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**GOVINDJEE. EFFECT OF COMBINING TWO  
WAVELENGTHS OF LIGHT ON THE PHOTO-  
SYNTHESIS OF ALGAE.**

**University of Illinois, Ph.D., 1960  
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**EFFECT OF COMBINING TWO WAVELENGTHS OF  
LIGHT ON THE PHOTOSYNTHESIS OF ALGAE**

**BY**

**GOVINDJEE**

**B.Sc., Allahabad University, 1952  
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**THESIS**

**SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY  
SUPERVISION BY GOVINDJEE  
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BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOPHYSICS

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## Chapter 1: INTRODUCTION

This investigation deals chiefly with the enhancing effect of shorter wavelengths of light on the photosynthetic yield of the long wave red light,  $> 650-680$  m $\mu$ , especially in relation to different photochemical activities of two (or more) forms of chlorophyll a. Action spectra of the enhancement effect (also called "second<sup>1</sup> Emerson effect") were examined in Chlorella pyrenoidosa, Navicula minima, Anacystis nidulans and Porphyridium cruentum. In addition, observations of the "negative Emerson effect" are described and evidence is given of the inhibitory effect of extreme red light<sup>2</sup> on the yield of photosynthesis produced by far red light.<sup>3</sup>

It is generally accepted that chlorophyll a is the primary sensitizer in photosynthesis, because of its universal occurrence in all true photosynthetic organisms and also because light energy absorbed by most accessory pigments (chlorophyll b in green algae, the phycoerythrins and the phycocyanins in red algae, the phycocyanins in blue green algae, fucoxanthol and chlorophyll c in diatoms) is re-emitted as fluorescence of chlorophyll a (cf. Vermeulen, Wassink and Reman, 79; Dutton, Manning and Duggar, 16; Wassink and Kersten, 83; Duysens, 18; French and Young, 39, cf. Rabinowitch, 66, 67, 68).

Measurements of the quantum yield of photosynthesis as a function of the wavelength of light, and its comparison with the fraction of light absorbed by the several pigments, gives information as to the role of accessory pigments in photosynthesis.

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<sup>1</sup>"Second" to distinguish it from the carbon dioxide burst at the beginning of a period of light exposure, also referred to as the "Emerson effect".

<sup>2</sup>Extreme red light is arbitrarily defined as comprising wavelengths from 720 m $\mu$  to 800 m $\mu$ .

<sup>3</sup>Far red light is arbitrarily defined as light with wavelengths ranging from 680 m $\mu$  to 720 m $\mu$ .

Engelmann (35, 36) was the first to have suggested, on the basis of crude experiments, the participation of accessory pigments in photosynthesis. Systematic measurements of the action spectra of photosynthesis in Nitzschia (Dutton and Manning, 16), Chröococcus (Emerson and Lewis, 31), Chlorella (Emerson and Lewis, 32), Navicula (Tanada, 75), and Porphyridium (M. Brody and Emerson, 6), showed that the quantum yield may be quite high in the region where most of the light is absorbed by accessory pigments (except for certain carotenoids). Relative efficiency of excitation energy transfer from these pigments to chlorophyll a, calculated from the yield of sensitized fluorescence of chlorophyll a, is found to parallel the relative efficiency of the light absorbed by these pigments,-- in bringing about photosynthesis (cf. Rabinowitch, 66, 67; Duysens, 18). Although the fluorescence yield of living cells is low, it was estimated at  $< 5\%$  by Latimer, Bannister and Rabinowitch (57)(see Brody and Rabinowitch, 9), it can be taken as a good indicator of the fate of excitation energy.

These results suggest that chlorophyll a is the only direct sensitizer of photosynthesis, whereas the accessory pigments sensitize it indirectly, by absorbing additional light energy and transferring it to chlorophyll a. However, this concept does not explain the so-called "red drop"--the decline of quantum yield of photosynthesis in the far red and the "second Emerson effect", i.e. the enhancement of this yield by simultaneous absorption of light of shorter wave length.<sup>1</sup>

Haxo and Blinks (46) and Yocum and Blinks (84) noted that in red algae the quantum yield declines beyond 650  $\mu$  in the main chlorophyll absorption

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<sup>1</sup>For sake of brevity, the second Emerson effect will be called simply Emerson effect throughout this thesis. The first one--the CO<sub>2</sub>-burst--is not discussed at all in the following pages.

band but is high in the region where phycoerythrin is the main absorber. This observation suggested that in these algae chlorophyll a may not play the primary role, usually assigned to it, but was, at least partly, inactive. Blinks and coworkers thought that phycoerythrin may take over, in the red algae, the primary role played in other organisms by chlorophyll a. Duysens (18), as well as French and Young (39), observed that in these algae, the fluorescence yield of chlorophyll a also was lower in the region of the chlorophyll a absorption band, and higher in the light absorbed by phycoerythrin. On the basis of this finding, Duysens (18) postulated that in red algae, part of chlorophyll a is associated with phycobilins and is "active", while another part is associated with some unknown pigment (which may be chlorophyll d) which acts as an energy "sink", and makes this part of chlorophyll a non-fluorescent and inactive in photosynthesis.

However, M. Brody and Emerson (6) showed that in the red alga Porphyridium cruentum, chlorophyll a has a photosynthetic efficiency as high as in Chlorella, Navicula and other green or brown algae, but only at wavelengths  $< 650 \text{ m}\mu$  (e.g., at  $644 \text{ m}\mu$ , where chlorophyll a contributes about 50% to the total absorption, the quantum yield is as high as at any other wavelength). However, at wavelengths  $> 650 \text{ m}\mu$ , the full quantum efficiency of chlorophyll a can be obtained only if phycoerythrin is excited simultaneously (Emerson effect). According to these newer findings, it is unnecessary to suppose that chlorophyll a in red algae is partly inactive, and that the pigment plays therefore, on the average a lesser role in the photosynthesis of the red algae than in that of the other algae.

Emerson (21) (also cf. Emerson and Chalmers, 25) pointed out that the drop in quantum yield of photosynthesis in the red or far red occurs when

chlorophyll a begins to absorb the predominant fraction of light. He concluded that to obtain the full yield of photosynthesis, it is necessary to excite, in the red algae, both phycoerythrin and chlorophyll a.

Emerson then looked back on his earlier experiments on Chlorella, in which he, together with Lewis (32) had observed a drop in the yield of photosynthesis in the far red light, beginning at 680 m $\mu$ ,--still well within the main red absorption band of chlorophyll a. Emerson, Chalmers and Cederstrand (27) restudied the phenomenon, which has become known as the "red drop". Emerson (21) speculated that the quantum yield drop which occurs in red algae at wavelengths  $> 650$  m $\mu$ , and in all other algal classes studied,  $> 680$  m $\mu$ , occurs quite generally when chlorophyll a begins to claim all the absorbed light energy.

In studying the "red drop", Emerson et al. (27) found another interesting phenomenon--the wavelength at which the red drop occurred was increased when shorter wave light of sufficient intensity was provided simultaneously. The quantum yield in far red light (at 700 m $\mu$ , in the region of the red drop) combined with shorter wavelength light given simultaneously was as high as 0.10, indicating full efficiency of both short and long waves. On the other hand, the quantum yield of the far red light alone was only .05 or less. The question was, whether this enhancement effect was due to the presence of higher-energy quanta, or to the absorption of light by some pigment other than chlorophyll a. Emerson and Chalmers (25; cf. 34) tried to solve this problem by studying the action spectra of the enhancement effect and then comparing them with the curves showing the fraction of light absorbed by the accessory pigments. Emerson et al. (25; 34, Figures 6-9) found that the action spectrum of the enhancement effect in Chlorella follows closely the curve showing the

fraction of absorbed light absorbed by chlorophyll b (prominent peaks at 480 m $\mu$  and 655 m $\mu$ ); in Navicula, it follows closely the similar curve for fucoxanthol (peak at 540 m $\mu$ ) and perhaps also chlorophyll c (peak at 645 m $\mu$ ); in Anacystis, that of phycocyanin, with a broad peak at 600 m $\mu$  and in Porphyridium, that of phycoerythrin, with a single peak at 546 m $\mu$ . Myers and French (61) have confirmed the findings of Emerson et al. (25, 34) in Chlorella and also emphasized the specific role of chlorophyll b.

Emerson (cf. 19, 20, 21) and Emerson et al. (cf. 25, 26, 27, and 34) concluded, from the similarity between the action spectra of the enhancement effect and the curves showing the fraction of light absorbed by the several pigments, that the 'red drop' occurs in the region where light is absorbed mainly or entirely by chlorophyll a, and that chlorophyll a alone must be ineffective in photosynthesis. The emphasis in the latter statement is on the word "alone", because the yield of photosynthesis in the region where chlorophyll a alone absorbs, can be raised to full value by simultaneous excitation of another pigment.

It therefore appeared that for photosynthesis to occur with maximum efficiency, it is necessary to excite both chlorophyll a and an accessory pigment. Emerson speculated that two different primary photochemical processes may be involved in photosynthesis, but he kept his results open to other explanations (cf. Emerson, 21).

Emerson's interpretation is consistent in itself, but it contradicts the results obtained by the study of sensitized fluorescence which support the earlier concept that excitation of chlorophyll a alone is the source of all photosynthesis, and that accessory pigments are present only to absorb additional light and transfer their excitation energy to chlorophyll a.

The occurrence of the red drop and of Emerson's enhancing effect was re-interpreted by James Franck (37), by postulating two forms of chlorophyll a, one associated with lipoids (hydrophobic molecules) and the other with hydrophilic molecules. In the first form (the nonfluorescent),  $^1(n-\pi)^*$  state lies below the lowest singlet excited state  $^1(\pi-\pi)^*$ ; and accelerates the transfer of  $^1(\pi-\pi)^*$  molecules into triplet state  $^3(\pi-\pi)^*$ ; this form is therefore non-fluorescent. The second form has its  $^1(n-\pi)^*$  excited state above the lowest  $^1(\pi-\pi)^*$  singlet excited state, so that the latter state is relatively more stable; this is the fluorescent form. Franck suggested that either a double excitation  $S \xrightarrow{+h\nu} S^* \rightarrow T \xrightarrow{+h\nu} T^*$ , (using simplified designations,  $S^*$  instead of  $^1(\pi-\pi)^*$  and  $T$  instead of  $^3(\pi-\pi)^*$ ), or simply a balanced formation of the two excited states ( $S^*$  and  $T$ ) is necessary for full photosynthesis. The triplet-forming, "hydrophobic" form absorbs at longer wavelengths; when light is absorbed by this form only, photosynthesis cannot occur efficiently and the yield goes down. If, on the other hand, accessory pigments are simultaneously excited, the energy is supposed to be transferred from them preferentially to the hydrophilic form, producing relatively stable singlet excited states ( $S^*$ ). Thus, a balanced formation of the two excited states takes place, and a full yield of photosynthesis becomes possible. Emerson's original work provided no spectroscopic confirmation of the existence of two forms of chlorophyll a, which, when excited simultaneously, produce full photosynthesis. This confirmation is provided by the present investigation; the main body of this thesis will deal with this subject.

Besides Franck's theory, other alternate or complementary theories, also based on the assumption of two (or more) forms of chlorophyll a, were suggested. On the basis of absorption studies on fluorescein and chlorophyll a in solution,

Lavorel (59) proposed that a variable fraction of chlorophyll a in living cells may be present in a dimeric, inactive form. The presence of a band belonging to the dimer, on the long wave side of the main red absorption band of chlorophyll, could explain the decline in the yields of photosynthesis, fluorescence and Hill reaction in the far red light. However, this hypothesis does not make it clear why simultaneous excitation of monomeric and dimeric molecules should enhance the photosynthetic activity of the dimer. The same can be said of Brody's (8) observations of the fluorescence spectrum of Chlorella, which lead him to a similar hypothesis. Krasnovsky and coworkers (cf. 52, 53 and 80) also suggested that the red drop is due to a dimer; they think that the photochemically active monomeric form absorbs around 670 m $\mu$ , whereas the form absorbing at about 680 m $\mu$  is the inactive dimer. Krasnovsky and coworkers (80) observed differences in the rate of bleaching of the two forms in vitro, Brown and French (11) confirmed it.

There exists experimental evidence in the spectroscopic literature suggesting the existence of two (or three) chlorophyll a forms in vivo. In isolated chloroplasts, Albers and Knorr (1) reported that they could observe, in addition to the main absorption peak at 682 m $\mu$ , another one at 670 m $\mu$ , and several minor maxima. Because of the asymmetric and broad nature of the chlorophyll a red band in vivo, Duysens (17) assumed that there may be more than one form of chlorophyll a. French & coworkers (11, 38), using differential spectroscopy, have revealed the existence of several peaks of chlorophyll a in vivo, at about 670 m $\mu$ , 680 m $\mu$  and 690 m $\mu$ . Complexity of the red absorption peak of chlorophyll a was noted also by Halldal (45) in Anacystis nidulans, by Thomas and Marsman (78) in Porphyra perforata, and by Cederstrand (14) and Thomas and Govindjee (77) in Porphyridium cruentum. Rajni Govindjee

(43) has observed two peaks--one at 670 m $\mu$  and the other at 680 m $\mu$  in the absorption spectrum of chloroplasts from spinach.

My experiments indicated the existence of a clear, sharp peak at 670 m $\mu$  in the action spectra of the Emerson effect in Navicula and Chlorella; a shoulder around 670 m $\mu$  was present in the corresponding spectra of Anacystis and Porphyridium. This peak (or shoulder) corresponds to a peak in the absorption spectrum of these algae, also located at 670 m $\mu$ , and probably due to a special form of chlorophyll a. My experiments thus provide evidence for the existence and different photochemical activities of two forms of chlorophyll a in vivo. Govindjee and Rabinowitch (42) and Rabinowitch and Govindjee (69) published preliminary reports describing this phenomenon. Detailed results will be discussed in Chapter 4 of this thesis. Action spectra of the Emerson effect in the Hill reaction of whole Chlorella cells (Rajni Govindjee et al., 44) also showed the existence of a 670 m $\mu$  peak. In addition, a peak in the blue region was observed in the Hill reaction study probably due to the same, photochemically active, form of chlorophyll a (Chl a 670).<sup>1</sup>

At certain wavelengths in Anacystis, Navicula and Porphyridium, Emerson's action spectrum had negative values. Emerson and Rabinowitch (34) pointed out that if this apparent negative effect turns out to be real, it could be due to reduction in the yield of the short wave light by the long wave light, rather than the other way around. It was found in the present investigation that the

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<sup>1</sup>Chlorophyll a form with an absorption peak at about 670 m $\mu$  will be referred to as Chl a 670, even if the actual peak lies at 672 m $\mu$ .

Other forms of chlorophyll a will be designated as Chl a 680 + 690, since no attempt has been made in this study to distinguish between the apparently existing two forms, absorbing at about 680 m $\mu$  and at about 690 m $\mu$  respectively.

intensity relation between the two participating beams has an important bearing on the occurrence of the negative Emerson effect. For example, in Anacystis, the "negative effect" described by Emerson and Rabinowitch (34) can be converted into a positive effect by a decrease in the relative intensity of the far red beam. It was also observed that the saturation of photosynthesis occurs at 700  $\mu$  in much weaker light, and at a much lower level than at 680  $\mu$  and, even more so, at 600  $\mu$ . This may explain, at least in part, the negative Emerson effect observed in this alga, as will be shown in Chapter 5, devoted to the discussion of negative effects.

Thomas and Govindjee (76, 77) studied the yield of photosynthesis in the red alga Porphyridium cruentum, and found no measurable photosynthesis when white light filtered through a dense phycobilin solution was used for illumination, although chlorophyll a molecules were excited by this light, at a significant rate (as proved by the occurrence of a positive Emerson effect when supplementary orange light was provided). On the face of it, this seemed to mean that chlorophyll a when excited alone, is entirely inactive in photosynthesis. However, it was soon found (cf. a preliminary report by Rabinowitch, Govindjee and Thomas, 70) that extreme red light ( $> 720 \mu$ ), transmitted by the phycobilin filter along with the far red light (680-720  $\mu$ ), was inhibiting photosynthesis produced by the far red light. This effect, too, has been studied in more detail; the results will be discussed in Chapter 6.

For comparison of cultures used in this investigation with those used by Emerson in the earlier work, the absolute quantum yield of photosynthesis of the former was determined. The red drop in the action spectra of photosynthesis was measured with Navicula and Anacystis, that of Chlorella and Porphyridium had been examined earlier by Emerson and coworkers (27), and by Brody and Emerson (6). These results will be discussed in Chapter 3.

## Chapter 2: MATERIALS AND METHODS

### A. Selection, Source and Characteristics of Organisms

Following organisms were studied: Chlorella pyrenoidosa, Navicula minima, Porphyridium cruentum and Anacystis nidulans. The Chlorella strain was isolated by R. Emerson, that of Navicula by Tanada. Porphyridium was obtained from Ralph Lewin (National Research Council of Canada, Halifax) and Anacystis from Jack Myers of the University of Texas. These organisms were selected for the following reasons: i) They are unicellular at least in turbulent medium, and easy to handle in manometric experiments. ii) They represent four important classes of algae - viz. Chlorophyta (Chlorella), Chrysophyta (Navicula), Myxophyta (Anacystis) and Rhodophyta (Porphyridium).

Unicellular organisms pose fewer problems of the diffusion of gases from and to the cell than do multicellular organisms or pieces of tissue from higher plants. In suspensions of unicellular organisms, light distribution is more even than in multicellular ones, or in plant tissues. In using a suspension of unicellular organisms, one uses millions of individuals and thus obtains a good statistical average.

A brief description of the algae used in this investigation is given in Table 1.

### B. Culturing of Algae

All four species were grown in 300 ml Erlenmeyer flasks with sealed-in tubes for the passing of gas (air, or air with 5% carbon dioxide). These flasks were filled with 200 ml of culture medium (see Table 2A and 2B), plugged with cotton wool and sterilized in an autoclave for 10-30 minutes at 15 lbs. per square inch. Flasks containing the culture medium were cooled, and inoculated. Care was taken to use always the same amount of inoculum, taken

Table 1

## DESCRIPTION OF THE ALGAE USED IN THIS INVESTIGATION

	<u>Chlorella</u> <u>pyrenoidosa</u> (Emerson's Strain 3) - a green alga	<u>Navicula</u> <u>minima</u> (var. <u>atmoides</u> ) - a diatom	<u>Anacystis</u> <u>nidulans</u> - a blue green alga	<u>Porphyridium</u> <u>cruentum</u> - a red alga
Description	unicellular	unicellular, sometimes forming short filaments	unicellular in turbulent cultures; sometimes form- ing two to four-celled filaments	unicellular, encased in a mucilage sheath
Size	average diameter = 5 $\mu$ (measured)	6 x 18 $\mu$ (Tanada, 75)	ca. 1.6 x 2.2 $\mu$ (Kratz and Myers, 54)	6-8 $\mu$ in diameter (Brody and Vatter, 7)
Pigments	chlorophyll <u>a</u> , chlorophyll <u>b</u> and carotenoids	chlorophyll <u>a</u> , chlorophyll <u>c</u> , fucoxanthol and other carotenoids	chlorophyll <u>a</u> , phycocyanins and carotenoids (traces of phycoerythrin ?)	chlorophyll <u>a</u> , phycoerythrins, and carotenoids (traces of phycocyanin ? and chloro- phyll d ?)

Table 2A

## CULTURE MEDIA

Main constituents are described in this table,  
for the micronutrients see Table 2B.

		<u>CHLORELLA</u>	<u>NAVICULA</u>	<u>ANACYSTIS</u>	<u>PORPHYRIDIUM</u>
As given by:		Emerson and Chalmers (24)	Tanada (75, modified by Emerson and Chalmers, 24)	Medium C of Kratz and Myers (55)(a different iron salt was used)	Brody and Emerson (5), with minor modifications in A <sub>5</sub> and B <sub>9</sub> solutions
		g/l	g/l	g/l	g/l
MgSO <sub>4</sub> · 7H <sub>2</sub> O		0.25	0.05	0.25	2.50
KH <sub>2</sub> PO <sub>4</sub>		1.875	--	--	--
Na <sub>2</sub> HPO <sub>4</sub>		0.025	--	--	--
K <sub>2</sub> HPO <sub>4</sub>		--	0.3125	1.00	0.50
KNO <sub>3</sub>		1.25	0.625	1.00	1.25
CaCO <sub>3</sub>		0.016	--	--	--
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O		--	0.01375	0.025	0.25
KCl		0.025	--	--	16.00
NaCl		--	--	--	12.50
KI		--	--	--	0.05
KBr		--	--	--	0.05
Sodium citrate		--	--	0.165	--
Na <sub>2</sub> SiO <sub>3</sub>		--	0.05	--	--
FeSO <sub>4</sub> · 7H <sub>2</sub> O		0.0057	0.00114	0.0057	0.0057
Micro- nutrients	* A <sub>2</sub>	1 ml/l	--	--	--
	*** A <sub>5</sub>	--	1 ml/l	1 ml/l	5 ml/l
	# B <sub>9</sub>	--	--	--	5 ml/l
	## ABC	0.05 ml/l	--	--	--

Table 2B

## MICRONUTRIENTS

*A <sub>2</sub>	MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.81 g/l
	H <sub>3</sub> BO <sub>3</sub>	2.86 g/l
**A <sub>3</sub>	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22 g/l
	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.079 g/l
	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	0.20 g/l
***A <sub>5</sub>	A <sub>2</sub> + A <sub>3</sub> (with slight modifications taken from Hoagland, 47)	
#B <sub>9</sub>	(after Brody and Emerson, 5, with slight modifications)	
		mg/l
	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·Na <sub>2</sub> SO <sub>4</sub> ·24H <sub>2</sub> O	.786
	KBr	.119
	KI	.2083
	Cd(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	.154
	Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	.145
	NiSO <sub>4</sub> ·6H <sub>2</sub> O	.131
	Cr(NO <sub>3</sub> ) <sub>3</sub> ·9H <sub>2</sub> O	.040
	Na <sub>2</sub> VO <sub>4</sub> ·16H <sub>2</sub> O	.041
	Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	.033
C <sub>10</sub>	As <sub>2</sub> O <sub>3</sub>	6.61
	SrSO <sub>4</sub>	10.49
	HgCl <sub>2</sub>	6.77
	PbCl <sub>2</sub>	6.71
	LiCl	30.55
	RbCl	14.20
	K <sub>2</sub> TiF <sub>6</sub> ·H <sub>2</sub> O	5.00
	NaSeO <sub>4</sub>	11.96
	Be(NO <sub>3</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	103.7
	Uranyl nitrate	10.0
##ABC	5 ml A <sub>3</sub> + 5 ml B <sub>9</sub> + 5 ml C <sub>10</sub> made up to 100 ml.	

from the same stock, when working with a given species. All the cultures used were single-strain pure cultures, free from bacteria and molds. After inoculation, culture flasks were capped with rubber caps and then placed in water baths, with the gas cylinders connected to their inlet side arms by means of rubber tubing. The flow of gas was adjusted to 100-120 bubbles per minute. Cultures were grown as described in Table 3.

Special care was taken to reduce the light intensity in the culture baths before the beginning of the manometric experiments, in order to reduce the rate of respiration and to obtain the maximum quantum yield of photosynthesis (cf. Emerson and Lewis, 30). In the case of Chlorella, the illumination was reduced to two 15 watt tungsten bulbs for the final 24 hours preceding the manometric experiments. In Navicula, the culture flask was exposed to only one 32 watt fluorescent tube at a distance of 6 inches for 24 hours before the experiment. Porphyridium cultures received, for the last 48 hours, only light from a single 15 watt fluorescent tube at a distance of about 6 inches. Illumination was reduced also in the Anacystis bath, to one 40 watt fluorescent tube at a distance of  $7\frac{1}{2}$  inches for the last 24 hours.

To prevent settling of the cells to the bottom of the flasks, Chlorella and Anacystis cultures required daily shaking; Navicula and Porphyridium cultures had to be shaken more often.

Navicula was grown in earlier experiments on glass wool inserted into the flask. Glass wool provided surface for the diatoms to stick to and this kept them from sticking to the culture flask. Later this method was given up in favor of the following: on the last day of culturing, a magnetic rod wrapped in Teflon sterilized with alcohol and dried by keeping it near a flame, was aseptically introduced into the flask. The culture flask was left overnight

Table 3  
SUMMARY OF GROWING PROCEDURES

Algae	<u>Chlorella pyrenoidosa</u>	<u>Navicula minima</u>	<u>Anacystis nidulans</u>	<u>Porphyridium cruentum</u>
Isolated by	Emerson	Tanada	Kratz and Allen	Lewin
Inoculum in $\mu$ l/200 ml	15-20	100	15-20	100
Culture medium described by	Emerson and Chalmers (24)	Tanada (75)	Kratz and Myers (55)	Brody and Emerson (5)
Illumination	four tungsten lamps and one white fluorescent ring	ten white fluorescent rods	four white fluorescent rods and one tungsten bulb	ten fluorescent rods, see Brody and Emerson (5) and Thomas and Govindjee (77) for details.
Wattage	60 watt tungsten lamp and 32 watt white fluorescent ring	15 watt white fluorescent rod	40 watt white fluorescent rod and 100 watt tungsten bulb	15 watt white fluorescent rod
Distance from the bottom of flask	6" (tungsten lamps) 4" (fluorescent ring)	6"	7 1/2"	6"
Axis to axis distance	3" (tungsten lamps)	1 1/16" (fluorescent rods)	3 1/16" (fluorescent rods)	2 1/4" (fluorescent rods)
No. of days algal cultures were on line	2-3	3-4	2-3	6-7
Temperature of culture bath	20°C $\pm$ 2°C	25°C $\pm$ 2°C	27°C $\pm$ 2°C	19°C $\pm$ 2°C
Gas mixture	5% CO <sub>2</sub> in air	Filtered room air or 5% CO <sub>2</sub> in air	Filtered room air or 5% CO <sub>2</sub> in air	5% CO <sub>2</sub> in air
Growth (x fold)	40 fold	20 fold	40 fold	40 fold

" means inches

(12-16 hours) on the magnetic stirrer. In addition, the flow of the gas was increased to 200 bubbles/min., and culture flasks were shaken at least twice a day.

With Porphyridium, homogenous cultures were obtained by shaking the cultures continuously by a wrist-action shaker. This method seemed not to work well with Navicula cells, because they stuck to glass in spite of continuous shaking. However, it was not tried out systematically enough, because the method described above was found to be sufficient to obtain homogenous cultures of Navicula.

#### C. Harvesting and Preparation of Samples for Manometry

Cells suspended in the culture medium were collected by centrifugation. It took about 5 minutes for Chlorella and Navicula, 10 minutes for Anacystis and 15 minutes for Porphyridium, for the cells to come down at about 1600 xg. Cells were washed twice with cold (10°C) buffer. Carbonate-bicarbonate buffer #9 of Warburg (81) was used, replacing  $\text{Na}_2\text{CO}_3$  with  $\text{K}_2\text{CO}_3$  (15 ml of  $\frac{M}{10} \text{K}_2\text{CO}_3$  and 85 ml of  $\frac{M}{10} \text{NaHCO}_3$ , in equilibrium with  $76 \times 10^{-6}$  mole  $\text{CO}_2$  per liter at 10°C) for washing cells of Chlorella, Anacystis and Navicula. Porphyridium cells were washed with buffer #11 (5.0 ml of  $\frac{M}{10} \text{K}_2\text{CO}_3$  and 95.0 ml of  $\frac{M}{10} \text{NaHCO}_3$  in equilibrium with  $274 \times 10^{-6}$  mole  $\text{CO}_2$ /l), with added NaCl in the following proportion: 16.0 g  $\text{NaHCO}_3$ ; 1.0 g  $\text{Na}_2\text{CO}_3$ ; 15.2 g NaCl, made up to 1 liter with glass-distilled water. After the second washing, cells were suspended in their respective buffers. Buffer #9 and Buffer #11 were selected because they gave higher yields than other buffers. The concentration of the suspension was adjusted to an optical density of approximately 0.500 at the chlorophyll a absorption peak (rather than to identical number of cells per ml). The samples were now ready for manometry.

#### D. Manometric Technique

7 ml of cell suspension was transferred to a rectangular vessel (size: 4 x 2.5 x 1.5 c.c.) and 7 ml buffer was transferred to the compensating vessel of the same size. The vessels were attached to the two arms of a differential manometer, which contained isocaproic acid as manometric fluid. Detailed description of the advantages of the closed differential manometer system and of its limitations was given by Emerson and Chalmers (23). The gas space above the cell suspension was flushed only when some CO<sub>2</sub> was lost during the handling of the buffer. No significant difference was observed when comparing the results obtained after the buffer was washed out with gas, and those obtained with a carefully handled sample which had not been gassed out.

The single vessel method of manometry was chosen because of easier calculation of results. The observed pressure change can be converted, in this case, to  $\mu$ l of oxygen exchanged simply by multiplying the pressure change with the "vessel constant" for oxygen (K<sub>O<sub>2</sub></sub>), determined for the same temperature. For the three sets of vessels used in this investigation, the constant K<sub>O<sub>2</sub></sub> varied at 10°C between 1.06 and 1.20.

The manometer was placed low enough for the vessels to be submerged in the water bath. The manometer was mounted on a shaking panel, which was started 15-20 minutes before the stop cocks of the manometer were closed; this was done to permit the establishment of the temperature equilibrium in the vessels. The temperature of the water bath was maintained at 10°C by means of a mercury thermoregulator, a heater and a refrigeration unit. The water was stirred vigorously. After closing the stop cocks, the vessels were shaken at 180 oscillations per minute, with an amplitude of 18 mm. After 5 minutes,

readings of pressure change were started. The starting and the stopping of the shaker was done gently by means of a variac attached to the motor. If the manometer is stopped suddenly, the fluid may block the cone of the manometer. In order to minimize this inconvenience, the inner walls of the cones were coated with paraffin. Readings of the pressure change were made while the manometer was shaking. Changes in pressure in both arms of the manometer were read simultaneously, to one hundredth of a mm, using the two telescopes of a double cathetometer.

#### E. Optical Arrangement

In the earlier experiments, far red light was isolated with the optical arrangement described by Thomas and Govindjee (77)(Figure 3). The source was an air-cooled, 1000 watt tungsten lighthouse lamp, operated through a variac at 750 watts (0-8 amps). Light from the lamp passed first through two heat-absorbing American Optical Co. filters, then through three sharp cut-off filters (Schott RG5, Schott RG8, and Corning "F glass"); only light with wavelengths longer than 680  $\mu$  was transmitted by this combination of filters (Figure 1). The filtered light beam diverted 90° by a prism, entered the thermostated bath, through a cylindrical plastic "light-pipe" employed to avoid scattering of the beam by the strongly agitated water. The beam left the light-pipe beneath the surface of the water and was reflected by a stainless steel mirror onto the bottom of the experimental vessel. In any given experiment, the intensity of the far red light was maintained constant (unless otherwise specified). In later experiments, the sharp cut-off red filters were replaced by an interference filter with a transmission peak at 700  $\mu$  (half band width 16  $\mu$ ). This was a Farrand #109556 filter; its transmission spectrum is also shown in Figure 1. Light  $> 700 \mu$  was obtained by using two (each one mm thick) Schott RG10 glass filters (Figure 2).

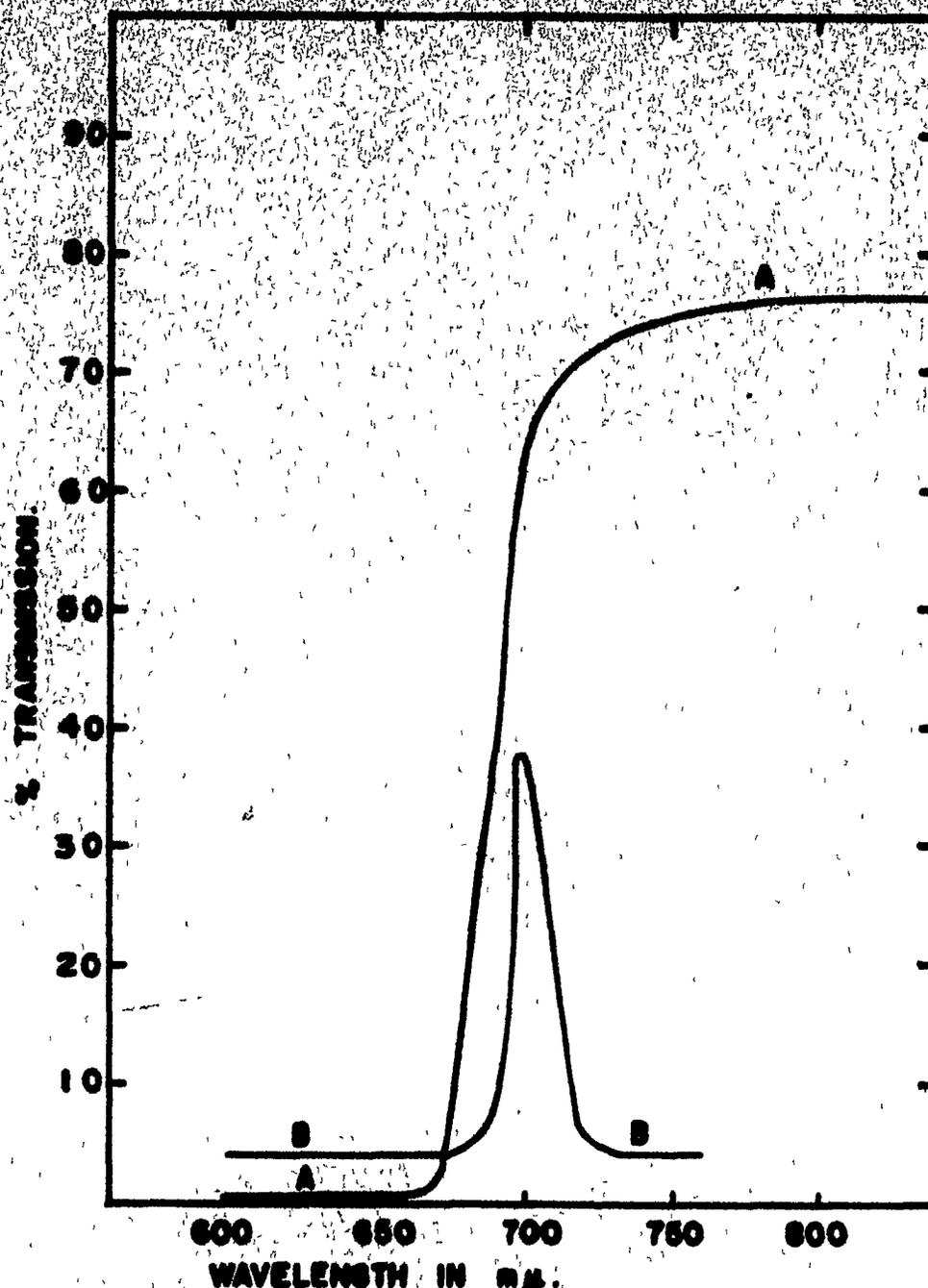


Figure 1. A: Transmission curve of the combination, of Schott glass filters RG5, and RG8, and a red Corning filter, to obtain light  $> 680$  mμ.  
 B: Transmission curve of a Farrand interference filter (#109556), to obtain light  $\sim 700$  mμ.

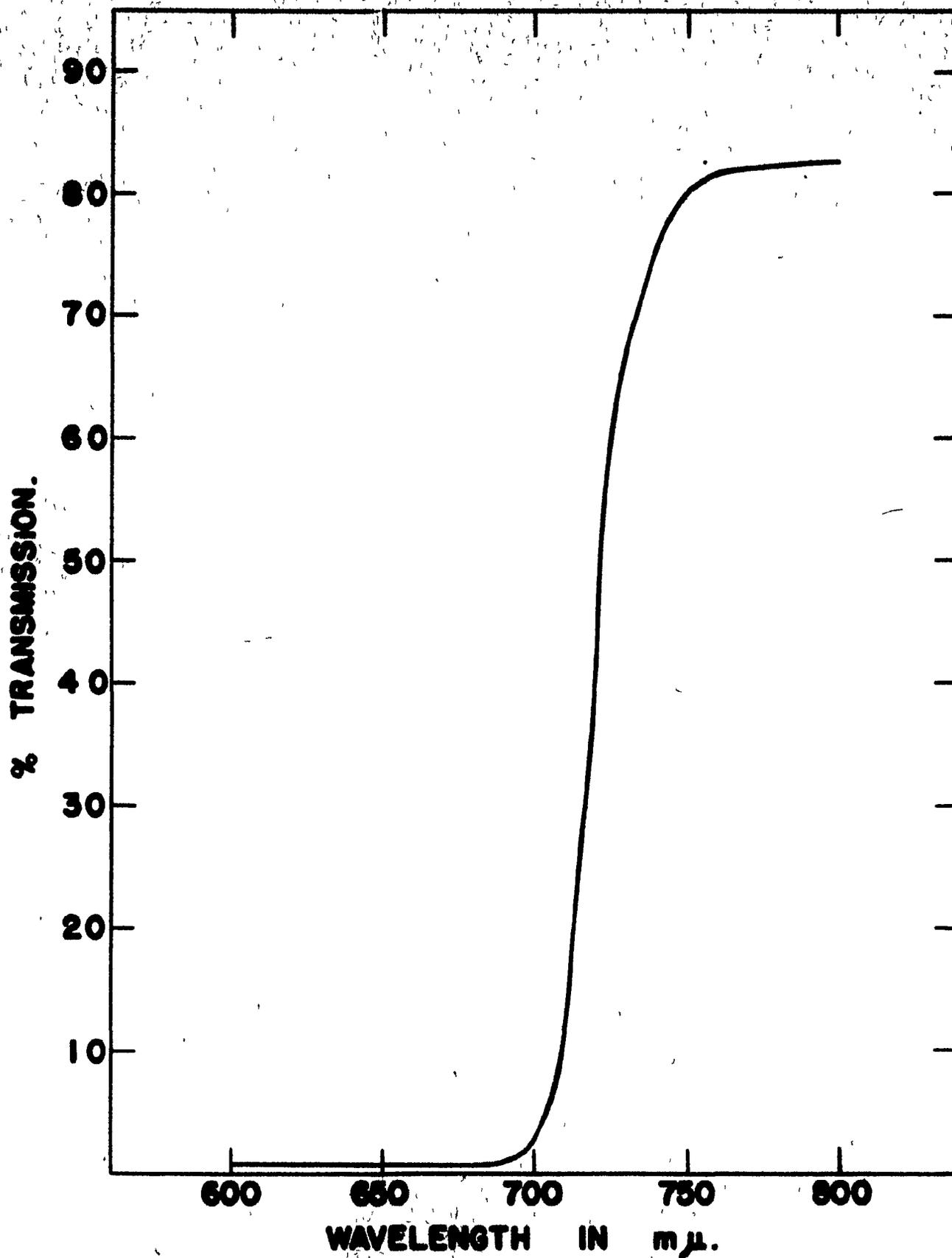


Figure 2. Transmission curve of two, one mm thick, Schott glass filters RG10.

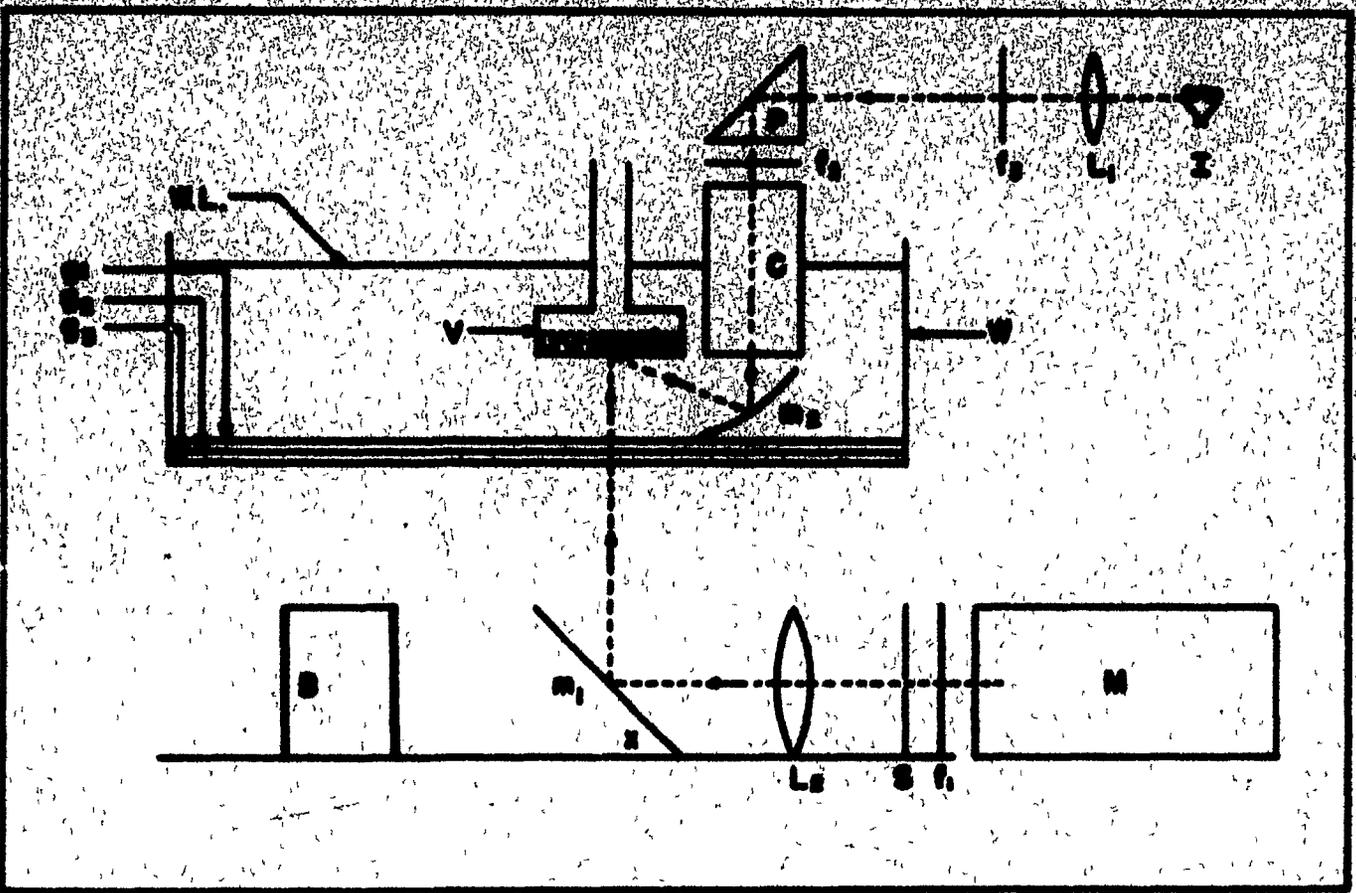


Figure 3. Optical arrangement to get two beams of light. M: Emerson-Lewis Monochromator.  $f_1$ : glass filter to cut off overlapping orders in the far red and the extreme red regions;  $f_2$ : interference filter to get 700 m $\mu$  or a combination of Schott glass filters RG5, and RG8 and a sharp cut off Corning glass;  $f_3$ : infra red absorbing American optical filter;  $L_1$  and  $L_2$ : lenses; I: Lighthouse 1000 Watt tungsten lamp; P: 45 degree reflection prism; C: lucite column (light pipe); V: manometer vessel; A: algal suspension;  $M_1$ : movable mirror along axis X;  $M_2$ : stainless steel concave mirror; W: water bath; W.L.: water line; B: Stenzell Bolometer; S: Exit slit of Monochromator;  $S_1$ ,  $S_2$ , and  $S_3$ : glass plates at the bottom of the water bath.

Note that the diagram has not been drawn to scale; distance between the monochromator light source and the bolometer is the same as that between the monochromator light source and the vessel (diagram after Thomas and Govindjee, 77).

Monochromatic supplementary light was obtained from Emerson's monochromator ( $f$  1.5) in combination with a General Electric 225 watt ribbon filament tungsten lamp operated at full capacity. If necessary, the current was controlled by a variac (range 0-30 amperes). This light source was run at 7.5 volts and 30 amperes on an A.C. Power supply<sup>1</sup> which was connected to the regular 110 volts 60 cycles power line. The monochromator was calibrated by using a standard cadmium source. The calibration was checked once every month. Entrance and exit slit widths were varied as needed, in different regions of the spectrum. In the 600 to 700  $m\mu$  region the band width was 10  $m\mu$ , but in the blue region, slits had to be opened to 20-30  $m\mu$ , when the tungsten lamp was used. Sometimes a Hg-Cd "HBO<sub>4</sub>" Osram lamp was used, which gives a line spectrum in order to obtain monochromatic light of greater purity in the blue region. This lamp was run on a D C. Power supply, because the light energy fluctuated too strongly with the A.C. Power supply. This lamp was used in particular to check the quantum yields in the blue region. Because of the wide slits that had to be used to get enough photosynthesis in this region while using a tungsten lamp as light source, precise determination of the quantum yields in this region is difficult. Monochromatic light was selected by turning the position of the grating in the monochromator by means of a screw gauge. The light beam coming out of the exit slit of the monochromator, was focussed by a lens to fall under  $45^\circ$  on an adjustable mirror situated beneath the water tank. The mirror reflected the monochromatic beam through a window made of 3 clear glass plates onto the bottom of the manometric vessel in the tank.

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<sup>1</sup>The power supply used in these experiments was built by Mr. Carl Cederstrand and the grating used in the monochromator was loaned to the late Dr. Emerson by the Mount Wilson Observatory.

## F. Photosynthesis Measurements

Photosynthesis was measured by observing pressure changes caused by the photosynthetic evolution of oxygen. Oxygen was also taken up because of respiration. Therefore, a correction had to be made. Respiration was measured in the dark and subtracted from the readings made in light. Unfortunately, the respiration correction is uncertain in the spectral region in which light is known to affect respiration (see Appendix 1). However, even at the wavelengths (mainly 480 m $\mu$ ) where light had the greatest effect on respiration, a steady level of respiration could be obtained by exposing the cells to light for about 10-30 minutes before beginning the measurements.

Quantum yield measurements were carried out with totally absorbing suspensions. A "totally absorbing" suspension is defined as one in which the absorption of light is practically 100% at all wavelengths within the absorption bands of the photosynthetic pigments. Totally absorbing suspensions were preferred to optically thin suspensions; to calculate the quantum yields in thin suspensions we have to know accurately the per cent absorption at all wavelengths. The ordinary available integrating devices do not allow the determination of absorption curves of scattering systems, such as algae, tissues, or cell suspensions with high precision. In these instruments the light sensitive element is located at a fixed point, and the energy measured varies with the distribution pattern of the scattered light. In order to reduce the errors, Warburg and Krippahl (82) have measured absorption also by comparison with a suspension of white cells, from which the pigments had been extracted as completely as possible. Even this procedure is not quite satisfactory, because extraction of the photosynthetic pigments changes the scattering properties of the cells (cf. Emerson, 21, for a detailed discussion).

All these inconveniences can be circumvented by using a totally absorbing suspension. The sequence of exposures was as follows: 7 min. dark, 7 min. light, 7 min. dark, 7 min. light, and 7 min. dark. From these data, the light action could be calculated twice at each given wavelength, and averaged. Pressure was recorded at intervals of one minute. A "beeper" produced a warning signal 5 seconds before the minute. First three readings were discarded because the steady state was not reached before the second minute of the exposure. Readings of the last four or five minutes were averaged. In nearly all experiments, when the steady state was reached, the fluctuations of the readings did not exceed 3% (except in rare cases; in these the whole set of experiments was discarded). Quantum yields of photosynthesis were calculated according to the formula:

$$Q.Y. = \frac{\text{moles of } O_2 \text{ evolved}}{\text{einsteins of light energy absorbed}}$$

However, the majority of the experiments reported in this thesis were not concerned with the measurement of absolute quantum yields. Rather, the relative yield in far red light (of fixed intensity) was measured, in the presence and in the absence of supplementary light of shorter wavelengths. The following sequence of readings was used in most experiments: 7 min. Dark, 7 min. Far Red, 7 min. Dark, 7 min. Far Red, 7 min. Dark, 7 min. Supplementary, 7 min. Supplementary + Far Red, 7 min. Supplementary, 7 min. Supplementary + Far Red, 7 min. Supplementary, etc. The light action of the far red light alone was checked at the end of each experiment. If necessary, a correction was made for the change in this yield. In some experiments, the action of the far red light was checked several times during the run, but this was found unnecessary. The light action due to far red light alone was arbitrarily

called 100. The light action of far red light during the same period in the presence of supplementary light was then determined and the "ratio":

$$R = \frac{\text{Light action of far red light (in presence of supplementary light)}}{\text{Light action of far red light (in absence of supplementary light)}} \times 100$$

calculated. R-values above 100 mean a positive Emerson effect, and below 100 mean a negative Emerson effect. These R-values<sup>1</sup> were plotted against the wavelength of the supplementary light. The resulting plot is the action spectrum of the Emerson effect.

The effect of extreme red light on photosynthesis by far red light was studied by the same method. Far red light was provided by an interference filter (Farrand #1322), the maximum transmission of which is at 700 mμ. The filter had been in the laboratory for a long time and had a broader transmission curve, than that shown in Figure 1. It had a half band width of about 20 mμ (or slightly more). Extreme red monochromatic light was provided by the Emerson-Lewis monochromator with a Schott RG8 filter in front of the exit slit. The grating in the Emerson-Lewis monochromator was blazed for observations in the visible region; therefore, its intensity in the extreme red light is not as high as in the 600-700 mμ region. In addition, the second order spectrum and the "ghosts" overlap the first order spectrum in the extreme red. Impurity of light in this region could be removed to some extent by using a red cut-off RG8 filter; but we could not eliminate all overlapping in this region. Action

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$$^1R' \text{ can also be written as, } \frac{R(R+S) - R_S}{R_R} \times 100 \text{ --- (1); } R_{(R+S)} =$$

rate of photosynthesis in far red light and supplementary light given simultaneously;  $R_S$  = rate of photosynthesis in supplementary light alone;  $R_R$  = rate of photosynthesis in far red light alone.

Intensity of far red light is kept constant throughout one set of experiments. Intensities of supplementary light of different wavelengths are varied such that they all give same rates of photosynthesis.

$R_{(R+S)} - R_S$  = rate of photosynthesis in far red light in presence of supplementary light; it is assumed that the rate of the latter does not change. If  $R_R$  is equated to 100,  $R_{(R+S)} - R_S$  becomes (1).

spectra of the effect of extreme red light on the photosynthesis produced by far red light (to be discussed in Chapter 6) are therefore somewhat distorted, although there are no doubts about the reality of the observed unhibition effect.

#### G. Absorption Measurements

Absorption measurement of the live cells has always been a problem because of ordinary and selective scattering (cf. Latimer, 56; Latimer and Rabinowitch, 58). The use of an integrating device is very helpful. In our laboratory, Cederstrand (14) has constructed an integrating device which has 12 light detectors on 12 sides of a dodecahedron instead of a single one, as in the usual Ulbricht sphere. He could therefore dispense with the usual baffle. The Cederstrand "sphere" (or rather, dodecahedron) is much more independent of scattering than the usual integrating spheres. Some of the absorption spectra in this study were measured by means of Cederstrand's integrating spectrophotometer.

Other absorption spectra were measured by a modified opal glass technique of Shibata (73, 74); the opal glass was replaced either by a wet filter paper, as described by Thomas and Govindjee (77), or by oiled filter paper. In the latter case, Whatman number 1 filter paper strips were oiled in liquid paraffin and placed against the cuvette on the photocell side. This proved an improvement over the usual technique of absorption measurements, for it lowered the 'valleys', and raised the peaks in the absorption curve, indicating a reduction in the contribution of scattering to the apparent absorption. However, the wet filter paper technique proved better (Figure 4), although still not suitable for quantitative measurement. In this method, two filter paper strips fitting into the Beckman cuvette were cut. One strip was placed on a sponge

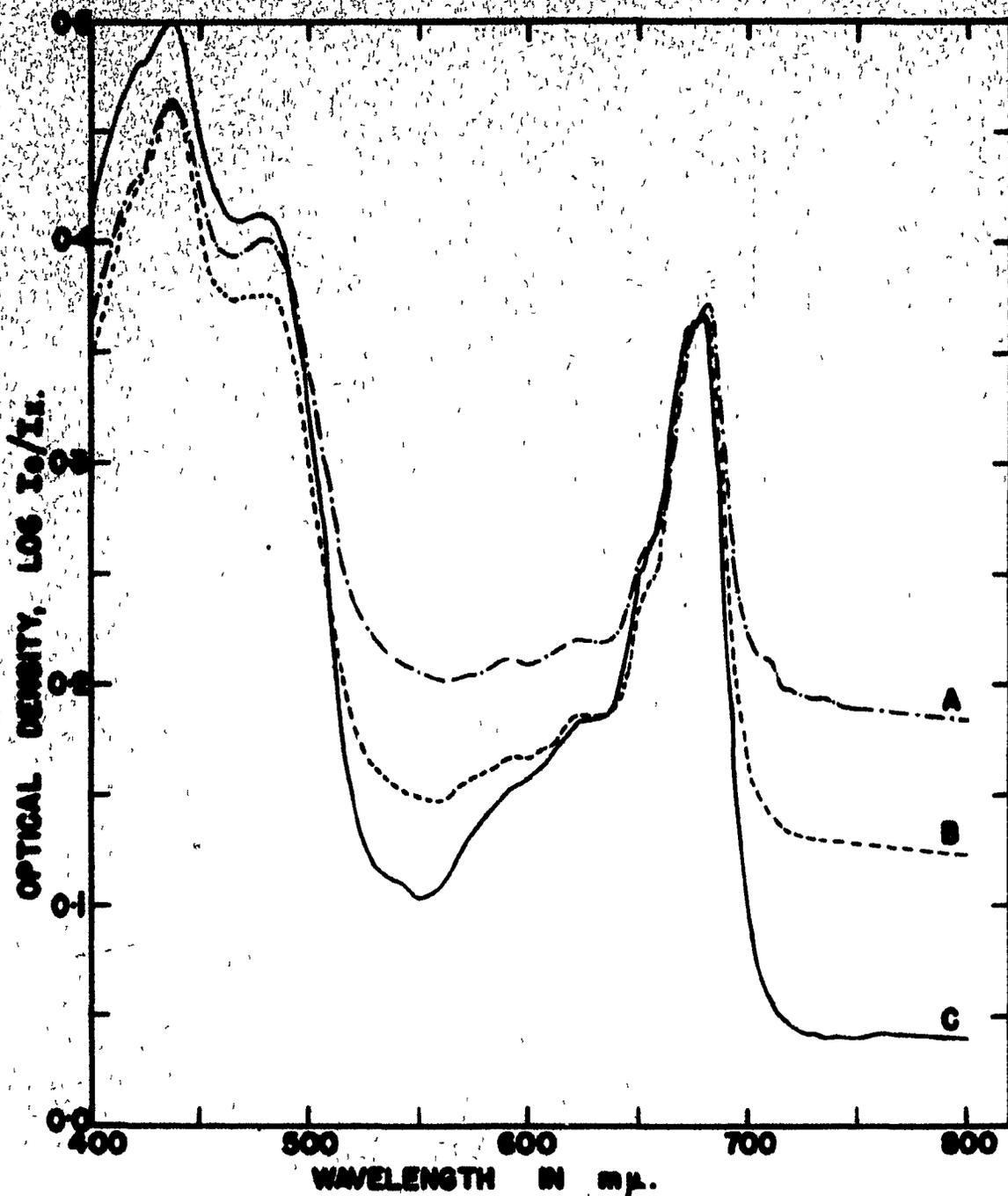


Figure 4. Absorption spectrum of *Chlorella pyrenoidosa* measured with Beckman DU Spectrophotometer. A: No special technique; B: oiled filter paper technique (oiled filter paper between cuvettes and photocell); C: Wet filter paper technique (algal cells soaked on Whatman #1 filter paper); note that the scattering in C is very low. Interval of measurement = 5 mμ and 2.5 mμ near the peaks in the red region. No corrections were made at 800 mμ; measurements were plotted directly on this graph.

and a suspension of algae was slowly poured on it, waiting each time for the liquid to pass through the filter paper. In this way, the algal cells re-accumulated on the filter paper. The strip was never allowed to dry up. The other strip was wetted with the culture medium (or the buffer) without the cells. These two strips were placed in two cuvettes along their corresponding walls. 2-3 drops of the buffer or the culture medium were placed at the bottom of the cuvettes to keep the paper strips wet; and covers were placed on the cuvettes. The absorption spectra were then measured. Readings at 800  $m\mu$  were taken as measure of the accidental difference in scattering power of the two strips of filter paper, and a corresponding correction was applied to the measurements at the shorter waves. This method gives very good absorption curves; there is no question of the settling of the algae, which is disturbing with Shibata's original method. However, this method is obviously not meant for quantitative purposes.

#### H. Measurement of Light Energy

For measurements of the quantum yield, it was necessary to know accurately the light energy absorbed by the cells. A large surface bolometer was used for this purpose because: i) it measures energy in absolute units, ii) it is equally sensitive to all wavelengths, iii) its surface is big enough for the full beam of light to fall within its surface. The bolometer was used in conjunction with a Wheatstone bridge potentiometer. Changes in the resistance of blackened platinum strips which are heated when light energy falls on them, are read by the instrument. A mirror galvanometer is used as a zero instrument. The bolometer was originally calibrated by Emerson and coworker in 1950 (33); the calibration was rechecked by Emerson, Cederstrand, Govindjee and R. Govindjee in 1958 (22), against a standard light source from the U. S.

Bureau of Standards. The sensitivity of the instrument used in the present investigation was 0.1895 volt/watt. It was impossible to place the bolometer at the spot where the algal suspensions were placed for manometric experiments. A correction for the difference in optical paths and for the passage through different media, therefore had to be applied. Published figures for losses of light energy at interfaces were not always used (partly because they do not take into account the absorption of ultraviolet light by glass). Instead, Emerson and coworkers in 1958 (22) measured the actual losses due primarily to reflection. A correction curve was drawn which took into account all differences due to the relative position of the bolometer and the algal suspensions (see Figure 33; cf. Appendix 2). Care was taken to check that light beam was totally intercepted by the bolometer, as well as by the algal suspensions. Light energy was measured according to the following schedule:

- i) Potentiometer was adjusted to zero on the standard cell circuit.
- ii) Potentiometer was adjusted to zero in the dark.
- iii) The mirror, which throws light onto the vessel containing the algal suspension, was moved out of the beam so that it now fell on the bolometer surface. By using a variac (or changing resistance), the galvanometer was adjusted to zero position. The voltage needed to bring the instrument back to zero was recorded and converted into the number of einsteins falling on the vessel per second by means of a calibration graph. When totally absorbing suspensions were used, this gave directly the amount of energy absorbed by the algae.

### Chapter 3: QUANTUM YIELD OF PHOTOSYNTHESIS AND THE RED DROP

Quantum yield of photosynthesis can be defined by the following expression:  $\frac{\text{number of molecules changed}}{\text{number of quanta absorbed}} = \frac{\text{moles changed}}{\text{einsteins absorbed}} = \Phi$

(where a mole =  $N$  molecules and an einstein =  $N$  quanta;  $N$  being the Avogadro number,  $6.02 \times 10^{23}$ ).

Before undertaking the study of the combined effect of two wavelengths of light on the photosynthetic yield, it was desirable to measure with the same cells, the yield at one wavelength. This permitted a comparison of the cultures used in the present study with those used earlier by Emerson and co-workers. Complete action spectra of photosynthesis ( $\Phi$  vs  $\lambda$ ) were not determined, but  $\Phi$  was measured in the blue region as well as in the region of the red drop.

#### A. Blue Region

Emerson and Lewis (32) measured the quantum yield of photosynthesis as a function of wavelength in Chlorella. The measurements were somewhat uncertain in the blue region because 30-40  $\mu$  wide bands had to be used to obtain sufficient energy from the tungsten lamp. There are no light sources available which would produce a smooth continuous spectrum of sufficiently high intensity in this region. A line source, which was readily available, and provided spectral lines in appropriate locations (e.g., the 436  $\mu$  line, very near the absorption peak of chlorophyll a, and the 480  $\mu$  line very near the absorption peak of the carotenoids) was used. This light source, a Hg-Cd arc lamp "HBO<sub>4</sub>", was obtained from Osram Co. in Germany. Working with this lamp did not solve the problem of obtaining complete accurate action spectra in the blue part of the spectrum, but, at least it permitted precise determinations of the quantum

yield at certain selected points. Emerson, Chalmers and Cederstrand (28) first tried these measurements, but at that time the lamp was unsteady. Cederstrand built a D.C. power supply to replace the previously used A.C. power supply, but even then, the Hg-Cd lamp remained unsteady until it had aged for several hours. The quantum yield of Chlorella at several wavelengths was then measured, and the results compared with those obtained earlier by Emerson and Lewis (32). Figure 5 shows the quantum yields plotted against the wavelength of light; these yields were measured in totally absorbing suspensions. A curve has been drawn somewhat arbitrarily through the measured points. Table 4 shows all the experiments done with Chlorella. The data suggests: i) that the quantum yield is lower at 480 m $\mu$  than at all other wavelengths used (except in Exp. #4). 480 m $\mu$  is the approximate position of the maximum absorption by the carotenoids. This result is in agreement with the conclusions of Emerson and Lewis (32). ii) Quantum yields at 436 m $\mu$  are always lower than at 644 m $\mu$  (cf. Emerson and Lewis, 32). iii) At 365 m $\mu$ , the quantum yield of photosynthesis is as high as, or even greater than, at 405 m $\mu$  (Emerson and Lewis made no measurements at 365 m $\mu$ ). iv) Quantum yield values obtained at 405 m $\mu$ , 436 m $\mu$ , 468 m $\mu$ , 480 m $\mu$ , and 509 m $\mu$  are in fair agreement with those of Emerson and Lewis (32). The quantum yield at 644 m $\mu$  was higher in the current experiments than in those of Emerson and Lewis (32).

Under the conditions of the present experiment, precise absolute quantum yields could be determined for Chlorella at seven wavelengths. It is important to point out that it cannot be claimed that these are the absolute yields at all possible conditions. It is known that the yields vary with culturing techniques, pre-illumination and several other factors (cf. Brody and Emerson, 6). However, results obtained here are important because until now, no precise

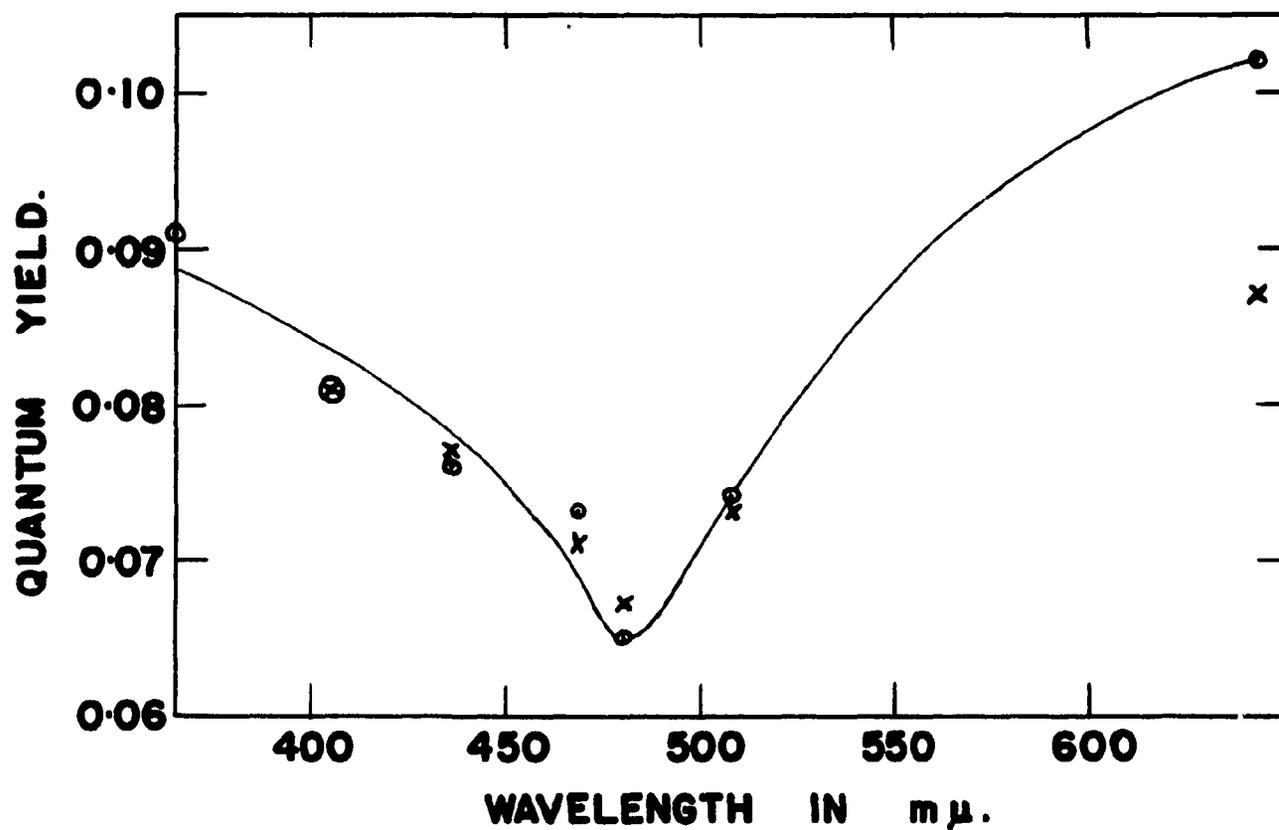


Figure 5. Quantum yield of photosynthesis of Chlorella pyrenoidosa measured with Hg-Cd monochromatic lines. Circles with dots indicate experimental points. Crosses indicate quantum yield values obtained by Emerson and Lewis, (32) using wide slits (30-40 mμ).

Table 4

**QUANTUM YIELDS OF PHOTOSYNTHESIS IN CHLORELLA--  
A REEVALUATION AT CERTAIN SELECTED WAVELENGTHS**

Wave-length in $\mu$	Exp.No. 1	Exp.No. 2	Exp.No. 3	Exp.No. 4	Exp.No. 5	Exp.No. 6	Average St. error* $\pm$	Emerson & Lewis (32)
365	0.074	0.075	0.080	-	0.120	0.107	0.091 $\pm 0.009$	-
405	0.073	0.065	0.075	- <sup>6</sup>	0.110	-	0.080 $\pm 0.010$	0.081
436	0.082	0.075	0.066	0.066	0.092	0.072	0.075 $\pm 0.004$	0.077
468	-	0.075	0.066	0.064	0.086	-	0.073 $\pm 0.004$	0.071
480	0.067	0.061	0.061	0.064	-	0.073	0.065 $\pm 0.003$	0.067
508	0.069	0.071	0.077	0.061	0.090	0.079	0.075 $\pm 0.004$	0.073
644	0.106	0.112	0.103	0.088	0.103	-	0.104 $\pm 0.004$	0.087

\* Standard Error =  $\sqrt{\frac{\sum \Delta^2}{n(n-1)}}$ , where  $\Delta$  = deviation and  
n = number of cases

We notice certain variations between the different experiments, even though they were done with cultures grown under the same general conditions. We believe that these differences are due to unintentional, minor variations in the culturing procedures.

values of  $\Phi$  were available for any point in the blue part of the spectrum. One may note that the quantum yield values given by Emerson and Lewis (32) on the basis of experiments with broad slits (30-40  $\mu$ ) were approximately correct, since they could be now confirmed by experiments with narrow spectral lines.

All these results show that the cultures in Chlorella used in this study were quite similar, as far as their photosynthetic capacity is concerned, to the ones used by Emerson and Lewis (32).

#### B. Red Drop

The quantum yield of photosynthesis in various algae, including Chlorella, Navicula and Porphyridium, is known to decline at the long waves well within the main red absorption band of chlorophyll a (cf. Emerson and Lewis, 32; Tanada, 75; Haxo and Blinks, 46). Emerson and coworkers (25, 26, 27, 34) pointed out that this decline occurs in the region where chlorophyll a becomes the predominant, and ultimately the sole, absorber of light energy. Emerson et al. (25, 27, 34) also showed that the red drop can be postponed to longer waves by simultaneous exposure to supplementary shorter-wave light. These findings called for a re-examination of the quantum yields in the red and the far red end of the spectrum. Emerson et al. (27) re-examined the quantum yields in the red drop region in Chlorella; Brody and Emerson (6) determined those of Porphyridium. The quantum yields of Anacystis in the red region were never measured, and those of Navicula needed re-examination.

Emerson, Chalmers and Cederstrand (27) found that the position of the threshold of the red drop depends on temperature; the drop occurs "earlier" at the higher temperatures. In order to determine the position of the red drop under the same experimental conditions under which the Emerson effect was

measured (see Chapter 4), the quantum yields were now measured at 10°C at various wavelengths, using cells grown under similar conditions. Figures 6, 7, 8, and 9 show the quantum yields as function of wavelength in the region of red drop, for the four organisms used, at 10°C.

Results of this study are tabulated in Table 5.

Quantum yield measurements were made considerably below the saturating light intensities, to ensure that the light action was in the linear part of the "light curve" of photosynthesis. However, recent findings by McLeod and French (60) and that reported in Chapter 5, showed that saturating intensities may be quite different at the different wavelengths; in particular, saturation occurs in much lower light and at a much lower level in the far red light. This early and low saturation may produce an apparent red drop. The two phenomena -- the red drop in the quantum yield and early saturation, may be due to the same cause. For example, if only the "long-wave" type of chlorophyll a is excited in the far red, this may produce both early saturation and a low quantum yield. Further work is needed to clarify these relationships.

### C. Rate of Respiration

As pointed out earlier (under "Materials and Methods"), respiration has to be measured in the dark, to correct for respiration during photosynthesis. Respiration rates of algae at the beginning of the experiment can be controlled, to a considerable extent, by growing the cultures under different conditions. High light intensity ( $> 10^4$  ergs/cm<sup>2</sup>/sec) maintained for 12 or 24 hours, prior to the measurements, can increase the amount of the photosynthates in the cells so much that the algae will respire at a high rate (0.5-1.5  $\mu\text{l O}_2$ /hr./ $\mu\text{l}$  cells). If the algae are starved before the measurements, by leaving them at low light intensity ( $< 10^2$  ergs/cm<sup>2</sup>/sec) overnight, the respiration rate goes down (0.1-0.5  $\mu\text{l O}_2$ /hr./ $\mu\text{l}$  cells).

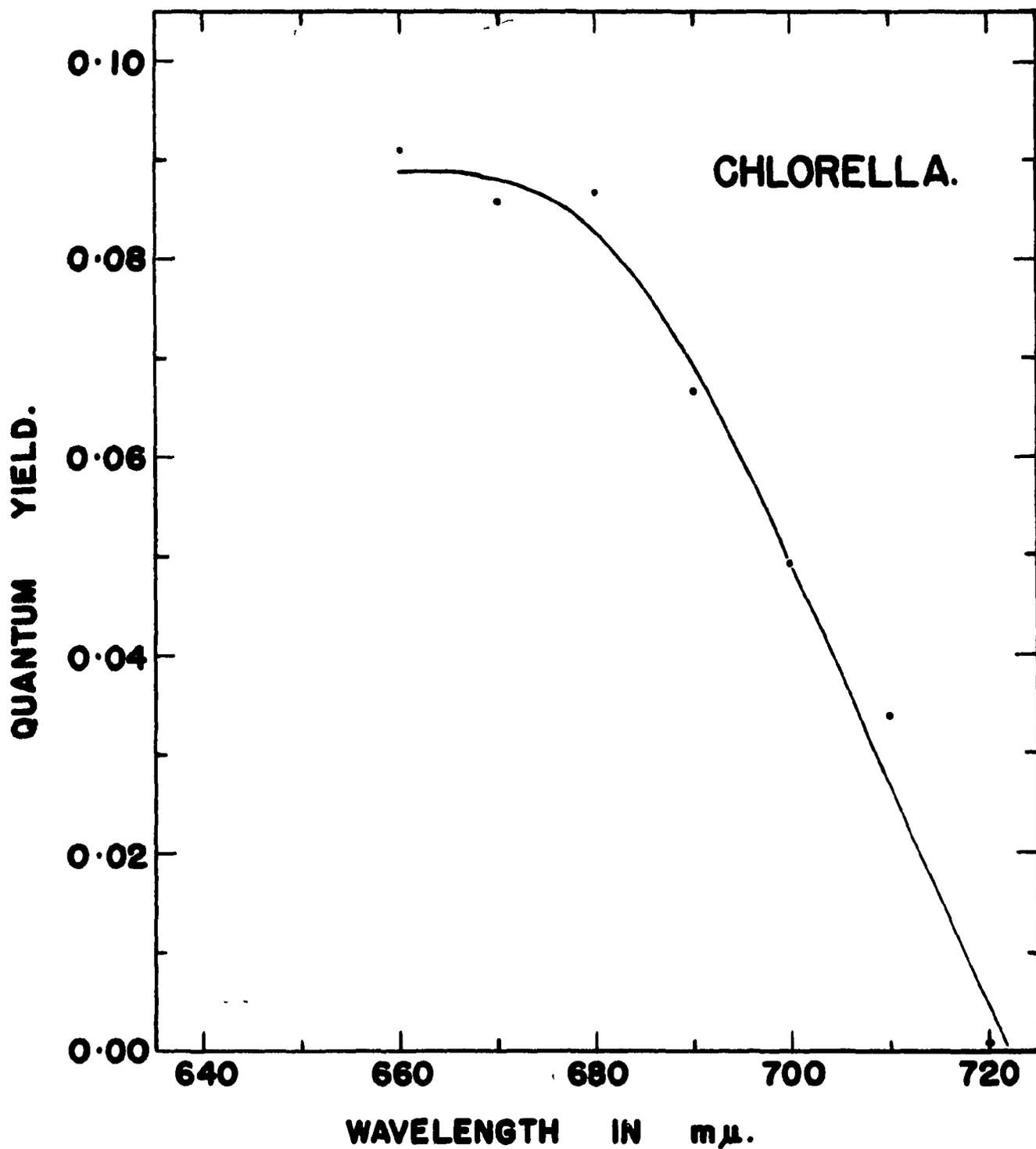


Figure 6. Quantum yield of *Chlorella pyrenoidosa* at 10°C, from 660 mμ to 720 mμ showing 'Red drop' (after Emerson, R. and Govindjee, R., 29).

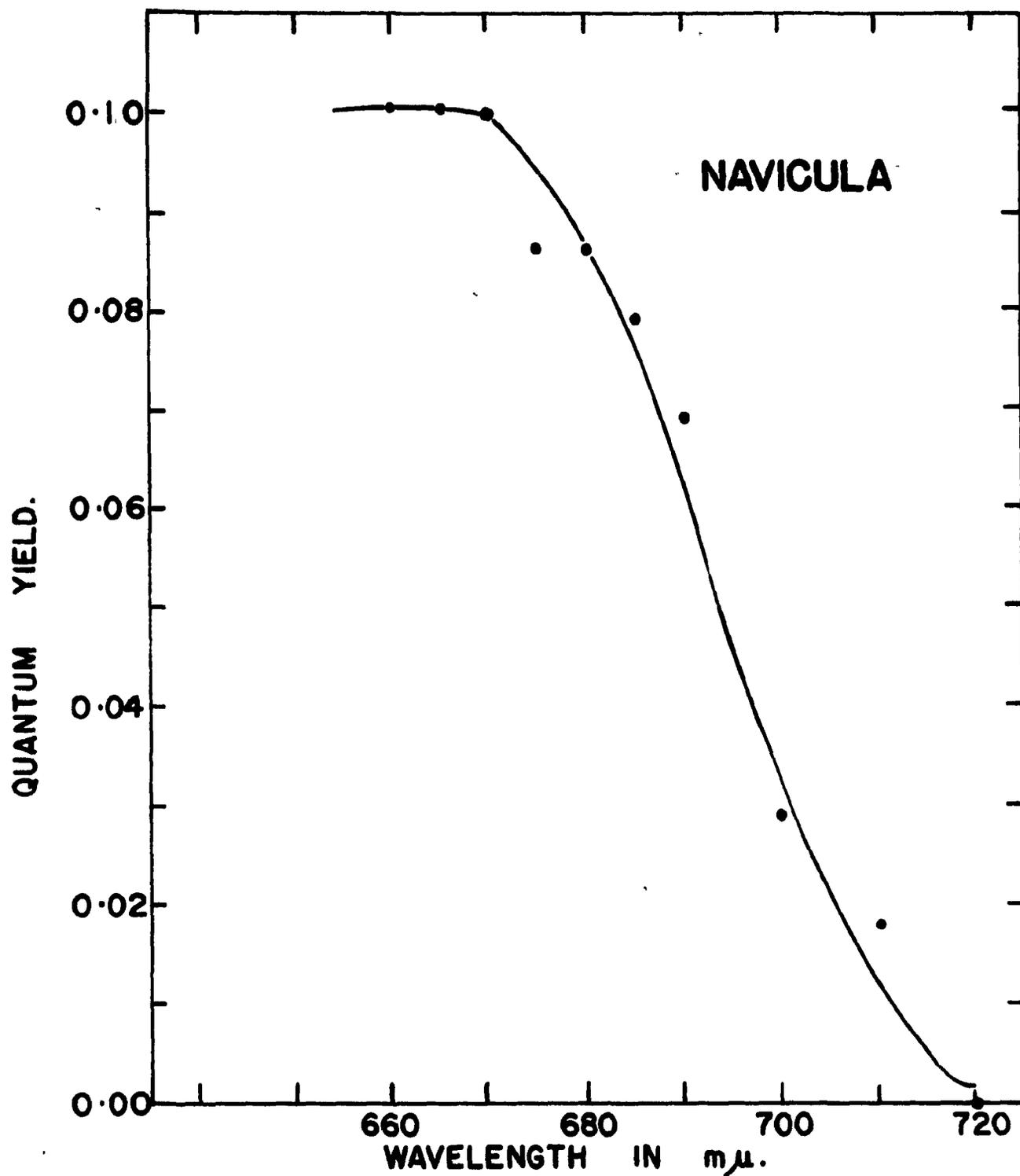


Figure 7. Quantum yield of Navicula minima at 10°C, from 660 mμ to 720 mμ showing 'Red drop'.

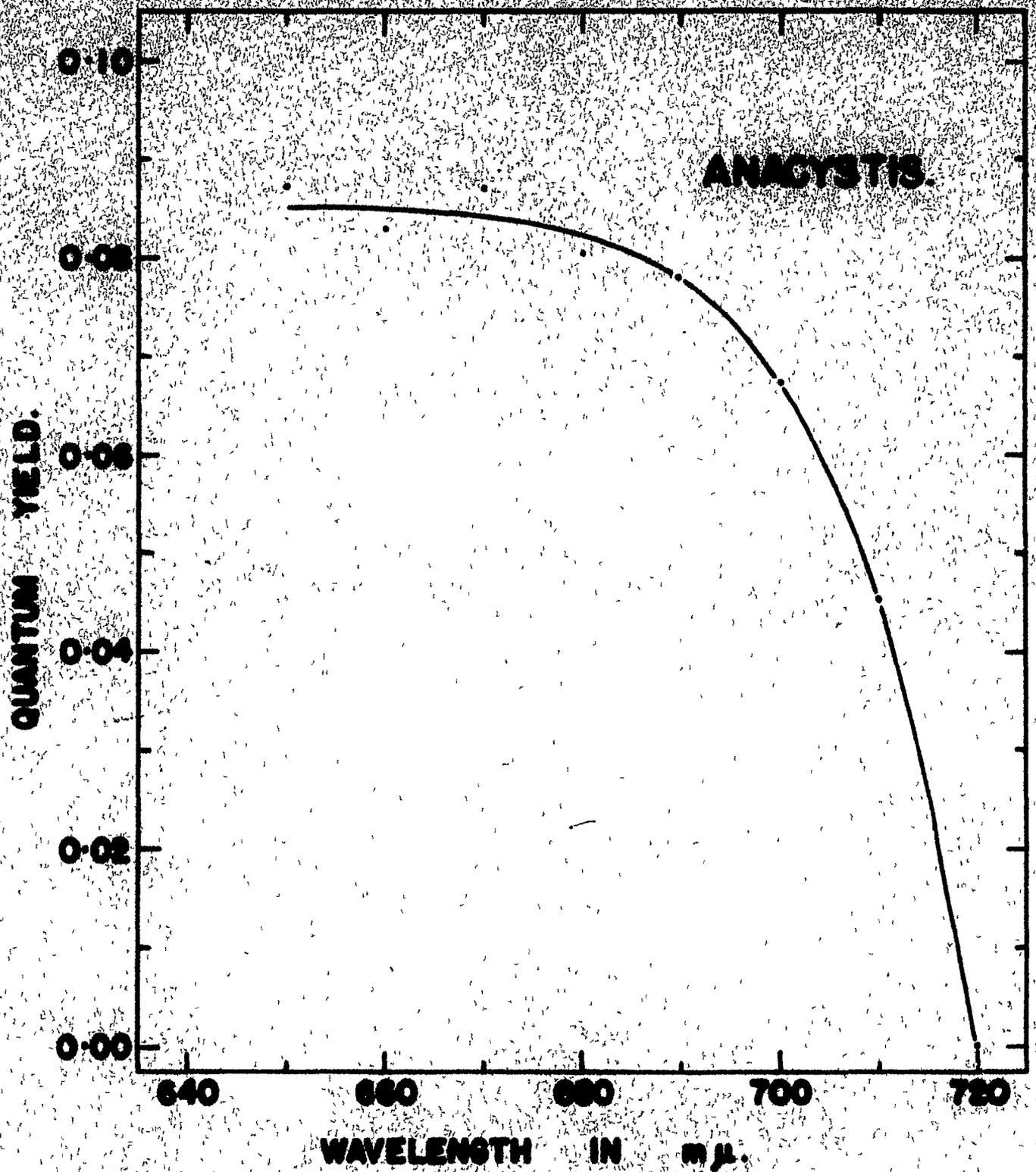


Figure 8. Quantum yield of Anacystis nidulans at 10°C, from 650 mμ to 720 mμ showing 'Red drop'.

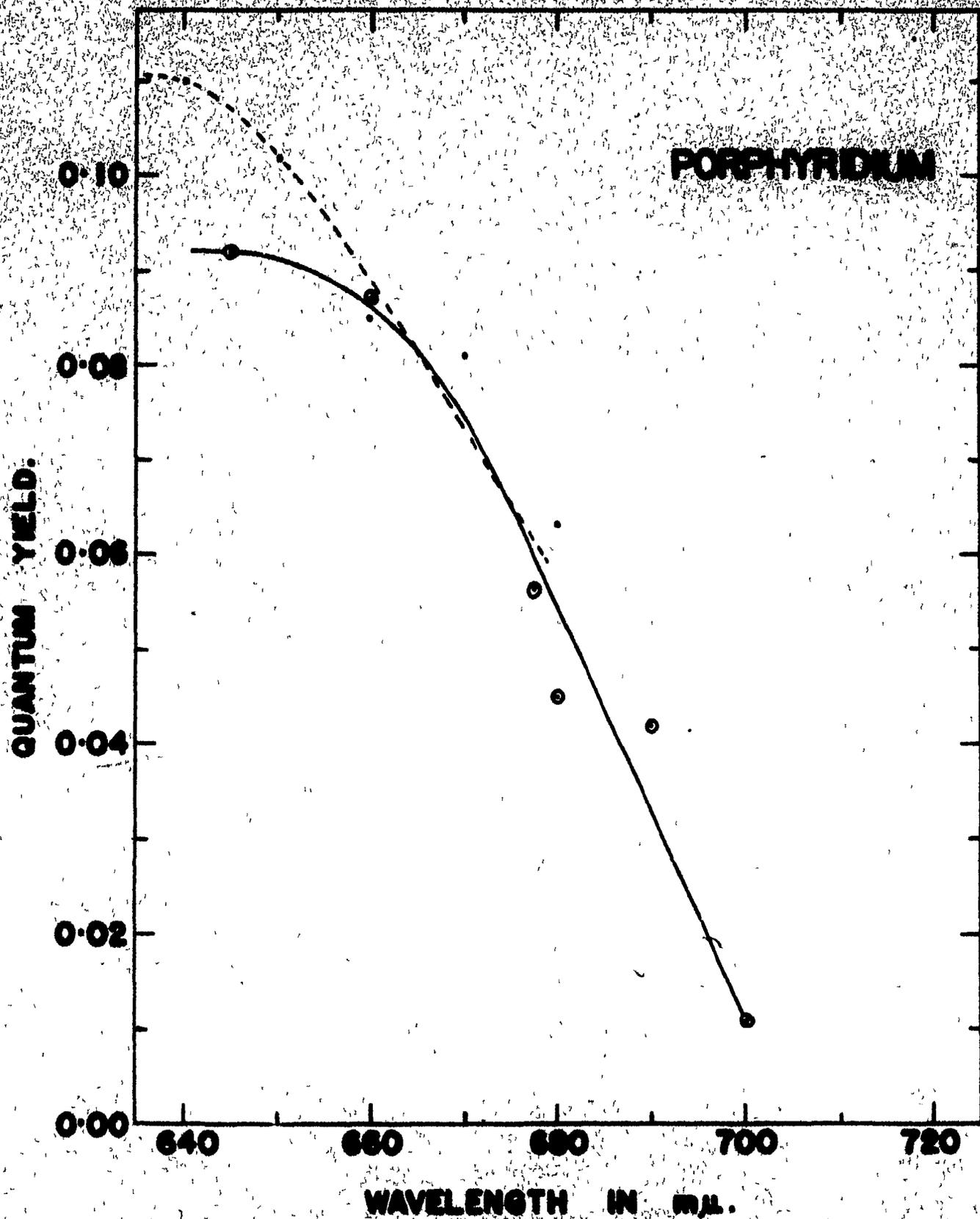


Figure 9. Quantum yield of Porphyridium cruentum at 10°C, from 640 mμ to 680 mμ -- dots and dashed line. Circles with dots and solid line -- quantum yield from 645 mμ to 700 mμ at 5°C, after (4).

Table 5

## RED DROP IN QUANTUM YIELD OF PHOTOSYNTHESIS

Organism	First Investigator(s)	Re-examined by	Temperature	Maximum quantum yield	$\lambda$ of the beginning of the red drop, $\mu$	$\lambda_{\frac{1}{2}}$ $\mu$
<u>Chlorella pyrenoidosa</u>	Emerson and Lewis (32)	Emerson et al. (27)	20°C	.08	680	696
			5°C	.12	685	702
		Emerson and R. Govindjee (29)	10°C	.09	680	700
<u>Navicula minima</u>	Tanada (75)	Govindjee	10°C	.09	675-680	693-696
<u>Porphyridium cruentum</u>	Brody and Emerson (6)	Govindjee	10°C	.09	650	685
<u>Anacystis nidulans</u>	Govindjee	Govindjee	10°C	.09	680-685	710

\*  $\lambda_{\frac{1}{2}}$  is the wavelength at which  $\Phi$  drops to  $\frac{1}{2}$  of  $\Phi$  max.

When quantum yield measurements were made, totally absorbing suspensions were used, and thus the number of cells in the vessel was very large. This means a relatively large volume of respiration. In this situation, it has to be seen that the respiration rate of each cell is low enough so that we do not need to open the manometer during the experiment. Also, it is inconvenient to readjust the cathetometers precisely every minute as would be needed in the case of rapid respiration. Procedures mentioned under Materials and Methods were followed to reduce respiration. These conditions not only give lower respiration rates, but also higher quantum yields of photosynthesis. Respiration rates of different organisms used in this investigation are tabulated in Table 6.

Table 6

RESPIRATION RATES IN DIFFERENT ALGAE  
(10°C)

Organism	$\mu\text{l O}_2/\text{hour}/\mu\text{l cells}$ "High Light" Cells*	$\mu\text{l O}_2/\text{hour}/\mu\text{l cells}$ "Low Light" Cells**
<u>Anacystis</u>	0.6 to 1.5	0.2 to 0.5
<u>Chlorella</u>	0.5 to 1.5	0.1 to 0.5
<u>Navicula</u>	0.6 to 1.5	0.1 to 0.5
<u>Porphyridium</u>	0.3 to 0.6	0.1 to 0.2

\* Cells which received  $> 10^4$  ergs/cm<sup>2</sup>/sec.

\*\* Cells which received  $< 10^2$  ergs/cm<sup>2</sup>/sec.

## Chapter 4: THE SECOND EMERSON EFFECT

The discovery by Emerson and coworkers (25, 26, 27, 34) that the low quantum yield of photosynthesis in far red light can be significantly raised by simultaneous excitation of auxiliary pigments, was interesting enough to be re-examined. The interpretation suggested by Emerson was in contradiction with the generally accepted view that the primary sensitizer of photosynthesis always is chlorophyll a, while other pigments are serving an auxiliary purpose of absorbing and transferring additional energy to chlorophyll a. A re-determination of the action spectra of the Emerson effect was especially necessary, because in some cases, the measured points were far apart, so that important peaks in the action spectrum may have been missed.

In some of the preliminary experiments to be described in this chapter, light actions of the two beams (far red and supplementary) were adjusted so that both gave about the same rate of photosynthesis, just below the compensation (of respiration). This procedure adopted by Emerson et al. (27) was followed, because, in this way, the combined light action of the two beams remained within the region of linear dependency of the rate of photosynthesis on light intensity. In many cases, however, it was possible to use 1:2 ratio or even more between the action of far red light and that of supplementary light, without entering into the non-linear part of the light curve of photosynthesis. Quite early in the study, it was realized that this ratio is not the only important factor: the action of far red light, by itself, is important, and the ratio loses its importance when the action of the far red becomes very low. Later, it was also realized that the saturation curves may not be the same for different wavelengths. In order to always obtain a positive Emerson effect, as well as to better study the location of the peaks, we had to adjust the "basic far red action to a certain low value which was kept constant throughout one set of experiments and the intensities of supplementary light of

different wavelengths were varied such that they all gave the same rates of photosynthesis. However, the location of the peaks in the action spectra of the Emerson effect could be confirmed, even when a great portion of the curve was below the 100 line.

The temperature of the bath was maintained at 10°C in all the experiments. Optically thin suspensions were used. Therefore, different heights of the peaks do not mean that the enhancement of the quantum yield due to the different accessory pigments is different, it may simply mean differences in absorption. These experiments were not designed to provide this information.

#### A. Chlorella pyrenoidosa

Figures 10 and 11 show the action spectrum of the Emerson effect in Chlorella. Supplementary light was varied in these experiments between 400 m $\mu$  and 700 m $\mu$  in steps of 10 m $\mu$ ; the band width in the 640-700 m $\mu$  region was 10-12 m $\mu$ ; in some experiments it was as small as 5 m $\mu$ . Going towards the blue region, the band width had to be increased until it reached 30 m $\mu$  at 400 m $\mu$ . Several peaks were found in the range studied, at 420 m $\mu$ , 480 m $\mu$ , 560 m $\mu$ , 600 m $\mu$ , 650 m $\mu$ , and 670 m $\mu$ . These results agree with Emerson's findings in respect to the peaks at 480 m $\mu$ , 560 m $\mu$ , 600 m $\mu$ , and 650 m $\mu$ . Thus, they support Emerson's suggestion that the enhancing effect is due to the specific participation of chlorophyll b. However, additional peaks occur at 670 m $\mu$  and at about 420 m $\mu$ ; shoulders at 440 m $\mu$  and 465 m $\mu$  also are seen. The peak at 670 m $\mu$  is very sharp; it was found in all seven experiments performed with Chlorella. The shape and position of the peak at 420 m $\mu$  (first observed by Rajni Govindjee in the work on the Emerson effect in Hill reaction), and the shoulder at 440 m $\mu$ , are not equally clear. However, a wide band had to be used in this region and only three experiments were performed. In Chlorella, no pigment except chlorophyll a is known to have an absorption peak beyond 650 m $\mu$ . The absorption spectrum

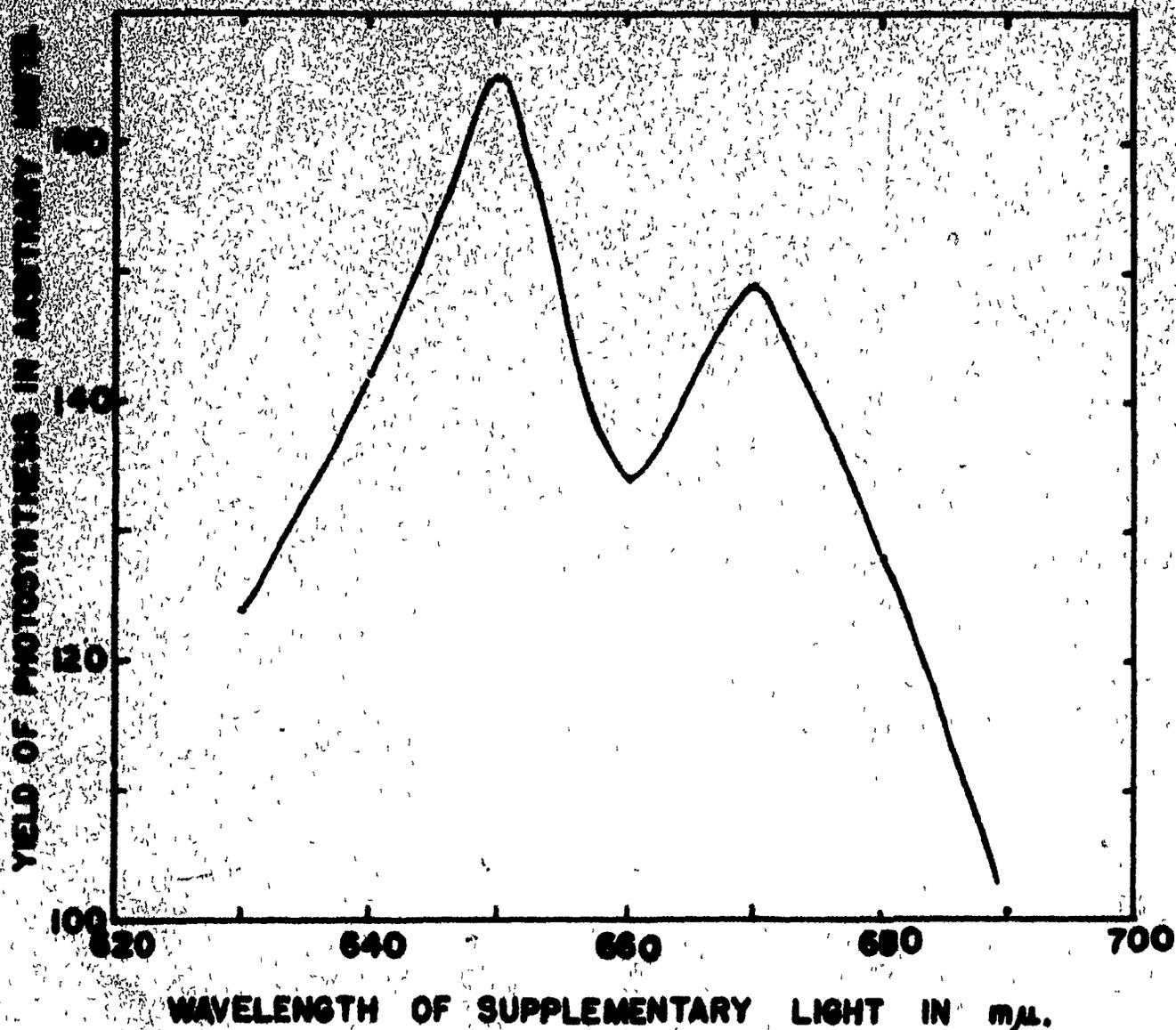


Figure 10. Action spectrum of the Emerson effect in Chlorella pyrenoidosa (630 mμ to 690 mμ). Rate of photosynthesis of far red light alone = 3.0 μl/hour; ratio of far red : supplementary light action = 1:1; photosynthesis was always below compensation of respiration. Far red light was > 680 mμ. Similar results were obtained by using far red light that was ~ 700 mμ.

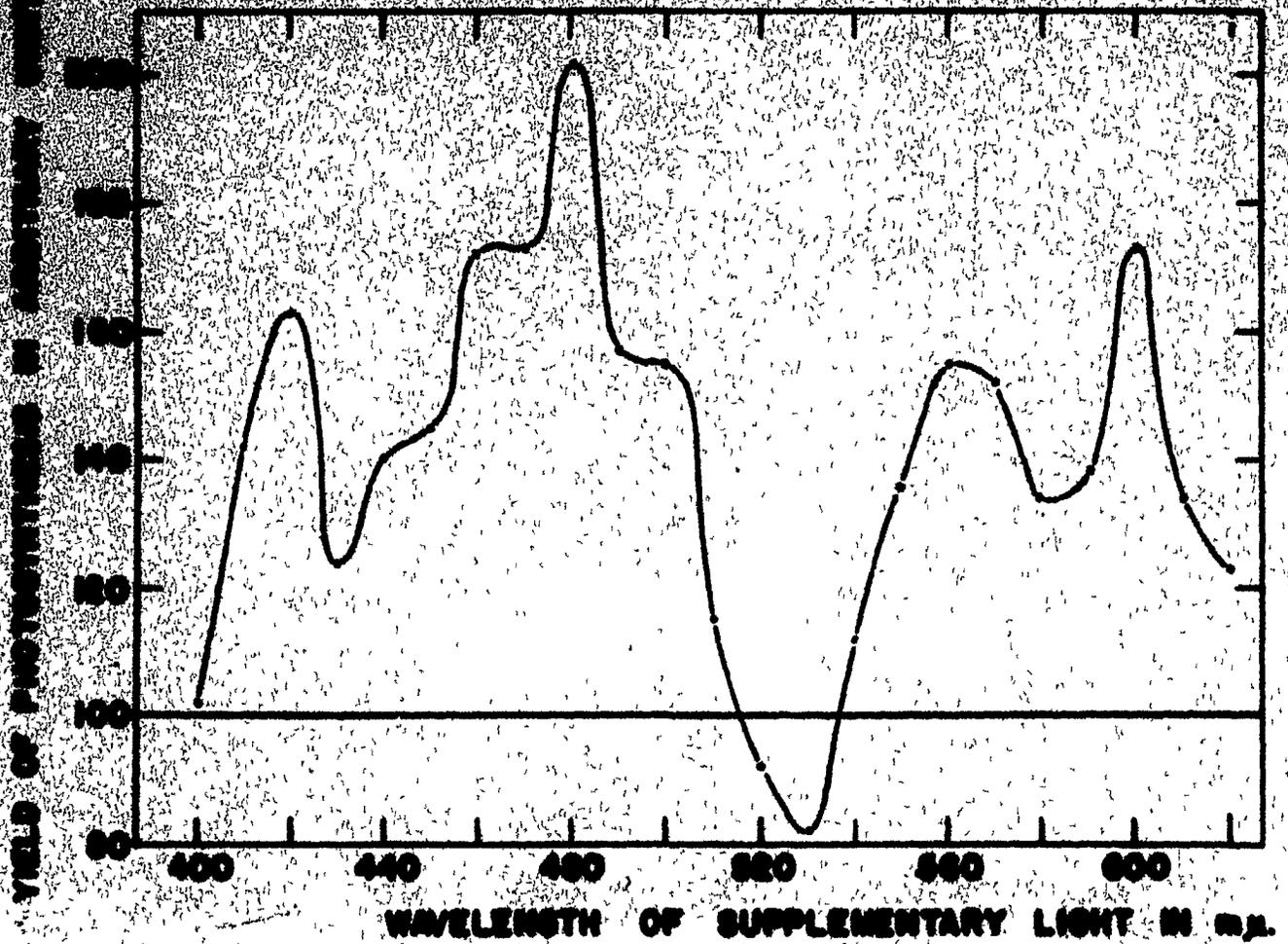


Figure 11. Action spectrum of the Emerson effect in Chlorella pyrenoidosa (400 mμ to 620 mμ). Rate of photosynthesis of far red light alone = 3.5 μl/hour; ratio of far red : supplementary light action = 1:1.5; rate of photosynthesis was always below compensation of respiration. Far red light was ~700 mμ.

of Chlorella is well known; I have re-determined it, and calculated the "first derivative" spectrum (cf. French, 38). Figure 13A shows the absorption spectrum of Chlorella obtained with the wet filter paper technique. Two peaks are seen in the red chlorophyll a absorption band--one at about 670  $\mu$  and the other at about 680  $\mu$  (see Figures 12 & 13A). Several determinations of this absorption spectrum were made, and all showed this double band. However, the sharpness of the two peaks and their exact locations differed from culture to culture. The whole absorption curve can be interpreted as the result of the super-position of three separate absorption bands, about 10  $\mu$  apart. Evidence for the existence of three peaks--at about 670  $\mu$ , 680  $\mu$  and 690  $\mu$ --was obtained earlier by French and coworkers (1,38). My results provide evidence of a specific photochemical function of one form of chlorophyll a, which can be designated as Chl a 670. The action spectra of the Emerson effect suggest that this function probably is not shared by Chl a 680+690.

It was noted that the relative heights of the peak at 670  $\mu$  and 650  $\mu$  in the action spectrum of the Emerson effect varied from culture to culture. Possibly, this may be due to variations in the relative amounts of Chl a 670 and of chlorophyll b. Table 7 gives the ratios obtained in different experiments.

The following consideration suggests that the peak at 420  $\mu$  and the shoulder at 440  $\mu$  also are due to Chl a 670. These features could be conceivably due either to an active form of chlorophyll a or to chlorophyll b or to a carotenoid pigment.

In order to estimate the fraction of light absorbed by each of these pigments, the absorption spectra of the extracts were shifted in such a way that their maxima and minima coincided with those in the absorption spectrum

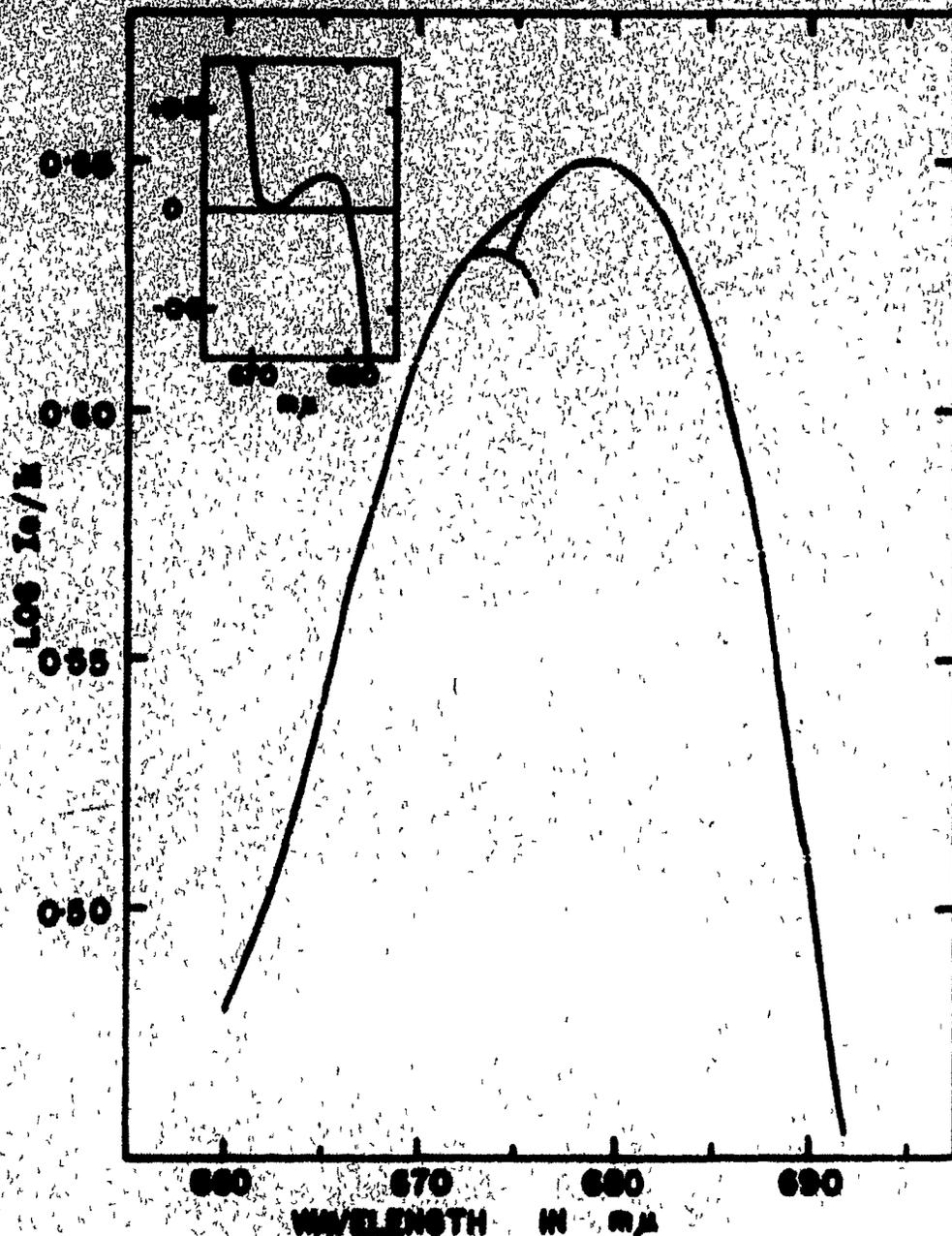


Figure 12. Absorption spectrum of Chlorella pyrenoidosa in the red absorption band of chlorophyll a, measured with Beckman DU Spectrophotometer using the oiled filter paper technique. Dotted lines are drawn to emphasize the existence of 2 peaks of chlorophyll a. Insert shows a first derivative absorption spectrum of Chlorella (a different sample).

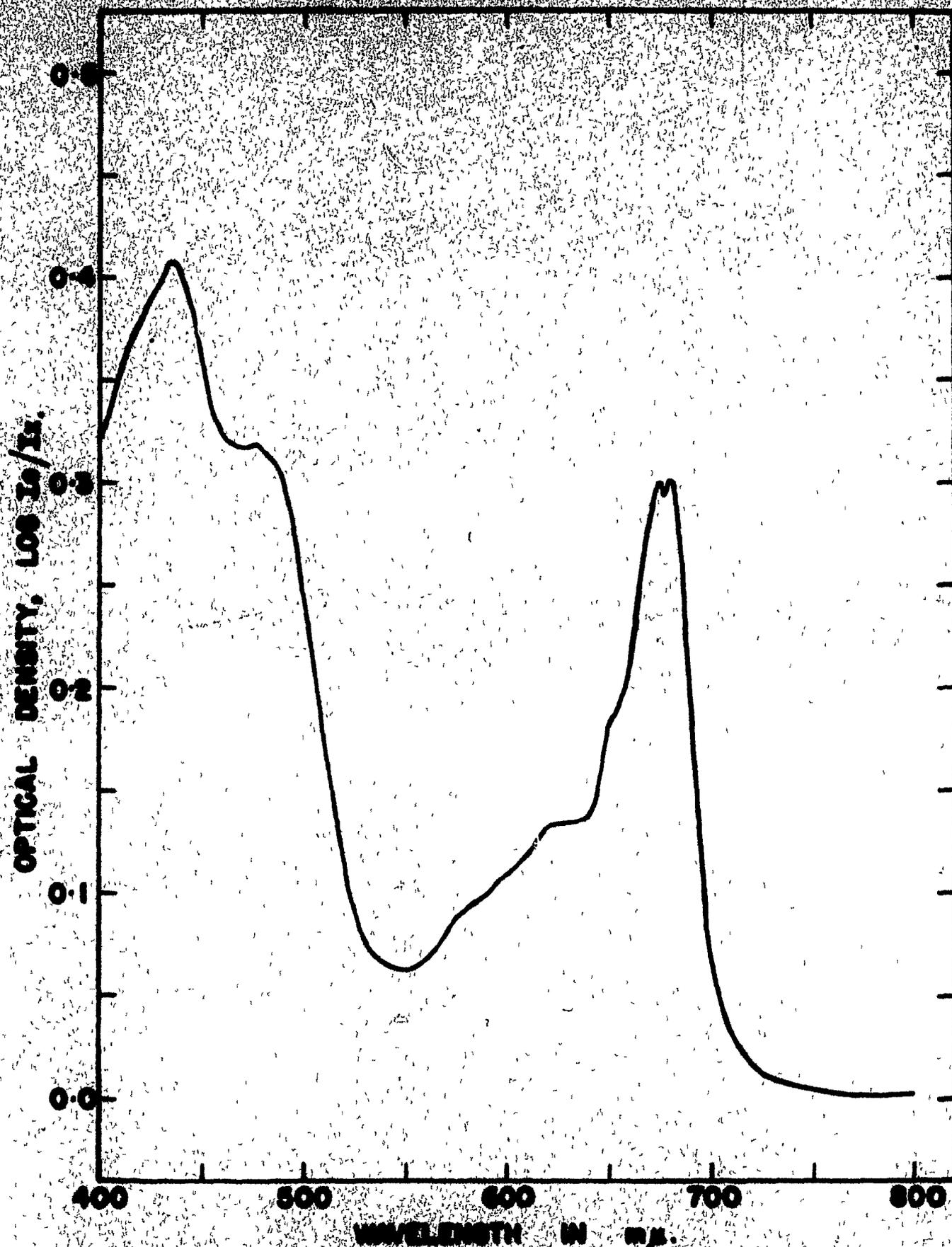


Figure 13A. Absorption spectrum of *Chlorococcoides pyrenoidosa* measured with Beckman DU Spectrophotometer using the wet filter paper technique. Note two peaks at 670 mμ and 685 mμ in the red absorption band of chlorophyll *a*.

Table 7

**RATIO OF THE PEAK HEIGHTS AT 650 AND 670  $\mu$  IN THE ACTION  
SPECTRUM OF THE EMERSON EFFECT IN CHLORELLA**

Experiment No.	Ordinate <sup>A</sup> of the action spectrum at 650 $\mu$	Same at <sup>B</sup> 670 $\mu$	Ratio $\frac{A}{B}$
1	165	137	1.20
2	130	120	1.08
3	126	120	1.05
4	152	176	0.86
5	150	148	1.01
6	133	113	1.18
7	135	120	1.13

of live cells. The chlorophyll a peak in the blue region had to be shifted to 440  $\mu$ , the chlorophyll b peak to 480  $\mu$ , and the first peak of the carotenoids (counting from the long wave side), to 484  $\mu$ . The fraction of light absorbed at each wavelength by each of these pigments was then estimated. These calculations (Figure 13B) suggested that the peak at 420  $\mu$  cannot be due to chlorophyll b, since this pigment probably has, in vivo, an absorption minimum at this wavelength. It could be due to either chlorophyll a or the carotenoids. The latter is less probable, because the absorption peak of the carotenoids lies not at 420  $\mu$ , but at 425-430  $\mu$ . If it is accepted that the 420  $\mu$  peak is due to chlorophyll a, a peak also should appear at 440  $\mu$ ; only a shoulder in this position was seen perhaps because the curves in this region are only rough approximations. Moreover, the "active" chlorophyll a form may well have a higher absorption peak at 420  $\mu$  than at 440  $\mu$ .

If it is assumed that the 420  $\mu$  band and the 440  $\mu$  shoulder are both due to chlorophyll a, the shoulder at 465  $\mu$  can be ascribed to chlorophyll b. A shoulder at about 460  $\mu$  can be, in fact, noted in the curve showing fractional absorption by chlorophyll b in Chlorella. An alternative way to explain the 420  $\mu$ , 440  $\mu$  and 465  $\mu$  peaks (or shoulders), would be to postulate at least a partial identification of the 420  $\mu$  peak with the 425-430  $\mu$  peak in the absorption curve of one (or several) carotenoids; the 440  $\mu$  peak could be due to an active form of chlorophyll a, and the 465  $\mu$  shoulder, to the carotenoids (and/or to chlorophyll b). The peak at 480  $\mu$  must be primarily due to chlorophyll b; but the shoulder at about 500  $\mu$  may be due in part to the carotenoids. Light absorbed by the carotenoids in Chlorella is active in photosynthesis with a low efficiency, as suggested by quantum yield measurements and sensitized fluorescence studies (cf. Emerson and Lewis, 32, Duysens, 18).

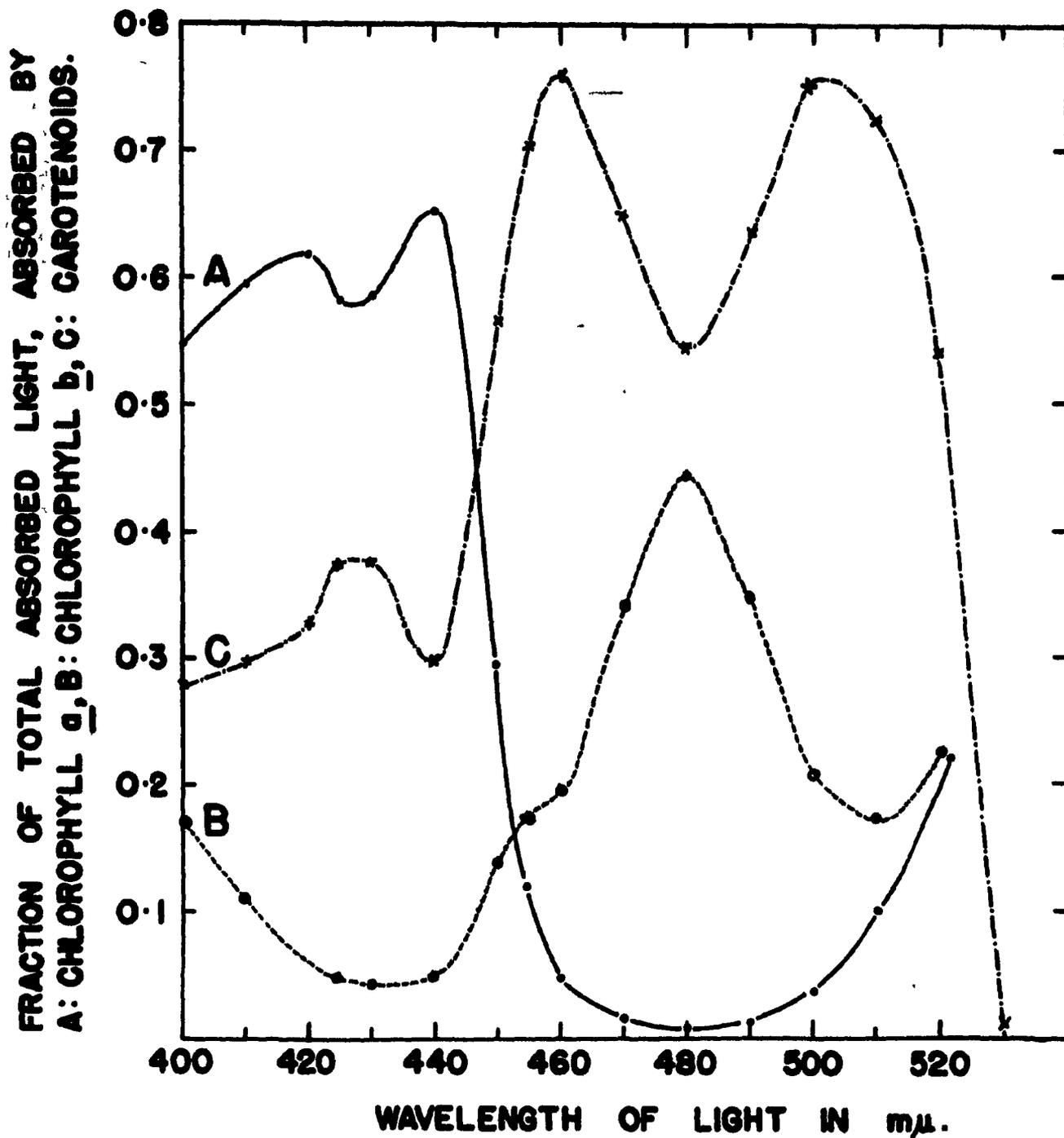


Figure 13B. Calculated fraction of total absorbed light, absorbed by A: chlorophyll a, B: chlorophyll b and C: carotenoids in a sample of Chlorella pyrenoidosa.

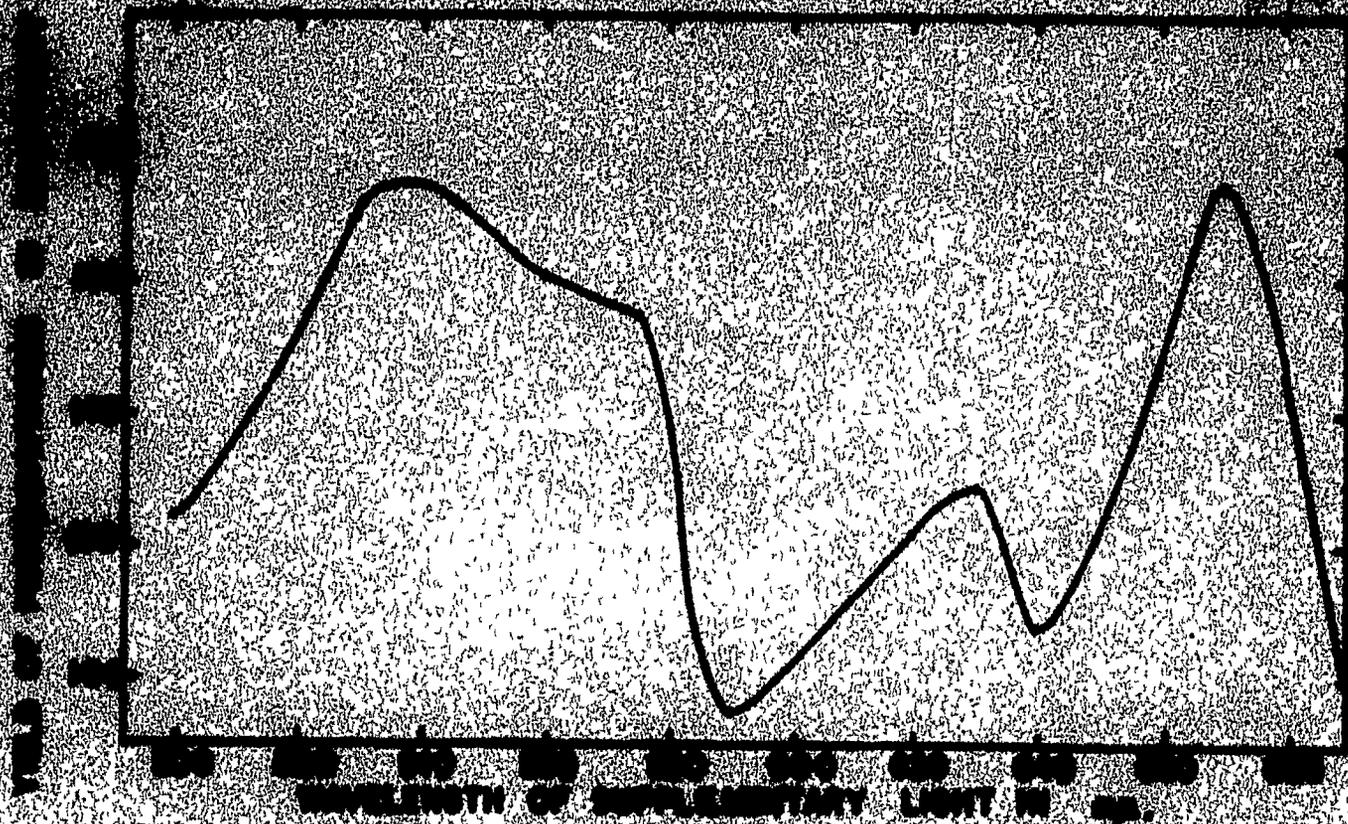
The results in the blue region are on the whole less certain than those in the red region, because of wide slits that had to be used. However, the existence of a peak at about 420  $m\mu$  and of shoulders at about 440  $m\mu$  and 465  $m\mu$ , can be considered as proven. As stated above, the peaks at 420  $m\mu$  and 440  $m\mu$  cannot be due to chlorophyll b, but must be due entirely to chlorophyll a (which is the more probable interpretation, because of the position of peaks in the fractional absorption curves), or to both chlorophyll a and carotenoids. To sum up, whatever the ultimate explanation will be, it seems difficult to escape the conclusion that a form of chlorophyll a does contribute to the Emerson effect in the blue region. It seems likely that this is the same form, Chl a 670, which we know to be active in the red. It seems plausible that in the blue region, as well as in the red one, certain peaks in the action spectrum of the Emerson effect can be ascribed to Chl a 670. It is also possible that excitation in the Soret bands of Chl a 680 + 690 can supplement that in their red bands, because some of it could be transferred by resonance, to Chl a 670. For the same reason, the carotenoids too, may possess some activity in the Emerson effect.

It can be postulated, on the basis of the above-described experiments, that to obtain a full yield of photosynthesis from the excitation of Chl a 680+690, the Chl a 670 must be co-excited in an appropriate proportion; failure to do so causes the "red drop" discussed in Chapter 3. It can be further postulated that when Chl a 670 alone is excited, it transfers enough energy by resonance to Chl a 680+690 to achieve a balanced excitation of both forms; while the reverse transfer is negligible for energetic reasons. Therefore, as soon as quanta become absorbed preferentially by Chl a 680+690, the quantum yield begins to decline (as suggested by Franck, 37; cf. also Emerson and Rabinowitch, 34).

After observing in Chlorella, the peak at 670 m $\mu$ , I re-examined the original curves of Emerson and coworkers, and noticed that if their curves were drawn through all the measured points, a peak would have become apparent at 670 m $\mu$ .

#### B. Navicula minima

In order to see whether the 670 m $\mu$  peak, presumably due to an active chlorophyll a form (Chl a 670) is present in other organisms, Navicula minima was first examined. Seven experiments were performed. Figures 14 and 15 show the action spectrum of the Emerson effect in this species. We clearly recognize a peak at 670 m $\mu$  in addition to the peaks at 630 m $\mu$  and 540 m $\mu$ , due to chlorophyll c and fucoxanthol, respectively. If we compare the action spectra obtained in the present investigation with those given by Emerson, we find that they agree in respect to the peaks at about 540 m $\mu$  and 630 m $\mu$ ; but Emerson's peak at 645 m $\mu$  is shifted, in the present curves to 670 m $\mu$ . This difference must be due to the fact that Emerson did no experiments at 660 m $\mu$  and 670 m $\mu$ . Emerson has attributed the 645 m $\mu$  peak to chlorophyll c and did not suggest any interpretation for the peak around 630 m $\mu$ . We attributed it above to chlorophyll c; that the 540 m $\mu$  peak is due to fucoxanthol, was already suggested by Emerson and coworkers (25, 34). However, the position and shape of the fucoxanthol band in the action spectrum has not yet been analyzed in terms of the contribution of this pigment to the total light absorption at different wavelengths. Emerson's calculations suggested a single peak in the "fractional absorption curve" at 535 m $\mu$ ; but they cannot be reliable because of the particularly strong change which the absorption spectrum of fucoxanthol in vivo undergoes when the pigment is extracted: its peak is shifted from around 550 m $\mu$  (in vivo) to almost 480 m $\mu$  in vitro. It has not



**Figure 14. Action spectrum of the Emerson effect in *Synechococcus elongatus*.** Measurements were made only in the region 400-700 mμ (the action spectrum of Emerson (cf. 34), is shown from 400 mμ to 600 mμ; the approximate shape of this portion has been estimated -- see Figure 13). Far red light action alone: 0.0 μl/hour; ratio of far red light : supplementary light action = 1:2; far red light was > 650 mμ; photosynthesis was always below compensation of respiration.

been possible to improve upon Emerson's calculation. The wide spectral shift may be due to complexing of the pigment in vivo with a protein. Extraction of a fucoxanthol-protein complex from Eiseneria arborea was tried, using the technique of Nishimura and Takamatsu (63) for the isolation of carotenoid-protein complexes, but it was not successful. Only when the shape of absorption curve of fucoxanthol in the living cell, will be known, will it be possible to calculate more precisely the fraction of light absorbed by fucoxanthol in vivo.

Figure 15 shows that besides 540 m $\mu$  peak (attributed to fucoxanthol), there are other peaks in the action spectrum of the Emerson effect in Navicula which may also be due to fucoxanthol. Peaks at 490 m $\mu$ , 515 m $\mu$ , 540 m $\mu$ , and 570 m $\mu$  may all be due to fucoxanthol. However, the possibility exists that some of the peaks (e g. at 490 m $\mu$  and 515 m $\mu$ ) may be partially due to other carotenoids also. Measurements in the 400-470(m $\mu$ ) region have not been made.

Having found a peak at 670 m $\mu$  in the action spectrum of the Emerson effect in Navicula, we wanted to know whether the absorption spectrum of Navicula contains a peak in the same region. An absorption spectrum was obtained with the integrating spectrophotometer. Figures 16 and 17 show in fact at least two peaks, in the region of the red chlorophyll a band, one at 670 m $\mu$  and the other at 680 m $\mu$ . The ratio of optical densities due to Chl a 670 and Chl a 680 appears to be even higher in Navicula than in Chlorella. As a rule, the relative height of the 670 m $\mu$  peak in the action spectrum of the Emerson effect studied under similar circumstances also is higher in Navicula than in Chlorella. This difference may be general for the two species, or it may apply only to the specific cultures which we have studied.

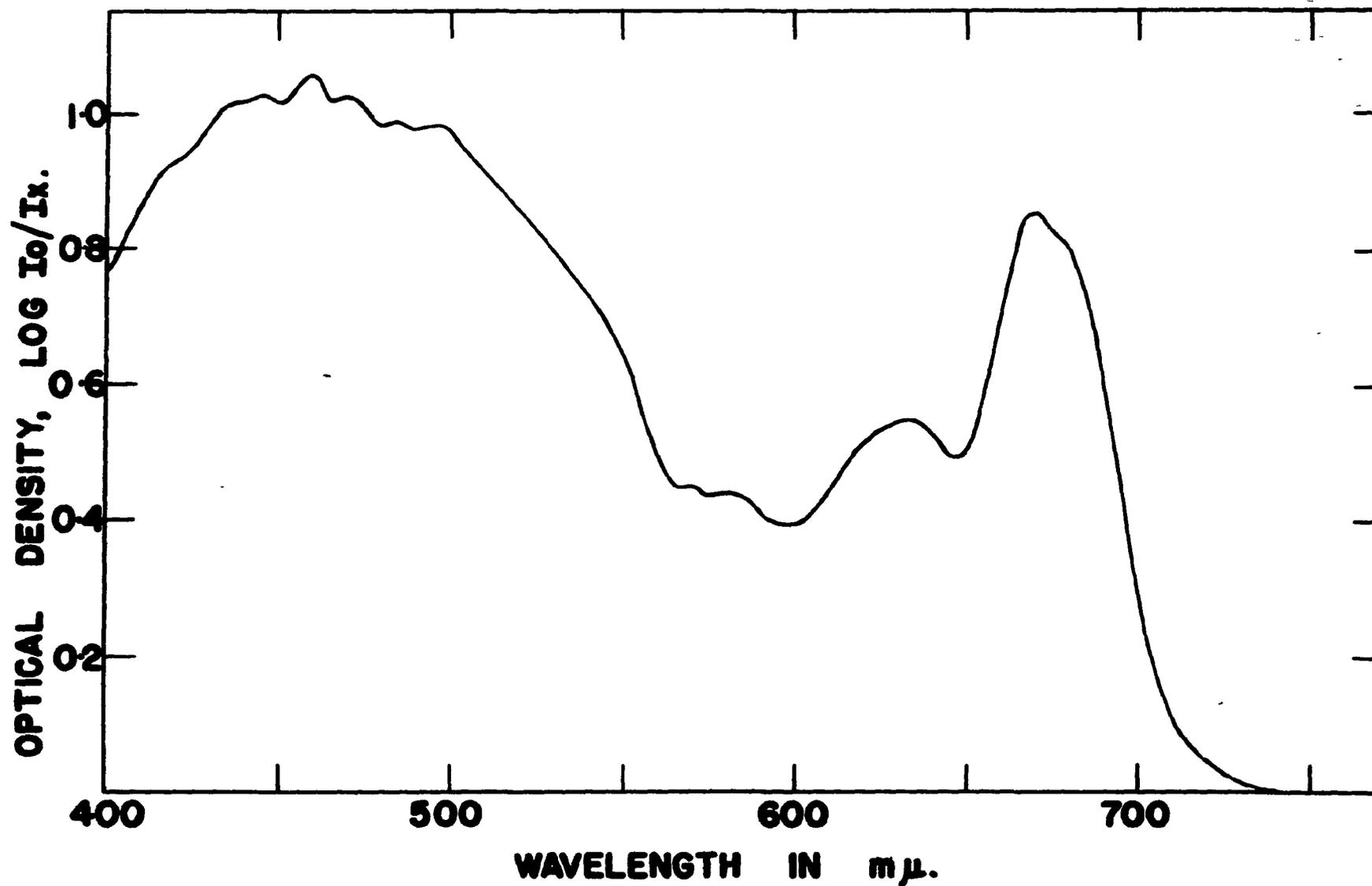


Figure 16. Absorption spectrum of *Navicula minima* measured with Cederstrand's integrating spectrophotometer. (Interval of measurement = 2 mμ).

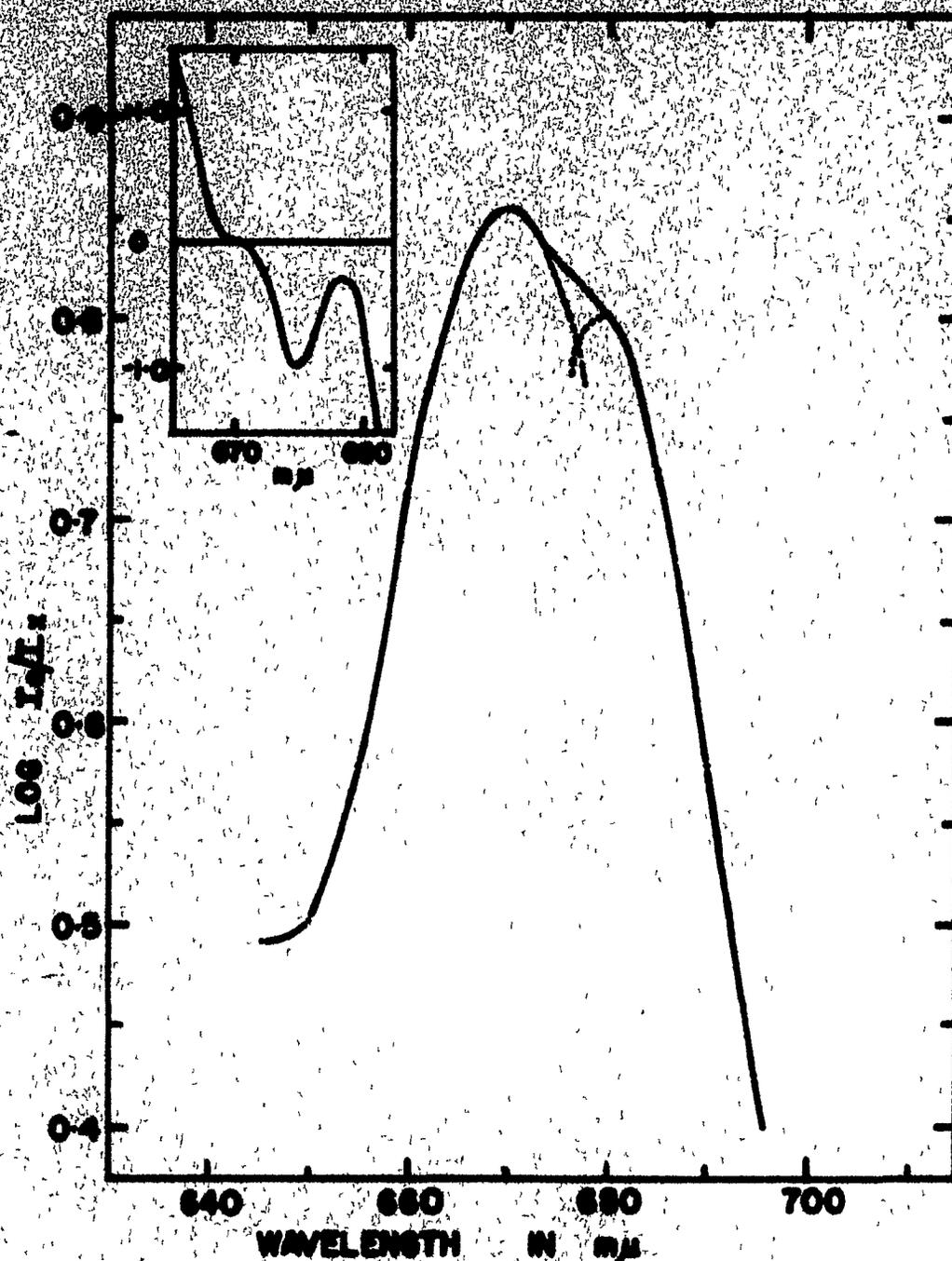


Figure 17. Red absorption band of chlorophyll a in Navicula minima enlarged from Figure 16. Dotted lines are drawn to emphasize the presence of two peaks of chlorophyll a. Insert shows a first derivative absorption spectrum of Navicula.

### C. Anacystis nidulans

Figure 18 represents the action spectrum of the Emerson effect in the blue-green alga Anacystis nidulans. The main peak at about 600 m $\mu$  is clearly due to phycocyanin. This confirms the results of Emerson and coworkers (25, 34). We note that the phycocyanin peak in the action spectrum of the Emerson effect is not single but triple (Figure 18), corresponding to three maxima in the curve showing the fraction of light absorbed by phycocyanin in the cell. Emerson's (25, 34) calculations showed these three peaks to be located at 570 m $\mu$ , 600 m $\mu$  and 640 m $\mu$ . However, Emerson did not notice the triple nature of the peak in the action spectrum of the enhancement effect in Anacystis, because of wide spacing of his measurements. No measurement with blue supplementary light has been made with Anacystis.

In nearly all ten experiments made with this alga, at least a shoulder could be noticed in the 670-680 m $\mu$  region. In some experiments (Figure 18C) it was observed at 665 m $\mu$ , in others (Figure 18D) at 675 m $\mu$ , and in two or three experiments, at 672 m $\mu$ . This suggests that the Chl a 670 form may exist and may also be active in this species. The variability of the action spectrum of the Emerson effect in Anacystis in this region (670-700 m $\mu$ ) may be related to the occurrence of a negative effect (to be discussed later) in this alga-- a relation which we do not yet fully understand.

Figure 19 shows the absorption spectrum of Anacystis nidulans in the red region. In addition to the absorption maximum at 627 m $\mu$ , (which is due to phycocyanin, but does not correspond to any one of the three peaks in the curve showing the fraction of light absorbed by phycocyanin as function of wavelength) it shows an apparently triple band of chlorophyll a, with peaks at approximately 672 m $\mu$ , 675 m $\mu$  and 680 m $\mu$ . However, such a triple peak was not observed in some cultures of the same strain (see Figure 20).

Figure 18. Action spectra of the Emerson effect in Anacystis nidulans (500 m $\mu$  to 700 m $\mu$ ).

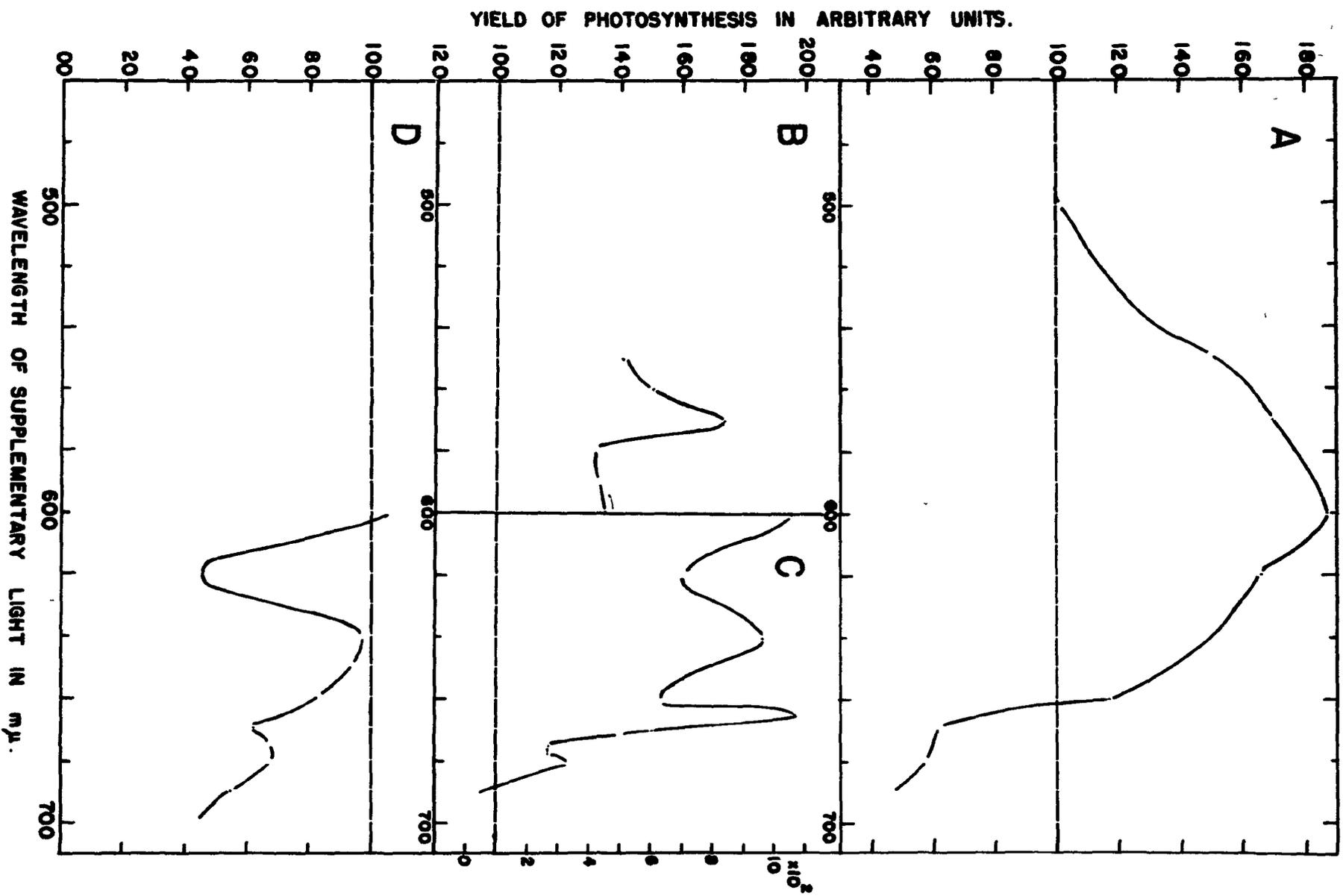
A: Intensity of far red light comparable to that used by Emerson (8.5  $\mu$ l/hour = rate of photosynthesis in far red light). Curve has been drawn to suggest a triple peak, although this experiment does not justify it. Note points which confirm Emerson's findings. Ratio of far red to supplementary light action = 1:1.

B: A different intensity of far red light (1.5  $\mu$ l/hour = rate of photosynthesis in far red light). Note peak at 570 m $\mu$ . Ratio of far red : supplementary light action = 1:2.

C: Intensity of far red light lower than in A, B, and D. (0.25  $\mu$ l/hour = rate of photosynthesis in far red light). Ratio of far red : supplementary light action = 1:20. Nearly all values are positive. Note peaks at 640 m $\mu$  and 665 m $\mu$ .

D: Intensity of far red light higher than in A, B, and C. All values are negative (Negative Emerson effect). There are peaks at 640 m $\mu$  and 675 m $\mu$ . Rate of photosynthesis of far red light : 15.0  $\mu$ l/hour. Far red : supplementary light action = 1:1.

Scale of ordinates is different for different curves. Far red light was  $\sim$  700 m $\mu$ ; photosynthesis was always below compensation of respiration.



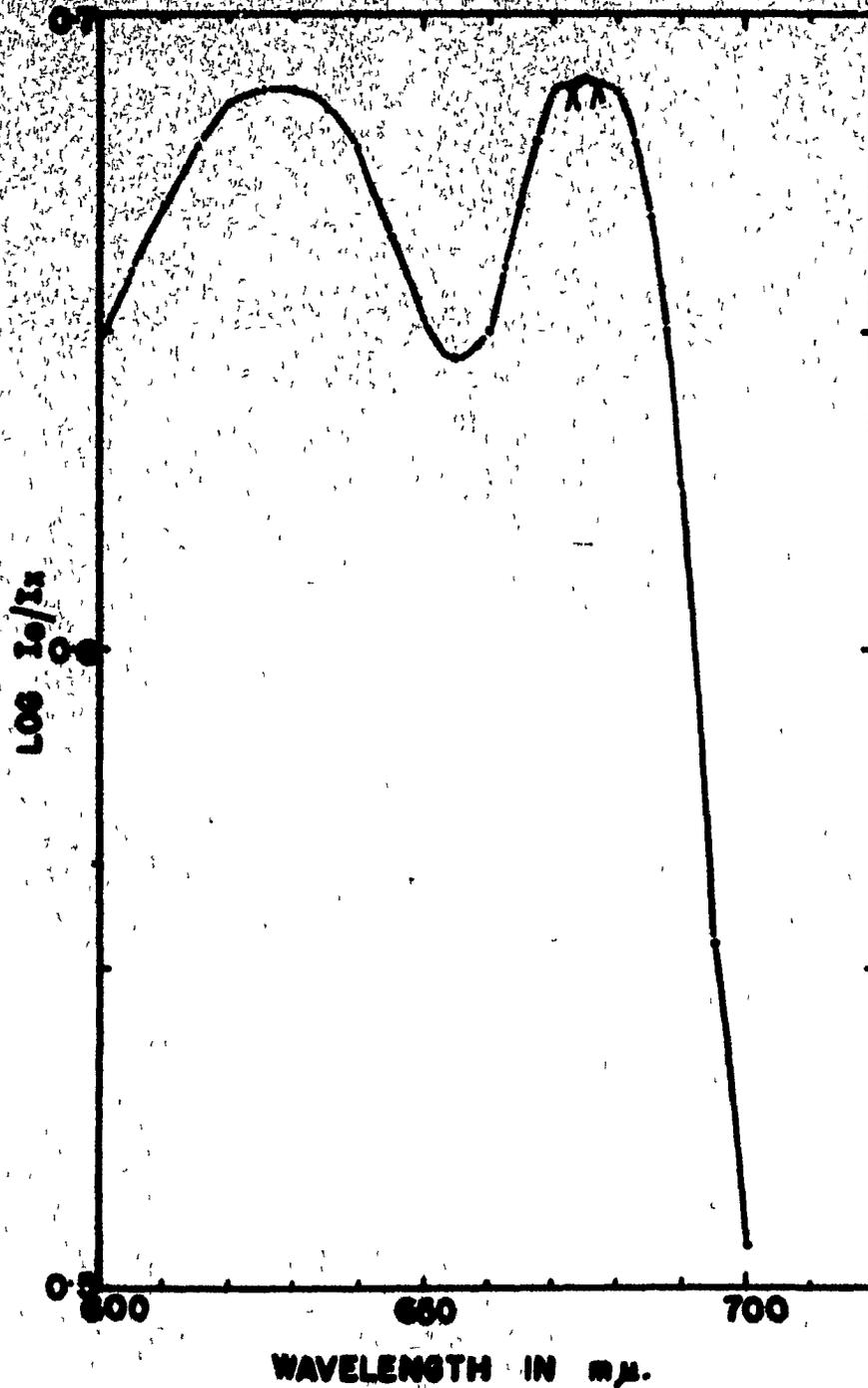


Figure 19. Absorption spectrum of *Anacystis nidulans* (red absorption band of chlorophyll a) measured with Beckman DU Spectrophotometer, using oiled filter paper technique. The spectrum was confirmed by wet filter paper technique. This spectrum shows three peaks in the chlorophyll a band. All samples did not show the triple peak (see Figure 20).

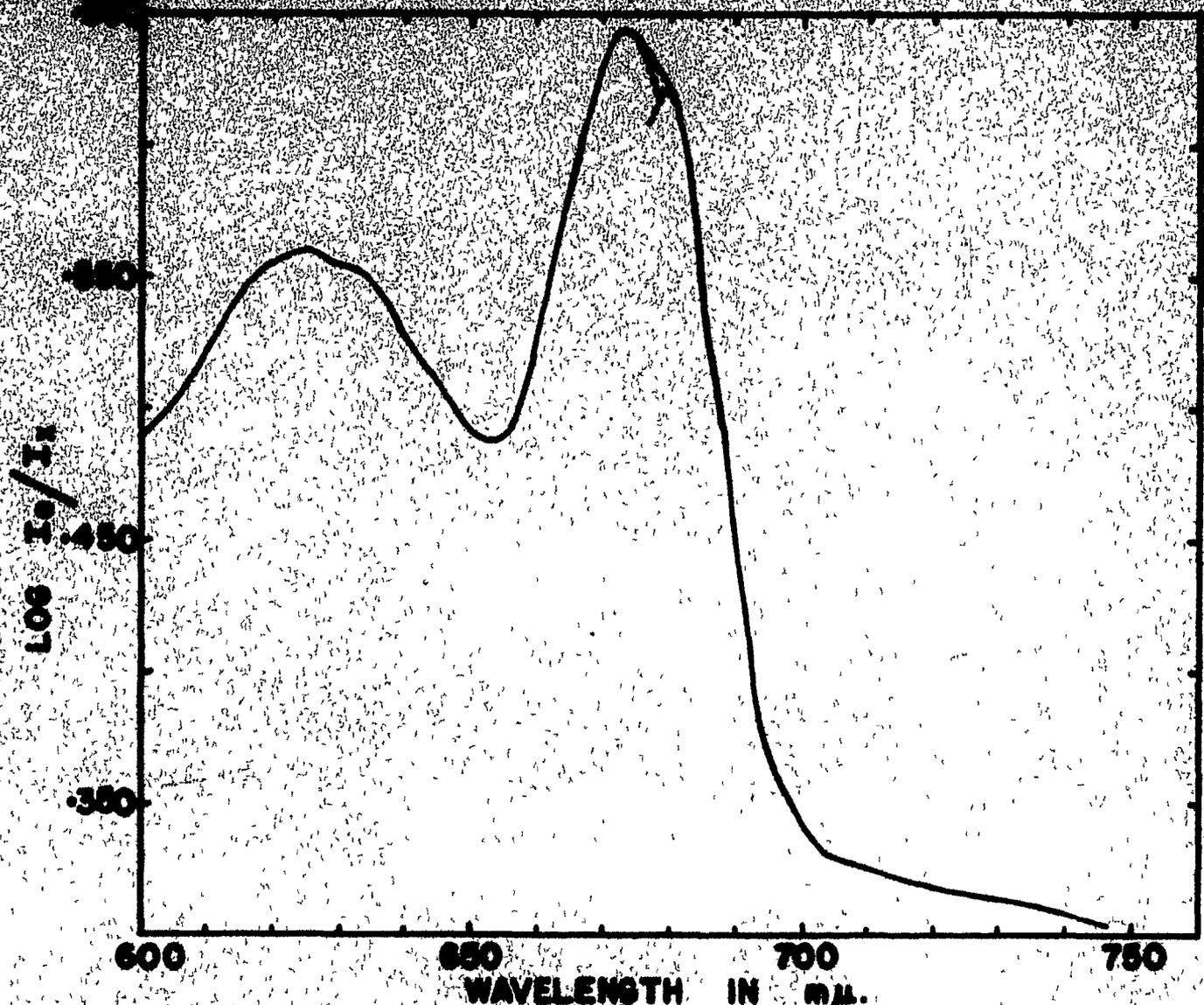


Figure 20. Absorption spectrum of *Anacystis nidulans* measured with Beckman Spectrophotometer, using oiled filter paper technique. Dotted lines emphasize the existence of two peaks in the red band of chlorophyll a (interval of measurement = 2.5 mμ).

#### D. Porphyridium cruentum

Figure 21 represents the action spectrum of the Emerson effect in the red alga Porphyridium cruentum. It, too, shows a very small shoulder at about 675 m $\mu$ , which may be due to the activity of the Chl a 670 form.

Red algae, such as Porphyridium, exhibit a "red drop" much earlier than Chlorella, Navicula and Anacystis (> 650 m $\mu$  instead of > 680 m $\mu$ ). Despite the early red drop, measurable photosynthesis of Porphyridium could be observed in monochromatic light at 680 m $\mu$ , 690 m $\mu$  and 700 m $\mu$  (cf. earlier findings of Brody and Emerson, '6). The reason for the earlier drop may be that the ratio of absorptions by Chl a 670 and Chl a 680+690 falls earlier, in these organisms, below the minimum required for full photosynthesis, than it does in Chlorella. This hypothesis should be checked by quantitative analysis of the red absorption bands of the different algae, and calculation of the relative contributions of the several chlorophyll a components to the total absorption. The amount of Chl a 670 in red algae may be sufficient for this pigment to serve as acceptor for energy transfer from auxiliary pigments, but not high enough for significant direct absorption. Alternatively, the difference in the position of the red drop may be due to spatial separation of Chl a 670 and Chl a 680 in Porphyridium, with phycoerythrin being closely associated with Chl a 670 (along the lines of Duysen's original interpretation). It is, however, not clear to me how Chl a 670 can cooperate with Chl a 680 + 690, if the former is spatially separated from the latter.

It may also be assumed that phycoerythrin is associated with a small portion of Chl a 670 molecules which are associated with Chl a 680 + 690. The majority of Chl a 670 molecules are assumed to be separated in space from Chl a 680 + 690. Direct absorption of light by majority of Chl a 670 molecules



is wasted because it cannot be transferred to Chl a 680 + 690 due to large inter-molecular distances. Even if both Chl a 670 and Chl a 680 + 690 are excited, full cooperation of two forms cannot occur because of large distances between the 'location' of these molecules. When light is absorbed by phycoerythrin, it is transferred to small number of Chl a 670 molecules, which are near Chl a 680 + 690; these Chl a 670 molecules may be enough in amount to serve as acceptors but not high enough for significant direct absorption.

Action spectrum of the Emerson effect in Porphyridium, in the blue region, shows several peaks--380  $\mu$ , 420  $\mu$ , 440  $\mu$ , and 460  $\mu$  (see Figure 22). Location of peaks at 420  $\mu$  and 440  $\mu$  is similar to that in Chlorella. All the above mentioned peaks may be due to active chlorophyll a form, Chl a 670, Soret bands of Chl a 680 + 690 or both chlorophyll a and carotenoids.

The absorption spectrum of Porphyridium (see Thomas and Govindjee, '77) also suggests a doublet nature of the chlorophyll a band (Figure 23). Cederstrand (14) observed, with his integrating-spectrophotometer, very sharp peaks at about 672  $\mu$  and 678  $\mu$ . Relative heights of the two absorption peaks in this organism do not support the suggested hypothesis that the concentration of Chl a 670 is particularly low in this organism. This seems to support the second or the third of the above mentioned hypothesis that in Porphyridium, the Chl a 670 molecules are physically remote from the Chl a 680 molecules. The situation can be affected by pre-illumination: Brody and Emerson (6) were able to increase the relative photosynthetic efficiency of green light by pre-illumination of red algae with green light, without perceptible change in the absorption spectrum.

Phycocyanin is not prominently present in Porphyridium. However, it reveals itself clearly in the occurrence, in the 600-660  $\mu$  region, of several peaks in the action spectrum of the Emerson effect. Only two of these peaks--

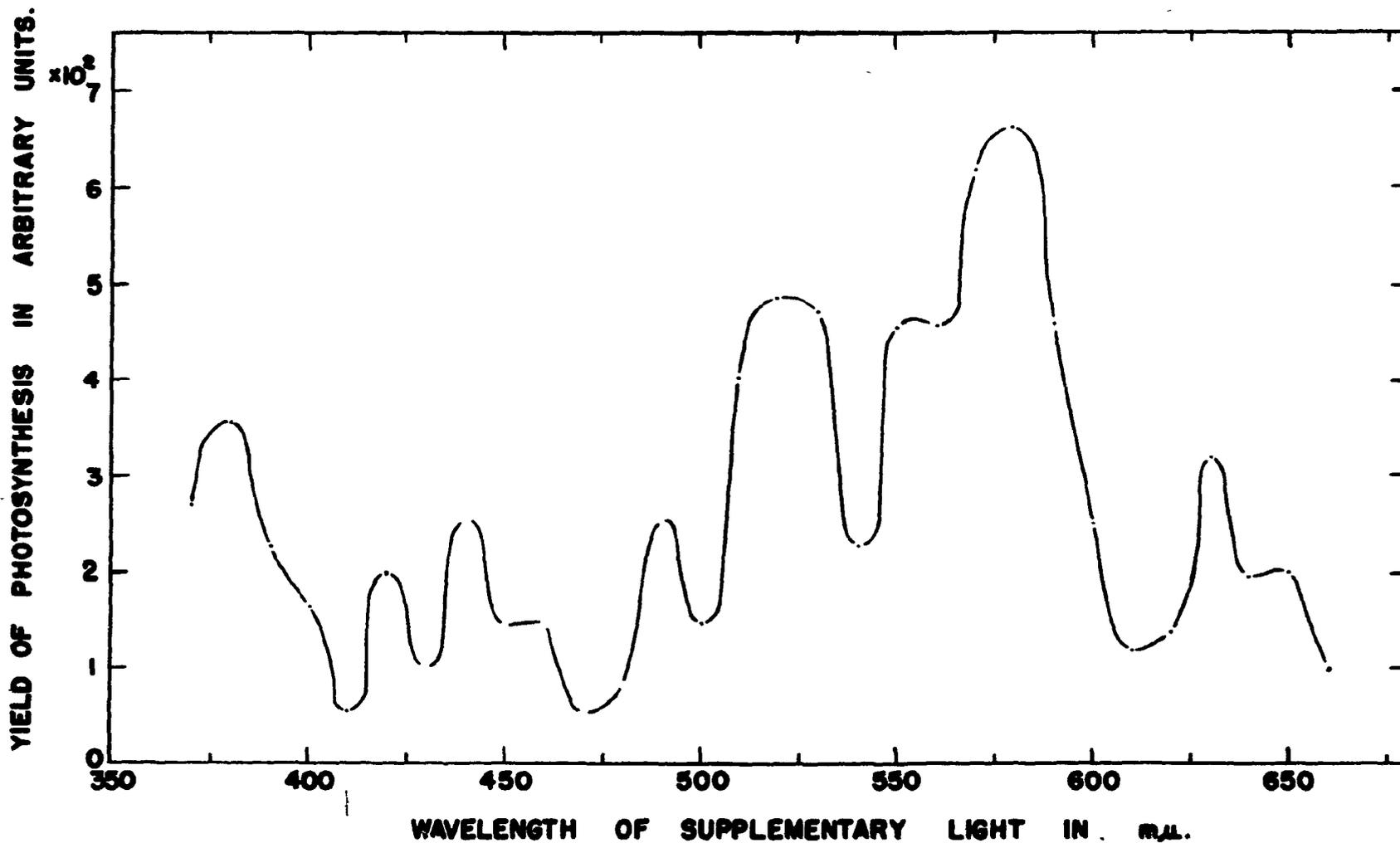


Figure 22. Action spectrum of the Emerson effect in Porphyridium cruentum. Far red light action alone = 1.0  $\mu$ l/hour. Ratio of far red to supplementary light action from 370  $m\mu$  to 500  $m\mu$  = 1:2; and from 500  $m\mu$  to 660  $m\mu$  = 1:6. Far red light  $\sim$  700  $m\mu$ .

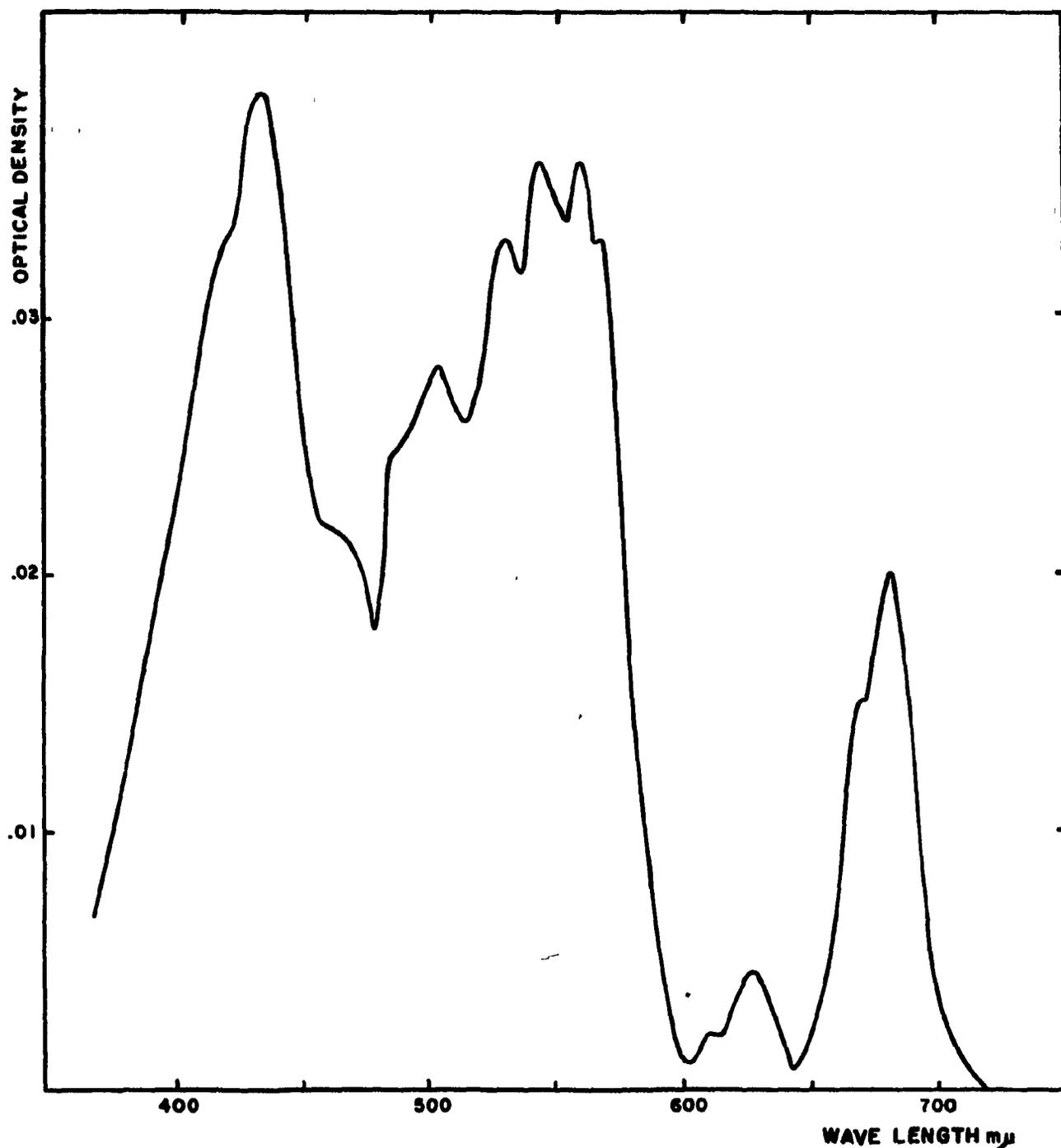


Figure 23. Absorption spectrum of *Porphyridium cruentum* measured with Beckman DU Spectrophotometer by the wet filter paper technique. Note two peaks of chlorophyll *a* at about 670 mμ and 680 mμ. Also note several peaks of phycoerythrin between 480 mμ and 600 mμ corresponding to several peaks in the action spectrum of the Emerson effect (see Fig. 22). (Interval of measurement = 5 mμ; 2.5 mμ near the peaks), after Thomas and Govindjee (77).

the one at about 630 m $\mu$  and 650 m $\mu$ --are sharp, although quite low. These peaks cannot be due to chlorophyll b, (which is absent) but can be due to phycocyanin (cf. O'hEocha, 64). The height of 650 m $\mu$  peak, relative to that of the peak at about 510 m $\mu$ , which is due to phycoerythrin, is usually 1:6. The exact absorption spectrum of the phycocyanin in Porphyridium is not known, and therefore, one cannot calculate the fraction of light absorbed by this pigment. However, this amount seems to be too small to raise the yield in the 650-680 m $\mu$  region to its maximum value, otherwise the red drop in Porphyridium would not have occurred as early as it does, even if Chl a 670 may be present in amounts insufficient for significant direct absorption of light in this region.

Figure 22 shows the action spectrum of the Emerson effect in Porphyridium also in the region of the phycoerythrin absorption. This spectrum differs from the spectrum of Emerson et al. (34) in that it shows several peaks for phycoerythrin, instead of a single one. These peaks are at about 570-585 m $\mu$ , 550-560 m $\mu$ , 510-530 m $\mu$ , and 490 m $\mu$ . These peaks may correspond approximately to several absorption peaks of phycoerythrin in Porphyridium. The phycoerythrin absorption band of Porphyridium is not single, as has been assumed before--cf. Brody, (4)--but has several peaks, as shown by Thomas and Govindjee (77) by the wet filter paper technique, (cf. Figure 23).

#### E. Reasons for Attributing the 670 m $\mu$ Peak to a Form of Chlorophyll a.

A complete analysis of the absorption curves of Chlorella, Navicula, Porphyridium and Anacystis is not easy, because the exact absorption curves of the different pigment complexes present in the cell are not known. How can it be made sure that the 670 m $\mu$  peak in the action spectrum of the Emerson effect (and in the absorption spectrum) of the various algae, is due to a form of chlorophyll a? Following possibilities exist: 1) It may be due to a form

of chlorophyll a. ii) It may be due to an unknown pigment. iii) It may be due to a form of chlorophyll b. The latter possibility seems to be ruled out because the 670 m $\mu$  peak is observed also in the action spectra of Navicula and Anacystis,--algae that contain no chlorophyll b. Complete extraction of the pigments from these algae did not reveal the presence of any unknown pigment. Therefore, the 670 m $\mu$  band has to be due to chlorophyll a. On extraction of chlorophyll a from algae, the extract has only one peak. No pigment other than a chlorophyll is known that could absorb in this region--only the chlorophylls b and a in Chlorella, the chlorophyll c and a in Navicula, and chlorophyll a in Porphyridium and Anacystis. Bacteriochlorophyll studies have shown that this pigment has a spectrum with three peaks in vivo, (attributed to three kinds of pigment-protein complexes) and only one peak in vitro; it can be suggested, by analogy, that there may be several complexes of chlorophyll a in vivo, with different absorption peaks.

A final proof that the 670 m $\mu$  band is due to a form of chlorophyll a, could come only from the extraction of this form as such, or from physical and chemical studies of its properties in vivo.

As noted earlier (in the Introduction), Emerson and coworkers have developed a theory, based on good experimental evidence, that excitation of chlorophyll a alone is ineffective in photosynthesis, unless one of the accessory pigments is excited simultaneously. The results reported in this chapter suggest a modification of this conclusion, as follows: one (or two) forms of chlorophyll a, which absorb at the long-wave side of the main absorption band, are inefficient in photosynthesis, unless sufficient amounts of light are simultaneously absorbed by another form of chlorophyll a (Chl a 670), or any accessory pigment. It remains to be seen whether the accessory pigments can

cooperate with Chl a 680 + 690 directly, or only via Chl a 670. One is inclined to think that they participate only through the intermediate of energy transfer to Chl a 670. This hypothesis can explain both Emerson's and results reported here. At the same time, it is not in contradiction with the generally accepted results of Duysens, French and Rabinowitch.

This conclusion does not mean an underestimation of the importance of the accessory pigments. In a wide region of the spectrum, where they absorb strongly, the yield of photosynthesis would be very low in their absence. For example, the absorption by chlorophyll a in green light is very low. In an organism such as Chlorella, where only the chlorophylls b and a are present, peaks found in the action spectrum of the Emerson effect in the region of 470-660 m $\mu$ , are due entirely to chlorophyll b. It seems that the absorption of Chl a 670 in this region is extremely weak, and peaks in the absorption spectrum due to chlorophyll b are not distorted by its presence. Thus, in a large portion of the spectrum, the yield of photosynthesis is high only because of the presence of accessory pigments.

Red algae, blue-green algae and diatoms provide very good evidence for the importance of accessory pigments--phycoerythrin, phycocyanin and fucoxanthol respectively--both from the action spectrum of photosynthesis and from that of the Emerson effect in these algae. In red algae where no clear evidence for a sharp peak of Chl a 670 in the action spectrum of the Emerson effect was observed, phycoerythrin seems to play a very important role.

## Chapter 5: THE NEGATIVE EMERSON EFFECT

With Anacystis, Navicula and Porphyridium, Emerson's original measurements showed, instead of an increase, a decrease in the quantum yield of photosynthesis attributable to far red light, when light of certain wavelengths was provided simultaneously. The corresponding curves can be found in Emerson and Rabinowitch (34). It was suggested, without proof, that the effect may be due to decrease in the quantum yield of supplementary light, rather than in that of far red light.

A negative effect could be explained if light saturation of photosynthesis were produced by the combination of the two light beams. Since the intensity of each of the two light beams used in these experiments was at or below the compensation point of photosynthesis, it was difficult to believe that saturation could occur by the superposition of two such beams. Nevertheless, we checked this hypothesis by measuring the rate of photosynthesis as function of intensity of the absorbed light at 700 m $\mu$ , 680 m $\mu$  and 600 m $\mu$  in Anacystis nidulans, where the negative effect was particularly pronounced. The saturation level at 600 m $\mu$ , was found to be higher than at 680 m $\mu$ , and that at 680 m $\mu$ , higher than that at 700 m $\mu$  (Figure 24). The saturation level at 700 m $\mu$  was very low, and was reached at very low intensity. These results ran contrary to the accepted belief that the saturation level of photosynthesis at all wavelengths is the same (cf. Rabinowitch, 66, p. 1161) being equal to the maximum rate of action of a certain limiting enzyme. This appears not to apply to the region of the red drop, where not only the maximum quantum yield is low, but also the saturation level is considerably reduced. It may be argued that the reduced quantum yield in weak light may be merely a consequence of the low saturation "ceiling".

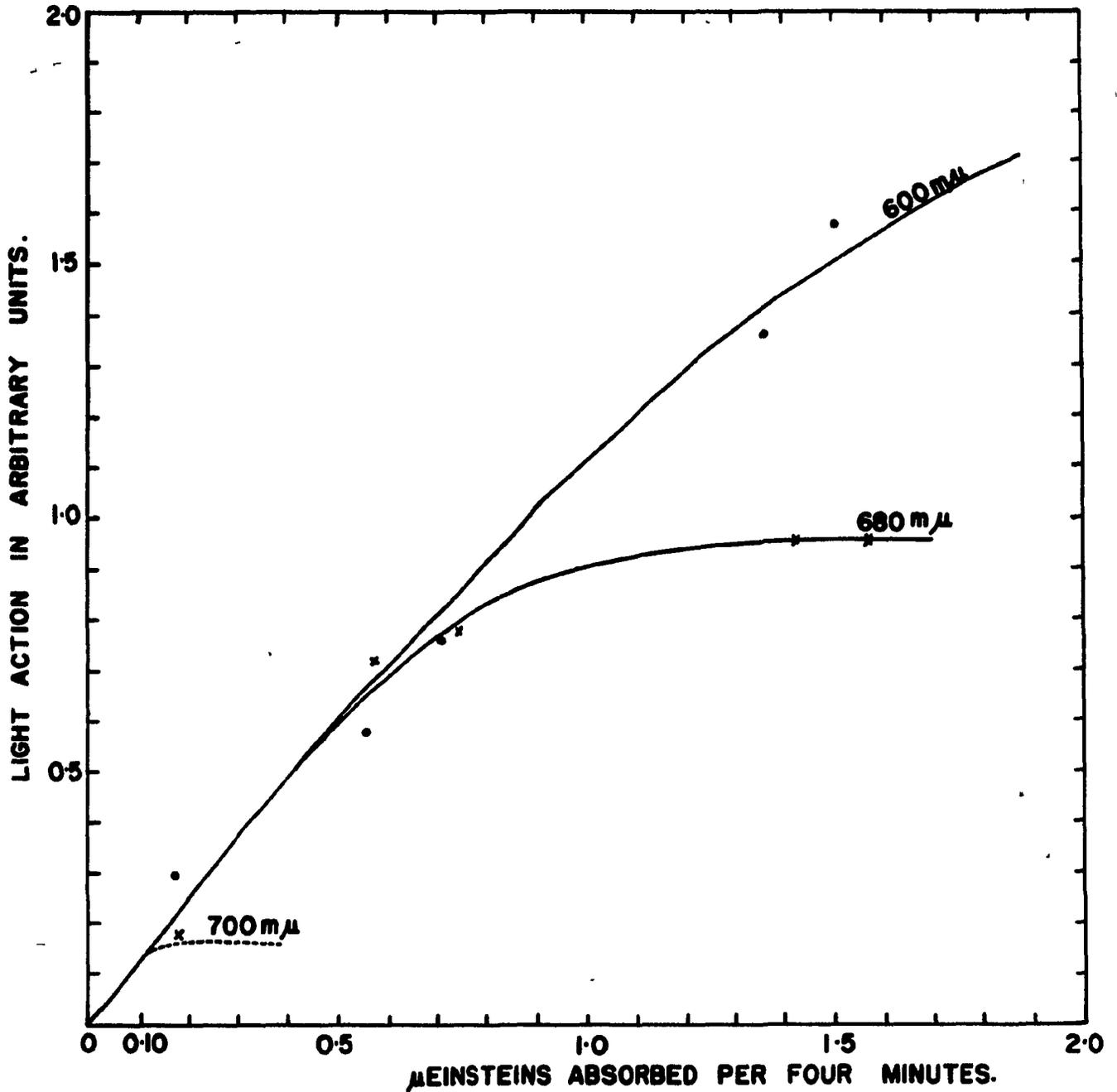


Figure 24. Light curves of *Anacystis nidulans* for 600 mμ, 680 mμ and 700 mμ. Different saturation levels are approached at these three wavelengths. Saturation at 700 mμ was reached at a very low level (as seen by plotting the light curve on a different scale--not shown here). In this Figure, 700 mμ curve is indicated by dotted line, to show its position relative to that of 680 mμ and 600 mμ.

It is clear that the light of a given wavelength could show a negative effect if, added to the background light of the same wavelength, it will bring the total light effect into the saturation range. It is less clear how the addition of light of a wavelength where the saturation level is low, to the light of a wavelength where the saturation level is high, will affect the saturation level, and thus also the quantum yield of the combined light.

The subject obviously requires a special, systematic study, and a new interpretation. Tentatively, one could suggest that the negative Emerson effect may be generally associated with the early light saturation of photosynthesis of the one of the two beams. Figure 25 shows the negative Emerson effect in Chlorella and Navicula. For the negative effect in Porphyridium and Anacystis, see Figure 6 and 9, Emerson and Rabinowitch (34).

It must be mentioned that McLeod and French (60) also have discovered that the saturation levels at the different wavelengths are not the same, as was previously assumed.

Experiments on the relation between light intensity and the sign of the Emerson effect were performed with Anacystis at 680  $\mu$ , in the region of the negative Emerson effect. The ratio of far red light action: to the supplementary light action was kept approximately constant (1:1), but the absolute intensities of both beams were changed. Table 8 summarizes the results. When the light action was sufficiently low (1.89  $\mu$ l/hr), a positive Emerson effect could be always obtained.

It was found that the Emerson effect at any wavelength and in every spectrum can be made positive by using sufficiently low intensity of far red light, and negative by choosing a sufficiently high intensity of far red light (cf. Figures 18C and 18D).

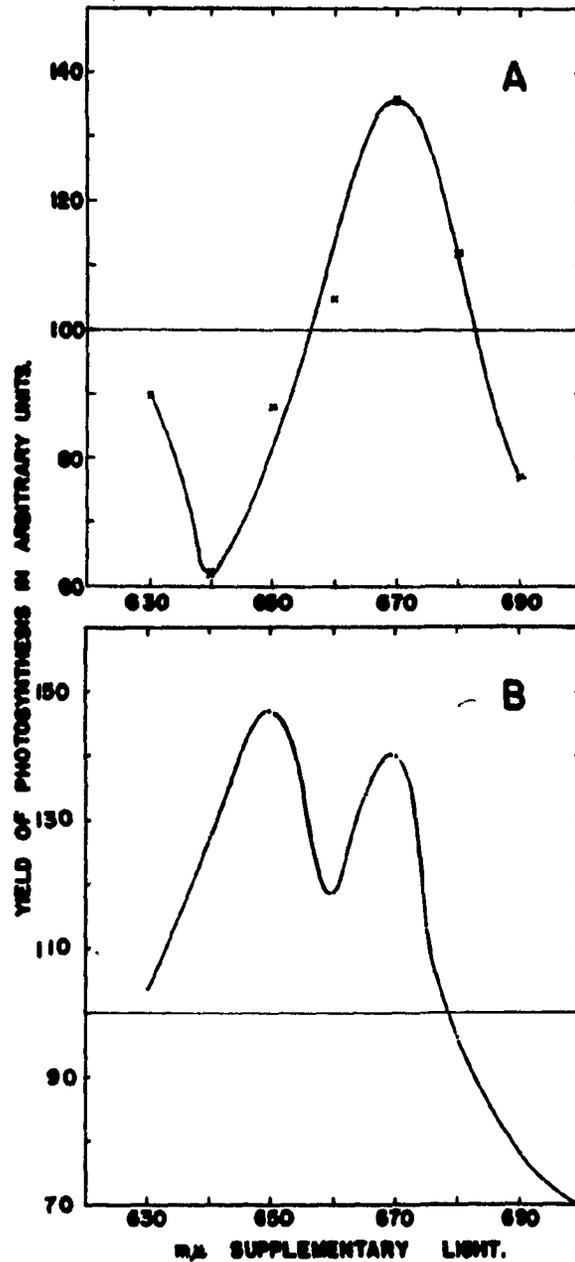


Figure 25. Action spectrum of Emerson effect in A: Navicula minima and B: Chlorella pyrenoidosa showing negative effect. Far red light action alone = 15.0  $\mu$ l/hour; ratio of far red : supplementary light action = 1:3; far red light was  $\sim$  700 m $\mu$ .

Table 8

## RELATION BETWEEN FAR RED LIGHT ACTION AND THE EMERSON EFFECT

	A	B	C	D	E	F	G
Experi- ment #	Light action of far red light alone $\mu\text{l}/\text{hour}$	Light action of supplemen- tary light (680 m $\mu$ ) $\mu\text{l}/\text{hour}$	Light action of far red light + 680 m $\mu$ light, given separ- ately. $\mu\text{l}/\text{hour}$	Light action of far red light + 680 m $\mu$ light given simul- taneously. $\mu\text{l}/\text{hour}$	Light action of far red light in pre- sence of sup- plementary light. $\mu\text{l}/\text{hour}$	Ratio of far red light act- ion: Supple- mentary light action	Emerson effect $\frac{D-B}{A} \times 100$
1	1.89	2.34	4.23	5.52	3.18	1:1.2	168
2	3.42	2.16	5.58	5.22	3.06	1:1.0	90
3	7.38	7.64	15.02	13.50	5.86	1:1.10	80
4	9.90	13.68	23.58	20.15	6.47	1:1.4	66
5	12.24	13.86	26.10	21.80	7.94	1:1.11	65
6	18.00	19.80	3.80	26.80	7.00	1:1.10	39

As mentioned above, the existence of a relation between the negative effect and low light saturation is plausible. Due to early saturation, addition of a certain amount of far red light (700  $m\mu$ ) to background light of the same wavelength can cause a negative effect (decline in the average quantum yield). It seems plausible that the same may result from a combination of two beams of different wavelength, one of which is characterized by early saturation. However, if the two beams have different photochemical functions--and this is what has been postulated as a working hypothesis--this conclusion becomes uncertain. Obviously, a satisfactory explanation would have to begin with explaining how the saturation level of photosynthesis can be different in light of different wavelengths, since this fact contradicts the simple hypothesis that the saturation level of photosynthesis is the measure of the maximum rate of a certain physical or enzymatic process, unrelated to the primary photochemical process.

The discovery (Rabinowitch, Govindjee and Thomas, 70) of the inhibitory effect of extreme red light (740-750  $m\mu$ ) on the rate of photosynthesis in the far red light (680-700  $m\mu$ ) suggests a possible relation between this effect and the negative Emerson effect, observed when monochromatic light with a wavelength of about 670  $m\mu$  is added to "far red light", obtained by means of a filter with a sharp cut-off on the short wave side (and therefore containing some of the inhibitory extreme red light). However, several experiments show that the negative Emerson effect is present even when the far red light is carefully freed from wavelengths longer than 700  $m\mu$ .

Chapter 6: INHIBITORY EFFECT OF LONG WAVELENGTH OF LIGHT ON  
PHOTOSYNTHESIS PRODUCED BY LIGHT OF SHORTER WAVELENGTHS

Thomas and Govindjee (76, 77) had observed that the rate of photosynthesis in Porphyridium cruentum is too low for manometric measurement when white light filtered through a very dense solution of mixed phycobilins (filter PB95) is used for illumination. However, if orange light is given as a supplement to this light, the contribution of far red light to the total photosynthetic activity becomes measurable. The filter of mixed phycobilins, used in these experiments, transmits waves longer than 660 m $\mu$ ; the absence of measurable photosynthesis in this light seemed to contradict the findings of Brody and Emerson (6), who reported that monochromatic light in the 660-710 m $\mu$  region can produce measurable photosynthesis in the same alga.

To further investigate this discrepancy, one had to make certain that the difference between the observed effects of filtered light and those of monochromatic light with  $\lambda = 660-710$  m $\mu$  was not due to differences in the algal cultures used in the two series of experiments. The measurement with monochromatic light of 680 m $\mu$  and with light filtered through a dense phycobilin filter (PB95) was repeated on one and the same sample of algae. Transmission spectrum of "PB-95" filter can be found in Thomas and Govindjee (77). The results are summarized in Table 9. There is no doubt that a real discrepancy exists. One possible source of it is that light transmitted through the phycobilin filter contains, in addition to the wavelengths 660-710 m $\mu$ , also "extreme red" light  $\lambda > 720$  m $\mu$ . No known algal pigment absorbs light beyond 720 m $\mu$ , so that a photochemical effect of light  $> 720$  m $\mu$  seemed improbable. However, it was obvious that either this extreme red light was responsible for the discrepancy, or light of certain specific wavelengths in the 660-710 m $\mu$  region interacted in such a way as to reduce the total rate of

Table 9

PHOTOSYNTHESIS OF PORPHYRIDIUM CRUENTUM  
 IN LIGHT OF 680 m $\mu$  AND IN LIGHT TRANSMITTED  
 BY DENSE PHYCOBILIN FILTER (5% TRANSMISSION)  
 (ADJUSTED TO EQUAL NUMBERS OF ABSORBED QUANTA)

Oxygen Evolution, $\mu$ l/hour	
In 680 m $\mu$ Light	In Light Transmitted by Dense Phycobilin Filter (PB 95)
+0.26	-0.55
+0.32	-0.26
+0.39	+0.22
+0.74	+0.18
+0.73	+0.21
mean +0.49 $\pm$ 0.10	-0.04 $\pm$ 0.15

photosynthesis to zero, a hypothesis which is even less plausible. To test the first alternative, monochromatic extreme-red light  $> 720 \text{ m}\mu$  was provided, as mentioned in Chapter 2, by means of a grating monochromator, and was added to far-red light obtained by means of an interference filter with maximum transmission at  $700 \text{ m}\mu$ , but covering the range from  $680$  to  $720 \text{ m}\mu$ . The wavelength of the extreme red light was changed in steps of 10 (and sometimes 5)  $\text{m}\mu$ .

The light action of far red light was determined in the presence of different wavelengths-- $720 \text{ m}\mu$  to  $780 \text{ m}\mu$ --and in their absence. The rate in the extreme red light alone was negligible; the rate of photosynthesis in the far red light alone was always greater than in the two lights together. The results obtained with Porphyridium, are presented in Table 10 and Figure 28, and those obtained with Chlorella, in Table 10 and Figures 26 and 27. They show that the extreme red light--wavelengths between  $720$  and  $780 \text{ m}\mu$ --has an inhibitory effect on the photosynthesis caused by far red light. Action spectra show that the maximum effect occurs at  $740$ - $750 \text{ m}\mu$ , and suggests the existence of a pigment with an absorption band in this region. The maximum inhibition varied in Porphyridium, from 22% to 85%. In Chlorella, however, not only the extent of the inhibition, but also the location of the maximum effect, was variable from culture to culture. In some experiments, the maximum effect was found at  $745 \text{ m}\mu$ , in others at  $740$  or  $750 \text{ m}\mu$ . It was noted in Chapter 2 that the purity of light derived from the monochromator decreased as we went from far red light toward the extreme red, because our grating was blazed for visible region. This purity could be improved by placing a sharp cut-off RG-8 filter at the exit slit of the monochromator, but overlapping could not be completely corrected by filters when it occurred between two close-by spectral regions. This overlapping may be the reason why the ordinates of the action spectra do

Table 10

INHIBITION BY EXTREME RED LIGHT OF PHOTOSYNTHESIS  
 PRODUCED BY FAR RED LIGHT. (THE PAIRS OF VALUES IN EACH  
 HORIZONTAL LINE ARE REDUCED TO EQUAL RATE OF ABSORPTION OF LIGHT QUANTA)

Rate of Photosynthesis, $\mu\text{l O}_2/\text{hour}$		
far red light (680-720m $\mu$ )	far red light + extreme red light (750 m $\mu$ )	Inhibition in %
<u>PORPHYRIDIDIUM CRUENTUM</u>		
4.01	2.23	44
1.87	0.29	85
1.85	0.29	84
3.30	2.04	38
1.08	0.84	22
<u>CHLORELLA PYRENOIDOSA</u>		
2.61	1.98	24
2.52	1.80	29
2.52	2.07	18
2.34	1.71	27
2.70	0.72	73
5.21	4.50	14

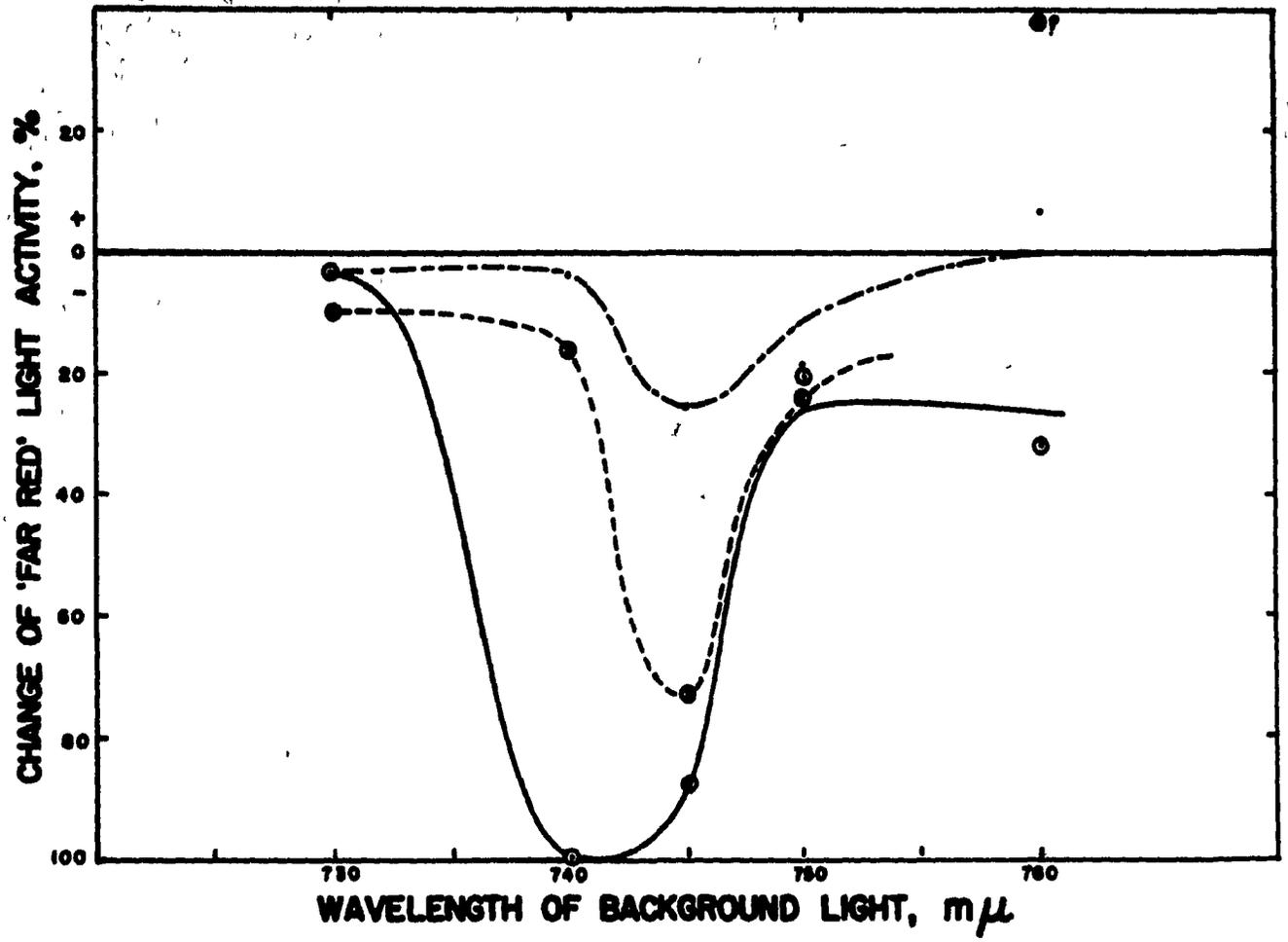


Figure 26. Action spectra of the inhibition of photosynthesis in *Chlorella pyrenoidosa* caused by the addition of "monochromatic" light (half band width = 10 mμ) of different wavelengths to "far red" light of about 700 mμ. x ? is a questionable point. Curves are drawn "through" the experimental points in as symmetrical way as consistent with the data. The suggested location of the minima is therefore arbitrary.

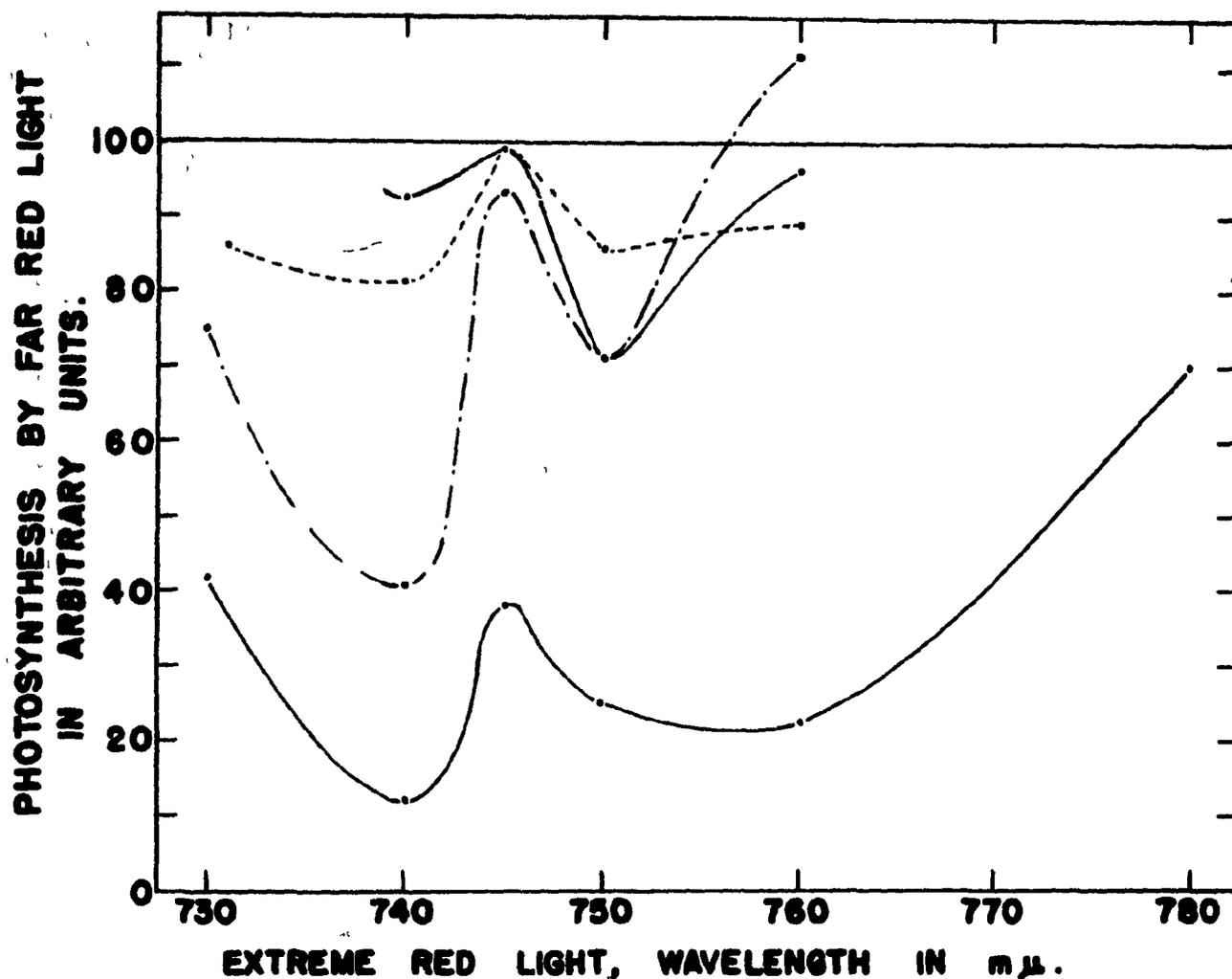


Figure 27. Action spectra of the inhibition of photosynthesis in *Chlorella pyrenoidosa* caused by the addition of "monochromatic" light (half band width = 10 mμ) of different wavelengths to "far red" light of about 700 mμ. (Four experiments are shown here; see other three experiments in Figure 26.) Variations are assumed to be due to different physiological age of the cultures used.

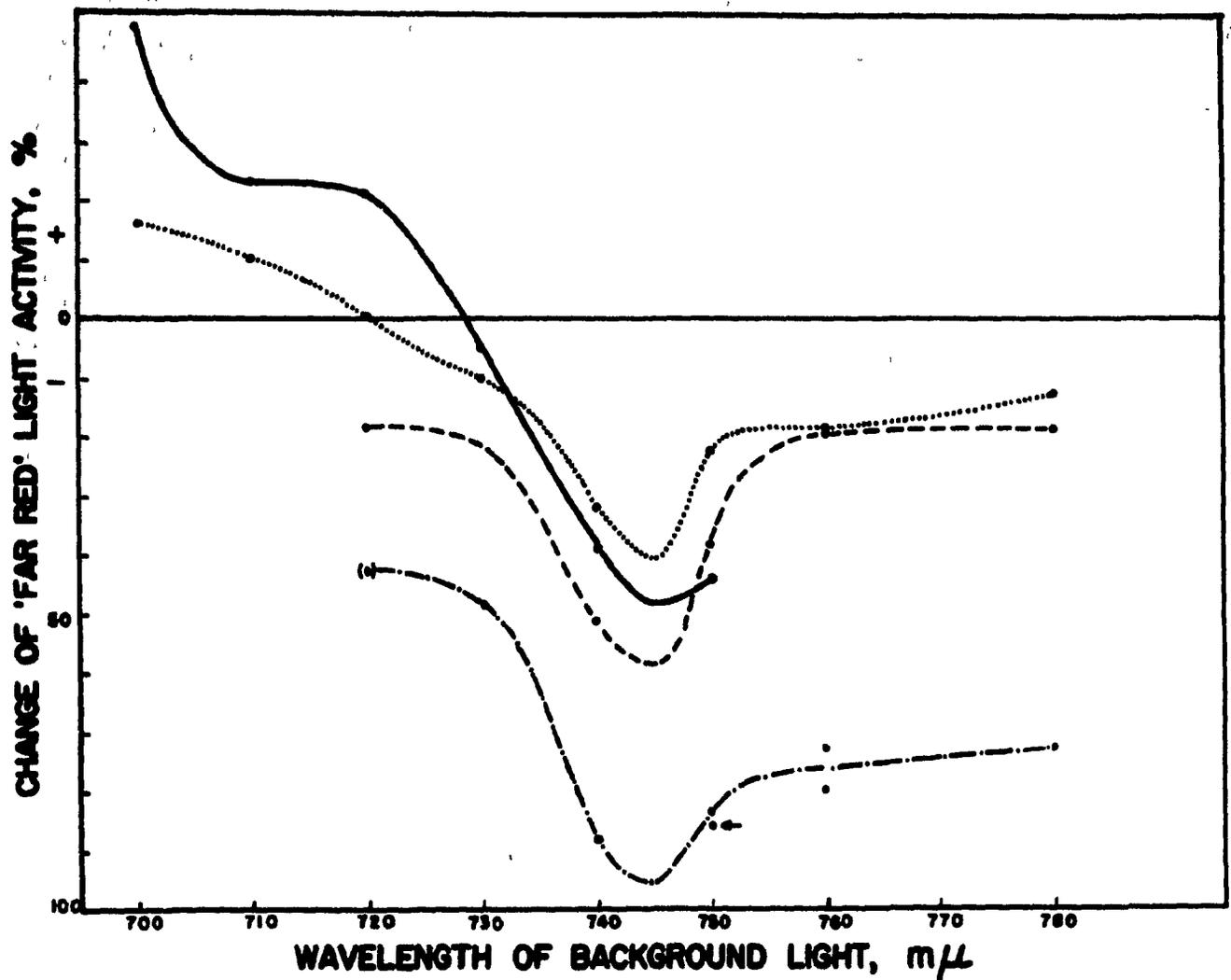


Figure 28. Action spectra of stimulation and inhibition of photosynthesis in *Porphyridium cruentum* caused by the addition of "monochromatic" light bands (half band width, 10 m $\mu$ ) of different wavelengths to "far red" light of about 700 m $\mu$ . Arrow indicates a separate experiment. (·) designates a less reliable measurement. Curves are drawn according to the same policy as of Figure 26.

not go back to 100% when we advance beyond 750  $\mu$ ; the 760-780  $\mu$  light may have been contaminated with some 740-750  $\mu$  light, and vice-versa. Action spectra observed under such conditions are liable to be distorted. However, there can be no doubt as to the reality of the observed inhibition effect. No better data could be obtained with the now available grating.

Variations in the extent of inhibition, observed in different cultures could be due to differences in the concentrations of the "inhibitory pigment"; or to differences in the relative intensities of the two beams to which the cells were exposed. A plot of the extent of the inhibition against wavelength of the extreme red light is shown for Porphyridium in Figure 28; it indicates an enhancement in the 700-720  $\mu$  region, and an inhibition  $>$  720  $\mu$ . The enhancement is simply the Emerson effect which occurs because in this region, the 700-720  $\mu$  light is being supplemented with light of shorter wavelengths ( $<$  700  $\mu$ ) transmitted through the 700  $\mu$  interference filter (half band width, 20  $\mu$ ).

Similar experiments were conducted also with Anacystis nidulans. Several cultures showed no inhibitory effect. However, these experiments do not prove a general absence of inhibition in this species, but merely its absence at the intensities used in the cultures examined.

If extreme red light inhibits photosynthesis, then how can the quantum yield be high in white light, which contains considerable amounts of the inhibitory extreme red light? This remains to be explained. We do not know as yet whether the inhibition occurs only in far red light, or also in light of shorter wavelengths. Two experiments were carried out in which the extreme red light was produced by means of two, one mm thick, Schott RG 10 filters (see Figure 2), and monochromatic far red light of different wavelength was added to this light.

Both experiments showed the inhibitory effect to decrease when the wavelength of the far red light became shorter. Possibly, inhibition may affect only photosynthesis produced by light in the 690-710 m $\mu$  region--i.e. in the region where the quantum yields are subnormal; inhibition may therefore make little difference for the total yield in white light. There may be even rays in the spectrum that counteract the extreme-red inhibition effect. In one experiment, blue light was combined with extreme red light; no inhibition was observed in this case. Experiments of Thomas and Govindjee (76, 77) with dense solution filters containing either the phycocyanins or the phycoerythrins but not both together, showed measurable amounts of photosynthesis behind such filters, although extreme red radiation was transmitted by them at least to the same extent as by the mixed-phycobilins filter, which reduced photosynthesis to zero. The difference could be due to the fact that the filters consisting of only one kind of phycobilin transmitted also wavelengths able to counteract the inhibitory effect. It is possible that the wavelengths which give the Emerson effect, also counteract the inhibitory effect, although the Emerson effect is certainly not based on the action of short-wave light on the "740-750 m $\mu$  pigment". This is shown by the fact that the Emerson effect occurs also when one uses monochromatic 700 m $\mu$  light (Figures 15, 21, 22) instead of a broad band > 700 m $\mu$ .

It has been already noted that the above-described experiments suggest the existence of a pigment absorbing light around 745 m $\mu$ . Absorption measurements with Chlorella and Porphyridium with the Beckman DU spectrophotometer failed to show a band in this region, but experiments with Anacystis showed a sharp band at 750 m $\mu$  (Figure 29). Absorption measurements with this alga were also done using Cederstrand's integrating spectrophotometer and confirmed the

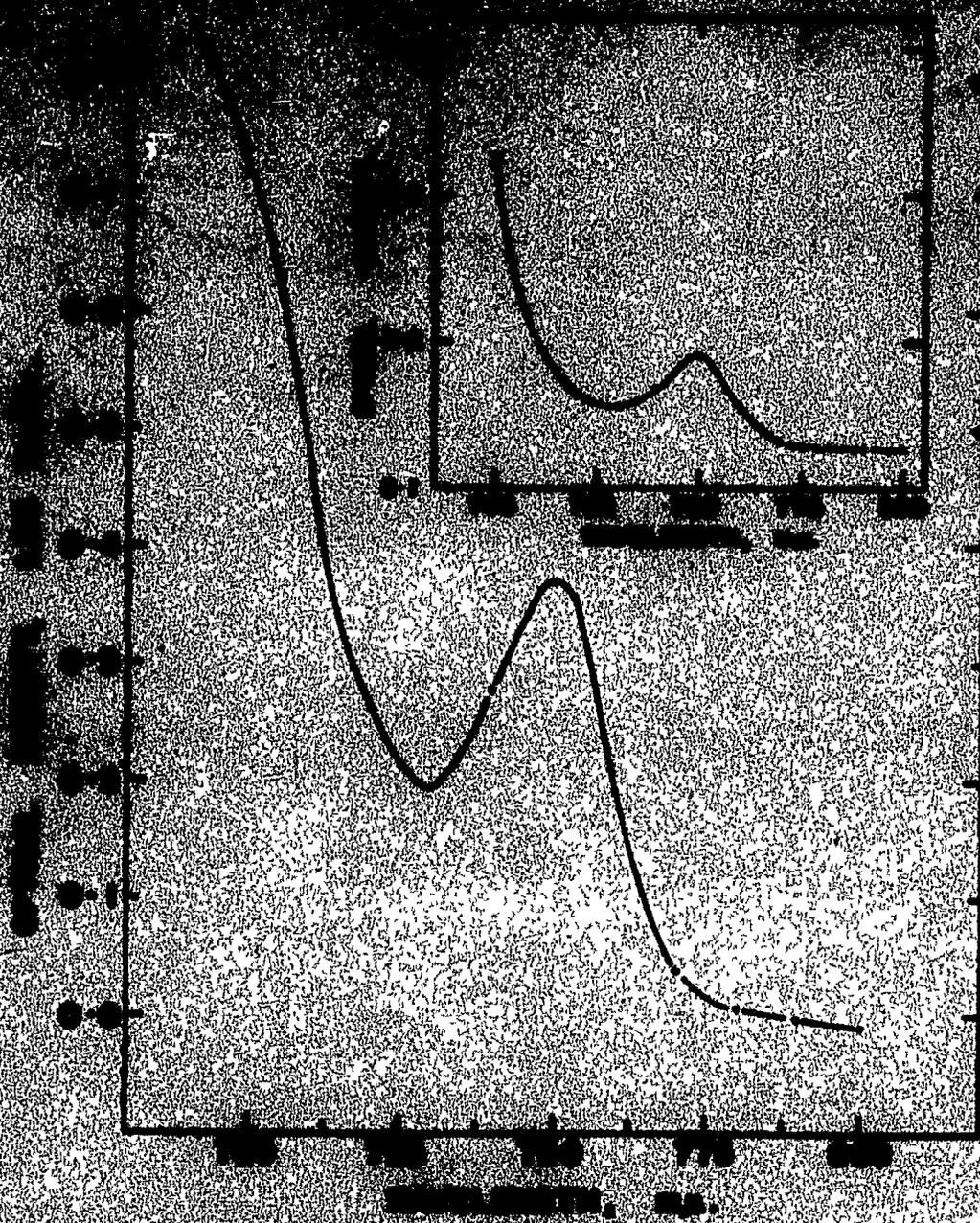


Figure 29. Infrared spectrum of a 10% composition of DMSO in polyethylene. The inset shows a magnified view of the 1700-1800 cm⁻¹ region. The peak at 1715 cm⁻¹ is due to the carbonyl group of DMSO, and the peak at 1735 cm⁻¹ is due to the carbonyl group of the polyethylene.

existence of the 750  $\mu$  band. We recall, however, that this was the species in which no inhibition effect was observed. Butler et al. (12, 13) found a photolabile pigment in higher plants which exists in two states ("730  $\mu$  state" and "660  $\mu$  state") and is supposed to be responsible for photomorphogenic phenomena. It could be responsible also for the above described photosynthesis effects. Experiments were also carried out with a double beam Cary's Recording Spectrophotometer, to see whether the existence of a photolabile pigment would be revealed by difference spectra between differently pre-illuminated cells. Thick suspensions of Chlorella, Porphyridium and Anacystis were divided into two portions 1 and 2. First a "dark run" was made using sample 1 against sample 2, this gave a "base line" (which may differ from the straight zero line because of differences in reflection between the two vessels). Sample 1 was then illuminated for 15 minutes by light of 660  $\mu$  (3mm wide slits); immediately afterwards their spectrum was recorded against sample 2, which had remained in dark. After this, sample 1 was irradiated with 745  $\mu$  (wide open slits; 3mm) for 15 minutes, and the difference spectrum between sample 1 and sample 2 was again recorded. Finally, a difference spectrum between the suspension pre-illuminated with 745  $\mu$  light and that pre-illuminated with 660  $\mu$  light was drawn. Figure 30B shows a curve obtained for Porphyridium. Three experiments of this kind were carried out, and they all showed qualitatively the same result. Two experiments with Chlorella and two with Anacystis gave similar general results. They all showed an increase in optical density in the extreme red region (745  $\mu$ ) after pre-illumination with 660  $\mu$  light and a decrease in optical density in the same region when the cells were pre-illuminated with 745  $\mu$  light, indicating the existence of a photolabile pigment absorbing at about 745  $\mu$ . However, two

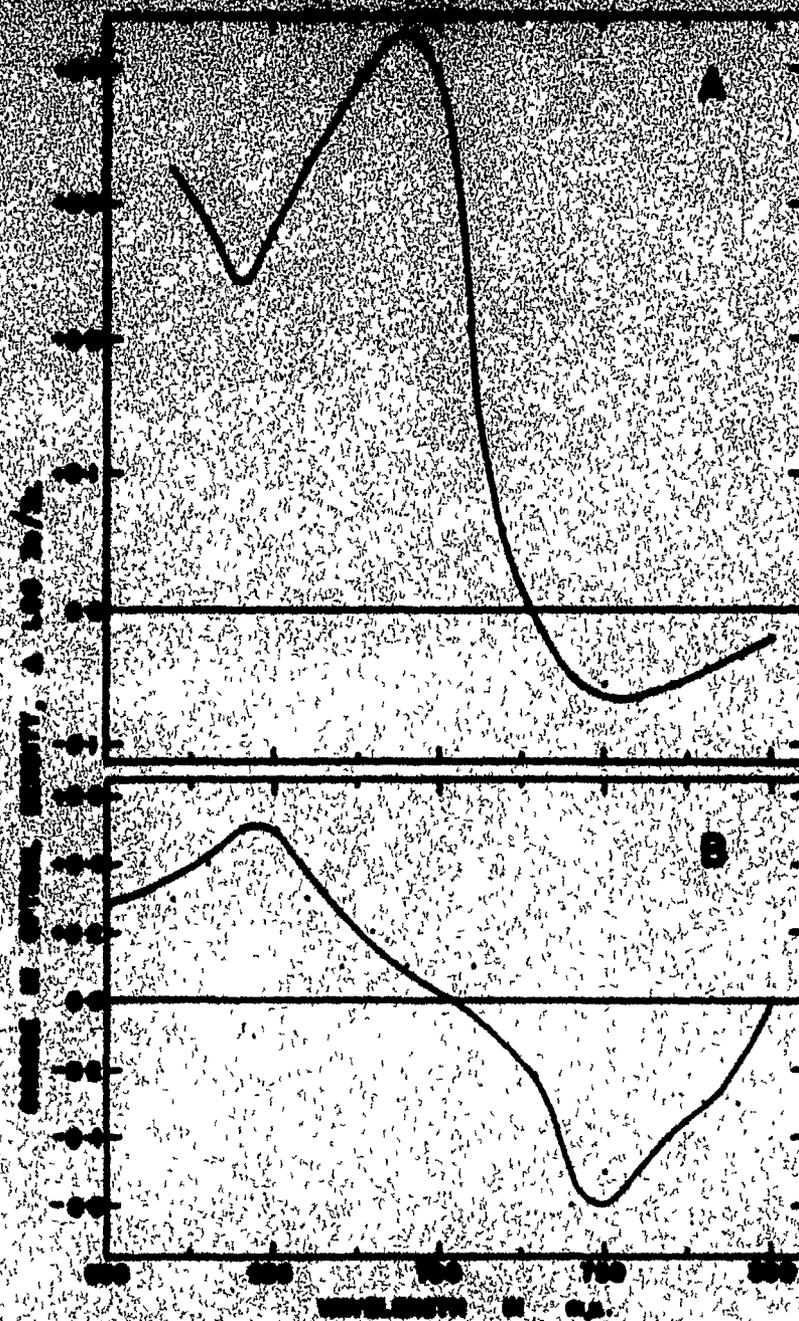


Figure 30. Difference absorption spectrum of A: Chlorella  
pyrenoidosa and B: Hydrocoleum sp.  
(spectrum measured after illumination with  
"745" mμ light against unilluminated sample minus  
spectrum measured after illumination with  
"660 mμ" light against unilluminated sample).  
Measurements were done with double beam Cary's  
recording spectrophotometer.

difficulties were encountered: 1) variability in the location of the absorption maxima, much wider than those found in the determination of the action spectra, and ii) variability of the base line. It was not possible to obtain better difference spectra with the Cary spectrophotometer, because of the wide slits that had to be used. Some results had to be discarded because of the settling of the algae; but suspending the cells in sucrose (1M) could be used to prevent settling.

To sum up, experiments with difference spectra only suggest the possible existence of a photolabile pigment, absorbing in one state at about 740-750 m $\mu$ . They do not prove that this is the pigment involved in the photosynthesis inhibition effect, although this appears to be a plausible working hypothesis.

## Chapter 7: GENERAL DISCUSSION

A. Role of the Several Forms of Chlorophyll a in Vivo

Chlorophyll a is known to undergo reversible photochemical reactions. Rabinowitch and Weiss (71, 72; also cf. Porret and Rabinowitch, 65) noted that methanolic solution of chlorophyll can be reversibly oxidized by ferric and ceric salts. Krasnovsky (49) and Krasnovsky et al. (50, 51) discovered that ascorbate in pyridine can be oxidized by chlorophyll in light, the chlorophyll being reversibly reduced to a pink compound (eosinophyll). Bannister (3) confirmed Krasnovsky's findings, but found that the presence of water is necessary for this reaction. The above observations pertain, however, to chlorophyll in solution. In living Chlorella cells, Coleman and Rabinowitch (15) found, with the aid of sensitive difference spectrophotometry, that at high light intensities, changes in absorption occur in the region where chlorophyll a absorbs. The intensity of the red band at 680 m $\mu$  decreases in light. On close examination, the changes at 670 m $\mu$ , 680 m $\mu$  and 690 m $\mu$  appear to be different, indicating that there may be three forms of chlorophyll a which change in a different way. Krasnovsky and coworkers (52, 53, 80) claimed that the rate of bleaching of two forms (Chl a 670 and Chl a 680) is different. Brown and French (11) have confirmed it. It has been shown earlier in this thesis that the two forms of chlorophyll a (Chl a 670 and Chl a 680+690), have different photochemical activity. Chlorophyll a (670 m $\mu$ ) can raise the quantum yield due to light absorption by chlorophyll a (680 m $\mu$  + 690 m $\mu$ ), if these two forms are simultaneously excited. This finding modifies Emerson's conclusion that chlorophyll a excitation alone is ineffective in photosynthesis. It is the excitation of the chlorophyll a forms (680 and 690 m $\mu$ ) which is ineffective. Perhaps, it is Chl a 690 form only which is ineffective. According to Emerson's conclusions, accessory

pigments have to be excited simultaneously with chlorophyll a. According to the present thesis, the function of accessory pigments can be taken over also by chlorophyll a 670 m $\mu$ ; this means that chlorophyll a does play the all-important role it was believed to play before the Emerson effect was discovered; it is the one pigment whose excitation can bring about photosynthesis. The following explanation of the Emerson effect can be suggested on the basis of the results described in this thesis. When both forms of chlorophyll a (Chl a 670 and Chl a 680 + 690) are excited, a properly balanced formation of two excited forms occurs, and full photosynthesis becomes possible.

When chlorophyll a absorbing at 670 m $\mu$  is alone excited, the excitation energy is transferred to the other forms of chlorophyll a, and properly balanced formation of the two excited states results, permitting full photosynthesis.

When the accessory pigments and the long-wave absorbing forms of chlorophyll a absorb light simultaneously, full photosynthesis occurs because accessory pigments transfer their energy preferentially to chlorophyll a 670 m $\mu$ , and properly balanced formation of two excited forms can take place.

When we excite long wave absorbing forms of chlorophyll a alone, full photosynthesis does not occur, because only one form is excited, and energy cannot flow from long-wave absorbers to short-wave absorbers for energetic reasons.

However, this statement should be made with some caution because in Porphyridium, we found no clear enhancement of yield in 680-700 m $\mu$  light by 670 m $\mu$  light, and had to invent ad hoc reasons for the absence of the Emerson effect in this spectral region.

## B. Purpose of the Existence of Accessory Pigments

Several already known facts about these pigments can be noted:

1) Chlorophyll a is always accompanied by some accessory pigments. In no photosynthetic organism it is found to be alone.

2) In organisms where chlorophyll a is accompanied only by carotenoids (other than fucoxanthol), e.g. in Ochromonas, the overall photosynthetic efficiency is poor. (Myers and Graham, 62). With Polyedriella, Emerson (cf. Emerson and Rabinowitch, 34) found that the quantum yield is poor, when the organism is cultured in inorganic medium. Allen (2) found that a chlorophyll b deficient mutant of Chlorella has very low photosynthetic efficiency.

3) Accessory pigments such as the phycocyanins in blue-green algae, the phycoerythrins in red algae, fucoxanthol and chlorophyll c in diatoms and chlorophyll b in green algae, have a very high efficiency of transfer of their excitation energy to chlorophyll a, as learned from studies of sensitized fluorescence.

4) The quantum yield of photosynthesis, in the region where these pigments absorb strongly, is high.

5) When these pigments are excited along with the long-wave absorbing forms of chlorophyll a, high rate of photosynthesis occurs, i.e. their excitation raises the yield of photosynthesis in far red light.

6) Fucoxanthol, phycocyanin, and phycoerythrin absorb mainly in a spectral region where the absorption by chlorophyll a is low. However, chlorophyll b does not markedly enhance absorption, because its absorption spectrum overlaps considerably that of chlorophyll a.

From observations of the fifth kind, described above, Emerson and Chalmers (25) concluded that accessory pigments must have a more important

purpose than simply to help plants to absorb more light in spectral regions where chlorophyll a absorption is weak. Emerson's arguments are very strong, but the fact that Chl a 670 also can raise the yield of photosynthesis in the light absorbed by the long-wave forms of chlorophyll a, calls for a modification of Emerson's conclusions. Appearance of peaks in the action spectra of the Emerson effect due to Chl a 670, as well as of those due to accessory pigments has been interpreted earlier in this thesis as evidence of a primary role of chlorophyll a 670; the quanta absorbed by the accessory pigments can be transferred by resonance to Chl a 670; and the excitation of the latter can be distributed in a "balanced way" (whatever this term may imply) between this pigment and Chl a 680 + 690. This "balanced" excitation of two chlorophyll a forms may be needed to bring about high rate of photosynthesis.

This hypothesis is proposed because it reconciles the action spectra of photosynthesis with the action spectra of the excitation of chlorophyll a fluorescence. If accessory pigments would participate in photosynthesis directly, one would have to ask: why do they contribute to the fluorescence of chlorophyll a with the same relative efficiency as they contribute to photosynthesis?

However, an alternate hypothesis is at least possible: When accessory pigments and the long-wave absorbing forms of chlorophyll a are excited simultaneously, a balanced formation of two excited states (one in the accessory pigments and the other in the long-wave forms of chlorophyll a) permits high rate of photosynthesis. A transfer of energy to chlorophyll a 670 may be unnecessary, (and perhaps impossible because of spatial separation of the pigments). Duysens' results only show that light absorbed by the accessory pigments causes sensitized fluorescence of chlorophyll a. Suppose that only one of the long-wave absorbing forms of chlorophyll a, say Chl a 680, is fluorescent, but is in itself inefficient in photosynthesis. The fluorescence band of chlorophyll

a that Duysens (and others) have observed lies at 685 m $\mu$ . This is the peak of the fluorescence band excited by absorption at 680 m $\mu$  (see Figure 31). We can postulate that accessory pigments sensitize the fluorescence of chlorophyll a 680, and when excited simultaneously with the 680 m $\mu$  form of chlorophyll a, produce photosynthesis because of balanced formation of two excited states.

When chlorophyll a 670 is excited alone, it too can transfer its energy to chlorophyll a 680 producing fluorescence of the latter. Balanced formation of two excited pigment states becomes possible and full-rate photosynthesis ensues. When Chl a 670 and Chl a 680 + 690 are excited simultaneously, the same thing happens. In this hypothesis, accessory pigments act as substitutes for chlorophyll a 670 in the region where the fraction of light absorbed by chlorophyll a 670 is small, and not as suppliers of energy to Chl a 670.

This hypothesis is somewhat less plausible, because it suggests that the same photochemical function can be carried out by several different excited pigments, whose only common property is that their excitation energies are higher than that of Chl a 680 + 690. It seems more plausible to postulate a common physical property--the capacity to transfer their excitation energy to Chl a 670 m $\mu$  as acceptor.

The question arises, however, why does efficient energy transfer stop at Chl a 670, and further transfer to Chl a 680+690 occurs only to the extent needed for "balanced" excitation of the two forms? Franck (37) proposed an interpretation of this "automatic balancing" in terms of a short-lived singlet and a long-lived triplet state, which are used in pairs for photosynthesis, so that singlet excited molecules can never accumulate.

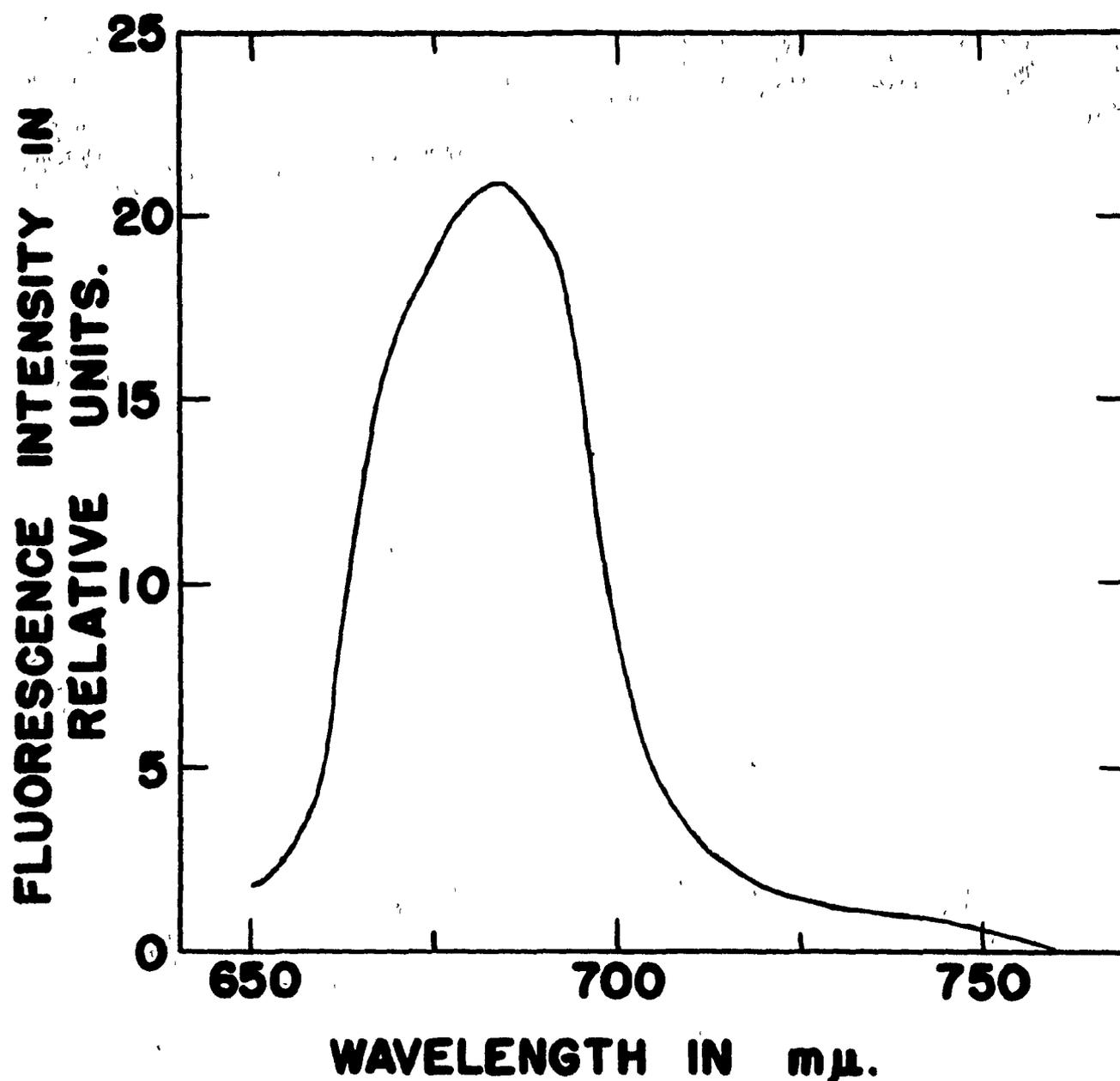


Figure 31. Fluorescence spectrum of *Chlorella pyrenoidosa*. A Bausch & Lomb monochromator, entrance slit of which was set at  $45^{\circ}$  to the exciting beam, was used to scan fluorescence. Exciting beam was 680 mμ obtained from another monochromator. RCA photomultiplier 6217 and Multiflex galvanometer was used to amplify and record fluorescence intensity. (Interval of measurement = 5 mμ).

Obviously, the hypothesis of Chl a 670 as a "catch basin" for all energy absorbed by the accessory pigments, has its difficulties, and only further investigations can decide whether it is correct.

C. Effect of Combined Lights of Different Wavelengths on Other Photochemical (or Photophysical) Processes

Effects of two beams of different wavelengths on some other processes have been studied in our laboratory. R. Govindjee, Thomas and Rabinowitch, (44) reported that the combination of far red light with supplementary light of shorter wave length has the same effect as in photosynthesis, also in the reduction of quinone by Chlorella (Hill reaction in whole cells). Existence of an Emerson effect in the Hill reaction is a strong indication--as pointed out by R. Govindjee et al. (44)--that this effect is associated with the part of photosynthesis which leads to the evolution of oxygen, rather than with the fixation and reduction of CO<sub>2</sub>. Their observations also indicate that the Emerson effect is not due to a decrease in the rate of respiration (which could conceivably simulate an increase in the rate of photosynthesis).

The effect of two beams of light on the fluorescence of Chlorella also was studied; for results see a preliminary report by Govindjee, Ichimura, Cederstrand and Rabinowitch (41). At the light intensities used, a quenching of fluorescence can take place when blue light (or red) light of 670 m $\mu$ , are applied simultaneously with 700 m $\mu$  light. This result suggests that a negative Emerson effect is possible in fluorescence.

It was also found by Ichimura (48, Figure 8) that depending on the relative intensities used, either a stimulation or a quenching of photoconductivity can take place in dried chloroplasts when accessory light is supplied simultaneously with 850 m $\mu$  light; only a stimulation was noted with 700 m $\mu$

light. We have noted in Chapter 5 that a negative Emerson effect can be replaced by a positive Emerson effect by changing the relative intensities of the two beams; this is true also for the Emerson effect in the Hill reaction (R. Govindjee, 43). The same seems to apply to fluorescence. Obviously, further work is needed in this new and interesting field.

#### D. Other Factors Influencing the Long-Wave Decline of the Yield of Photosynthesis

Emerson et al. (27) observed that the "red drop" occurred earlier (i.e., shorter wave lengths) at higher temperatures (20°C) than at lower temperatures (5°C). The decline began at longer wavelengths also when Chlorella was grown in a medium containing earth extract or beef broth (cf. Emerson and Rabinowitch, 34). Polydriella, grown in a medium containing earth extract, did not show any red decline at all. The effects of organic nutrition and of temperature indicate that the long-wave absorbing forms, which are inefficient unless Chl a 670 or auxiliary pigments are excited simultaneously, can be made more efficient by chemical treatments or that these treatments change the distribution of chlorophyll between the several forms. It may also be that the presence of certain intermediate metabolites (derived from organic nutrients) may make "balanced excitation" of two chlorophyll forms unnecessary, or at least reduce the relative need for one of the two forms.

Precise studies of the shape of the red absorption band of the living cells seems to be called for to reveal possible changes in the relative amounts of the several Chl a components, caused by different pre-treatments, changes in temperature, light conditions, etc. Such studies are now being carried out in our laboratory by Mr. C. Cederstrand.

## APPENDIX 1

## LIGHT AND RESPIRATION

As mentioned in Chapter 2, measurement of photosynthesis required respiration correction. During Emerson's early experiments with Chlorella, it was realized that blue light causes an increase in respiration, which persists in darkness (Emerson and Lewis, 32). This did pose a problem for the determination of the respiration correction in photosynthesis. One cannot be sure of respiration in light, if it changes considerably (sometimes up to 50%) in the time between the beginning and the end of the light exposure. According to a suggestion of Emerson and Lewis (32), the cells were exposed for 20-30 minutes to blue light before measuring the photosynthesis in that light. This gave unchanged rates of respiration before and after the light period used for the measurement of photosynthesis. Similar pre-illumination was not necessary when green, orange or red light was used, because these did not affect respiration significantly. The action spectrum of the blue light effect on respiration has a peak at about 480 m $\mu$ ; Emerson and Lewis (32) suggested that it may be due to the carotenoids. Figure 32 shows a plot of the maximum change (in per cents) in respiration (caused by seven minute exposure of light), against wavelength of light; it confirms that the carotenoids are the most likely compounds to be made responsible for it. Alternatively, the blue light effect may be due to some other pigment, such as a flavin.

It was observed that all cultures of algae did not behave the same way as regards effect of light on respiration was concerned. In Chlorella, some cultures did not show any effect at all. Percent increase in respiration caused by 480 m $\mu$  light varied from 0 to 50%. Light energy used was of the order of  $1-10 \times 10^{-8}$  einsteins/cm<sup>2</sup>/min.

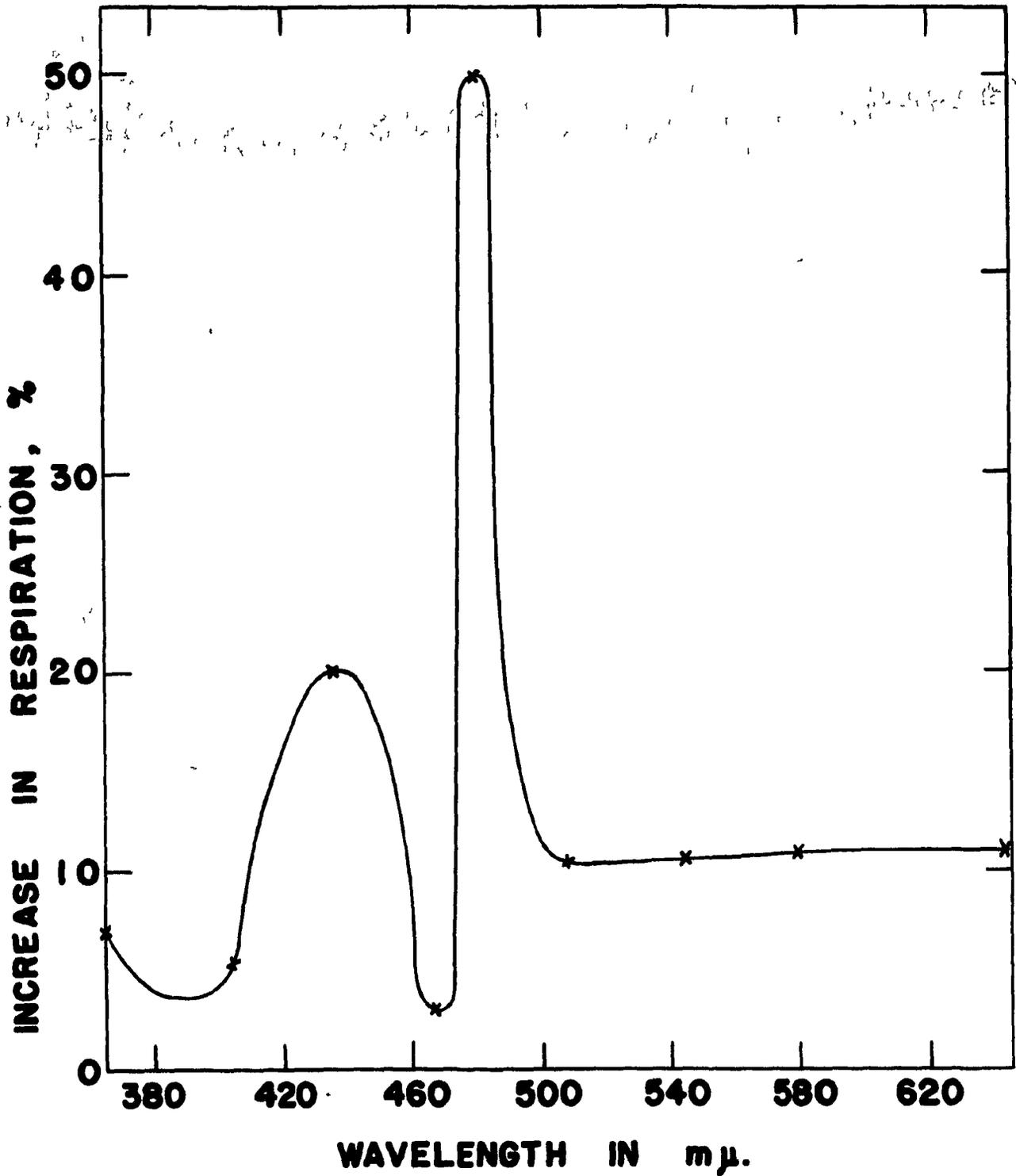


Figure 32. Percent increase in respiration of Chlorella pyrenoidosa, after a preillumination period of seven minutes, with different wavelengths of light.

The effect of blue light on the respiration of Chlorella lasted for almost 15 minutes after the end of irradiation; it was not exactly the same after the second and third exposures.

It appears that if cells were kept in darkness for sometime and then exposed to light, effect was stronger although 'dark adaptation period' was not necessary.

In Anacystis also, an effect of light on respiration could be noted. In this case, too, pre-illumination was an effective means to stabilize respiration. Porphyridium (cf. Brody, 4) and Navicula (cf. Tanada, 75) were affected in the same way. To sum up, the effect could be observed in all organisms studied (cf. Gaffron, 40 and Brown, 10). No further study of this subject was carried out because I was not directly interested in the problem, and my only concern was with obtaining cells with a steady rate of respiration.

## APPENDIX 2

STANDARDIZATION OF BOLOMETER<sup>1</sup>A. Calibration of Bolometer Against a Radiation Standard

The objective was to find the number of micro volts required to balance the wheatstone bridge when a known number of watts was absorbed per second by the bolometer surface. According to specifications of the U. S. Bureau of Standards, the bolometer was placed at a distance of two meters from a specified mark on a lamp. First, using a secondary standard, the response of the bolometer was established in relation to the portion of its surface that was illuminated. Diaphragms of different sizes were used for this determination. The following table (Table 11) shows the result.

Table 11

RESPONSE OF BOLOMETER IN RELATION TO  
THE AREA OPEN TO LIGHT

Diameter of the diaphragm A, mm	Area of the diaphragm B, cm <sup>2</sup>	Output C, $\mu$ volts	Response D = C/B
☒ 32.1 x 29.1	9.33	109.4, 109.1	11.70, 11.74
	30.1	86.0, 86.8, 86.5	12.08, 12.14, 12.18
	26.8	68.6, 69.1	12.16, 12.25
☒ 21.2 x 21.9	4.64	59.6	12.84
	22.8	49.1	12.03
	19.8	37.7	12.20
	16.2	26.8	13.01

☒ Square diaphragms, length and breadth given instead of diameter.

It was noticed that, except for the largest and the smallest diaphragm, all others led to almost the same sensitivity. Once this was determined, one diaphragm was selected (area = 7.12 cm<sup>2</sup>, diameter = 30.1 mm) and the sensitivity of bolometer (volt/watt) was determined at three different currents (see Table 12).

<sup>1</sup>I am specially grateful to late Professor Robert Emerson and Mr. Carl Cederstrand for their help.

Table 12

## SENSITIVITY OF BOLOMETER AT DIFFERENT CURRENTS

A	B	C	D	E	F
Current in Amperes	Energy flux in micro watts/cm <sup>2</sup> according to the U. S. Bureau of Standards	Energy flux in micro watts/cm <sup>2</sup> going through the fluorite window*	Energy flux x exposed area of the bolometer	E. M. F. in micro volts needed to balance the bridge when the bolometer was illuminated	Sensitivity = E/D μ volt/μ watt
0.300	45.90	42.3	301.1	57.24	0.1903
0.350	64.3	59.0	420.0	79.30	0.1888
0.400	85.6	78.6	559.8	106.00	0.1895
				average	0.1895

\* Bolometer had a fluorite window when in use. Some light energy was lost on two interfaces and had to be taken into account.

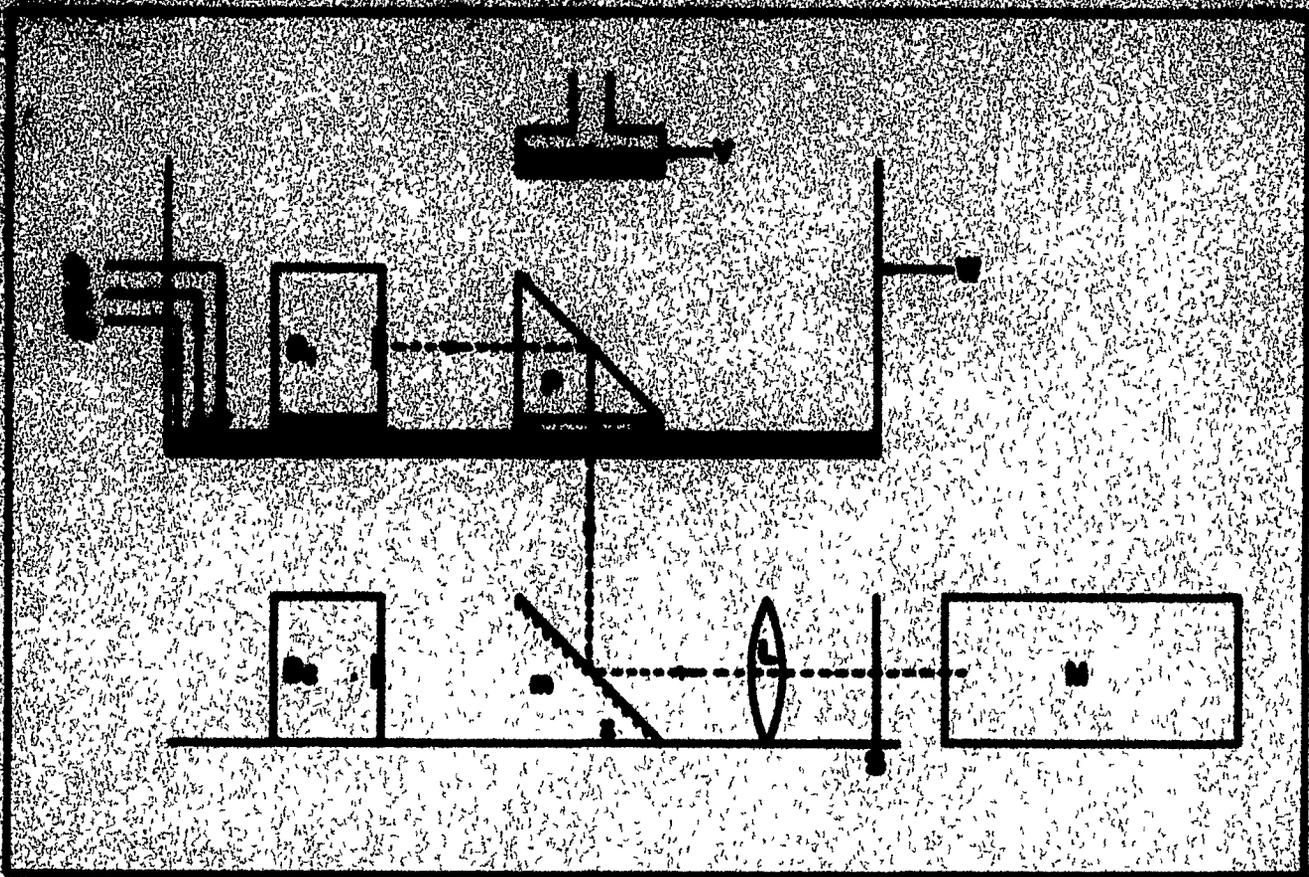
The sensitivity of Stenzell Bolometer used in all the experiments was determined to be 0.1895  $\mu$  volt/ $\mu$  watt in November, 1958 by Emerson, Cederstrand, Govindjee, and R. Govindjee (22); a previous determination, made in 1950 by Emerson and Morgan (33), gave 0.206. Thus, in 8 years, only a small loss of sensitivity has occurred.

**B. Evaluation of the Conversion Factors for the Conversion of the Bolometer Output in Micro Volts into Energy Flux Incident on the Algal Suspension Contained in the Manometric Vessel, in Micro Einsteins/Second.**

Step 1. The bolometer output (read by bolometer at position  $B_2$ ; see Figure 33) was converted into ergs/second and then into microeinsteins/second-- as explained below.

The sensitivity of the bolometer (obtained in Part A) was 0.1895 microvolt/microwatt. This meant that an output of 1 microvolt was due to 5.29 (=  $1 \div 0.1895$ ) microwatts falling on the bolometer surface. If the bolometer output at position  $B_2$  was  $Q$ , the energy falling on the bolometer at the same position was 5.29  $Q$  microwatt or 52.9  $Q$  ergs/second (as 1 microwatt = 10 ergs/sec.). The energy in ergs/second was easily converted to energy in einsteins/second by using a proper factor. This factor can be represented as  $E = \text{ergs/einstein} = Nhc/\lambda$ , where  $N = \text{Avogadro's number} = 6.023 \times 10^{23}$ ,  $h = \text{Planck's constant} = 6.624 \times 10^{-27}$ ,  $c = \text{wavelength of light} = 2.998 \times 10^{10}$  cm/sec.,  $\lambda = \text{wavelength of light (in } \text{\AA} \text{ units)} \times 10^{-8}$  cms. If numerical values for  $N$ ,  $h$  and  $c$  are substituted in the equation given above,  $E$  becomes  $\frac{1.196 \times 10^8}{\lambda}$  ergs/einstein. The energy falling on the bolometer surface, (at position  $B_2$ ) thus becomes  $\frac{Q \times 52.9}{1.196 \times 10^8 \lambda (\text{\AA}) \times 10^{-8}}$  einsteins/second. This equals  $Q \times \lambda (\mu\mu) \times 4.423 \times 10^{-8}$  microeinsteins/second and is referred as  $M_2$ .

$$M_2 = Q \times \lambda (\mu\mu) \times 4.423 \times 10^{-8} \text{ microeinsteins/sec.}$$



**Figure 33.** Optical arrangement for corrections to be made for differences in optical path between measurement of energy at position  $B_2$  and energy incident on algal suspension (A). M: monochromator; S: exit slit of monochromator; L: lens; M: movable mirror on axis x;  $B_1$  and  $B_2$ : two positions of Bolometer;  $G_1$ ,  $G_2$ , and  $G_3$ : glass plates; P:  $45^\circ$  prism; W: water tank; V: vessel; A: algal suspension.

Note that the diagram has not been drawn to scale; distance between monochromator light source and the algal suspension (A) is the same as that between the former and the bolometer ( $B_2$ ).

Step 2. The energy flux on the bolometer (at  $B_2$ ) in microeinsteins/second was then converted to give the energy flux incident on the algal suspension (A) contained in vessel (V) (see Figures 3 and 33).

First, difference in optical path between light reaching  $B_2$  (Figure 33) or B (see Figure 3) and A was noted. It can be seen from Figure 3 (set up for photosynthesis measurements) that when light reaches A, it has to pass through three glass plates ( $g_1$ ,  $g_2$  and  $g_3$ ), water in the water bath (W) and the bottom of the vessel (V). In doing so, some energy is lost at the interfaces. Loss of light energy through the glass plates was measured and losses at other interfaces was calculated by using Fresnel's formula,  $\alpha = \left(\frac{n-1}{n+1}\right)^2$ , where  $\alpha$  = fraction of light reflected at the interface and  $n$  = refractive index.

Let  $Y$  be the light energy in einsteins/second inside the glass plate ( $g_1$ ) just before it has come out in the water bath (W), when a beam of light is being reflected up by the mirror M. A prism (P) made of quartz was placed as shown in Figure 33. The energy read by the bolometer at  $B_1$  is equal to  $Y \times f$  ( $f$  = fraction transmitted by prism)  $\times .87$ , where .87 is the fraction transmitted after the losses at the interfaces described below. When the measurement at position  $B_1$  was made, the water bath was dry and the interfaces were: glass ( $g_1$ ) to air, air to quartz prism (P) and prism (P) to air. Losses at these interfaces were calculated from Fresnel's law. They were .05 for glass to air, .046 for quartz to air and .046 for air to quartz. From these values, the fraction transmitted (= .87), noted above, was obtained.

For the same beam of light, measurements were made both at position  $B_1$  and  $B_2$  and a fraction called  $F$  was determined.  $F = \frac{M_1}{M_2}$ , where  $M_1$  = energy read by bolometer at position  $B_1$  and  $M_2$  = energy read by bolometer at position  $B_2$ . From the above relation,  $M_1 = F \times M_2$ ;  $M_1$  is also equal to  $Y \times f \times .87$ , as

noted in the previous paragraph. Thus,  $Y = \frac{F \times M_2}{f \times .87}$ . A relation between Y and the measured quantities F,  $M_2$  and f was obtained. The quantity f was measured directly by using two prisms; first energy was measured when light passed through both the prisms and then when the experimental prism was removed from the light path. The fraction of light transmitted through the experimental prism was measured at different wavelengths. A curve was plotted which gave values of f at any desired wavelength. The prism had a higher absorption in the ultra-violet ( $\sim 50\%$ ) than in the red ( $\sim 15\%$ ).

After a relation between Y and the measured quantities F,  $M_2$  and f was determined, one needed to spell out a relation between the energy flux incident on the algal suspension (A) and the measured parameters. Let Z be the incident energy flux on the algal suspension. Now,  $Z = Y (.987)$ , where .987 is the fraction of Y that gets transmitted up to A. There are three interfaces at which energy is lost in this light path- glass ( $g_1$ ) to water (because when photosynthesis measurements are made and that is when energy measurements are required, tank (W) is filled with water), water to glass (of vessel, V), and glass to water (vessel to algal suspension). The fraction transmitted (i.e. .987, given above) was calculated by correcting for losses at these three interfaces; at each interface the loss was .05. Then,

$$Z = Y \times (.987) = \frac{F \times M_2 \times .987}{f \times .87}, \text{ (as Y is equal to } \frac{F \times M_2}{f \times .87} \text{ )}.$$

In case  $M_2$  was measured through a cellophane window, the correct value for Z is --  $\frac{F \times M_2 \times .987}{f \times .87 \times .91}$ , as .91 is the calculated fraction transmitted by the

cellophane window. From Step 1,  $M_2$  equals  $Q \times \lambda_{\mu} \times 4.423 \times 10^{-8}$ . Therefore,  
 $Z = \frac{F \times Q \times \lambda_{\mu} \times 10^{-8} \times 4.423 \times .987}{.87 \times .91 \times f}$  The correction factor at wavelength,

$\lambda_1$ , to convert the bolometer output in  $\mu$  volts (denoted as Q here) to energy flux in  $\mu$  einsteins/second incident on algal suspension, equals--

$$\frac{F \text{ (measured)} \times \lambda_1 \text{ (m}\mu\text{)} \times 4.423 \times 10^{-8} \times .987}{.87 \times .91 \times f \text{ (at } \lambda_1\text{)}} (= K).$$

This correction factor (K) was plotted against wavelength of light and a calibration curve was obtained. Using the latter curve one can convert microvolts produced by a light beam when the bolometer is at position B<sub>2</sub> (Figure 33) or B (Figure 3) into microeinsteins per second incident on the algal suspension (A).

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## VITA

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