange separation of the water soluble and extra-cellular release fractions of Chlorella vulgaris.

		Neutral	Basic	Acidic
		(dpm 10^6 cells^{-1})		
White				
Water soluble*	1	4973	9594	5556
	2	5164	6669	3627
Excreted*	1	168	114	98
	2	233	79	45
Blue				
Water soluble	1	13642	7254	3892
	2	20198	8894	4076
Excreted	1	66	48	46
	2	123	86	52
Green				
Water soluble	1	13038	3011	1626
	2	10363	2191	1244
Excreted	1	183	52	47
	2	105	35	38
Red				
Water soluble	1	19802	6205	2283
	2	14978	5697	2086
Excreted	1	107	78	62
	2	102	75	54

* 20 min. sample - all other 40 min. samples.

Table 35. Ion exchange separation of the water soluble and extracellular release fractions of Chlamydomonas reinhardtii.

		Neutral	Basic	Acidic
		(dpm 10^6 cells^{-1})		
White				
Water soluble	1	35325	21440	28401
	2	39752	20967	28932
Excreted	1	5805	4460	5750
	2	4984	3697	3961
Blue				
Water soluble	1	10629	27845	20395
	2	13084	30692	26760
Excreted	1	4102	3019	3616
	2	5700	3086	4302
Green				
Water soluble	1	7004	2539	5787
	2	8046	6069	7787
Excreted	1	3265	1782	1579
	2	5451	4456	3228
Red				
Water soluble	1	37294	27230	39456
	2	48401	32834	46642
Excreted	1	5855	1958	4419
	2	5682	2351	2053

being 12 - 15% of the water soluble fraction and lowest in red light (2.2 - 2.5%). An intermediate amount (8.4%) was released in blue or green light. In this case a slight increase in the amount released with time was observed (Tables 30 and 31).

In the diatom, Navicula, highest amounts of extracellular release were found in white light. The amount released by the 20 min. samples apparently formed 35.6% of the activity of the water soluble fraction, however, the activity of this latter fraction would appear to be unusually low. The value of 7.4% for the 40 min. sample appears to be more realistic. The lowest amount of radioactivity was released in red light (0.6 - 0.7%) with intermediate levels of release found in blue or green light. Again the amounts released are very low considering the high percentage of activity in the water soluble fraction of this alga. A modest increase in activity released with time was observed, (Tables 28 and 31).

As a percentage of activity of the water soluble fraction extracellular release was highest in white grown cells of Anacystis. Extracellular release decreased in a regular manner in green, blue and red light grown cells. The lowest percentage release, i.e. 1/4 the amount in white light was found in red light cells. Most treatments showed a fairly strong increase in extracellular release with time (Tables 27 and 31).

If the activity of the extracellular release fraction is considered as a percentage of the total ^{14}C-incorporated into whole cells at 40 min., it can be seen that cellular loss of carbon via this fraction does not exceed 4.1% (Table 36). Chlorella has the lowest carbon loss due to extracellular release, activity in this fraction ranging from

Table 36. Carbon losses due to extracellular release and $^{14}CO_2$ release in the light (based on 40 min. samples).

	Net $^{14}CO_2$ uptake	Extracellular release	%	$^{14}CO_2$ light release	%
	(dpm 10^6cells^{-1})			(dpm 10^6cells^{-1})	
CHLORELLA					
White 1	70899	545	0.8	578	0.8
2	68815	381	0.6	316	0.5
Blue 1	59639	115	0.2	960	1.6
2	63222	393	0.6	640	1.0
Green 1	26880	111	0.4	1088	4.0
2	26257	98	0.4	271	1.0
Red 1	52895	163	0.3	328	0.6
2	38223	190	0.5	273	0.7
CHLAMYDOMONAS					
White 1	852265	20767	2.4	6367	0.8
2	711826	15231	2.1	6267	0.9
Blue 1	584222	9616	1.6	12062	2.1
2	603121	7566	1.3	13898	2.3
Green 1	83360	1798	2.2	23500	28.2
2	207905	2773	1.3	20520	9.9
Red 1	875882	4460	0.5	9680	1.1
2	872643	3455	0.4	3539	0.4
NAVICULA					
White 1	182402	7440	4.1	3713	2.0
2	187347	6642	3.6	2213	1.2
Blue 1	192453	3021	1.6	5367	2.8
2	187279	3496	1.9	4729	2.5
Green 1	97488	617	0.6	12396	12.7
2	104222	714	0.7	6777	6.7
Red 1	200440	743	0.4	3600	1.8
2	210242	828	0.4	1967	0.9
ANACYSTIS					
White 1	64910	1180	1.8	405	0.6
2	57648	1238	2.1	625	1.1
Blue 1	9958	320	3.2	4727	47.5
2	10655	349	3.3	6542	61.4
Green 1	15557	481	3.1	1460	9.4
2	14135	487	3.4	1940	13.7
Red 1	222313	1021	0.5	37	0.02
2	218153	1108	0.5	20	0.01

0.2 to 0.8% of total C^{14}-fixation. Light quality does not appear to affect extracellular release at this level in Chlorella. In Chlamydomonas, carbon loss as a percentage of total ^{14}C-incorporated into whole cells is lowest in red light (0.4 - 0.5%) and fairly similar in white, blue and green light (1.3 - 2.4%). In Navicula, carbon loss was 0.4 to 0.7% of the whole cell value in red and green light, this rose to 1.9% in blue light and 4.1% in white light. In Anacystis, extracellular release as a percentage of whole cell activity was lowest in red light (0.5%), intermediate in white light (2.1%) and highest in blue and green light (3.1 - 3.4%).

To further investigate the influence of light quality on the partitioning of carbon in algal cells, the radioactivity in the neutral, basic and acidic fractions of the water soluble and extracellular release extracts was examined (Tables 32 to 35). In Chlorella, with the exception of the white light treatment, the highest percentage of radioactivity was found in the neutral fraction of the water soluble extract. This was followed in decreasing order of magnitude by the basic and acidic fractions. In general, a similar trend in percentage activity was found in the extracellular release fractions. Light quality did not seem to affect this distribution of radioactivity (Table 34).

In Chlamydomonas, a more even distribution of radioactivity was found in the neutral, basic and acidic fractions of the water soluble extract. With the exception of blue light, the level of activity in the neutral and acidic fractions was similar with a lower amount of activity in the basic fraction. These distributions of activity were generally mirrored in the extracellular release fraction (Table 35). In blue light, the radioactivity of the basic water soluble fraction was

increased apparently at the expense of the neutral fraction.

The highest level of activity was found in the neutral fraction of the water soluble extract from Navicula. In green and red light, the distribution of activity in the carbon released by the cells generally paralleled the activity in the water soluble extract. This was not so in white or blue light. In white light, the high level of activity in the neutral fraction of the water soluble extract was not observed in the carbon released by the cells. While only 20% of the carbon was fixed in the acidic fraction of the water soluble extract, over 60% of the released activity was found in this fraction. In blue light, the high level of activity in the neutral water soluble fraction and rather low levels of activity in the basic and acidic fractions was not reflected in the rather even distribution of activity in these fractions released by the cells (Table 33).

An interesting feature of the Anacystis results is that while the neutral fraction never exceeded 40% of the activity of the water soluble fraction, it always formed 50% or greater levels of the activity in the extracellular release fraction (Table 32). In white and green light, activity in the basic fraction of the water soluble extract was low. The activity in the acidic fraction of this extract was greater than 60% in both these light treatments, however, the acidic fraction activity of the extracellular release fraction was only 14 to 15%. In red and blue light grown cells no major assymetry in the fractions was noticed apart from the above mentioned effect in the neutral fraction.

5.3 Photorespiration

In attempts to assess the carbon loss due to photorespiration,

$^{14}CO_2$ released by the cells was examined as described in Materials and Methods. The results of these experiments are expressed as dpm released per 10^6 cells per 40 min. interval for comparison with $^{14}CO_2$-uptake over a similar time interval (Tables 36 to 40). If one compares the amount of $^{14}CO_2$ released in the light with the amount taken up over a 40 min. time period, photorespiration would appear to be a very minor process in Chlorella. The $^{14}CO_2$ lost by this alga appears to range from 0.5 to 4% of the total $^{14}CO_2$-uptake (Table 36). Losses appear to be lowest in white or red light and highest in blue and green light. In Chlamydomonas, losses were 1% or less in white and red light, about 2% in blue light, but rose considerably in green light (over 10%) Table 36).

In Navicula, $^{14}CO_2$ losses ranged from 0.9 to 2.8% of the total $^{14}CO_2$ incorporated in white, blue and red light. In green light, losses ranged from 6.7 to 12.7% (Table 36).

In red light, losses of $^{14}CO_2$ in the light were very low, i.e. 0.01 to 0.02% for Anacystis. These losses rose to about 1% in white, 9.4 to 13.7% in green light and extremely high losses of 47.5 to 61.4% were observed in blue light (Table 36).

It should be pointed out that the highest $^{14}CO_2$ losses were observed under conditions where the algae showed low $^{14}CO_2$-uptake rates in the light, e.g. green light for Chlamydomonas and Navicula and blue light for Anacystis (Table 36). In these cases a high percentage of the incorporated $^{14}CO_2$ was found in the water soluble fraction. Since this may represent a pool of highly labelled photorespiratory substrate, $^{14}CO_2$ of higher specific activity may be released under these conditions and thus complicate interpretation of these results.

Table 37. Comparison of the release of $^{14}CO_2$ in the light and dark of Anacystis nidulans cells grown in white, blue, green and red light (550 μW cm^{-2})

Light quality	Sample	Total $^{14}CO_2$ fixed (dpm 10^6 $cells^{-1}$ 40 $min.^{-1}$)	Rate of $^{14}CO_2$ release* (dpm 10^6 $cells^{-1}$ 40 $min.^{-1}$)		L/D Ratio
			Light	Dark	
White	1	64910	405	1573	0.19
	2	57648	624	1851	0.10
Blue	1	9958	4727	9969	0.47
	2	10655	6542	14697	0.45
Green	1	15557	1460	3973	0.37
	2	14135	1940	4360	0.44
Red	1	222313	37	196	0.19
	2	218153	20	200	0.10

* The rate of $^{14}CO_2$ release was calculated from the steady rates determined in time course experiments.

Table 38. Comparison of the release of $^{14}CO_2$ in the light and dark of Navicula pelliculosa cells grown in white, blue, green and red light (550 μW cm^{-2}).

Light quality	Sample	Total $^{14}CO_2$ fixed (dpm 10^6 $cells^{-1}$ 40 $min.^{-1}$)	Rate of $^{14}CO_2$ release* (dpm 10^6 $cells^{-1}$ 40 $min.^{-1}$)		Light/Dark Ratio
			Light	Dark	
White	1	182402	3713	5553	0.67
	2	187347	2213	3383	0.65
Blue	1	192453	5367	9187	0.58
	2	187279	4729	9204	0.51
Green	1	97488	12396	12380	1.0
	2	104222	6977	7720	0.9
Red	1	200440	3600	5603	0.64
	2	210242	1967	2720	0.72

*The rate of $^{14}CO_2$ release was calculated from the steady rates determined in time-course experiments.

Table 39. Comparison of the release of $^{14}CO_2$ in the light and dark of Chlorella vulgaris cells grown in white, blue, green and red light (550 μW cm^{-2}).

Light quality	Sample	Total $^{14}CO_2$ fixed (dpm 10^6 cells^{-1} 40 min.$^{-1}$)	Rate of $^{14}CO_2$ release* (dpm 10^6 cells^{-1} 40 min.$^{-1}$)		Light/Dark Ratio
			Light	Dark	
White	1	70855	578	2004	0.29
	2	68815	316	1327	0.24
Blue	1	59639	960	2094	0.46
	2	63222	640	1620	0.40
Green	1	26880	1088	1453	0.75
	2	26257	271	1173	0.23
Red	1	52895	328	1273	0.26
	2	38223	273	760	0.36

* The rate of $^{14}CO_2$ release was calculated from the steady rates determined in time-course experiments.

Table 40. Comparison of the release of $^{14}CO_2$ in the light and dark of Chlamydomonas reinhardtii cells grown in white, blue, green and red light (550 μW cm^{-2}).

Light quality	Sample	Total $^{14}CO_2$ fixed (dpm 10^6 $cells^{-1}$ 40 $min.^{-1}$)	Rate of $^{14}CO_2$ release* (dpm 10^6 $cells^{-1}$ 40 $min.^{-1}$)		Light/Dark Ratio
			Light	Dark	
White	1	852265	6367	15333	0.42
	2	711826	6267	17293	0.36
Blue	1	584222	12062	28160	0.43
	2	603121	13898	40920	0.34
Green	1	83360	23500	30529	0.76
	2	207905	20520	27167	0.76
Red	1	875882	9680	9680	1.0
	2	872643	3539	3539	1.0

* The rate of $^{14}CO_2$ release was calculated from the steady rates determined in time-course experiments.

Figure 13. Time-course of $^{14}CO_2$ release into CO_2-free air by Anacystis nidulans in white light (550 μW cm^{-2}) and darkness.

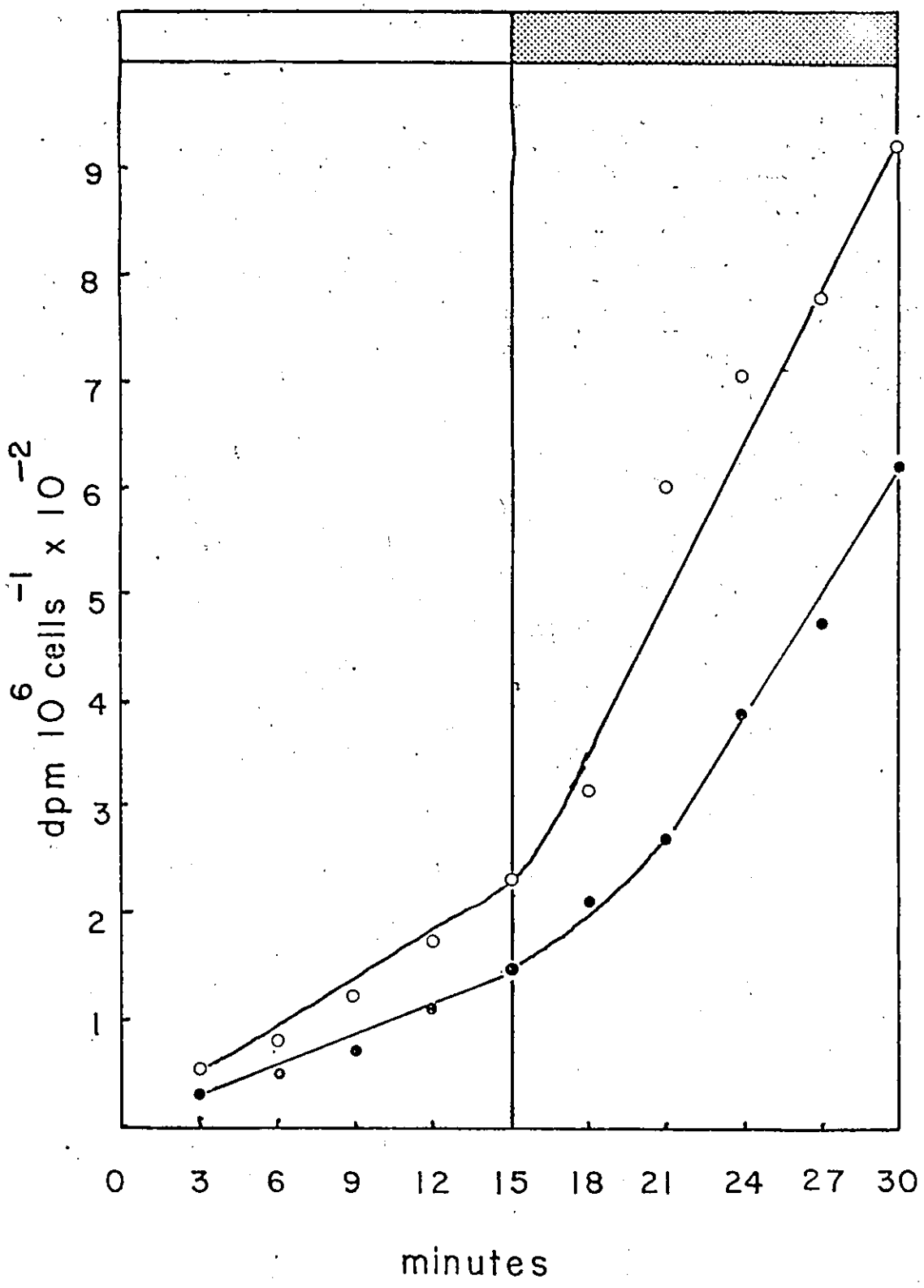
dpm 10^6 cells^{-1} x 10^{-2}
1
2
3
4
5
6
7
8
9
0
3
6
9
12
15
18
21
24
27
30
minutes

Figure 13.

Figure 14. Time-course of $^{14}CO_2$ release into CO_2-free air by Navicula pelliculosa in white light (550 μW cm^{-2}) and darkness.

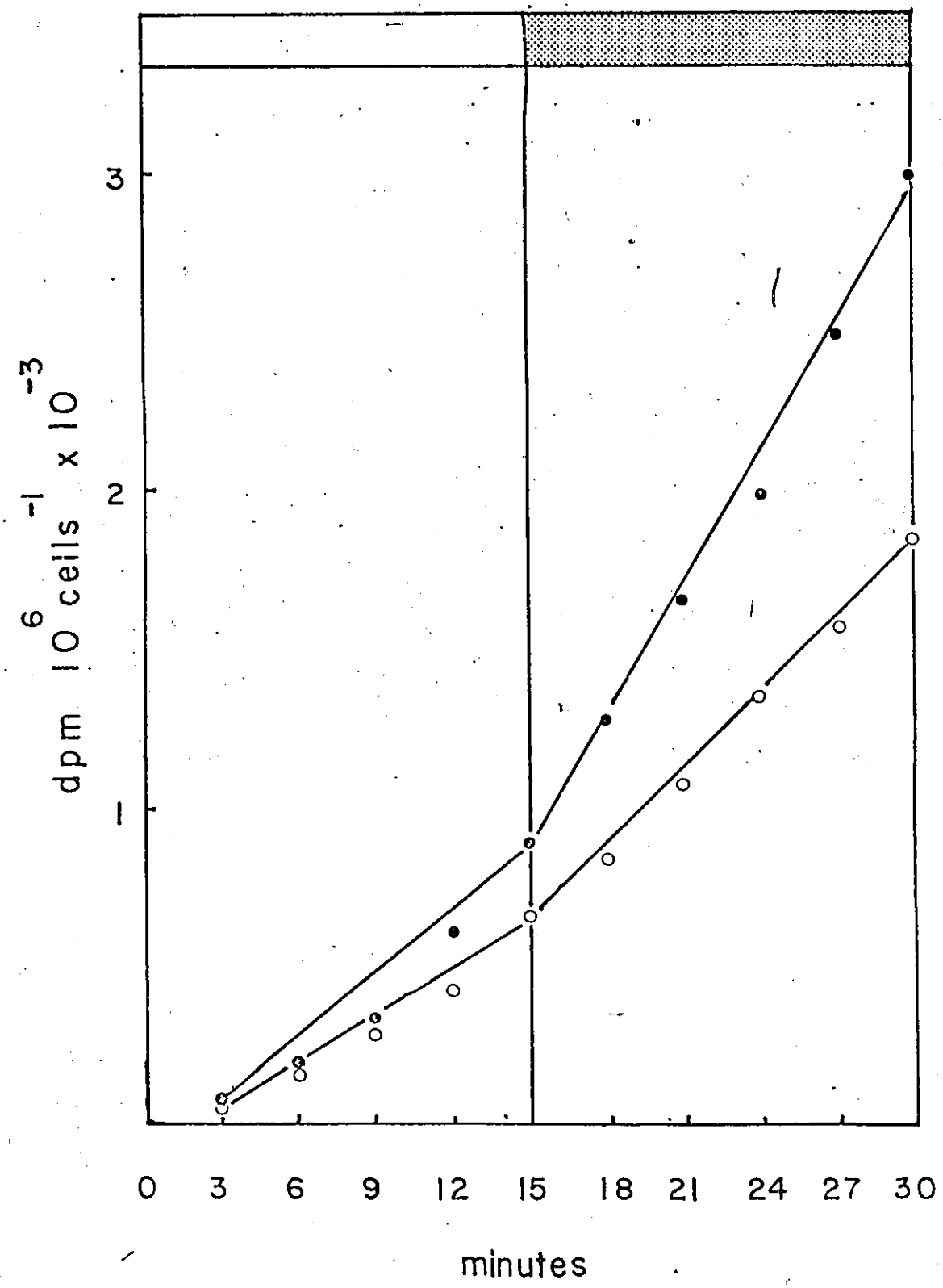

Figure 14.

Figure 15. Time-course of $^{14}CO_2$ release into CO_2-free air by Chlorella vulgaris in white light (550 μW cm^{-2}) and darkness.

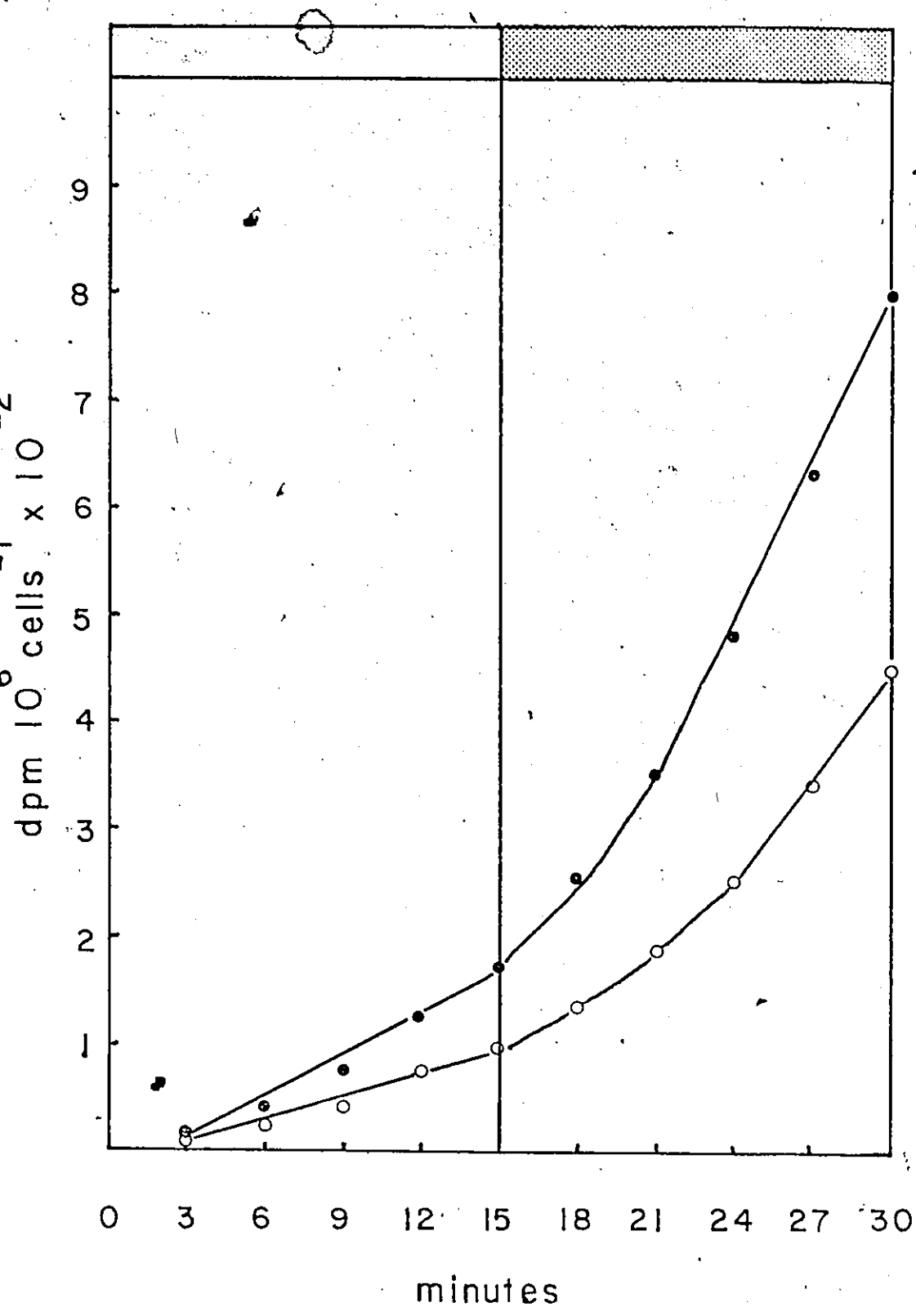
dpm 10^6 cells^{-1} x 10^{-2}
1
2
3
4
5
6
7
8
9
0
3
6
9
12
15
18
21
24
27
30
minutes

Figure 15.

Figure 16. Time-course of $^{14}CO_2$ release into CO_2-free air by Chlamydomonas reinhardtii in white light (550 μW cm^{-2}) and darkness.

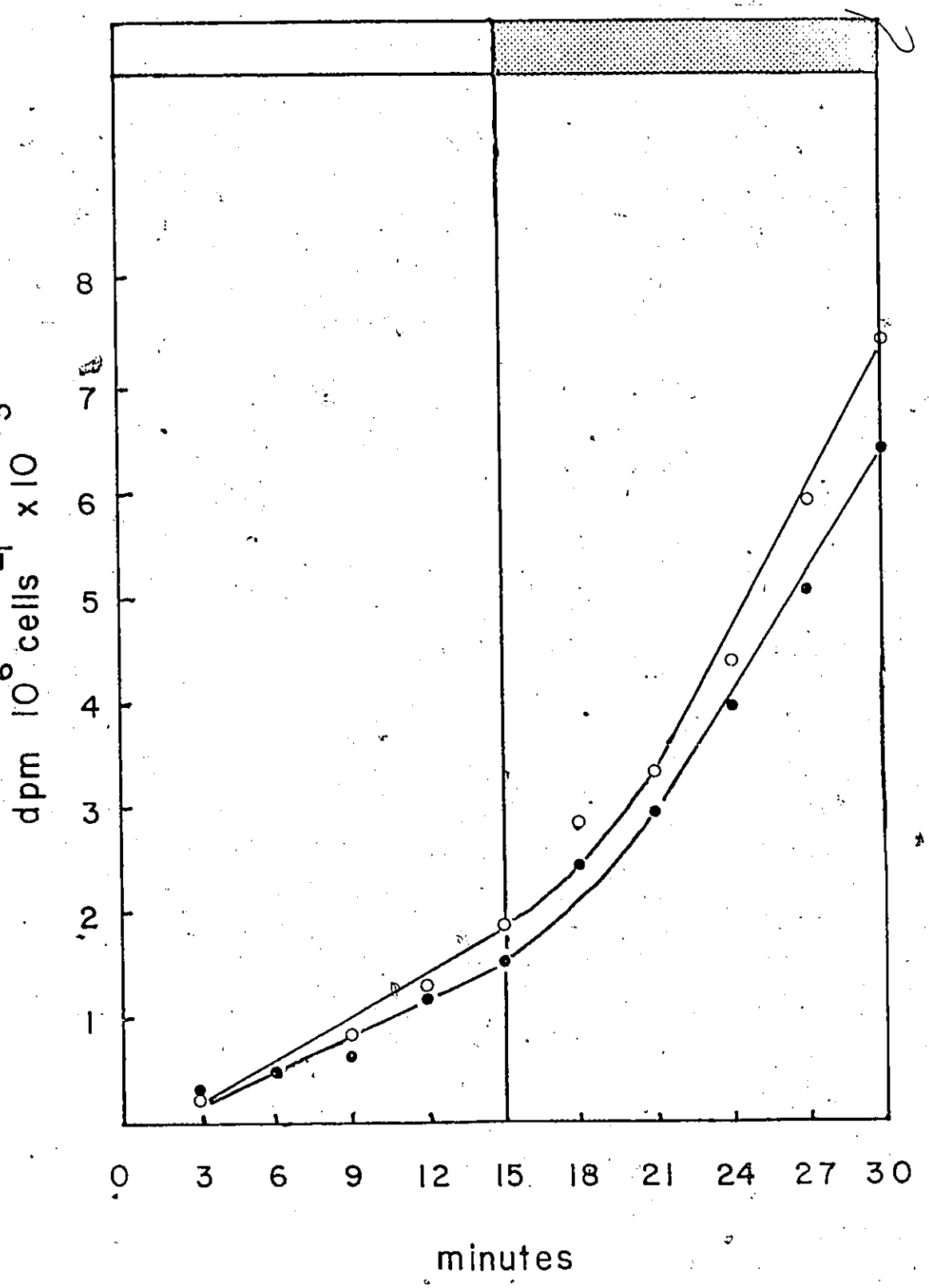

Figure 16.

It has been suggested that the ratio of $^{14}CO_2$ release in the light to that released in the dark provides a means of comparing the degree of photorespiration among various plant species (383). This assumption is based on $^{12}CO_2$-exchange studies with terrestrial plants where it has been shown that this ratio is very low (<< 1) in C_4 plants and > 1 in C_3 plants. Except for two cases (in Tables 38 and 40), the ratio of light release to dark release was less than one, which suggests at a very minimum estimate that dark respiration does not continue unabated in the light and that algae do not photorespire to the same degree as terrestrial C_3 plants. However, since the ratio is dependent on the relative rates of $^{14}CO_2$ release in the light and dark somewhat anomalous results can arise. For example, Chlamydomonas in green light has an L/D ratio of 0.76 whereas in red light the ratio is 1.0, however, the amount of $^{14}CO_2$ lost in red light is substantially less on an absolute or percent total $^{14}CO_2$-uptake basis (Tables 36 and 40). In other instances, the $^{14}CO_2$ loss in the light may rise with no change in the L/D ratio. For example, the $^{14}CO_2$ loss in blue light is twice the rate for white light Chlamydomonas cells though the same ratio is observed (Table 40). A similar situation exists for white and red light grown Anacystis cells where $^{14}CO_2$ losses in red light are 1/10 the white light losses (Table 37). A major factor contributing to this variation in L/D ratio is the rate of dark $^{14}CO_2$ release. In Chlorella, dark $^{14}CO_2$ release does not vary dramatically, though rates are lowest after red light and highest after blue light (Table 39). In Chlamydomonas, a 3- to 4-fold variation in dark $^{14}CO_2$ release rates was observed, the highest and lowest rates occurring after exposure to blue and red light respectively (Table 40). Rates of $^{14}CO_2$

release in the dark varied about two-fold in Navicula, the highest dark rate occurred following green light (Table 38). $^{14}CO_2$ release in the dark varied by a factor of at least 5 in Anacystis, the rate was lowest after red light and highest after blue light (Table 37).

Because of the complications induced by the spectral dependence of $^{14}CO_2$-uptake and release, both on an absolute or ratio basis, it would seem that the ^{14}C-assay technique is not the appropriate method for measuring the effect of light quality on photorespiration within an algal species. However, this assay has been used to compare photorespiration in white light between different species of algae (43). If we compare the four algae used in this study on the basis of their L/D ratio in white light, the lowest ratios occur in the blue-green alga Anacystis (0.10 - 0.19) followed in ascending order of magnitude by Chlorella (0.24 - 0.29) Chlamydomonas (0.36 - 0.42) and Navicula (0.65 - 0.67) (Tables 37 to 40 and Figs. 13 to 16). These results are of the same order of magnitude as those reported for similar algal species (43).

In an attempt to get some measure of the actual amount of CO_2 loss due to photorespiration the method of Hew et al (139) was adapted for use in aqueous systems. This method estimates the rate of CO_2-evolution in the light from the decrease in the specific radioactivity of $^{14}CO_2$ in a closed system.

The sensitivity and accuracy of the gas chromatographic technique developed in this study is shown in Fig. 17. This standard curve of peak area against dissolved inorganic carbon (DIC) concentration was obtained by injecting µl amounts of 1 mM Na_2CO_3. The standard deviation at the 2nM Na_2CO_3 level was 8.5% (n = 31). Generally 1 ml of cell-free medium was injected onto the gas chromatograph so that peak area could

Figure 17. Recorder response (peak area) vs. volume (µl) of 1 mM Na_2CO_3 injected.

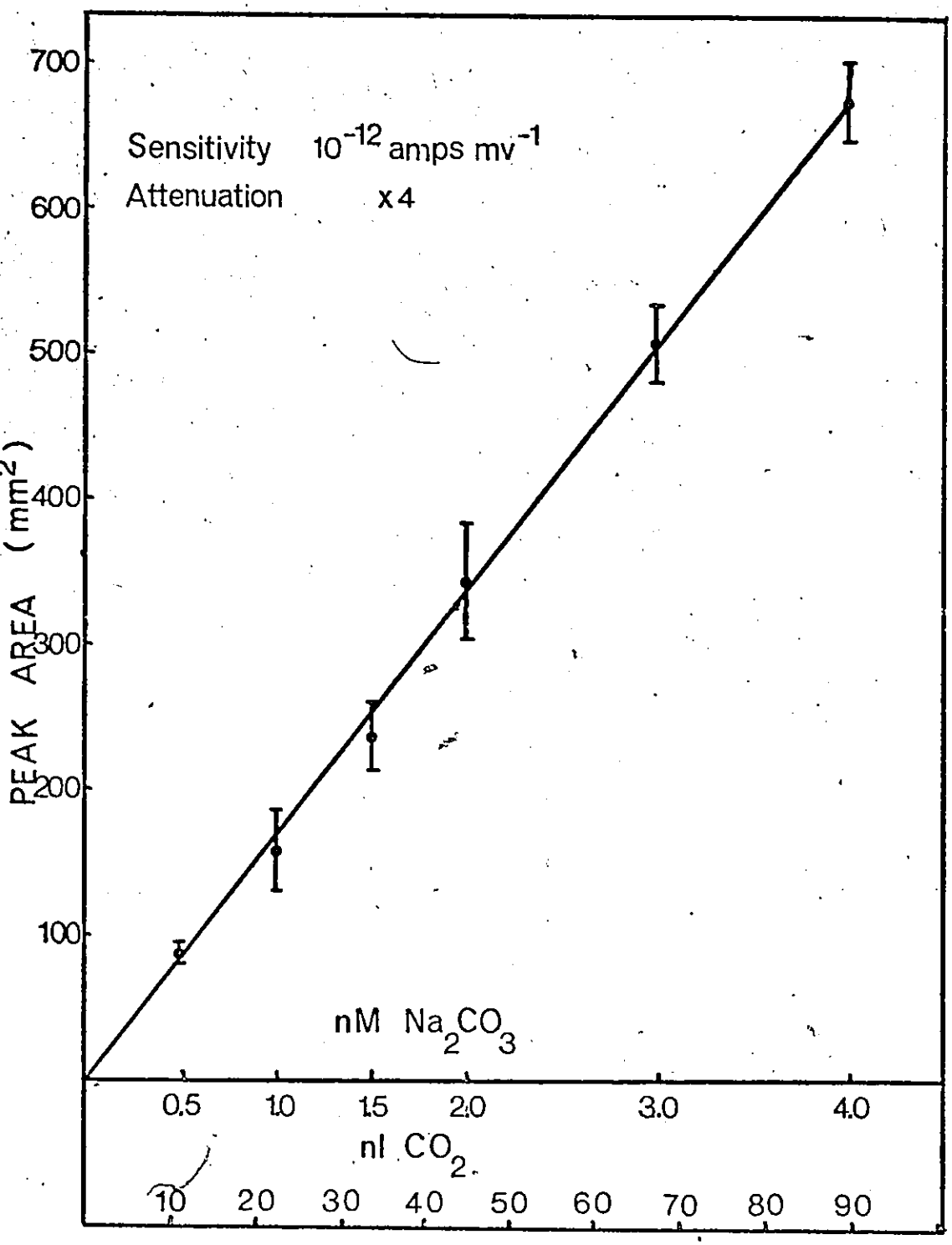
Sensitivity 10^{-12} amps mv^{-1}
Attenuation x4
PEAK AREA (mm^2)
700
600
500
400
300
200
100
nM Na_2CO_3
0.5
1.0
1.5
2.0
3.0
4.0
nl CO_2
10
20
30
40
50
60
70
80
90

Figure 17.

be read off directly as nl CO_2/ml or µl CO_2/l. This CO_2-hydrogenation technique is extremely sensitive with a detection limit of 5nl/l CO_2.

The principle was first used to measure CO and CO_2 separated by gas chromatography by Schwenk et al (303) and Porter and Volman (269). Since then it has been used to measure organic carbon in water (63, 71), CO_2 in hyperbaric atmospheres (375) total CO_2 in biological fluids (326), fungal respiration (315), net CO_2 exchange in lichens (62), and CO_2 compensation points in freshwater algae (Birmingham and Colman, in press).

It should be pointed out that since the sample was acidified all forms of DIC, i.e. dissolved CO_2, H_2CO_3, HCO_3^- and CO_3^- are converted to CO_2. The use of a gas stripping column to transfer all DIC to the gas chromatograph following acidification (334) avoids problems involved with headspace sampling encountered in earlier applications of this technique (326).

The results of experiments using this CO_2-hydrogenation technique in conjunction with liquid scintillation counting to measure changes in specific radioactivity of $^{14}CO_2$ external to white light-grown freshwater algae in a closed system, are shown in Tables 41 to 44. The results are presented graphically in Figs. 18 to 21. In Fig. 20 the total CO_2 and $^{14}CO_2$ concentration and specific radioactivity changes caused by Chlorella are shown. The initial total CO_2 concentration is less than the CO_2 level that might be expected if the water was in equilibrium with the atmosphere, i.e. about 300 µl CO_2/l. Low CO_2 concentrations were used to simulate the low CO_2 surrounding cells being gassed with CO_2-free air as in the ^{14}C-assay technique. In the light the CO_2 level dropped rapidly to CO_2 compensation point levels.

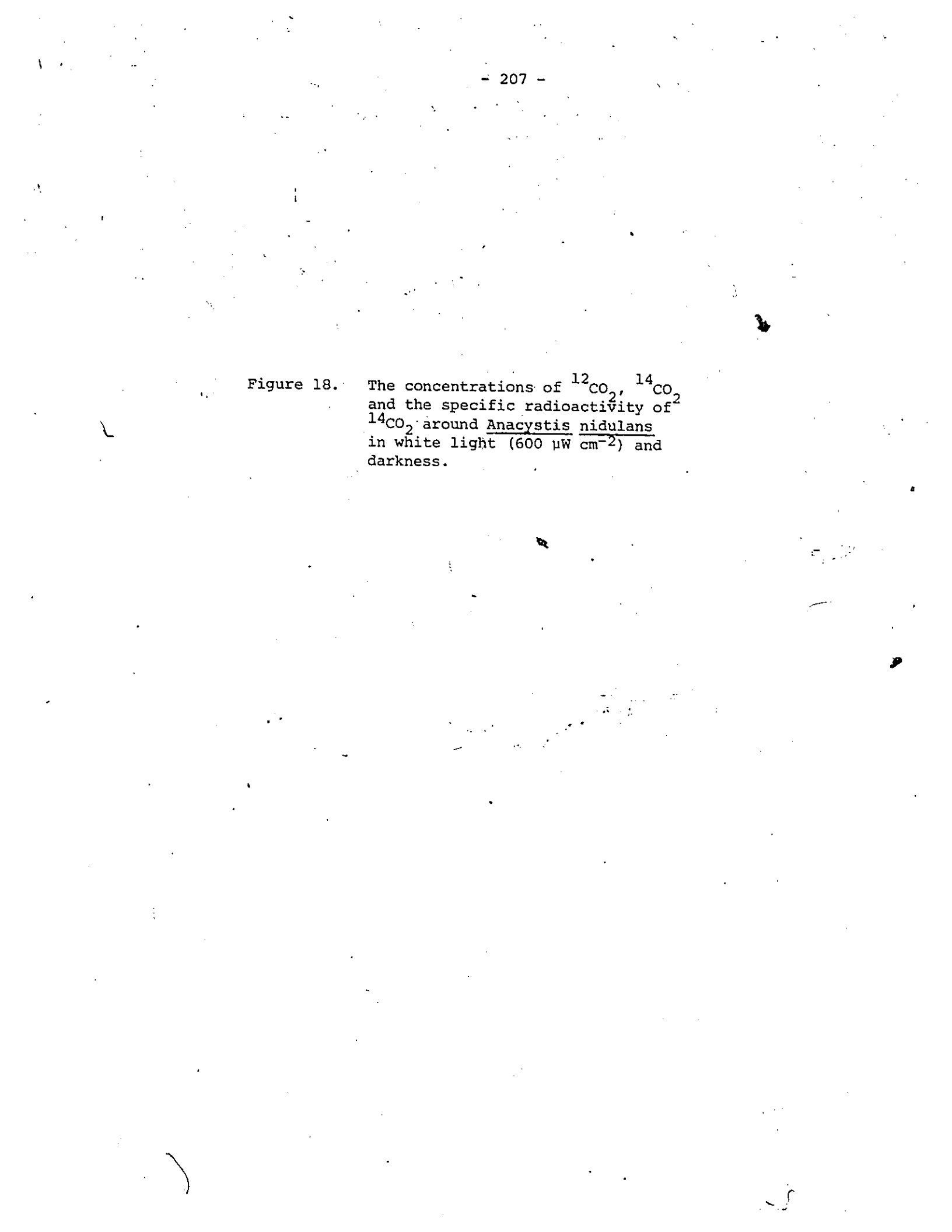

Figure 18. The concentrations of $^{12}CO_2$, $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Anacystis nidulans in white light (600 μW cm^{-2}) and darkness.

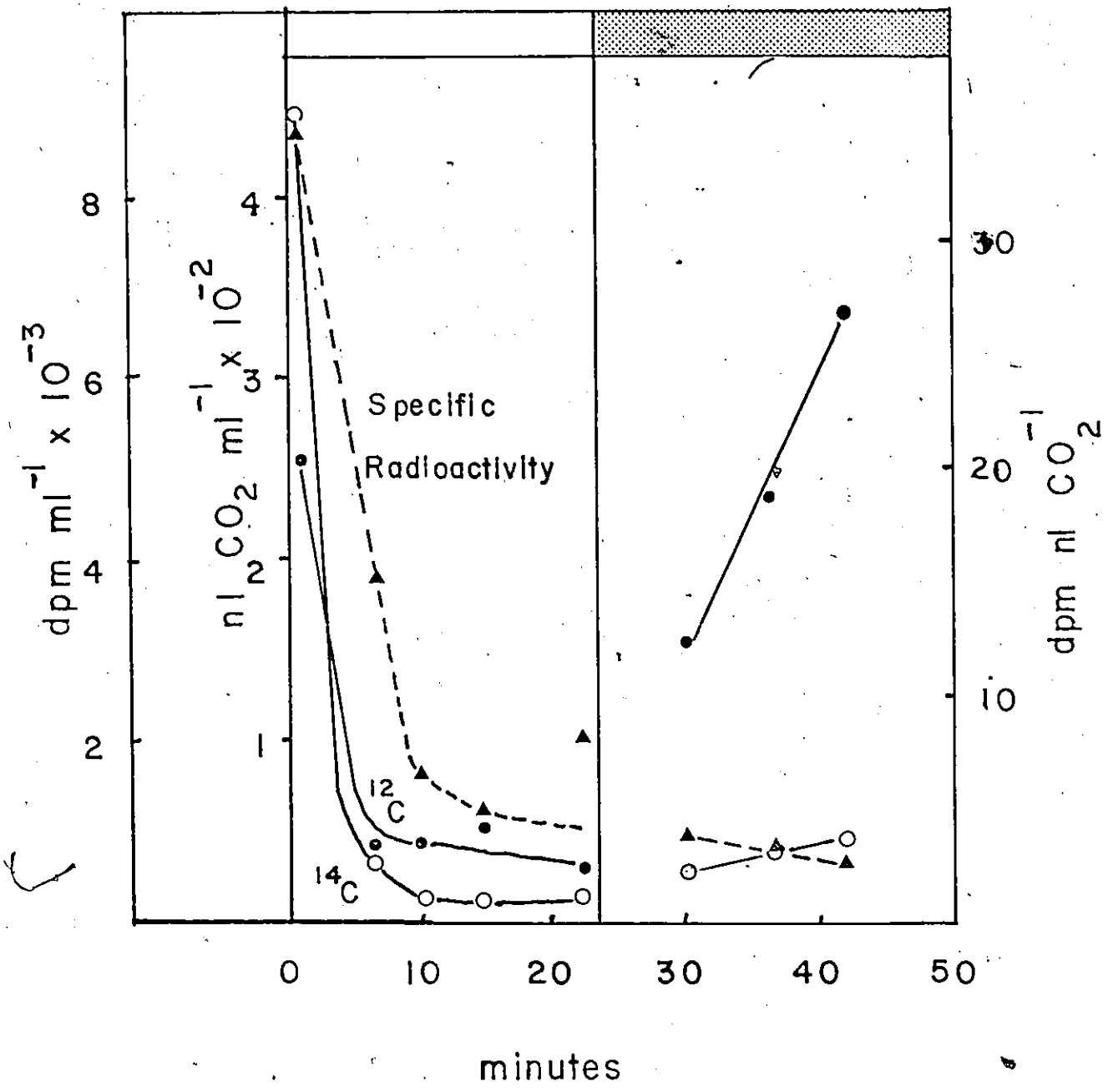
dpm ml^{-1} x 10^{-3}
nl CO$_2$ ml^{-1} x 10^{-2}
dpm nl CO$_2$ $^{-1}$
Specific
Radioactivity
^{12}C
^{14}C
8
6
4
2
4
3
2
1
30
20
10
0
10
20
30
40
50
minutes

Figure 18.

Figure 19. The concentrations of $^{12}CO_2$ and $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Navicula pelliculosa in white light (600 μW cm^{-2}) and darkness.

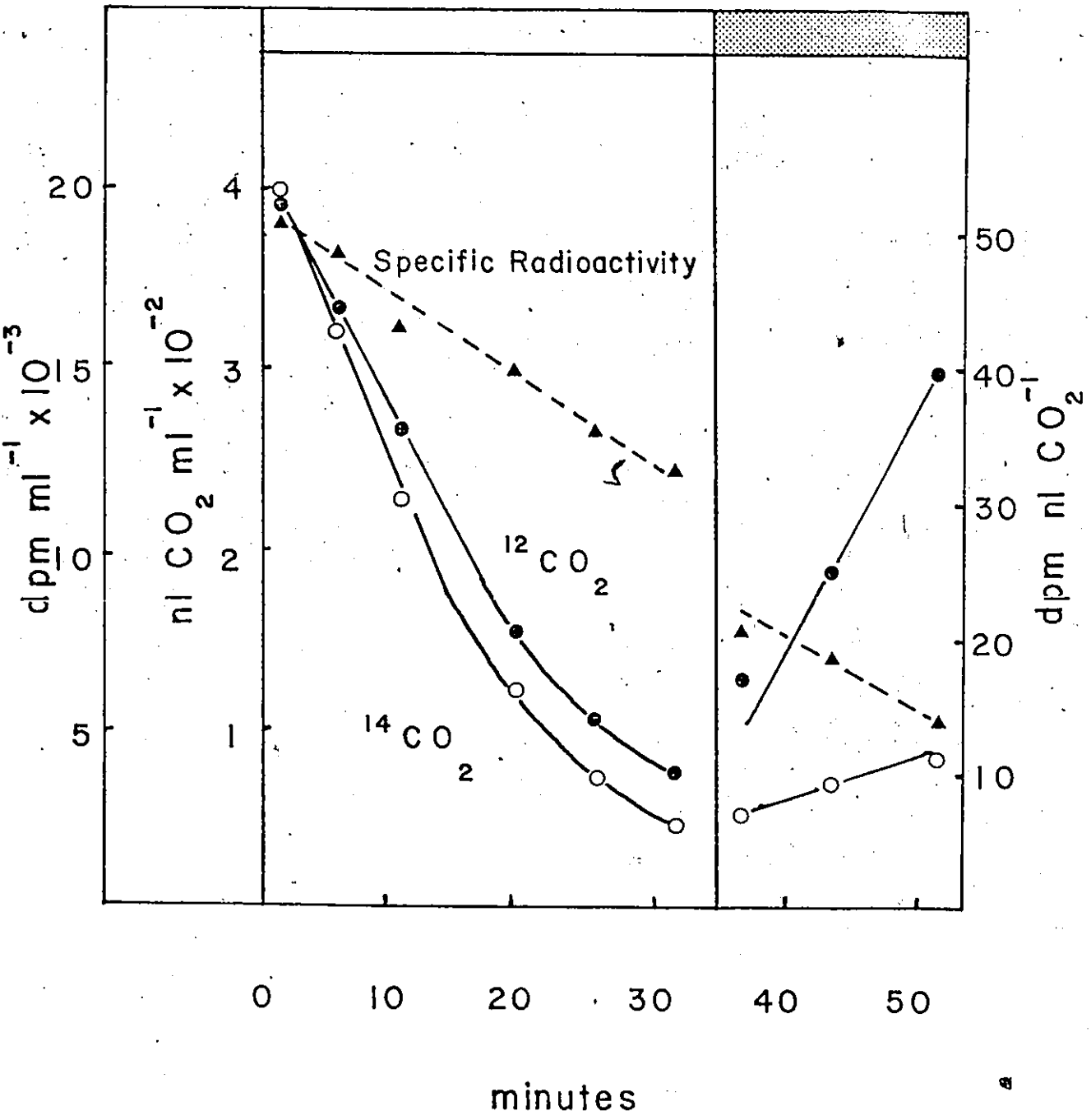
Specific Radioactivity
$^{12}CO_2$
$^{14}CO_2$
dpm $ml^{-1} \times 10^{-3}$
nl CO_2 $ml^{-1} \times 10^{-2}$
dpm nl CO_2^{-1}
minutes
0
10
20
30
40
50
5
10
15
20
1
2
3
4
10
20
30
40
50

Figure 19.

Figure 20. The concentrations of $^{12}CO_2$ and $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Chlorella vulgaris in white light (600 μW cm^{-2}) and darkness.

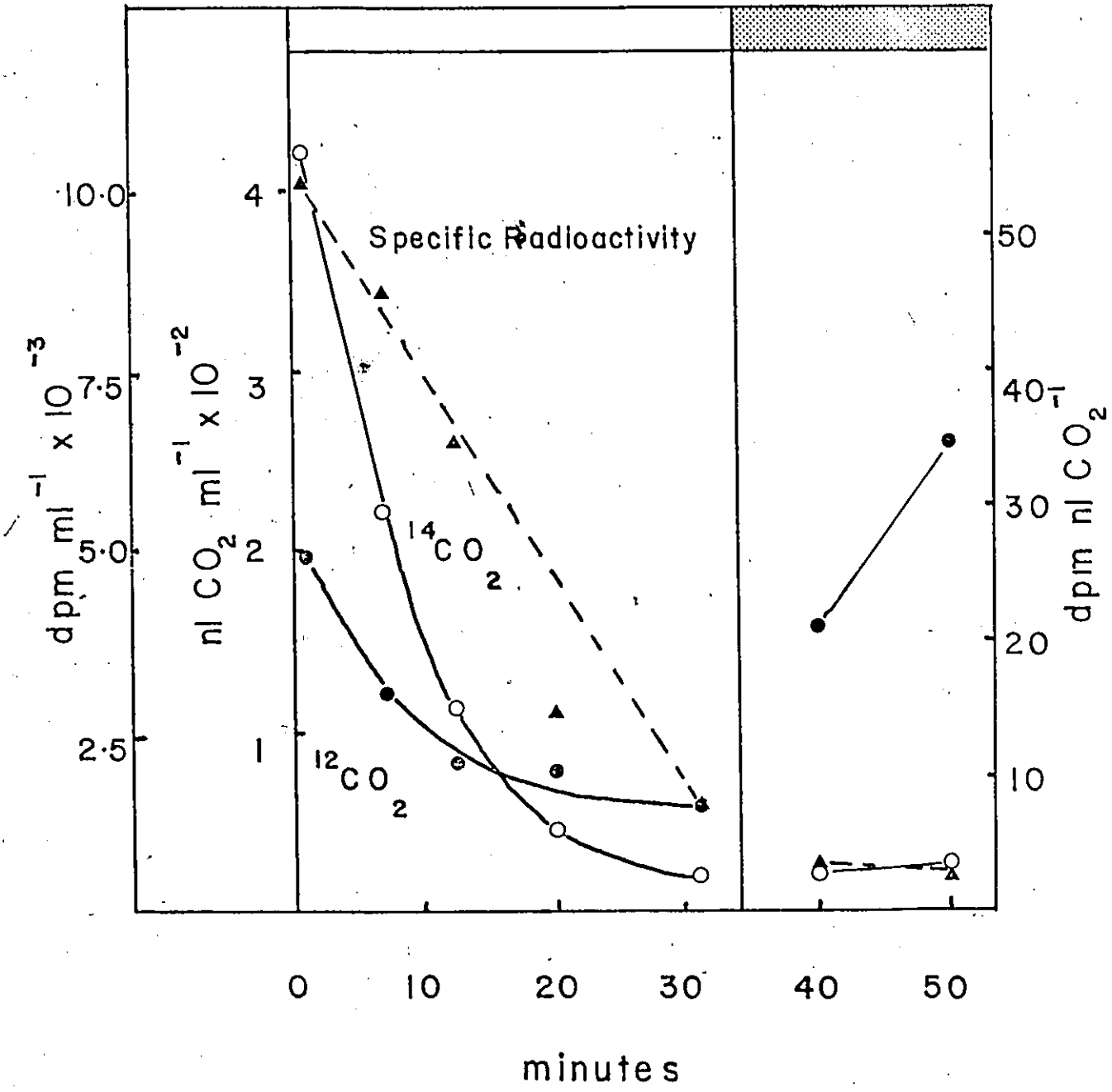
Specific Radioactivity
$^{14}CO_2$
$^{12}CO_2$
dpm ml^{-1} x 10^{-3}
nl CO_2 ml^{-1} x 10^{-2}
dpm nl CO_2^{-1}
10·0
7·5
5·0
2·5
4
3
2
1
50
40
30
20
10
0
10
20
30
40
50
minutes

Figure 20.

Figure 21. The concentrations of $^{12}CO_2$ and $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Chlamydomonas reinhardtii in white light (600 μW cm^{-2}) and darkness.

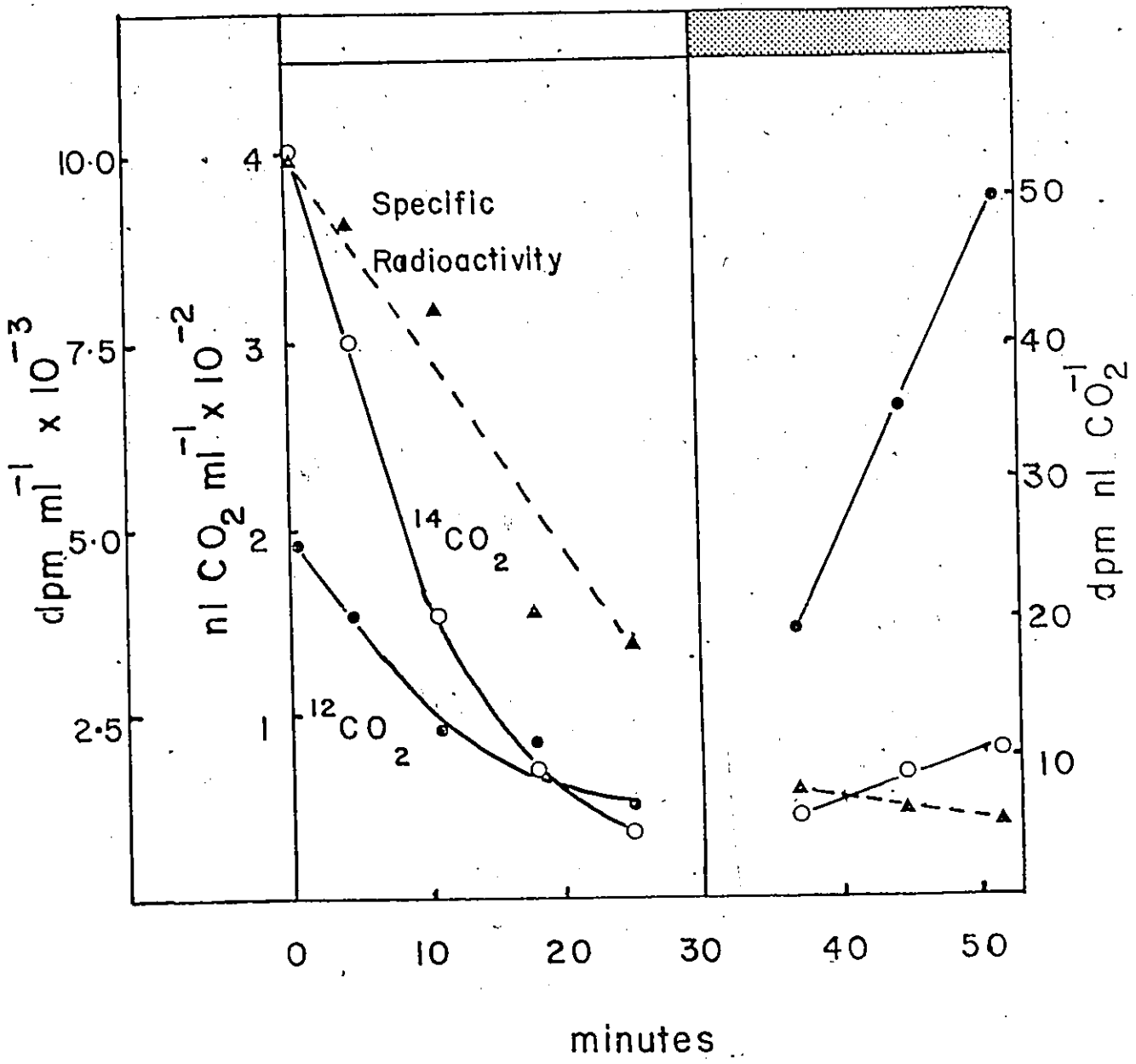
Specific
Radioactivity
$^{14}CO_2$
$^{12}CO_2$
dpm ml^{-1} x 10^{-3}
nl CO_2 ml^{-1} x 10^{-2}
dpm nl CO_2^{-1}
10·0
7·5
5·0
2·5
4
3
2
1
50
40
30
20
10
0
10
20
30
40
50
minutes

Figure 21.

The corresponding decrease in specific radioactivity indicated that unlabelled CO_2 was being released in the light and hence photorespiration was occurring. Photorespiration rates were calculated using the following rationale: a) the nl CO_2 ml removed from the medium represents a net process and can be equated with net photosynthesis (N.P.); b) the dpm ml^{-1} removed from the medium over the same time period divided by the initial specific radioactivity gives initial rates of $^{14}CO_2$-uptake and measures something approaching gross photosynthesis (G.P.)

If we assume that the difference between gross photosynthesis and net photosynthesis is due to photorespiration, i.e.

$$G.P. - N.P. = P.R.,$$

then the following equation should hold;

$$\frac{1}{RT}\left[\left(\frac{\text{dpm ml}^{-1}_{a} - \text{dpm ml}^{-1}_{b}}{\text{dpm nl CO}_2^{-1}_{a}}\right) - \left(\text{nlCO}_2\text{ml}^{-1}_{a} - \text{nlCO}_2\text{ml}^{-1}_{b}\right)\right] \cdot \frac{1}{\mu\text{gchlml}^{-1}} \cdot \frac{1}{(a-b)\text{hrs}}$$

$$= \text{P.R.(nmoles CO}_2\ \mu\text{gchl}^{-1}\ \text{hr}^{-1})$$

$$= \text{P.R.(}\mu\text{moles CO}_2\ \text{mgchl}^{-1}\ \text{hr}^{-1})$$

where R = 0.08206 l atm $mole^{-1}$ deg^{-1} and T = ^{o}Kelvin. The results of applying this equation are shown in the lower part of Tables 41 to 44. Due to the rapid recycling of photoassimilated $^{14}CO_2$, only the initial changes in dpm ml^{-1} and nl CO_2 ml^{-1} can be used to estimate photorespiration. The calculated rates of G.P. and N.P. are all low, due to the use of subsaturating CO_2 concentrations to simulate condition during the $^{14}CO_2$ release assay. The rate of photosynthesis of these algae approaches saturation over the range 400 to 1000 nl CO_2 ml^{-1} (i.e. 20 to

50 µmoles CO_2 l^{-1}). Examples of the effect of lowering the initial CO_2 concentration on the photosynthetic rate of the algae can be seen in all the Tables. Under these conditions, P.R. as a percentage of G.P. was 18.3 to 19.5% in Chlorella, 27.1 to 28.4% in Chlamydomonas, 10.0 to 26.2% in Anacystis and 17.0 to 32.3% in Navicula. Dark respiration was calculated from the increase in nl CO_2 ml^{-1} in the dark. A comparison of the P.R./D.R. ratio obtained in these experiments with white light grown cells to the L/D ratio of white light cells in the previous ^{14}C-assay experiments (Table 45) shows reasonably close agreement at this level. However, comparison of P.R. as a percentage of G.P. with $^{14}CO_2$ release in the light as a percentage of total $^{14}CO_2$-uptake, indicates that the $^{14}CO_2$ assay grossly under-estimates the magnitude of P.R. in these algae (Table 45). The specific radioactivity of $^{14}CO_2$ released by cells subsequent to $^{14}CO_2$-uptake would be quite low as indicated by our experiments and this presumably is the main reason for the discrepancy oberved between the two methods.

Table 41. The concentration of CO_2 and $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Anacystis nidulans cells in white light (600 μW cm^{-2}) and darkness.

	Time (min.)	nl CO_2 ml^{-1}	dpm ml^{-1}	Specific radioactivity dpm nl CO_2^{-1}
Sample 1	1.0	255	8851	34.7
	6.5	42	639	15.2
	10.0	44.1	285	6.5
	14.5	52.5	265	5.05
	22.0	30.2	250	8.30
	23.0	-----	darkness	-----
	30.0	156	586	3.8
	36.5	232	768	3.3
	42.0	335	903	2.7
Sample 2	1.0	150	4883	32.6
	6.0	54.6	692	12.7
	12.0	23.9	451	18.9
	18.0	35.3	508	14.4
	25.0	54.2	472	8.7
	27.0	-----	darkness	-----
	35.0	161	959	6.0
	43.0	298	1172	3.9
	57.0	472	1240	2.6

Sample	G.P.*	N.P.*	P.R.*	P.R. (% G.P.)	D.R.*	P.R./D.R.
1	16.2	14.6	1.6	10.0	5.7	.28
2	11.2	8.3	2.9	26.2	6.4	.46

* μmoles CO_2 mg chl^{-1} hr^{-1} (G.P. and N.P. based on initial rates).

Table 42. The concentration of CO_2 and $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Navicula pelliculosa cells in white light (600 μW cm^{-2}) and darkness.

	Time (min.)	nl CO_2 ml^{-1}	dpm ml^{-1}	Specific radioactivity dpm nl CO_2^{-1}
Sample 1	1.5	392	19896	50.8
	5.5	331	16162	48.8
	10.5	266	11460	43.1
	19.0	154	6145	39.9
	25.5	105	3712	35.4
	31.5	76	2467	32.5
	34.5	---	darkness	---
	36.5	127	2593	20.4
	43.5	188	3507	18.7
	51.5	300	4180	13.9
Sample 2	1.0	284	16684	58.8
	13.0	218	8366	38.4
	20.0	139	3772	27.1
	30.0	57.2	1107	19.4
	37.0	49.0	591	12.1
	45.0	36.9	305	8.3
	47.0	---	darkness	---
	56.0	159	598	5.5
	63.5	212	1246	6.6

Sample	G.P.*	N.P.*	P.R.*	P.R. (% G.P.)	D.R.*	P.R./D.R.
1	10.0	8.3	1.7	17.0	4.8	0.35
2	6.2	4.2	2.0	32.3	3.9	0.51

* μmoles CO_2 mg chl^{-1} hr^{-1} (G.P. and N.P. based on initial rates).

Table 43. The concentration of CO_2 and $^{14}CO_2$ and the specific radio-activity of $^{14}CO_2$ around Chlorella vulgaris cells in white light (600 μW cm^{-2}) and darkness.

	Time (min.)	nl CO_2 ml^{-1}	dpm ml^{-1}	Specific radio-activity dpm nl CO_2^{-1}
Sample 1	1.0	258	8398	32.6
	6.5	167	4773	28.6
	14.5	87	2173	24.9
	21.0	67	913	13.6
	31.5	35.4	279	7.9
	32.5	---	darkness	---
	42.5	155	469	3.3
	51.5	194	614	3.2
	59.5	268	679	2.5
Sample 2	1.0	197	10590	53.8
	7.0	122	5578	45.7
	12.5	83	2855	34.5
	20.0	78	1146	14.7
	31.0	57.4	459	8.0
	34.0	---	darkness	---
	40.0	158	553	3.5
	50.0	259	646	2.5
	60.0	310	743	2.4

Sample	G.P.*	N.P.*	P.R.*	P.R. (% G.P.)	D.R.*	P.R./D.R.
1	15.0	12.3	2.7	18.3	5.5	0.5
2	7.9	6.4	1.5	19.5	5.1	0.3

* μmoles CO_2 mg chl^{-1} hr^{-1} (G.P. and N.P. based on initial rates).

Table 44. The concentration of CO_2 and $^{14}CO_2$ and the specific radioactivity of $^{14}CO_2$ around Chlamydomonas reinhardtii cells in white light (600 μW cm^{-2}) and darkness.

	Time (min.)	nl CO_2 ml^{-1}	dpm ml^{-1}	Specific radioactivity dpm nl CO_2^{-1}
Sample 1	0.5	190	10082	53.1
	4.5	154	7469	48.5
	11.0	90	3809	42.3
	18.0	85.3	1750	20.5
	25.0	49.9	906	18.2
	30.0	---	darkness	---
	37.0	146	1085	7.4
	45.0	267	1679	6.3
	52.0	375	1960	5.2
Sample 2	1.0	329	9333	28.4
	6.0	203	4342	21.4
	13.5	94	2056	21.9
	20.5	74.5	891	12.0
	27.5	65.2	406	6.2
	30.0	---	darkness	---
	38.5	315	1210	3.8
	44.5	444	1419	3.2
	51.0	493	1825	3.7

Sample	G.P.*	N.P.*	P.R.*	P.R. (% G.P.)	D.R.*	P.R./D.R.
1	9.4	6.9	2.5	27.1	11.7	.22
2	19.7	14.1	5.6	28.4	12.0	.47

*μmoles CO_2 mg chl^{-1} hr^{-1} (G.P. and N.P. based on initial rates).

Table 45. A comparison of the Light/Dark ratios and the P.R./D.R. ratios obtained for cells grown in white light.

	L/D Ratio	P.R./D.R. Ratio
Chlorella vulgaris	0.29 0.24	0.50 0.30
Chlamydomonas reinhardtii	0.42 0.36	0.22 0.47
Navicula pelliculosa	0.67 0.65	0.35 0.51
Anacystis nidulans	0.19 0.10	0.28 0.47

	$^{14}CO_2$ release/ $^{14}CO_2$ uptake	P.R. (% G.P.)
Chlorella vulgaris	0.8 0.5	18.3 19.5
Chlamydomonas reinhardtii	0.8 0.9	27.1 28.4
Navicula pelliculosa	2.0 1.2	17.0 32.3
Anacystis nidulans	0.6 1.1	10.0 26.2

DISCUSSION

1. Light Quality and Growth

An interesting result of the present study is the high growth rate of Navicula pelliculosa and Anacystis nidulans in red light. In the case of the two green algae used in this study, the complete spectrum of white light was more effective in promoting growth than any part of the spectrum (Tables 1 and 2). In terrestrial plants, red light clearly enhances growth, i.e. dry weight production (17, 194, 335, 367). On the contrary, the growth of marine unicellular green algae is clearly enhanced by blue light (49, 164, 217, 361). In freshwater forms of unicellular green algae, red light supports higher growth rates (181, 182, 217).

The growth rates of Chlamydomonas, under the various light qualities used in this study, are different from the results of Brown and Geen (29) using the same strain of alga and similar light sources. The major difference between the two studies was the much higher growth medium with NH_4NO_3 as the major nitrogen source, a slightly lower temperature (18°C) and less than half the light intensity (240 μW cm^{-2}) used in the present study. Since many blue light responses appear to saturate at low light intensities, it is suggested that the influence of blue light may have been greater at the low light intensities used by Brown and Geen.

An examination of the turnover times suggests that short wavelengths i.e. blue and green light are more growth promoting for Chlorella vulgaris. Kowallik (182) grew Chlorella pyrenoidosa on high CO_2 in white

light and measured their growth rate (dry weight accumulation) immediately following transfer to blue or red light. Growth rates in white light were not reported. He also showed that even though dry weight accumulation could be adjusted to equal rates by lowering the intensity of red light, the daughter cells produced in blue light were larger and fewer in number than in red light (267). The ability of Chlorella vulgaris to utilize blue light is further emphasized by the fact that the red light used in this study has a 40.6% higher quantum flux than blue light at equal intensity. Carroll et al (40) have also shown that in contrast to other green algae such as Protosiphon botryoides and Chlamydomonas gymnogama, blue light (460 nm) stimulated rather than inhibited daughter cell formation in Chlorella pyrenoidosa.

The higher quanta flux in red light should also be taken into account when considering the growth of Navicula and Anacystis in red light. Work with marine diatoms clearly suggests that blue or green light favours their normal growth (5, 134, 158, 361). Apart from the work of Baatz (5), no studies of diatom growth in continuous red light have been reported. The red light used in the present study not only had a greater quantum flux than other light qualities but also had peak energy emission between 625 and 650 nm, the absorption band for chlorophyll c.

The higher quantum flux and the peak emission band of the red light source also favoured the growth of Anacystis nidulans since phycocyanin is the major light harvesting pigment of unicellular blue-green algae. Compared with red light the 5-fold increase of turnover time in blue and green light is not entirely explained by the lower quantum flux of these light sources, especially in the case of blue light where the number of quanta

absorbed by the cells equalled the amount absorbed in red light (Table 16).

2. Light Quality and Chemical Composition

In contrast to other reports (49, 182, 183, 263, 266, 267, 304, 320, 360) strong effects of blue or red light on the chemical composition of these freshwater algae were not observed. The lack of statistically significant changes in the nucleic acid, protein, carbohydrate and lipid content of these cells may be due to the large degree of variation and the sample population (3-6 replicates). To get a sufficient cell density, cultures were sampled during the mid to late exponential phase of growth. Major changes in the relative concentrations of various cellular components can occur during this phase of growth (64). Some of the best demonstrations of light quality effects on protein, carbohydrate and RNA content have occurred in synchronized cultures (182, 183, 266, 267). In this study, no attempt was made to synchronize the cultures, though regular light-dark periods can entrain algae (339).

The best evidence for the effects of blue or red light on nucleic acid, protein or carbohydrate content comes from studies of higher plants and the green alga Chlorella (4, 182, 183, 263, 266, 270, 327, 328, 358) and some other green algae (49, 320, 360). Apart from the study of Wallen and Geen (360) using the marine diatom Cyclotella nana, virtually no reports on the effect of light quality on the chemical composition of other algal groups exist. In the case of the two green algae used in the present study levels of RNA and carbohydrate were similar in red or blue grown cells (Tables 5 and 6). The level of protein in blue light grown cells was higher than in red light, especially so in

Chlamydomonas reinhardtii. These differences in protein level are reflected in the carbohydrate:protein ratios (Table 7), where in the case of Chlamydomonas the ratio was significantly higher in red light. This supports the previous literature on this subject (Introduction, section 3.1.2). Green light depressed the levels of carbohydrate and protein significantly in this alga, though the carbohydrate:protein ratio was not significantly different from that in blue or white light.

Levels of protein and carbohydrate were higher in blue light grown Anacystis nidulans compared with red light cells (Table 3) and this is reflected in the carbohydrate:protein ratio (Table 7). Though these trends are not significant they do reflect the large and significant differences in the phycocyanin content of blue and red light grown cells (Table 8).

While the RNA and protein content of Navicula pelliculosa cells grown in red or blue light was quite similar, the carbohydrate content of blue light grown cells was significantly higher than in red light. This result is in marked contrast to most reports of blue light effects on the carbohydrate content of green algae and higher plants and emphasizes our lack of knowledge of light quality effects on the chemical composition of algal groups other than the Chlorophyceae.

If it is assumed that the ethanol-insoluble fraction in algae is mainly protein with some carbohydrates (362), then some information about the influence of light quality on the synthesis of protein can be obtained from the ^{14}C-incorporation experiments, (Table 26). White and blue light enhanced the percentage ^{14}C-incorporation into this fraction in Chlorella, Chlamydomonas and Navicula. This suggests that short wavelength light or this component of white light was enhancing protein

production in these algae even though the actual protein content of cells grown under these conditions was not significantly increased.

There are no published reports on the effect of light quality on the lipid content of algae. Lipids form 21 to 34% of the dry weight of chloroplasts (177). Light quality-induced changes in chloroplast number or structure have been reported for algae and higher plants (35, 151, 158, 166). No significant effect of light quality on the lipid content of Chlorella, Chlamydomonas and Navicula was observed (Tables 4 and 6). Variation in the lipid content of Navicula was large. Lipid levels in Anacystis were highest in green light and lowest in white light (Table 3). ^{14}C-radioactivity of the chloroform-soluble fraction during the ^{14}C-incorporation experiments suggests that white light enhanced lipid synthesis in Navicula (Table 26). This is in marked contrast to the results of Opute (248) with Nitzschia palea, where the lipid fraction was labelled more rapidly in the presence of red or blue light. In the other three algae, the percentage ^{14}C-incorporated into this fraction was two to three times higher in red light. This red light enhancement of lipid synthesis was not reflected in the lipid content of these algae during growth.

3. Light Quality and Photosynthetic Pigments

In this study, large changes in the pigment content or composition in the cells of the two green algae were not observed. Highest mean total chlorophyll concentrations were found in white and blue light grown cells, however, due to a large amount of natural variation these differences were not significant at the 95% confidence level (Tables 10 and 11). No significant changes in the concentration of chlorophylls a

and b and total carotenoid content, or the chlorophyll b:a or total carotenoid:chlorophyll a ratios occurred following growth of Chlamydomonas in any of the light treatments. This was generally confirmed by the in vivo absorbance spectra, except in the case of blue light grown cells which showed increased absorption in the blue part of their spectrum (Figs. 7 and 8).

Cells of Chlorella grown in green and red light showed highest and lowest chlorophyll b concentrations respectively. These changes were significant ($P < 0.05$) when compared to the other three light treatments. The chlorophyll b:a ratio was significantly higher in green light cells compared with the other three light treatments. This is mainly due to a lowered chlorophyll a content. The higher chlorophyll b content of blue light cells also lead to a high chlorophyll b:a ratio, however, it was not significantly different from the ratio in green light cells when blue and green cells were compared with white and red cells. Changes in the carotenoid composition of Chlorella in response to light quality were indicated by the in vivo absorption spectra. No changes in the colour of the green algal cultures comparable to those observed in Navicula or Anacystis were observed.

More dramatic changes in chlorophyll content and/or pigment ratios have been reported for green algae grown under different light qualities (137, 354, 360) or intensities (165, 177, 236, 284, 306, 313). Changes in the pigment content or ratio of leaves of various higher plants in response to changes in light quality or intensity have also been observed (18, 20, 35, 177, 180, 302, 336).

It should be pointed out that very little evidence for chromatic adaptation in green algae has been found, especially in freshwater

situations (78).

While total pigment (chlorophylls a and b and total carotenoids) production was highest in green algae grown in white light, highest pigment production (chlorophyll a and phycocyanin and total carotenoids) in the blue-green alga, Anacystis occurred in blue light. The changes in phycocyanin content were reflected in visual colour changes of the cultures. In a recent report Myers et al (225) found higher levels of phycocyanin relative to chlorophyll in Anacystis cells grown in parts of the spectrum where phycocyanin is a poor absorber, i.e. in blue light and the red part of the spectrum at wavelengths > 670 nm. The lowest ratio of phycocyanin/chlorophyll in this study (225) was observed in cells grown under red fluorescent lamps. The results of Nicholls and Bogorad (231) showing maximum phycocyanin formation in chlorophyll-free mutant cells of Cyanidium caldarum grown in blue light also support the results of the present study. The increased phycocyanin content of blue light grown cells suggests that the cells were trying to optimize light absorption by this pigment and some form of inverse chromatic adaptation had occurred. The high phycocyanin content of blue light grown Anacystis also suggests that nitrogen was not limiting under blue light (1, 191). This contrasts sharply with the results of Steven and van Baalen (329) and Pulich and van Baalen (273) who found that nitrate reduction was inhibited in blue light in unicellular marine blue-green algae.

Highest total pigment production (chlorophyll a + c + total carotenoids) in Navicula was observed in red light. The effect of red light on pigment content of a diatom has only been reported for one

other species (166, 167). Unfortunately, the red light was only 1/10 that used to grow white light cells and hence the role of light quality or intensity in producing the pigment changes observed is confounded. Visual colour changes in diatoms adapted to various light qualities have been reported. Mothes & Sagromsky (223) found that Chaetoceros was dark brown in green light and golden-brown in red light. Jeffrey & Vesk observed that Stephanopyxis was dark chocolate brown in blue-green light (158). In our case Navicula was greenish-brown in red light, golden brown in green and white light and dark chocolate brown in blue light. Increased pigment production has been reported for marine diatoms in blue or blue-green light when compared to white or green light (158, 354, 360). Consequently, the higher pigment production in red and green light relative to white and blue light in Navicula is another notable result of the present study. It should also be noted that Navicula is a benthic freshwater diatom whereas the majority of diatoms used in light quality studies have been planktonic marine forms.

Major light quality effects on growth rate, rate of photosynthesis and pigment content were observed in the present study. To a lesser extent changes were observed in the levels of protein and carbohydrate. Dramatic changes in nucleic acid content were not observed and this reflects the minor effects of light quality on protein content. Compared with the effects of nutrient deficiency (1, 64, 87, 131, 173, 191, 227, 248, 310, 318) it is not expected that changes in light quality would have such a dramatic effect on the chemical composition of algae. It is more likely that the changes observed reflect the adjustments of the light harvesting and photosynthetic machinery to growth in restricted portions of the PAR spectrum.

4. Light Quality and Photosynthetic Adaptation

As pointed out in the Introduction, the effect of light quality on photosynthesis has mainly been investigated in algal cells originally grown under white light and then exposed to various parts of the spectrum, usually without an adaptation period (24, 69, 85, 86, 239, 265, 289, 316, 340). Furthermore, various workers have used different light qualities of either equal energy or equal quanta flux. In theory equal quanta fluxes should be used since photochemical reactions are proportional to numbers of quanta. In the present study light sources of equal energy were used. Under those conditions, 550 $\mu W\ cm^{-2}$ represented 1.67×10^{15}, 1.28×10^{15}, 1.47×10^{15} and 1.80×10^{15} quanta $s^{-1}.cm^{-2}$ for white, blue, green and red light respectively. Red light had about 40% greater quanta flux than blue light.

On the other hand, calculations of the amount of incident light absorbed by the algae based on the in vivo absorbance spectra and the spectral energy distribution of the light sources (Tables 12 to 15) indicate that algae have differing abilities to absorb various parts of the spectrum. Blue light energy was absorbed with almost twice the efficiency of red light for all cells examined. Green light was absorbed least efficiently in Chlorella, Chlamydomonas and Anacystis (Tables 12, 14, and 15). In the case of Navicula, (Table 13) green light was absorbed as efficiently as red light. Broad spectrum white light was generally absorbed with an efficiency intermediate between red and blue light. Thus not only must the incident energy or quanta flux be considered but also the ability of the cells to absorb the incident light must be taken into account when considering the effect of light quality on growth and photosynthesis. The analysis is further complicated by the fact that the

light absorbed by the cells in different parts of the spectrum is transferred to photosynthesis with differing degrees of efficiency.

This is highlighted by the fact that white light adapted cells respond differently to changes in light quality than cells adapted to the part of the spectrum being investigated. Only four other studies (137, 158, 223, 273) appear to have noticed this aspect of light quality adaptation. The changes in pigment content and in vivo absorbance spectra, especially in Anacystis and Navicula, suggest that the cells have attempted to optimize light absorption and/or photosynthetic efficiency when grown in restricted parts of the spectrum. This is emphasized by the higher photosynthetic rates in white light and the light that the cells have adapted to when compared to the responses of white light-adapted cells (Tables 21 to 24) under the same conditions. This was a property of all algae examined in this study. This response, i.e. increased rate of photosynthesis, is especially interesting since adaptation to blue, green and red light by Chlorella and Chlamydomonas did not involve increases in pigment content. Increases in light absorption based on in vivo spectra were not observed either, except in the case of the blue light adapted green algae. Since the growth light intensity was already low (ca. 1% full sunlight) large increases in pigment content would not be expected and the increased photosynthetic efficiency may represent light quality mediated changes in chloroplast structure. The significant changes in chlorophyll b content and the chlorophyll b:a ratio that occurred in Chlorella following light quality adaptation give some support to this idea. While Hess and Tolbert (137) observed a large increase in chlorophyll b content of blue light-adapted Chlamydomonas

reinhardtii, the pigment composition of this alga was remarkably invariant during the present study (Table 11).

Apparent I_k was lowest for green light adapted Anacystis, Chlorella and Chlamydomonas and for blue light adapted Navicula (Figs. 9 to 12). Since these algae were grown at equal light intensities, this result suggests that light quality strongly influences photosynthetic adaptation to the low light conditions in the lower part of the photic zone.

It is also interesting to compare the effects of light quality on growth rate with its effects on rates of photosynthesis. In the case of Navicula red light was more effective for growth whereas blue light was least effective (Table 1). When CO_2-fixation is measured on a cell number basis, rates of photosynthesis are highest in red light and lowest in blue light in this alga. When photosynthesis is expressed on a chlorophyll basis, the increased chlorophyll content of red light cells lowers the rate in red light and the photosynthetic rate in various parts of the spectrum is not dramatically different (Table 20). This would tend to agree with action spectra and quantum yield experiments with diatoms which suggest that this group of algae can harvest light efficiently over most of the PAR spectrum (79, 213, 340). In the case of this freshwater diatom, the green to red part of the spectrum clearly promotes growth and photosynthesis.

The growth rate of Chlamydomonas was highest in white light and lowest in blue light (Table 1). On the contrary, CO_2-fixation rates were highest in blue and red light adapted cells on both cell number and unit chlorophyll bases (Table 20). The photosynthesis data appears to follow the action spectrum of green plant photosynthesis i.e. maxima in

the red and blue parts of the spectrum, however, the growth data suggests that metabolic processes such as photorespiration, dark respiration or extracellular release may be diverting assimilated carbon from cell building processes.

The growth rate of Chlorella is highest in white light, and lowest in red light. CO_2-fixation rates per cell number are highest in blue light, followed by white, then red light adapted cells. This reflects to a limited degree the slightly higher growth rate of Chlorella in blue light compared with red light. On a chlorophyll basis, typical green plant photosynthetic maxima in red and blue light are observed. Again when comparing photosynthetic rates with growth rates, processes antagonistic to buildup of cellular material appear to be operating in red and blue light. This discrepancy between the growth rates and rates of photosynthesis of the green algae in blue and red light is an outstanding result of this study, especially since these cells absorb red and blue light maximally, both in terms of energy and quanta flux (Tables 14, 15, 18,19). In two other reports (29, 361) where the growth rates and rates of photosynthesis of green algae were examined following growth in white, blue, green and in one case (29) red light, the growth rates parallelled the photosynthesis rate. Hess and Tolbert (137) found that white light grown Chlorella and Chlamydomonas grew slowly for several days following transfer to red or blue light. After 10 days the growth rate in red or blue light was similar to the rate in white light. Brown and Geen (29) also noticed a five day lag in the growth of Chlamydomonas following transfer to red light. Growth was followed for up to seventeen days in the present study with no apparent improvement to the growth rate in blue light.

Growth of Chlorella and Chlamydomonas under the present growth conditions appears to be favoured by the spectral output of cool-white fluorescent lamps. The spectrum of these lamps is relatively deficient in red light (wavelengths > 650 nm). Peak energy emission occurs around 580 nm and only one third of the light output occurs between 400 and 525 nm. This spectral energy distribution resembles that found in eutrophic waters (15, 160, 211, 293, 300, 317).

In the blue-green alga Anacystis there is a direct relationship between photosynthetic rate and growth rate (Tables 1 and 20), the high rate of photosynthesis in red light adapted cells reflecting a high growth rate in red light. The spectral dependence of growth and photosynthesis clearly reflects the importance of phycocyanin to the cellular economy of this alga. This fact is emphasized in Table 16, which shows that approximately equal numbers of quanta were absorbed by the cells in white, blue and red light.

While some reports (76, 273, 330) suggest that this type of blue-green alga is selected against by blue light, my data suggests that these cells can adapt to blue light conditions though rates of photosynthesis and growth are suboptimal. Clearly this type of cell would be most successful in shallow, greenish-red, highly eutrophic waters.

5. Light Quality and Photosynthetic Carbon Metabolism

An attempt was made to assess the effects of light quality on the carbon metabolism of four freshwater algae. The partitioning of incorporated ^{14}C revealed different cellular incorporation strategies in terms of soluble versus insoluble fractions (Table 26). In the eukaryotic algae, most radioactivity was found in the soluble fraction in red

light cells and correspondingly radioactivity in the insoluble fraction was lowest in this light quality. In contrast, radioactivity was higher in the insoluble fraction in blue and white light cells, and in the case of Chlamydomonas and Navicula insoluble radioactivity was also higher in green light adapted cells. Brown and Geen (29) also showed that insoluble radioactivity was highest in blue light adapted Chlamydomonas. Olive and Morrison (246), Olive et al (245) and Wallen and Geen (362) all reported an increase in the radioactivity of the insoluble fraction with depth in freshwater and marine phytoplankton. The data of the present study lends support to the suggestion of Wallen and Geen (362) that light quality might account for these depth-dependent changes in cell composition.

Considering the number of reports indicating that blue light enhances the synthesis of organic and aminoacids (Introduction, section 5.5.2) no large changes in the ^{14}-C activity of the acidic and basic components of the water-soluble fractions of Anacystis, Navicula or Chlorella were observed in blue light (Tables 32 to 34). A possible effect of blue light was observed in Chlamydomonas, where the radioactivity of the basic water-soluble fraction was increased at the expense of the neutral fraction. These blue light effects are most pronounced at very low light levels in white light adapted cells. It is possible that following adaptation to blue light and at light levels approaching light saturation of photosynthesis that blue light effects on organic and amino acid and subsequent protein formation may not be so pronounced.

The percentage of extracellular organic carbon released was less than 1% of the total $^{14}CO_2$-fixed by Chlorella (Table 36) and in this respect it resembles the strain of Chlorella pyrenoidosa used by

Lord and Merrett (204) that did not normally extrete glycolate. No strong effect of light quality on extracellular release by Chlorella vulgaris was found. The lowest release rate of extracellular organic carbon was found in red light adapted cells of Chlamydomonas, Navicula and Anacystis (Table 36). This is in marked contrast to published reports of high rates of glycolate excretion in red light by white light adapted cells (14, 203).

Higher rates of extracellular release were found in white and blue light adapted cells of Chlamydomonas, Navicula and Anacystis. This result would tend to support the data of Watt (368) who found that percentage extracellular release tended to increase towards the bottom of the photic zone in freshwater situations. Wallen and Geen (362) on the contrary found that extracellular release decreased under these conditions in marine phytoplankton. It should be pointed out though that the percentage extracellular release of organic carbon encountered in this study is extremely low compared with other published reports.

The very low excretion rates are much closer to those predicted by Sharp (308) i.e. < 5% total C-fixation, and support his contention that healthy phytoplankton do not lose significant portions of their photo-assimilated carbon as organic excretion during normal growth. Other factors affect carbon excretion and have been reviewed (135, 308). There are two factors pertinent to my results which lead to lowered rates of excretion; one, the fact that the cells were allowed to equilibrate for one hour under growth conditions following centrifugation and resuspension, and, two, the use of uniformly high cell densities. The equilibration period may have avoided any abnormal excretion episodes immediately after cell manipulation (308) and percentage excretion is

reported to be lower at higher cell densities (29, 357).

As mentioned above, Wallen and Geen (362) reported that the percentage release of dissolved organic carbon as a percentage of total C-fixation decreased with depth. This was directly related to the depth dependent decrease in the size of the ethanol-soluble fraction. Since ^{14}C-fixation also decreased with depth, the lowered excretion at the bottom of the photic zone is a reflection of this as well as the decreased ethanol-soluble fraction.

Some effect of light quality on extracellular release as a percentage of the ^{14}C-activity in the water-soluble fraction was observed in the present study. Extracellular release as a percentage of water soluble fraction was highest in white light grown cells and lowest in red light grown cells in all four algae examined (Table 31). This red light effect was quite pronounced since ^{14}C-activity of the water soluble fraction was high in red light grown Chlorella, Chlamydomonas and Navicula (Table 26). Extracellular release as a percentage of the water soluble fraction was also high relative to the red light value for blue and green light adapted cells of Anacystis and Chlamydomonas and for blue light adapted cells of Navicula. These results suggest that the relationship between the ^{14}C-activities of the water-soluble and extracellular release fractions is not direct and that two depth-dependent or light quality related processes may occur, one, a decrease in ^{14}C-activity of the water-soluble fraction, and two, an increase in the amount of the water-soluble fraction released by the cells. Depending on the ratio of these two processes, more or less carbon would be released at the bottom of the photic zone. The nature of the algal species present would also affect the extent of these processes.

Brown and Geen (29) also observed no direct relationship between the size of the ethanol-soluble fraction and the extracellular release fraction in Chlamydomonas reinhardtii. Extracellular release as a percentage of ^{14}C-activity in the ethanol-soluble fraction was highest in white light adapted cells, lower in blue light cells and lowest in red light grown cells. Their reported excretion value in white light was high (35.5% total ^{14}C-fixation) however, this may have been related to use of a lower cell density than in red or blue light. They suggested that the release rate equivalent to the higher cell density was < 10% total ^{14}C-fixation. Even allowing for this, the ^{14}C-activity of the extracellular release fraction was still highest in white light.

The distribution of ^{14}C-activity in the neutral, basic and acidic fractions of the water-soluble fraction was generally mirrored in the same fractions of the extracellular release fraction of the two green algae (Table 34 and 35). This suggests that extracellular release was a passive process and there was no strong evidence of light quality causing a selective release of any particular fraction in these algae. Brown and Geen (29) reported a strong effect of light quality on membrane permeability of Chlamydomonas reinhardtii. In white light adapted cells the extracellular products included amino-acids, organic acids and sugars, whereas only sugars were released by blue light cells and only organic acids were released by red light cells. My results with the same alga do not confirm their report(29).

Some possible effects of light quality on membrane permeability were observed in cells of Navicula grown in white or blue light (Table 33). In white light grown Navicula the water soluble ^{14}C-activity was highest in the neutral fraction and lowest in the acidic fraction. This

situation was reversed in the same fractions released by these cells. In blue light, the high level of ^{14}C-activity in the neutral water-soluble fraction was not reflected in the activity of this fraction released by the cells. Further possible light quality effects on membrane permeability were observed in white or green light grown Anacystis cells (Table 32). The ^{14}C-activity of the acidic water soluble fraction in both these light treatments was greater than 60%, however, only 14% of this activity was in the same fraction released by the cells. These light quality effects on the class of compounds released by Navicula and Anacystis would appear to be separate from light quality related changes in the internal pattern of ^{14}C-labelling. Light quality mediated changes in the permeability of plant membranes have been proposed by several authors (29, 192, 288, 357) and may be a mechanism for controlling pools of metabolites. Most studies of light quality effects on ^{14}C-labelling patterns in algae have ignored the extracellular release component. It is possible that differential retention or release of various metabolites by the cell membrane may influence these ^{14}C-labelling patterns. This aspect of light quality effects on photosynthesis has not been explored and may have important implications in the natural environment.

Another aspect of carbon metabolism which may have a controlling influence on algal growth is that of photorespiration. Only a limited number of studies have attempted to estimate the magnitude of this process in algae (cf. Introduction, section 5.4). This is mainly due to the difficulties involved in measuring CO_2 exchange in aqueous systems, especially at alkaline pH. In fact algae, and green algae in particular, are in the anomalous position of having very low CO_2

compensation points like low photorespiration plant species such as C_4 plants on one hand and having the CO_2-fixation pathway of C_3 plants on the other hand (45). The enzymes of the glycolate pathway have also been demonstrated in green algae (30).

Investigations of CO_2 release in the light by, and CO_2 compensation points of algae have been difficult to measure because of the buffering effect of CO_3^- and HCO_3^- in aqueous media and the slow equilibration of dissolved CO_2 between the gas phase and the aqueous medium. A major criticism of dynamic gas analysis experiments where dissolved CO_2 is measured in the gas phase, i.e. by IRGA or when dissolved $^{14}CO_2$ is removed from solution by gassing as in the Zelitch ^{14}C-assay is that only the gaseous component is measured. CO_2 in the gas phase only approaches equilibrium with CO_2 dissolved in the aqueous phase at acid pH (pH 4 to 5). With increasing alkalinity, the reaction $CO_2 + OH^- \rightarrow HCO_3^-$ becomes important (373) and HCO_3^- formation acts as a sink for free CO_2. The CO_2 in equilibrium with the gas being bubbled through the medium decreases. This is probably a major reason why CO_2 compensation points reported for algae have been so low. The measurement of all DIC using the gas chromatographic technique avoided these gas-exchange problems.

Another factor which further complicates the situation is the fact that many algae have been shown to utilize HCO_3^- directly at alkaline pH (6, 90, 207, 282, 338). The impact of this process on measurements of photorespiration or CO_2 exchange by methods relying on CO_2 equilibrating with gases being bubbled through the medium is unknown.

The experiments based on $^{14}CO_2$ release in the light clearly show that some degree of photorespiration was occurring in the algae used

in this study. While the $^{14}CO_2$ release method seriously underestimated the magnitude of photorespiration in algae and some anomalous results arose when the influence of light quality on this process was investigated, the impact of light quality on the rates of $^{14}CO_2$ release both in the light and the dark cannot be ignored. In all algae examined rates of $^{14}CO_2$ release both in the light and the dark were highest during or following exposure to blue or green light (Tables 37 to 40). The well known enhancement of dark respiration (185, 264) decreased photosynthesis at low light intensities (329, 287, 289) and inefficient absorption of light at shorter wavelengths (86) documented for green algae clearly must have some bearing on this result. Photorespiration and dark respiration may be more important processes than extracellular release for the carbon economy of cells when only shortwavelength light is transmitted to the lower part of the photic zone. Carbon losses due to photorespiration and dark respiration were much lower in cells adapted to white or red light and this is reflected in the higher rates of growth and photosynthesis observed in the four algae under these light conditions. If one only considers dpm lost in the light by $^{14}CO_2$ release this does not seem to be an important process, however, the differential CO_2 and $^{14}CO_2$ influx measurements point out the larger magnitude of this process. These measurements of the differential CO_2 and $^{14}CO_2$ influx are highly significant in that they represent the first direct evidence for photorespiration rates in algae approaching those reported for C_3 higher plants (139, 208, 209).

The $^{14}CO_2$ release and differential carbon isotope uptake measurements using Navicula pelliculosa also represent the first direct evidence that photorespiration occurs in diatoms. Previous reports

measured glycolate metabolism or enzymes associated with glycolate metabolism (60, 258, 260, 261). The influence of light quality on HCO_3^- uptake and refixation rates of photorespired CO_2 at alkaline pH are also unknown and are factors that light quality may also affect.

SUMMARY AND CONCLUSIONS

Four unicellular freshwater algae were grown in equal intensities of white light and broad waveband light from the blue, green and red parts of the spectrum. The intensity and spectral distribution of these light sources simulated to some degree the underwater light climate in the lower part of the photic zone of natural waters.

Contrary to previous literature where blue or blue-green light was shown to favour the growth of marine forms of green algae and diatoms, the results of this study suggest that shortwavelength radiation was not utilized as efficiently by freshwater algae. The complete spectrum of white light was most effective in promoting the growth of Chlorella and Chlamydomonas. The growth rate of Anacystis and Navicula was highest in red light. Red light also enhanced the growth of Chlamydomonas. Blue light was more important for the growth of Chlorella.

Following adaptation to the various light sources, rates of photosynthesis of the two green algae were highest in red or blue light grown cells and lowest in green light grown cells. White light adapted cells generally had photosynthetic rates slightly lower than the red and blue photosynthetic optima. A similar pattern of photosynthetic adaptation was observed in the diatom Navicula, but rates of photosynthesis in green light adapted cells, in comparison with the green algae, were only slightly less than the rates observed in the other light qualities. Rates of photosynthesis were highest in red light adapted cells of Anacystis and uniformly low in blue or green light adapted cells.

All the algae examined showed increased photosynthetic efficiency following adaptation to the various light qualities when

compared to white light adapted cells exposed to the same light source. The lowest apparent I_k using white light was found in green light adapted cells of Anacystis, Chlorella and Chlamydomonas and blue light adapted cells of Navicula. Clearly green or blue light represents an extreme shade habitat for these algae.

These increases in the photosynthetic efficiency of the two green algae were not reflected in large changes in the total pigment content of the cells. Mean total chlorophyll concentrations were highest in white or blue light grown cells but these differences were not statistically significant, though blue light grown cells showed increased light absorption in the blue part of their in vivo absorption spectrum. Chlorophyll b concentration was significantly lower in red light adapted cells of Chlorella and this was reflected in the higher chlorophyll b:a ratio of these cells, however, the pigment composition of Chlamydomonas was remarkably invariant in response to light quality. This lack of change in total pigment content may reflect the already low light intensities used in the study and may be more related to light quality mediated changes in chloroplast structure.

Chlorophyll a content was highest in red and green light grown Navicula and this resulted in a decreased chlorophyll c:a ratio in these cells. Blue and white light grown cultures of Navicula were visibly browner than green or red light grown cultures and the total carotenoid/chlorophyll a ratio was significantly higher in these cells. Changes in the in vivo absorption spectra of Navicula at 460 and 490 nm suggest that changes in the carotenoid composition and content had occurred during light quality adaptation.

While the chlorophyll a content of Anacystis was relatively

unaffected by light quality, the phycocyanin content of blue light grown cells was 4.5-fold greater than red light cells. The phycocyanin content of blue light adapted cells was 32% of the total cellular protein and resulted in visibly blue cells. The phycocyanin content of white and green light adapted cells was about twice that of red light cells. The total carotenoid/chlorophyll a ratio was also significantly higher in blue light adapted Anacystis cells. Changes in the phycocyanin peak at 625 nm were the dominant feature of the Anacystis in vivo absorption spectra and suggest that some form of inverse chromatic adaptation had occurred in these cells.

Since there is some controversy regarding the use of equal intensities or quanta flux in studies of this nature, the ability of the cells to absorb light energy or quanta from the waveband of the light sources was calculated from in vivo spectra before and after adaptation. Though the red light used in this study had about 40% greater quanta flux than the blue light, blue light energy was absorbed with almost twice the efficiency of red light for all cells examined. Blue quanta were absorbed with equal or greater efficiency than red quanta. Green light was absorbed least efficiently by all cells. Blue light adapted Chlamydomonas and Chlorella cells showed 18% and 37% increases in the ability to absorb blue light, respectively, when compared with white light adapted cells. Apart from these two cases small or no increases in light absorption efficiency following adaptation to blue, green or red light were observed in the other algae.

No strong effect of light quality on the protein, carbohydrate, nucleic acid or lipid content of these algae was observed. This may reflect sampling during the mid to late exponential phase growth and

the use of unsynchronised cultures. Slightly higher levels of protein in blue light grown cells of Chlorella, Chlamydomonas and Anacystis when compared with red light are reflected in the carbohydrate:protein ratio of these cells. The carbohydrate content of blue light grown Navicula was significantly higher than in red light, suggesting that this diatom may respond differently to the blue light in comparison with green algae. ^{14}C-incorporation in the insoluble fraction (mainly protein) of Chlorella, Chlamydomonas and Navicula was enhanced in the presence of white or blue light and suggests that short wavelength light or its component of white light enhanced protein production in these algae even though the actual protein content of the cells was not significantly increased.

Use of the phosphovanillin lipid test revealed significant changes in the lipid content of Anacystis cells but no significant effect of light quality on the lipid content of Navicula, Chlorella and Chlamydomonas was observed. ^{14}C-incorporation into the chloroform soluble fraction suggested that lipid synthesis was enhanced by white light in Navicula and by red light in the other three algae. Again this enhanced lipid synthesis was not reflected in the actual lipid content of these algae.

Dramatic changes in nucleic acid content were not observed and this reflects the minor effects of light quality on protein content.

Compared with the effects of nutrient deficiency, the changes in the chemical composition induced by light quality were relatively minor, and probably reflect adjustments of the cells light harvesting and photosynthetic mechanisms in response to growth in restricted spectral wavebands.

The carbon metabolism of these algae was investigated by following the fate of $^{14}CO_2$ taken up in the light. The cells were partitioned into water, chloroform and insoluble fractions following hot ethanol extraction. The water-soluble and extracellular release fractions were further separated into neutral, basic and acidic fractions using ion-exchange resins. Photorespiration was measured using a $^{14}CO_2$ release assay. The magnitude of photorespiration in white light was later determined using a differential carbon isotope uptake method.

The partitioning of incorporated ^{14}C revealed light quality effects in terms of the ^{14}C-activity of the soluble and insoluble fractions. In the eukaryotic algae most radioactivity was found in the soluble fraction in red light cells. This situation was reversed in blue and white light where ^{14}C-activity was higher in the insoluble fraction. Radioactivity of the insoluble fraction has been shown to increase with depth in freshwater and marine phytoplankton. These results support the suggestion of Wallen and Geen (362) that light quality might account for these depth-dependent changes in cell composition.

Rates of extracellular release as a percentage of total ^{14}C-fixation were < 5% and support the contention that healthy phytoplankton do not excrete significant portions of recent photosynthate during normal growth (308). Chlorella released > 1% of the total ^{14}C-fixed and no extracellular release by this alga was found. Extracellular release as a percentage of total CO_2-fixation was lowest in red light and highest in white or blue light in the other three algae. This result supports the data of Watt (368) who found that percentage extracellular release increased towards the bottom of the photic zone in freshwater situations.

No direct relationship between the size of the soluble fraction and extracellular release was observed. The red light effect on extracellular release was quite striking in this regard since ^{14}C-activity of the soluble fraction was high in red light grown cells. Extracellular release as a percentage of ^{14}C-activity in the soluble fraction showed the same trend as percentage total $^{14}CO_2$-fixation, being highest in white light, lower in blue and green light and lowest in red light. The distribution of ^{14}C-activity in the neutral, basic and acidic fractions of the soluble fraction were generally mirrored by the same fractions of the extracellular release fraction in most cases. Some possible effects of light quality on membrane permeability in terms of selective release of the neutral and acidic components of the soluble fraction were observed in white light adapted Navicula and white or green light grown cells of Anacystis. It was proposed that two light quality and depth related processes may occur in algae, one, a decrease in ^{14}C-activity of the soluble fraction and, two, an increase in the amount of ^{14}C-activity released from this fraction. The ratio of these two processes may control organic carbon release in the lower part of the photic zone.

An attempt was made to measure the effect of light quality on photorespiration using the $^{14}CO_2$ release assay of Zelitch (383). While this method seriously underestimates the magnitude of photorespiration and some anomalous results were obtained, light quality clearly influenced rates of $^{14}CO_2$ release. In all algae examined rates of $^{14}CO_2$ release, both in the light and the dark, were highest during or following exposure to blue or green light. $^{14}CO_2$ release rates were lowest in red or white light. Using a differential carbon isotope uptake method, the rate of

photorespiration in white light as a percentage of the total CO_2-fixation rate) was shown to range from 17 to 32% for the three eukaryotic algae and from 10 to 26% for the blue-green alga. It is suggested that photorespiration and dark respiration may be more important processes than extracellular release for the carbon economy of algal cells deep in the photic zone.

In relating the growth of freshwater algae to underwater changes in the spectral distribution of light, this study has shown light quality related effects on their rate of photosynthesis, carbon metabolism and chemical composition. The importance of the red part of the spectrum for the growth of freshwater algae is an outstanding result of this study. The high growth rate in red light is strongly correlated with high rates of photosynthesis and minimal losses of fixed carbon via extracellular release, photorespiration and dark respiration. This may reflect a long-term adaptation to the red-shifted λ_{Hmax} of many eutrophic freshwaters analogous to the importance of blue and blue-green light to marine phytoplankton in their environment where λ_{Hmax} is often shifted to the blue and blue-green part of the spectrum. The experimental verification of this hypothesis would require simultaneous measurement of the in situ spectral composition and intensity of light, and rates of CO_2-fixation and loss via photorespiration and extracellular release at various depths in freshwater situations where the phytoplankton have had to adapt to the available light, i.e. during stratification.

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