

CHAPTER 12

LIGHT ABSORPTION, EMISSION AND PHOTOSYNTHESIS

GOVINDJEE AND BARBARA ZILINSKAS BRAUN

Department of Botany,
University of Illinois,
Urbana, Illinois 61801, U.S.A.

1	Introduction	346	5	Light-induced absorption changes and photosynthesis	373
2	Absorption Spectra	353	5.1	P680-P690	375
2.1	Pigments in solution	353	5.2	C550	376
2.2	Chlorophylls	356	5.3	P430	376
2.3	Carotenoids	361	6	Adaptation	377
2.4	Phycobilins	363	7	References	379
3	Separation of algal photosynthetic systems	365			
4	Composition of the two pigment systems	369			

1 INTRODUCTION

Solar radiation provides the energy to maintain life on earth through photosynthesis—the process by which green plants convert light energy into chemical energy. The first act of photosynthesis is light absorption. Out of all the electromagnetic radiation falling on photosynthesizing plants (including algae) only the visible light (wavelength (λ) range, 400 to 720nm) is absorbed and used for photosynthesis. This information is obtained by the measurements of the absorption spectra (absorbance as a function of wavelength of electromagnetic radiation) and of the action spectra of photosynthesis (O_2 evolution per incident quantum as a function of wavelength of light).

Algae have evolved various pigments for the purpose of light absorption; these can be classified into three major groups: (1) *chlorophylls* (Chl) that strongly absorb blue and red light (see Chapter 5, p. 161)—examples are Chl *a* (present in all algae) and Chl *b* (present in green algae); (2) *carotenoids* that absorb blue and green light—examples are β -carotene (present in all algae) and fucoxanthin (present in brown algae) (see Chapter 6, p. 176); (3) *phycobilins* that

absorb green, yellow and orange light (see Chapter 6, p. 194). Examples are R-phycoerythrin (present in red algae) and C-phycocyanin (present in blue-green algae). These bulk pigments, called such as they are present in large quantities, provide the algae with *antennae* to capture the light energy.

Absorption spectra of algae provide us with the knowledge of the types and concentrations of pigments present in them. Such a knowledge is necessary for the complete understanding of photosynthesis, and it can also be used for taxonomic and phylogenetic purposes. Likewise, absorption spectra have furnished information regarding the adaptability of algae to varied environments. The photochemist has used absorption measurements in determining the natural lifetime of the excited state as well as the energy levels of the molecules under study. Absorption measurements are absolutely essential for calculations of quantum yields of photosynthesis (number of molecules of O_2 evolved or of an intermediate phototransformed into its oxidized or reduced form per absorbed quantum) and of Chl *a* fluorescence (number of quanta emitted per quanta absorbed) as we need these measurements for the calculation of the number of absorbed quanta (N_a) from the number of incident quanta (N_i) ($N_a = N_i \times$ fractional absorption). The quantum yield of oxygen evolution (ϕ_{O_2}) sets up the framework for the feasibility of various theories of photosynthesis; the quantum yield of a specific intermediate reaction allows us to judge whether it is in the main path of the process or not. Quantum yields of Chl *a* fluorescence (ϕ_f) and of O_2 evolution as a function of wavelength are necessary for calculations of the efficiency of excitation energy transfer from one pigment to another.

In the first approximation, absorption spectra can be used to predict photosynthetic spectral response as in the case of green algae, brown algae, diatoms and dinoflagellates (Emerson & Lewis 1943, Haxo & Blinks 1950, Tanada 1951, Haxo 1960). However, in these cases, the predictions are approximate because of the relative inefficiency of the carotenoids and the existence of the 'red drop' phenomena, that is, decline in the quantum yield of O_2 evolution (ϕ_{O_2}) and of Chl *a* fluorescence (ϕ_f) in the red end of the spectrum (Emerson & Lewis 1943, Duysens 1952, Emerson *et al.* 1957, Govindjee 1960, Govindjee 1963a, Das & Govindjee 1967, Szalay *et al.* 1967, Das *et al.* 1968, R. Govindjee *et al.* 1968, Williams *et al.* 1969). In red and blue-green algae, absorption spectra cannot be used at all for these purposes as the red drop begins well before the decline of the main absorption band of Chl *a* and is accompanied by a 'blue drop', that is, a drop in the yield in the blue end of the spectrum (Haxo & Blinks 1950, Duysens 1952, Brody & Emerson 1959a, Blinks 1960, Haxo 1960, Hoch & Kok 1961, Fork 1963, Papageorgiou & Govindjee 1967a).

Two Light Reactions. In photosynthesis, there are two primary photoreactions (I and II) and two pigment systems (photosystem I and photosystem II) that 'sensitize' them. This idea originated in the discovery of the Emerson enhancement effect (referred to by most authors as 'enhancement effect'; in earlier work it was called 'second Emerson effect' or simply 'Emerson effect'), that is, the rate of photosynthesis being greater when short wavelength light (absorbed mainly

by Chl *b* or another accessory pigment) and far red light (absorbed mainly by Chl *a*) are used simultaneously than the sum of the two when they were used separately (Emerson *et al.* 1957, Emerson & Rabinowitch 1960, French *et al.* 1960, Govindjee & Rabinowitch 1960, Govindjee 1963a, Blinks 1963, Myers 1963, R. Govindjee *et al.* 1964, Myers 1971). It is widely accepted that two light reactions, arranged in series, are involved in this process (Hill & Bendall 1960, Kautsky *et al.* 1960, Duysens *et al.* 1961, Witt *et al.* 1961, see Chapter 13, p. 391). The first light reaction—arbitrarily called light reaction II—leads to the reduction of a cytochrome and oxidation of H_2O to molecular O_2 ; the second light reaction (called light reaction I) results in reoxidation of the cytochrome and reduction of NADP. Along the electron transport pathway from H_2O to NADP, a fraction of light energy is utilized to synthesize ATP from ADP and inorganic phosphate (Fig. 12.1). With sufficient NADPH and ATP available, enzymatic reduction of CO_2 to the carbohydrate level (Calvin-Benson cycle) becomes possible. Such a scheme and its details have been confirmed and elaborated and can be found in various reviews: Hoch and Kok 1961 (general), Rabinowitch

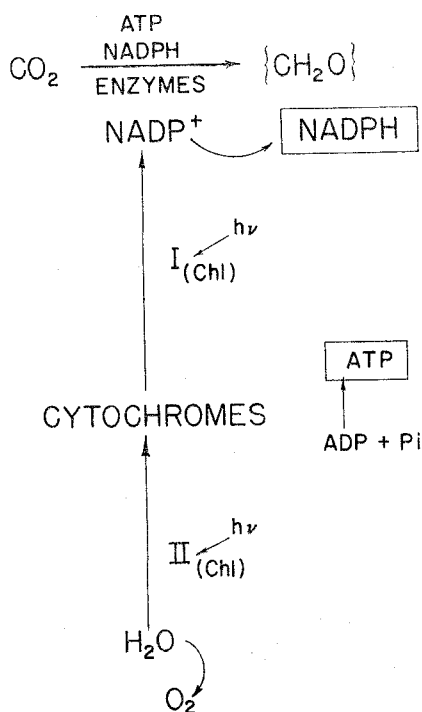


Fig. 12.1. Transfer of electrons (or hydrogen atoms) from water to carbon dioxide involving two light reactions. ADP, adenosine diphosphate; Pi, inorganic phosphate; ATP, adenosine triphosphate; NADP⁺, nicotinamide adenine dinucleotide phosphate; NADPH, reduced NADP⁺; I and II, light reactions; Chl, chlorophyll; (CH_2O), carbohydrate moiety; $h\nu$, light quanta.

1963 (general), Smith and French 1963 (pigments); Duysens 1964 (biophysical aspects), Robinson 1964 (physico-chemical aspects); Vernon and Avron 1965 (biochemical aspects), Witt 1967 (fast absorbance changes), Avron and Neumann 1968 (photophosphorylation), Bendall and Hill 1968 (haem proteins), Hind and Olson 1968 (electron transport), Weaver 1968 (electron spin resonance studies), Fork and Ames 1969 (energy transfer), Levine 1969 (mutants), Rabinowitch and Govindjee 1969 (general mechanisms), Boardman 1970 (physical separation of pigment systems), Cheniae 1970 (O_2 evolution), Packer *et al.* 1970 (ion movements and structural changes), Bishop 1971 (intermediates), Clayton 1971 (general, cooperation of pigment systems), Govindjee and Papageorgiou 1971 (chlorophyll fluorescence), Park and Sane 1971 (structure and function), Govindjee and Mohanty 1972 (photosynthesis of blue-green algae); see also Chapter 13, p. 391.

However, several other models have been proposed in the last ten years. Franck and Rosenberg (1964), Govindjee *et al.* (1967) (see Jackson & Volk 1970), Arnold and Azzi (1968) and Knaff and Arnon (1969) have proposed alternate models. References are given here so that the reader may consult them to keep an open mind toward future developments in this field. In this connexion, the experiment of Rurainski *et al.* (1971) is worth mentioning in which they could not find a relationship between NADP reduction and the energy trap of photosystem I in chloroplasts treated with $MgCl_2$ —the implication being that NADP can be reduced by a system (photosystem II) independent of photosystem I!

Two Pigment Systems. It appears that most of the pigments are present in both photosystems but in different proportions; for example, photosystem II of the green algae has a lower ratio of Chl *a/b* than photosystem I, and those algae containing phycobilins usually have a larger proportion of these pigments in photosystem II. Another complexity is that as Chl *a* does not exist as a single homogeneous species *in vivo*, but rather in several spectroscopically distinguishable forms labelled Chl *a* 660, Chl *a* 670, Chl *a* 680, Chl *a* 690, Chl *a* 695, and Chl *a* 705, according to the location of the absorption maxima in the red end of the spectrum (see Butler & Hopkins 1970a,b, and French 1971). The chemical nature of these forms is not yet known. It has been suggested (e.g. by Seely 1971) that the existence of so many forms increases the efficiency of excitation energy transfer to the reaction centres; this may be the reason why algae evolved them.

Many experiments, especially the spectral measurements of separated photosystem II and photosystem I fractions from algae and higher plants, suggest rather strongly that a larger proportion of the long wavelength Chl *a* forms is found in photosystem I, while the reverse holds for photosystem II. The fact that the long wavelength form of Chl *a* is non-, or weakly, fluorescent at room temperature (being an aggregate form, it loses more energy by radiationless transitions as it has more modes of interaction than do its monomer molecules) makes the localization of these Chl *a* forms in one or the other photosystem amenable to fluorescence techniques that are often far more sensitive than absorption measurements. For instance, Brown (1969) has shown for algae of

several groups a heavy, photosystem II enriched fraction that is significantly more fluorescent than the light, photosystem I fraction, and has correctly inferred from these and other data that the long wavelength Chl *a* forms are preponderant in the photosystem I fraction. Emission spectra are equally helpful. Thus certain Chl *a* forms at low temperatures (77°K or 4°K) fluoresce strongly at 710 to 740nm, while fluorescence at 685 and 695nm is due mainly to the short wavelength Chl *a* forms. There are some specialized pigment molecules (traps) to which the energy absorbed by the bulk pigments is transferred. These traps, present in small quantities (one per several hundred bulk Chl molecules), are simply Chl *a* molecules in specialized environments. The trap molecules, also called *reaction centres*, perform the primary photochemical reactions of photosynthesis transforming light energy, now in the form of 'excitons', into chemical energy. The primary oxidation-reduction reaction is:



where D is the primary donor of electrons, A the primary acceptor of electrons $h\nu$ the photon or exciton, T the energy trap, and T* the excited energy trap. Two types of reaction centres have been implicated: P700 (Kok 1957, 1959, 1961, Rumberg & Witt 1964, Witt 1967) and P680-P690 (Döring *et al.* 1967, 1968, 1969, Govindjee *et al.* 1970, Floyd *et al.* 1971), where P stands for pigment and the number following for the location of the long wavelength absorption band, in nanometres (nm). P680-690 has only been measured in chloroplasts from higher plants. However, its existence in algae has been inferred from a new fluorescence band at about 695nm appearing under conditions when photosynthesis is saturated or absent (Bergeron 1963, Brody & Brody 1963, Govindjee 1963a, Krey & Govindjee 1966, Cho & Govindjee 1970a, Govindjee & Briantais 1972, see also Satoh 1972). Most bulk pigments, except carotenoids (although there are conflicting reports), emit some light as fluorescence upon absorption of light, i.e. all the absorbed energy is not used for chemistry. In solution, extracted algal pigments have a high yield of fluorescence, but *in vivo* the yield is low as most energy is used for chemistry (Latimer *et al.* 1957, Weber 1960). The fluorescence yield of Chl *a* (ϕ_f) in algae is related to the chemical reactions of photosynthesis by the following relationship:

$$\phi_f = \frac{k_f}{k_f + k_r + k_t + k_c[T]} \quad (1)$$

where k_f is the rate constant of fluorescence (i.e. the number of transitions per sec. leading to fluorescence), k_r for radiationless loss, k_t for energy transfer from fluorescent to non- or weakly fluorescent Chl *a*, k_c for chemical reactions, and $[T]$ is the concentration of the 'open' traps, i.e. traps that are in a state ready to perform chemistry. This relationship tells us that fluorescence yield can be used as a monitor of photochemistry in algae. (For background in the physical aspects of the interaction of light with matter, see Clayton 1965, 1971, and Rabin-

owitch & Govindjee 1969, and for a detailed discussion of Chl fluorescence studies in photosynthesis see, Rabinowitch 1951, 1956, Weber 1960, Duysens 1964, Butler 1966a,b, Goedheer 1966a, Govindjee *et al.* 1967, Hoch & Knox 1968, Fork & Ames 1969, Govindjee & Papageorgiou 1971).

Table 12.1. The quantum efficiency* of each decay process of the excited first singlet state of Chl *a* *in vivo* for photosystem II (after Mar *et al.* 1972).

Sample	ϕ_f	ϕ_h	ϕ_t
<i>Chlorella</i>	0.04	0.30	0.66
<i>Porphyridium</i>	0.02	0.30	0.68
<i>Anacystis</i>	0.02	0.38	0.60

* $\phi_f = \frac{k_f}{k_f + k_h + k_t}$; $\phi_h = \frac{k_h}{k_f + k_h + k_t}$; $\phi_t = \frac{k_t}{k_f + k_h + k_t}$, where *k*'s refer to the rate constants, and the subscripts *f*, *h* and *t* are for fluorescence, heat loss and energy transfer to weakly fluorescent photosystem I, and trapping respectively.

Excitation Energy Migration. Light energy absorbed by carotenoids, phycobilins and Chl *b* is transferred to Chl *a* leading to fluorescence of Chl *a*. This 'sensitized fluorescence' has been used in determining the efficiency of excitation energy transfer in photosynthesis from the various accessory pigments to Chl *a* (heterogenous energy transfer) (Dutton *et al.* 1943, Wassink & Kersten 1946, Duysens 1952, French & Young 1952, Tomita & Rabinowitch 1962, Ghosh & Govindjee 1966, Cho & Govindjee 1970b,c). Energy migration within molecules of the same kind (homogeneous energy transfer) is often demonstrated by the depolarization of fluorescence (Arnold & Meek 1956, Mar & Govindjee 1971), by the concentration quenching of fluorescence or by the observation that the quantum yield of the primary photochemical reaction of photosynthesis is close to 1.0. However, the quantum yield of O₂ evolution in algae is 0.12 as there are 8 primary photochemical reactions in the evolution of one molecule of O₂ when CO₂ is used as the oxidant (Emerson 1958, R. Govindjee *et al.*, 1968).

Lifetimes of Excited States. Strongly absorbing compounds have short radiative lifetimes while weakly absorbing compounds have long ones (see Rabinowitch 1957, Calvert & Pitts 1966). The radiative life of chlorophyll *a* in solution is about 15 nsec. (Brody & Rabinowitch 1957). It is difficult to calculate the exact radiative life for chlorophyll *a* *in vivo* because of the presence of different forms of Chl *a* as well as Chl *b* or other accessory pigments, and the absence of definite information regarding the extinction coefficient *in vivo*, but making certain assumptions we can calculate an order of magnitude of about 20 nsec. However, the measured lifetime of the excited state of Chl *a* in algae (*Chlorella*, *Porphyridium* and *Anacystis* sp.) is of the order of 0.5 to 2 nsec. (Brody & Rabinowitch 1957, Tomita & Rabinowitch 1962, Müller *et al.* 1965, Murty & Rabinowitch 1965, Nicholson & Fortoul 1967, Singhal & Rabinowitch 1969, Merkelo

Table 12.2. Spectral bands of chlorophylls (after Smith & Benitez 1955, also see Klein & Cronquist 1967).

Pigment and Solvent	Absorption Maxima and Specific Extinction (Base 10) coefficients						Fluores- cence*	
	Chl <i>a</i> (Ethyl Ether)	410 (85.2)	430 (131.5)	534 (4.22)	578 (9.27)	615 (16.3)	662 (100.9)	669, 723
Chl <i>b</i> (Ethyl Ether)	430 (62.7)	455 (174.8)	549 (7.07)	595 (12.7)		644 (62)	649, 708	
Chl <i>c</i> (Ethyl Ether)	(417)	444 (277)	(545)	578 (20.6)		626 (22)	629, 690	
Chl <i>d</i> (Ethyl Ether)	392 (58.4)	447 (97.8)	512 (1.98)	549 (4.03)	595 (9.47)	643 (14.3)	688 (110.4)	696, 752
	UV-Blue		Green-Yellow		Orange-Red		Red or I.R.	

* Italic figures correspond to the location of the main peak, the others to the location of the vibrational satellite band.

et al. 1969, Müller *et al.* 1969, Briantais *et al.* 1972, Mar *et al.* 1972). The observed discrepancies between the calculated and experimentally measured ϕ_f are due to the presence of non- or weakly fluorescent chlorophyll *a* in algae (see Govindjee *et al.* 1967 for refs.). From measurements of the lifetime of the excited state of Chl *a* in several algae, in the presence and the absence of photosynthesis, Mar *et al.* (1972) calculated the quantum efficiencies of fluorescence, of heat loss and of transfer to weakly fluorescent photosystem I (ϕ_h), and of energy trapping (ϕ_t) to be 0.02–0.04, 0.30–0.38, and 0.60–0.68 respectively (Table 12.1). Mohanty (1972) has calculated the efficiency of energy transfer from photosystem II to photosystem I to be of the order of 0.10 from the increase in the fluorescence yield by the addition of Mg^{2+} to 3-(3, 4-dichlorophenyl)-1, 1 di-methylurea-treated chloroplasts.

2 ABSORPTION SPECTRA

2.1 Pigments in solution

Pigments in intact cells can only be identified if we know the absorption spectra of known pigments in solutions. Thus, a brief discussion follows. No attempt is made here to cite and discuss all the work. Such data are reviewed elsewhere (Rabinowitch 1951, 1956, Rabinowitch & Govindjee 1969 (all pigments), O'hEocha 1965 (phycobilins), Goedheer 1966b (chlorophylls), Davies 1965 (carotenoids)). Recently, Singhal *et al.* (1968) have presented absorption spectra of chlorophyll and its derivatives at 77°K and have reviewed the past work in this area. The location of the absorption peaks of various chlorophylls are presented in Table 12.2 and Fig. 12.2.

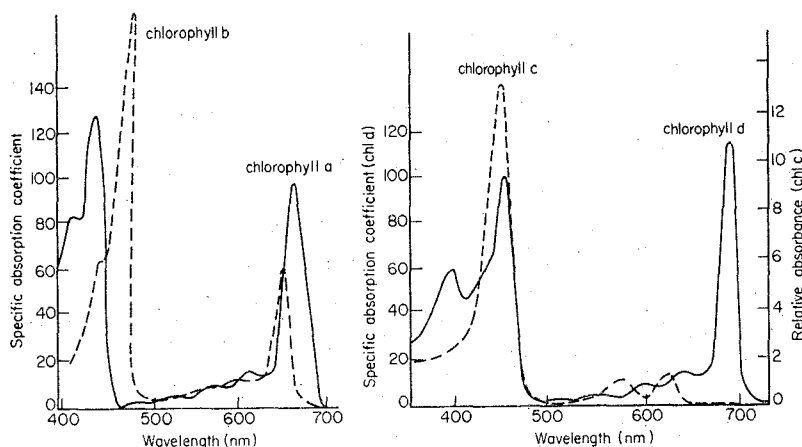


Fig. 12.2. Absorption spectra of chlorophylls in diethyl ether. Curves on the left, ———, Chl *a*; - - - - -, Chl *b*. Curves on the right, ———, Chl *d*; - - - - -, Chl *c* (after Smith & Benitez 1955, redrawn from Allen *et al.* 1960).

Table 12.3. The photosynthetic pigments (after Rabinowitch & Govindjee 1969).

A. The Chlorophylls*			
Type of Chlorophyll	Characteristic absorption peaks		Occurrence
	In organic solvents, nm	In cells, nm	
Chl <i>a</i>	420, 662	435, 670–680	All algae
Chl <i>b</i>	455, 644	(several forms) 480, 650	Green algae
Chl <i>c</i>	444, 626	(two forms?) Red band at 645	Diatoms and brown algae
Chl <i>d</i>	450, 690	Red band at 740	Reported in some red algae (?)
B. The Carotenoids***			
Types of Carotenoids	Characteristic absorption peaks, nm**		Occurrence
	I. Carotenes		
α -carotene	In hexane, at 420, 440, 470		In red algae and in siphonaceous green algae it is the major carotene Major carotene of all other algae
β -carotene	In hexane, at 425, 450, 480 (the 480nm band may be shifted to 500nm <i>in vivo</i>)		
II. The Xanthophylls			
Lutein	In ethanol, at 425, 445, 475		Major carotenoid of green algae and red algae Major carotenoid of diatoms and brown algae
Fucoxanthin	In hexane, at 425, 450, 475 (<i>In vivo</i> , absorption extends to 580nm)		
C. The Phycobilins***			
Types of Phycobilins	Absorption peaks		Occurrence
Phycocerythrins	In water, and <i>in vivo</i> : 490, 546, and 576nm		Main phycobilin in red algae; also found in some blue-green algae
Phycocyanins	At 618nm, in water and <i>in vivo</i>		
Allophycocyanin	At 654nm, in phosphate buffer (at pH 6.5) and <i>in vivo</i>		Found in blue-green and red algae

* For fuller details see Chapter 5, p. 161.

** It has been difficult to establish the exact location of carotenoid bands *in vivo* (except in the case of purple bacteria) because of their strong over-lapping with the blue-violet bands of chlorophylls. The bands *in vivo* are estimated to be shifted by about 20–40nm to the long wavelength side from their position in solution.

*** For fuller details see Chapter 6, p. 176.

The main feature to remember is that the spectra of chlorophylls as well as of carotenoids are shifted upon extraction (Emerson & Lewis 1943). For example, the red peak of Chl *a* shifts from 675nm *in vivo* to 662nm in diethyl ether, whereas the blue peak shifts from 430nm to 435nm. In carotenoids, the shifts could be larger, e.g. one of the three peaks shifts from 470nm to 500nm. Fig. 12.3 shows

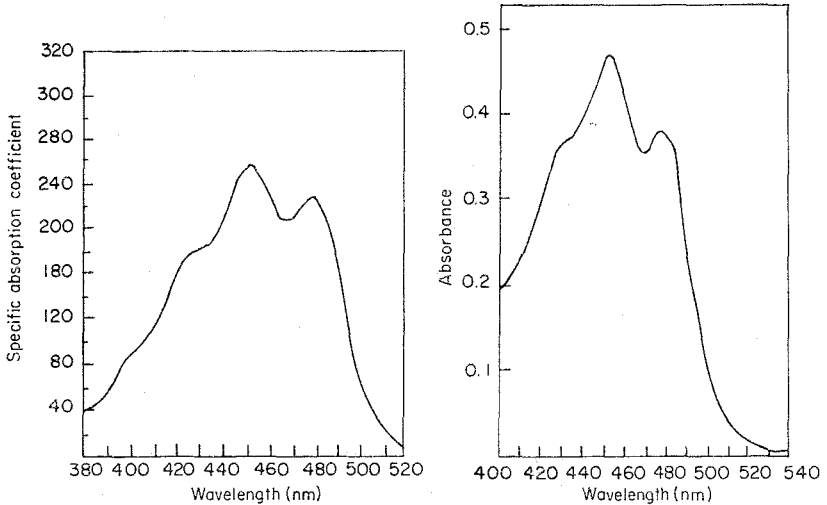


Fig. 12.3. Absorption spectra of β -carotene (left) and fucoxanthin (right) in hexane. Redrawn from Rabinowitch (1951); see the latter paper for references to original work.

absorption spectra of two well known carotenoids *in vitro*. Water soluble phycobilins, however, do not show any shifts. (See Table 12.3 for shifts observed in various pigments.) Fig. 12.4 shows absorption spectra of two phycoerythrins

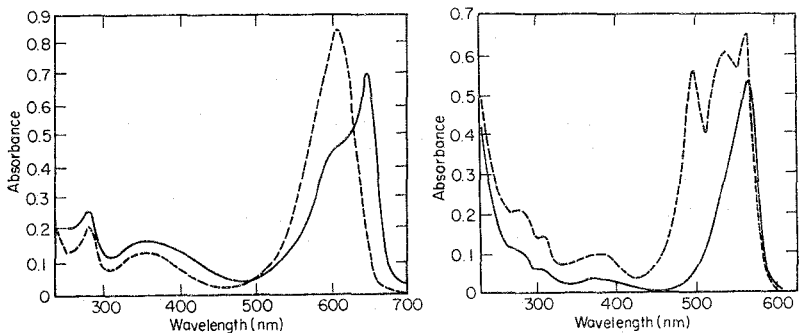


Fig. 12.4. Absorption spectra of aqueous solutions of phycoerythrins and phycocyanins (pH 6-7). Curves on the left, — — —, C-phycocyanin; ———, allo-phycocyanin (both from *Nostoc muscorum*). Curves on the right, — — —, R-phycoerythrin (*Ceramium rubrum*); ———, C-phycoerythrin (*Phormidium persicinum*) (redrawn from O'hEocha 1965).

Table 12.4. Physical properties of biliproteins (after O'hEocha 1965, also see Klein & Cronquist 1967, and references cited therein).

Biliprotein	Organism	Absorption λ_{\max} (nm), and $E^{1\%}_{1\text{cm}}$					Fluorescence (λ_{\max} , nm)
		where available indicated by brackets					
C-phycoerythrin	Predominant in Cyanophyceae	275	305	~370		562 (125)	575
Cryptomonad phycoerythrin (type II)	Cryptomonads	274	310			556	580
B-phycoerythrin	Predominant in Bangiophycideae	278	307	~370		546 (82)	578
R-phycoerythrin (various forms)	Predominant in Florideophycideae	278	307	~370	498	540 (81)	578
C-phycocyanin	Cyanophyceae Rhodophyceae	278	~350			615 (65)	647
Allophycocyanin	Cyanophyceae Rhodophyceae	278	~350			610 (65)	660
Cryptomonad (HV-) phycocyanin	Cryptomonads	270	~350	583		625 643	660
R-phycocyanin	Rhodophyceae Cyanophyceae	278	~350	583	551	615 (66)	565 637

and two phycocyanins, and Table 12.4 lists characteristics of various phycobilins in several algae.

2.2 Chlorophylls

The physical state of chlorophyll in algae is not yet clear. There are two, not mutually exclusive, views: (a) chlorophyll forms are different states of aggregation of Chl (see Brody & Brody 1963); and (b) chlorophyll is complexed with proteins. Recent success by Thornber (1971) in isolating chlorophyll-protein complexes from blue-green algae lends support to the latter possibility.

Chlorophyll *a*, existing as several different forms, occurs in all algae (see also Chapter 5, p. 161). Two forms (Chl *a* 670 and Chl *a* 680) can be directly observed in several algae at room temperature (Cederstrand 1965) by using narrow band widths for measurements (for references to the existence of several forms of Chl *a*, see Halldal 1970, French 1971). Usual room temperature absorption spectra show in most algae only a broad 675nm band for chlorophyll *a* in the red. Cooling the algae to 77°K allows the resolution of the band into two components, Chl *a* 670 and Chl *a* 680 (Fig. 12.5) (Frei 1962, Kok 1963, Butler 1966a, Cho & Govindjee 1970b,c). Also a new band appears at 705 to 710nm which is thought to be different from P700 (Allen 1961 (in apple green mutant of *Chlorella pyrenoidosa*), Kok 1963 (in *Scenedesmus*)). Fig. 12.6 shows 77°K absorption spectra of *Euglena* chloroplasts with peaks at 672, 681, 693 and 706nm. Cho

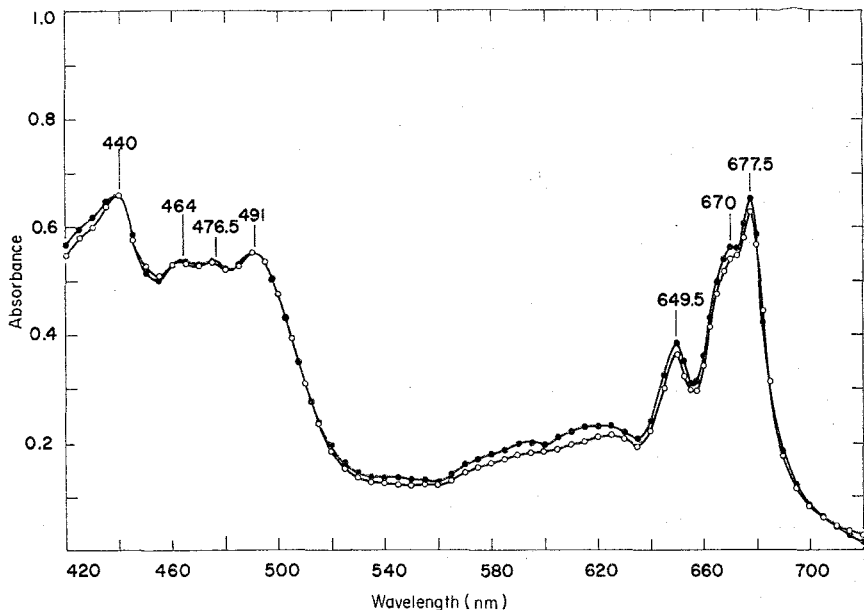


Fig. 12.5. Absorption spectra of *Chlorella pyrenoidosa* measured at 4°K (●—●) and 77°K (○—○). Note peaks at 440, 670 and 677.5nm due to Chl *a*, 476.5 and 649.5nm due to Chl *b*, and 464 and 491nm due to carotenoids (Cho & Govindjee 1970b).

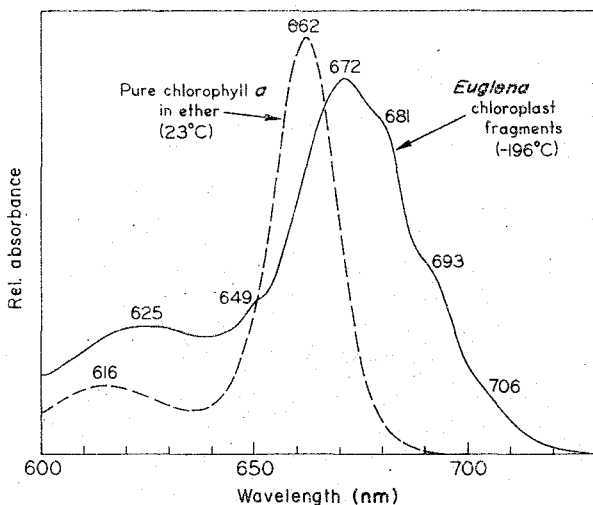


Fig. 12.6. Absorption spectra of *Euglena* at -196°C (77°K) and Chl *a* in ether at 23°C. The spectrum of *Euglena* shows bands at 672, 681, 693 and 706nm due to various forms of Chl *a*; the small band at 649nm is due to Chl *b*, and the band at 625nm is due to the vibrational bands of all Chl *a* forms (French 1971).

(1969) found an additional shoulder at 686nm due to Chl *a* in *Anacystis* at 77°K. Two additional bands have been implicated: Chl *a* 660 and Chl *a* 695. A plot of the quantum yield of O₂ evolution versus wavelength of light shows a clear dip at 660nm (Emerson & Lewis 1943, R. Govindjee *et al.* 1968). Although earlier workers (Thomas 1962, Metzner 1963) reported a band, in addition to several other bands, at about 665nm, more definitive presence of this Chl *a* form was recently reported by French (1971) and Butler and Hopkins (1970a,b). A band at 695nm was observed in several algae by Allen (1961) in *Ochromonas*, by Brown and French (cited in Allen 1961) in *Euglena* and by Das and Govindjee (1967) in *Chlorella*.

Detailed studies of the forms of Chl *a* has come from Litvin's group in the U.S.S.R. (see Litvin & Gulyaev 1969) and from French's group in California (French 1971) from their extensive spectral analyses of derivative spectra at 77°K and more recently from Butler and Hopkins (1970a,b) by taking the fourth derivative of the spectra at 77°K. In all algae studied as well as in higher plants, six major bands can be distinguished as Chl *a* 660, Chl *a* 670, Chl *a* 680, Chl *a* 685, Chl *a* 690, and Chl *a* 705. The Chl *a* 705 may be composed of Chl *a* 702 and Chl *a* 710 (Litvin & Gulyaev 1969). Table 12.5 explains the differences in the general shapes in the red absorption band of algae, due to the presence of varying proportions of each of the forms of Chl *a*.

Only the Chlorophyceae, Prasinophyceae and Euglenophyceae contain Chl *b*; absorption peaks appear at about 480 and 650nm (Fig. 12.5). Recently Thomas (1971) has shown that two forms of Chl *b* may exist, absorbing at 640 and 650nm. French (1971) and Butler and Hopkins (1970a,b) have both confirmed this observation.

Members of the Dinophyceae, Bacillariophyceae, Cryptophyceae, Rhaphidophyceae, Chrysophyceae, Haptophyceae, Xanthophyceae and Phaeophyceae contain minor amounts of Chl *c* in addition to Chl *a*. The weak absorption maximum in the red is at 645nm; a very high Soret band of this Chl has suggested some differences in the structure of this Chl species as compared to other chlorophylls (Allen *et al.* 1960), but as yet the structure is not fully known. It is a chlorophyllide and not chlorophyll (see Holt 1966).

The existence of Chl *d* *in vivo* is uncertain; it has been suggested to be simply an oxidation product of Chl *a*, an artifact of extraction (Allen 1966). The chemical structure of Chl *d* has been described by Holt & Morley (1959) and Holt (1961). The absorption spectra of several species of Rhodophyceae, the one group that is thought to contain Chl *d*, do not show any evidence of a pigment that absorbs at ~740nm (Allen *et al.* 1960). Considering the sizeable concentrations of Chl *d* that have been detected *in vitro*, one should expect to see an *in vivo* absorption peak if the pigment exists in living algae (see Holt 1966).

Strain (1951) extracted a pigment from a species of the Xanthophyceae, *Tribonema bombycinum*, that absorbs maximally at 415nm and 654nm in methanol, and labelled it Chl *e*. Whether it exists *in vivo* is not certain.

Govindjee (1960, 1963b), Govindjee *et al.* (1961), and Gassner (1962), have

Table 12.5. Half band widths and proportions of chlorophyll *a* components in algae (after French *et al.* 1972).

Curve No. and Material	Half band widths of Chl <i>b</i> forms		Half band widths of Chl <i>a</i> forms; Proportions of Components*					
	Chl <i>b</i> 640	Chl <i>b</i> 650	Chl <i>a</i> 662	Chl <i>a</i> 670	Chl <i>a</i> 677	Chl <i>a</i> 683	Chl <i>a</i> 691	Chl <i>a</i> 704
Fraction 1 preparations								
Very sharp spectra								
C34G, <i>Stichococcus</i>	8.3	11.0	10.8; 24.7	8.9; 21.4	8.4; 30.7	11.3; 15.8	20.0; 7.4	... ; 0
C71C, <i>Scenedesmus</i>	12.6	11.7	11.1; 21.9	9.8; 26.1	8.8; 27.4	9.9; 14.9	13.0; 8.0	(14.2)**; 1.7
Typical green algal spectra								
C27D, <i>C. pyrenoidosa</i>	12.1	12.4	11.6; 23.3	10.1; 24.4	9.8; 27.2	10.6; 16.2	13.1; 6.9	(15.1); 2.0
Fraction 2 preparations								
Very sharp spectra								
C35H, <i>Stichococcus</i>	(7.3)	10.5	10.7; 27.3	8.9; 24.2	7.6; 34.6	8.8; 8.3	19.3; 5.6	... ; 0
C72A, <i>Scenedesmus</i>	(11.3)	11.2	11.6; 24.6	10.4; 32.0	8.0; 30.6	8.2; 7.5	14.9; 5.3	... ; 0
Typical green algal spectra								
C28E, <i>C. pyrenoidosa</i>	11.3	11.2	12.0; 26.3	10.2; 26.3	9.3; 28.5	11.0; 12.4	17.1; 5.8	... ; 0
Unfractionated preparations								
Typical green algal spectra								
C76J, <i>C. vulgaris</i>	(7.0)	11.8	10.9; 25.4	9.6; 29.6	8.4; 31.1	10.6; 7.3	18.8; 6.7	... ; 0
C61B, <i>Scenedesmus</i> mutant 8	(13.1)	12.1	11.3; 24.5	9.8; 27.3	8.6; 29.2	10.1; 11.8	18.8; 7.2	... ; 0

* Proportions are given after the semicolon. ** Parentheses indicate curves too small to be significant.

found an absorption band at 750nm (P750) in two species of blue-green algae (Fig. 12.7) which could be due to a long wavelength form of Chl *a* or to bacteriopheophytin. However, Fischer and Metzner (1969) have shown that it is not a bacteriopheophytin, and it lacks chlorin characteristics. They suggest that P750 may be an open chain tetrapyrrole pigment, perhaps, of the bile pigment type.

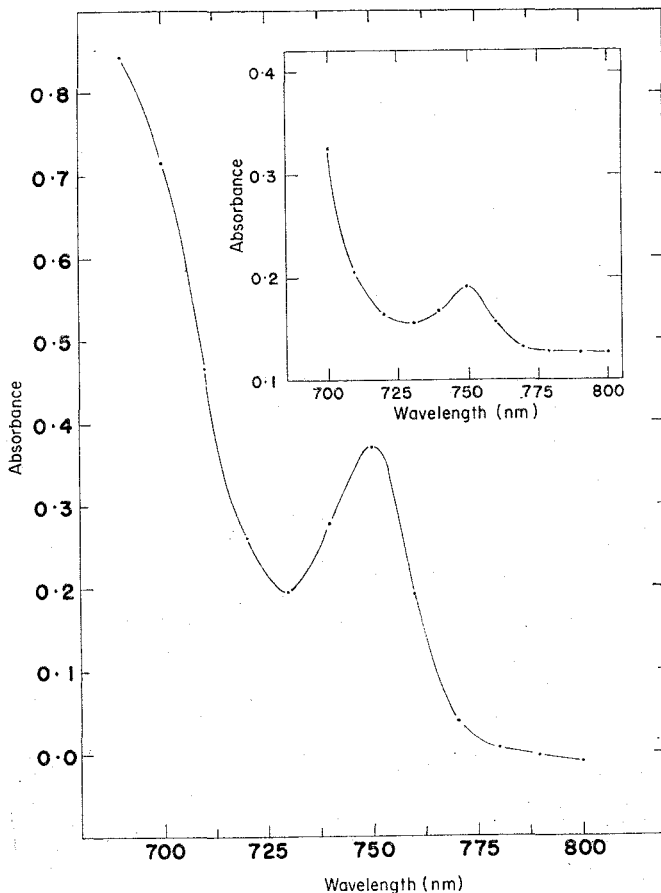


Fig. 12.7. Absorption spectrum of a thick suspension of *Anacystis nidulans* measured with an integrating dodecahedron spectrophotometer showing a peak at 750nm. Insert shows absorption spectrum of a similar sample measured with a Beckman DU spectrophotometer (Govindjee 1960).

Efficiency of energy transfer from Chl *b* to Chl *a* in algae, as measured by the excitation of Chl *a* fluorescence by Chl *b* is almost 100% both at room and liquid nitrogen temperature, and the efficiency of energy transfer from the various forms of Chl *a* to the reaction centre is also very high (Duysens 1952, Tomita & Rabinowitch 1962, Cho & Govindjee 1970b,c). Cho *et al.* (1966) and Cho and

Govindjee (1970b), however, found that energy transfer from the various forms of Chl *a* to the reaction centre was temperature dependent being lower at 4°K than at 77°K. This can, perhaps, be taken to mean that the mechanism of energy transfer is by Förster's resonance 'slow' transfer.

2.3 Carotenoids

The variety of carotenoids present in algae is greater than in higher plants. Several excellent reviews on carotenoids are available: see Weedon (1965) for chemistry, Goodwin (1965a) for distribution, Goodwin (1965b) for biosynthesis, Burnett (1965) for function other than photosynthesis, Davies (1965) for chemical and spectral identification and this book (Chapter 6, p. 176). Several groups of algae have acquired common names that reflect their carotenoid content, e.g. the brown algae, Phaeophyceae, which contain several xanthophylls, most notably fucoxanthin. The carotenoids are usually yellow or orange in colour, but their colour is masked by the more predominant chlorophyll. β -Carotene, existing in fairly high concentration, is the only universal carotenoid of the algae. Chlorophyceae and Rhodophyceae, similar in this respect to the higher plants, also contain significant amounts of α -carotene, while only one species of diatom is known to have ϵ -carotene.

The types of xanthophylls are numerous in the algae. The similarity between higher plants and Chlorophyceae is again attested by the high lutein content of these algae. The Rhodophyceae and Cryptophyceae likewise contain considerable amounts of this pigment. The other xanthophylls of importance are myxoxanthin and myxoxanthophyll, characteristic only of the blue-green algae; peridinin, the primary xanthophyll of the Dinophyceae; and fucoxanthin, present primarily in members of the Phaeophyceae and Bacillariophyceae.

Often it is difficult to determine the precise absorption band of any one carotenoid in algae because in addition to there usually being more than one type of carotenoid present, there is also an overlap of absorption bands of the various chlorophylls present. In general, *in vivo* absorption bands for the carotenoids are to be found (apart from chlorophyll) between 400 and 540nm. Fig. 12.8 shows the calculated fractional absorption by carotenoids along with that by Chl *a* and Chl *b* in *Chlorella* (Govindjee 1960).

Fucoxanthin is unique in that its absorption *in vivo* is extended to 590nm and this pigment is very efficient, unlike most carotenoids, in transferring energy to Chl *a*. It is thought that fucoxanthin is probably complexed to some protein *in vivo*, as a wavelength shift of 40nm results when this pigment is extracted with organic solvents or when the algal cells are treated with detergents (Mann & Myers 1968). This shift can likewise be seen when cells are heated to 70°C for ten seconds (Goedheer 1970).

According to Goedheer (1970), β -carotene is the only carotenoid, with the exception of fucoxanthin, that can really be seen in the *in vivo* absorption spectra of algae. Making use of the fact that β -carotene can be extracted preferentially

from algal cells and chloroplasts with light petroleum ether, Goedheer (1969a) showed that the bands seen at 465 and 495 nm at room temperature in blue-green and red algae are due primarily, if not entirely, to β -carotene; at 77°K, these bands are shifted to 471 and 504nm.

Cho and Govindjee (1970b) presented absorption spectra of *Chlorella* at 77°K and at 4°K which showed shoulders at 465 and 491nm due to carotenoids (Fig. 12.5). Goedheer (1969b), working with a Chl *b*-less species of the Chlorophyceae and applying the same extraction technique as described above, suggests that the bands at 460 and 485nm at room temperature (465 and 493nm at 77°K) are due to β -carotene. However, it must be pointed out that there is never complete disappearance of absorption from 460 to 500nm in any algae by extraction with petroleum ether, which can be explained by either incomplete extraction of the β -carotene or by the presence of additional minor carotenoids. The brown algae show shoulders at 495 and 545nm at 77°K for β -carotene and fucoxanthin respectively.

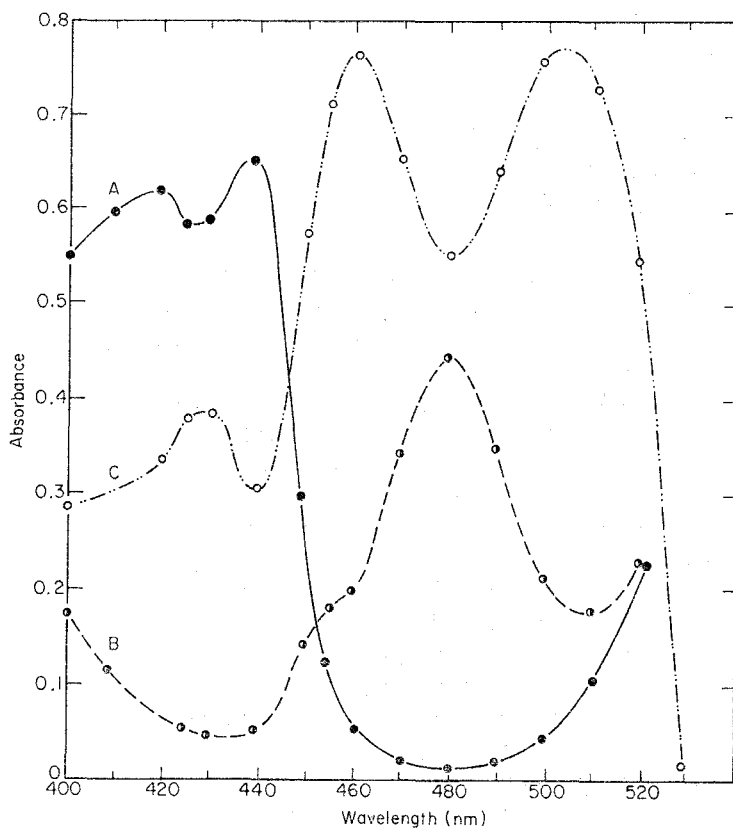


Fig. 12.8. Calculated fraction of total absorbed light, absorbed by Chl *a* (A), Chl *b* (B) and carotenoids (C) in a sample of *Chlorella pyrenoidosa* (Govindjee 1960).

From measurements of *in vitro* quenching of fluorescence of Chl *a* by various carotenoids, Teale (1958) concluded that β -carotene transfers energy to Chl *a* with 10% efficiency, lutein to Chl *a* with 60% efficiency and fucoxanthin to Chl *a* with 100% efficiency. In intact algae, energy transfer from the carotenoids to Chl *a* is 50% in green algae (Emerson & Lewis 1943, Duysens 1952, Cho & Govindjee 1970b), 10 to 15% in blue-green algae (Emerson & Lewis 1942, Duysens 1952, Papageorgiou & Govindjee 1967a, Cho & Govindjee 1970c), and 70 to 80% from fucoxanthin to Chl *a* (Tanada 1951, Duysens 1952). In view of the existence of two pigment systems (one strongly fluorescent photosystem II, and the other weakly fluorescent photosystem I) there is a need for reinvestigation of the energy transfer efficiencies in separated photosystem I and photosystem II particles. It is likely that carotenes transfer energy as efficiently as xanthophylls to photosystem I and photosystem II chlorophylls respectively.

2.4 Phycobilins

The water-soluble pigments, the phycobilins, are found in abundance only in the blue-green and red algae, although trace amounts of chemically different types of phycobilins have been found in isolated species of other algal groups (see Chapter

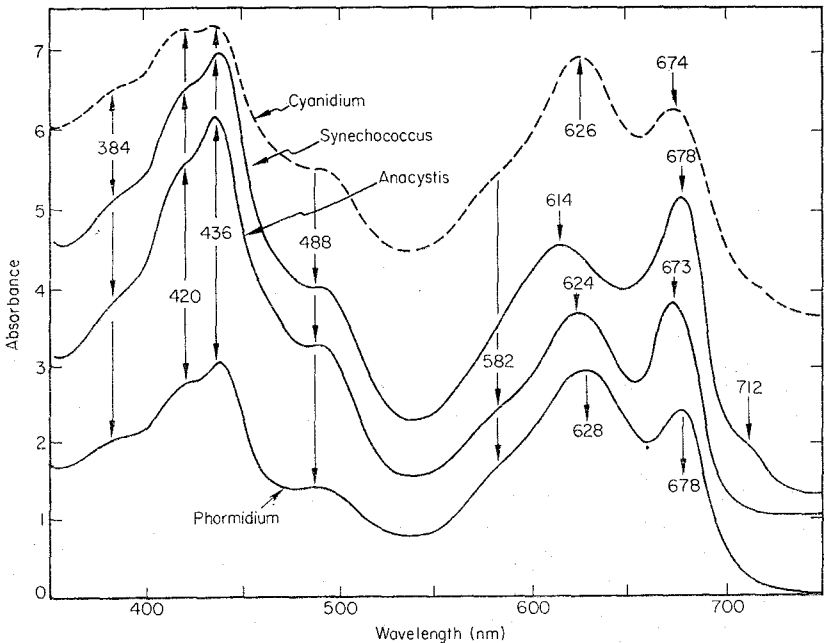


Fig. 12.9. Absorption spectra of several blue-green algae (solid curves) and of *Cyanidium* (dashed curve). The baselines for the different algae were shifted on the ordinate scale. The bands at 384, 420, 436, 673–678 and 712nm are due to Chl *a*; at 582 and 614–628nm due to phycocyanin, and at 490nm due to carotenoids (Govindjee & Mohanty 1972).

6, p. 194). Chemical and spectral characteristics of phycobilins have been extensively reviewed by O'hEocha (1960, 1962, 1965, 1971). Members of the Cyanophyceae contain largely the blue pigment, phycocyanin (see peaks around 620nm in Fig. 12.9), although several species may contain the red phycoerythrin in addition to, or in place of, phycocyanin; the reverse is true of members of the Rhodophyceae (see Hattori & Fujita (1959c). Both groups often also contain small amounts of allophycocyanin. (For chromoproteid pigments of cryptomonads, see Allen *et al.* 1959.) The existence of these pigments is very important

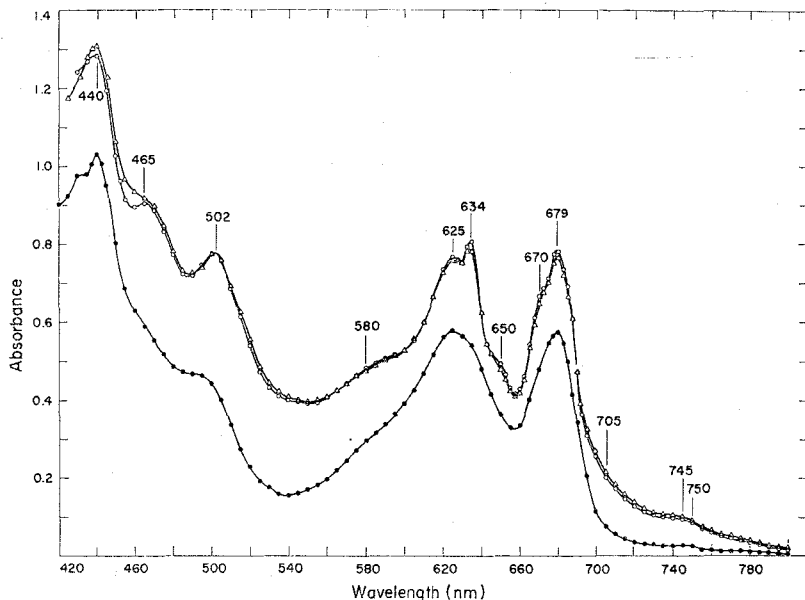


Fig. 12.10. Absorption spectra of *Anacystis nidulans* measured at 4°K (○), 77°K (△) and 295°K (●). Bands at 440, 670 and 679nm are due to Chl *a*; 580, 625 and 634nm due to phycocyanin; 650nm due to allophycocyanin; 465 and 502nm due to carotenoids; and 745nm due to P750 (Cho & Govindjee 1970c).

for the light-harvesting capabilities of these algae, as (excluding carotenoids) both groups in actuality contain only Chl *a* (if Chl *d* is considered to be an artefact of isolation). The phycobilins fill in, or at least narrow, much of the light energy gap left by Chl *a* and the carotenoids, allowing the algae to use the solar radiation much more efficiently in photosynthesis, in a manner much like that of fucoxanthin in the brown algae. This is possible because of the absorption characteristics of phycobilins: phycocyanins usually have a broad absorption band around 620nm, phycoerythrins around 545nm, and allophycocyanin around 650nm *in vivo* (Allen 1959, Brody & Emerson 1959b (*Porphyridium*); Thomas & Govindjee 1960 (*Porphyridium*), Ghosh & Govindjee 1966 (*Anacystis*),

Papageorgiou & Govindjee 1967a,b (*Anacystis*), Cho & Govindjee 1970c (*Anacystis*), Govindjee & Mohanty 1972 (*Cyanidium*)).

At room temperature, a phycocyanin peak can be seen in absorption spectra of blue-green and some red algae at about 625nm with shoulders at 580 and 635nm; at 4° and 77°K, the broad phycocyanin band becomes resolved into two maxima at 625 and 634nm, and a shoulder at 650nm due to allophycocyanin can be seen (Fig. 12.10). Absorption peaks of phycoerythrin can be seen *in vivo* and *in vitro* at 490, 546, and 576nm (Fig. 12.4) which may indicate why the red algae are so successful in sub-littoral marine habitats.

Excitation energy transfer from phycoerythrin to Chl *a* (via phycocyanin) and from phycocyanin to Chl *a* is very efficient (about 70 to 80%) (Emerson & Lewis 1942, French & Young 1952, Duysens 1952, Brody & Emerson 1959a, Brody & Brody 1959, Tomita & Rabinowitch 1962, Ghosh & Govindjee 1966) but varies with experimental conditions. In fact, in blue-green and red algae a large proportion of the chlorophylls are in photosystem II, and thus one observes main peaks due to phycobilins in the action spectra of Chl *a*₂ fluorescence (for *Anacystis*, see Papageorgiou & Govindjee 1967a,b, Shimony *et al.* 1967, Cho & Govindjee 1970c; for *Chlorella*, see Cho & Govindjee 1970b). The energy transfer from phycocyanin to Chl *a* is temperature dependent and is about 20% lower at 4°K than at 77°K, perhaps, due to the operation of Förster's resonance 'slow' energy transfer mechanism. In *Cyanidium caldarium*, there is a relative increase in phycocyanin fluorescence (at 655nm) with respect to that in allophycocyanin (at 665nm) which allows the two bands at 77°K to be observed. This information may also be taken, with certain reservations to indicate that the energy transfer from phycocyanin to allophycocyanin is temperature dependent and is in agreement with 'slow' Förster type transfer (Mohanty *et al.* 1972).

3 SEPARATION OF ALGAL PHOTOSYNTHETIC SYSTEMS

The discovery of two pigment systems operating in photosynthesis has led to various attempts to physically fractionate the chloroplasts into photosystem II and photosystem I particles. This work has proceeded much more slowly in algae than in higher plants (see Boardman 1970), probably due to the relative ease of procuring ample higher plant material and breaking their cell walls. Nevertheless, fractionation procedures have been devised for several blue-green and green algae, two red algae, a diatom and a euglenoid.

Allen *et al.* (1963) were the first to attempt a separation of the photochemical systems. They subjected *Chlorella pyrenoidosa* to repeated freezing and grinding, followed by sonication and density gradient centrifugation and obtained two fractions: a heavy fraction with an absorption maximum at 680nm indicating that it was probably only broken cells, and a light fraction absorbing maximally at 672nm and containing also a large amount of Chl *b*. This light fraction is

probably similar to the photosystem II particles isolated from higher plants, as they have been shown to contain most of the short wavelength form of Chl *a* as well as Chl *b*.

Brown (1969), using the French press fractionation method of Michel and Michel-Wolwertz (1968), was able to obtain two fractions from three different species of the Chlorophyceae. It has been shown (Sane *et al.* 1970) that in higher plants this means of separation results in two particles, the lighter one, derived from the stroma lamellae, being photosystem I, and the heavier one being both photosystem I and photosystem II as it is the unseparated grana segment of the chloroplast membrane. Generally speaking, Brown found that the spectra of the green algae fractions resembled those obtained from higher plants. The light fraction contained more of the long wavelength Chl *a* and less Chl *b* compared to the heavy fraction (Fig. 12.11). In addition, the absorption spectra of the chloroplasts and the light fraction of *Scenedesmus obliquus* showed a band at 698nm, that was absent from the heavy fraction. These data suggest that there must be enough differentiation in green algae between stacked and unstacked regions in the chloroplast lamellae to allow the French pressure technique to be effective in fractionation. The electron micrographs of freeze-etched preparations from *Chlamydomonas* indicate that this is the case (Goodenough & Staehelin 1971), at least for this green alga. It is, however, not clear whether *Chlorella*, where most of the thylakoids appear stacked, has any unstacked thylakoids (Reger & Krauss 1970).

Treatment of *Euglena gracilis* cells with the French press and sodium deoxycholate followed by differential centrifugation results in the appearance of two fractions, i.e. a supernatant containing primarily Chl *a* 670 and a sediment enriched in Chl *a* 680 and Chl *a* 695. If the latter fraction is indeed photosystem I, then it is not clear why a large proportion of Chl *b* was found to be associated with it (Brown *et al.* 1965).

Brown (1969) also fractionated a red alga, *Porphyridium cruentum*, and a diatom, *Phaeodactylum tricorutum*, by the French pressure technique. (Neushul 1970, from a freeze-etching study of *Porphyridium*, suggests that both 'free' and 'stacked' thylakoids may be present in this alga.) There was no clear separation of the red alga into a long wavelength Chl *a* enriched fraction and a short wavelength Chl *a* fraction as witnessed by the absorption spectra, but the fluorescence emission spectra of the light fraction at 77°K showed a higher ratio of fluorescence from the long wavelength forms of Chl *a* than from the short wavelength Chl *a* forms, although the relative levels of Chl *a* fluorescence at 685nm were drastically reduced in the light fraction. The effectiveness of fractionating *Phaeodactylum* is equally questionable. Breaking the cells alone resulted in a drastic change in the absorption spectrum with most of the long wavelength Chl *a* forms being lost as well as some of the carotenoids. No visible separation in pigments could be achieved by a French press treatment although the relative fluorescence yield from the heavy fraction was several times greater than from the light fraction. This is an indication that there must be some

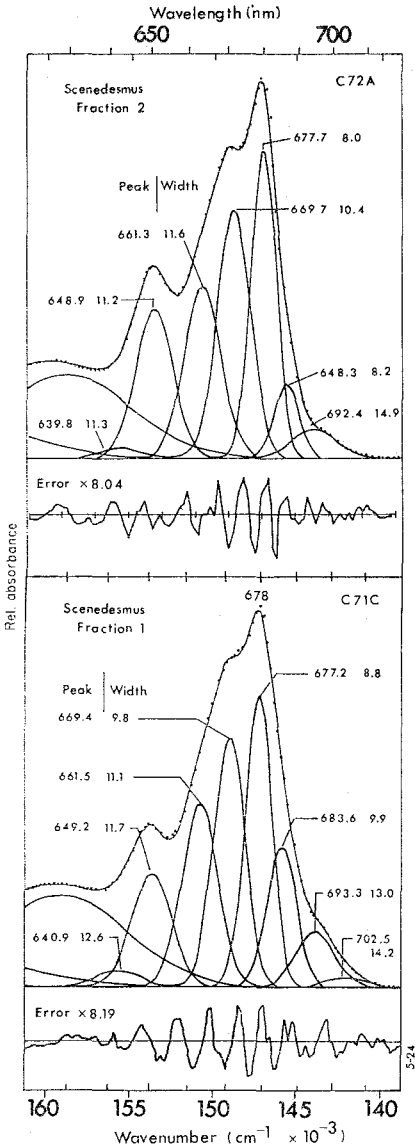


Fig. 12.11. Absorption spectra of fraction 2 (photosystems I + II) and fraction 1 (photosystem I) at -196°C (77°K) from *Scenedesmus* chloroplasts, fitted by the sums of Gaussian components due to the different forms of Chl *a* and of Chl *b*. The error of fit is shown below each diagram with the designated magnification; peak locations and half widths are also indicated on the graph (French 1971).

difference in the proportion of weakly fluorescent long wavelength to strongly fluorescent short wavelength Chl *a* in the two fractions.

Ogawa *et al.* (1968) subjected the red alga, *Porphyra yezoensis*, and the diatom, *Phaeodactylum tricorutum*, to breaking by grinding or sonication respectively, followed by differential centrifugation and polyacrylamide-gel electrophoresis of their chloroplasts solubilized with sodium dodecyl sulphate. They found two 'components' of interest in each alga. One fraction (analogous to photosystem I) contained more long wavelength Chl *a* and carotenes (especially β -carotene), while the other had a larger proportion of Chl *a* 670 and more oxidized carotenoids, i.e. xanthophylls. Noteworthy is the fact that the component of *Phaeodactylum* corresponding to photosystem II is enriched in Chl *c*, in much the same way as Chl *b* is in green algae and higher plants.

Considerably more work has been done with the physical separation of the two photosystems in blue-green algae. The French pressure technique has been the least successful approach (Brown 1969); the net result has been only the removal of phycocyanin from the thylakoids. The difficulty is probably due to the fact that these algae have no thylakoid stacking, a necessity, at least in higher plants (where grana have both photosystem I and photosystem II, and stroma only photosystem I), for separation by the French press (Sane *et al.* 1970). Shimony *et al.* (1967) incubated a suspension of *Anacystis nidulans* in 0.6% digitonin and followed this treatment with differential centrifugation. Four fractions were obtained: one of solubilized phycocyanin, one of broken cells, a light fraction containing a large proportion of long wavelength Chl *a*, and a heavy fraction enriched in the short wavelength Chl *a*. These latter two fractions are probably comparable to photosystem I and photosystem II enriched particles respectively obtained from higher plants. Goedheer (1969a) prepared particles from *Synechococcus* cells treated with deoxycholate and subjected to differential centrifugation. The heavy fraction (10,000 \times g) abounds in short wavelength Chl *a* and xanthophylls absorbing maximally at 525, 485 and 460nm (presumably myxoxanthophyll and lutein), while the light fraction (144,000 \times g) contains mainly the long wavelength Chl *a* and β -carotene.

Ogawa *et al.* (1969), incubating sonicated *Anabaena variabilis* cells in 0.75% Triton-X-100 and separating the resulting suspension by sucrose density centrifugation, showed separation into two bands—a heavy, blue-coloured band and a light, orange-coloured band. Contrary to other photosystem separations, their heavy fragment corresponded chemically and spectrally to photosystem I (a preponderance of long wavelength Chl *a*, the presence of P700, and a higher ratio of β -carotene to total xanthophylls), while the light fraction (containing mainly short wavelength Chl *a* and a large amount of carotenoids, especially echinenone, zeaxanthin and myxoxanthophyll) looked more like photosystem II. These authors have suggested that unlike most other plants, Chl *a* of photosystem II of some blue-green algae such as *Anabaena* may be localized close to the outside of the thylakoid membrane such that it might be in contact with the phycobilisomes which are found on the exterior of the membrane (Gantt &

Conti 1966). However, unlike *Anabaena*, *Anacystis* which may by comparison also have 'exterior' phycobilisomes, shows the usual separation (Shimony *et al.* 1967). Obviously, more work on the structure-function relationships in algae is needed (see also Chapter 4, p. 124).

Lastly, in the way of particle preparations, Ogawa and Vernon (1969) treated lyophilized, carotenoid-extracted fragments from *Anabaena variabilis* with Triton-X-100 and then used sucrose-gradient centrifugation to isolate a fraction highly enriched in P700 (three P700 molecules per 100 bulk Chl *a* molecules). In addition, Thornber and Olson (1971) reported a Chl *a*-protein complex, isolated from sodium dodecyl sulphate treated lamellae of the blue-green alga *Phormidium luridum* lamellae, which contains one molecule of P700 per 60 to 90 Chl *a* molecules and has a red absorption band at 677nm at room temperature. This complex has a main emission band at 682nm and shoulders at 692, 720 and 740nm at room temperature, and two main emission bands at 681nm and 720nm with shoulders at 695nm and 730nm at 77°K (Mohanty *et al.* 1972). A similar Chl *a*-protein complex has been isolated from the red alga *Porphyridium cruentum*, although its red absorption maximum is at 671nm. Similar treatment of green algae and higher plants yields two Chl-protein complexes: one very similar to that of the blue-green algae and the other, containing equal amounts of Chl *a* and Chl *b* absorbing at 672 and 653nm respectively, which corresponds with the bulk pigments of photosystem II.

4 COMPOSITION OF THE TWO PIGMENT SYSTEMS

On the basis of action spectra of photosynthesis and fluorescence of the two pigment systems and spectral analyses of the physically separated photosystems (references and details given in the text; see Mohanty *et al.* 1972), it is commonly accepted that photosystem I and photosystem II of all plants, with the possible exception of blue-green and red algae, contain both Chl *a* 670 and Chl *a* 678 (and Chl *b* in Chlorophyceae, Euglenophyceae and higher plants), although the proportions of Chl *a* 670 (and Chl *b*) are somewhat greater in photosystem II. However, the long wavelength forms of Chl *a* (685-705) are mainly found in photosystem I (Fig. 12.12).

Cederstrand *et al.* (1966), using a precision integrating spectrophotometer, obtained absorption spectra of *Anacystis nidulans*, *Chlorella pyrenoidosa*, and *Porphyridium cruentum*. Comparing these spectra with those derived for photosystem I and photosystem II by Duysens (1963) from data of French *et al.* (1960) on action spectra of photosynthesis in the presence of strong background photosystem II or photosystem I light, Cederstrand *et al.* concluded that in *Chlorella* much of Chl *a* 668 and Chl *b* occurs in photosystem II, and a larger portion of Chl *a* 683 is found in photosystem I. However, in the phycobilin-containing algae, most of the Chl *a* 668 and Chl *a* 683 is associated with photosystem I; in fact, it is very difficult to see a peak at 670nm in the action spectrum of the

Emerson effect in blue-green and red algae, as there is a larger proportion of Chl *a* in photosystem I than in photosystem II (Govindjee & Rabinowitch 1960, Jones & Myers 1964). Moreover, there is four fold more phycocyanin than Chl *a* in photosystem II, and approximately an equal ratio of the two pigments in photosystem I. In addition, Cho and Govindjee (1970c), looking at the excitation spectra of *Anacystis nidulans* for fluorescence at 685 and 705nm (approximately photosystem II and photosystem I fluorescence respectively), showed a higher ratio of the excitation band of phycocyanin (637nm) to that of Chl *a* (436nm) for fluorescence at 685nm than for 715nm fluorescence. Using a similar approach with *Chlorella pyrenoidosa*, Cho and Govindjee (1970a,b) confirmed that Chl *b*, Chl *a* 670, and Chl *a* 678 were present in both photosystems, photosystem II being enriched in Chl *b* and photosystem I in Chl *a* 685–705 (Fig. 12.12).

The curve analyses of the first and fourth derivative absorption spectra of photosystem I and photosystem II enriched fractions, from a number of algae and higher plants into various Chl *a* components (Butler & Hopkins 1970a,b, French *et al.* 1971) show quantitatively the distinction of pigment forms existing in photosystem II and photosystem I (see Tables 12.5 and 12.6, and Fig. 12.11). Analyses of room and low temperature absorption spectra of fractions obtained from various green algae (French *et al.* 1971) indicate that the enrichment of Chl *a* 670 in photosystem II fractions is 10 to 20%; moreover, there is 10% more Chl *a* 678 in the photosystem II enriched fraction than in the photosystem I fraction. The latter, however, is enriched in the longer wavelength forms of Chl *a* (i.e. Chl *a* 684, Chl *a* 693 and Chl *a* 705). The placement of almost similar quantities of Chl *a* 693 in the two photosystems raises some contradiction with fluorescence data which suggests that photosystem II contains much less of this Chl *a* form than photosystem I (see Govindjee *et al.* 1967). As indicated in Table 12.6, this curve analysis assigns approximately 10% more Chl *b* to photosystem II enriched particle fractions.

French's data on the placement of the various Chl *a* forms and Chl *b* in the two photosystems should be improved by changing the method used for separation of the photosystems because fractions prepared by the French pressure technique are actually not photosystem II and photosystem I fractions, but rather grana photosystem II *and* photosystem I and stroma lamellae (photosystem I alone) fractions (Sane *et al.* 1970). Curve analysis applied to absorption spectra obtained from a more purified photosystem II preparation rather than from grana would be valuable.*

* Recently R. A. Gasanor and C. S. French (personal communication, 1973) have analyzed absorption spectra of pigment systems I and II separated from grana. They have indeed shown the absence of Chl *a* 693 in pigment system II. The fractions of different forms of Chl *a* in pigment systems II and I from grana are: Chl *a* 660 (25; 23), Chl *a* 670 (30; 31), Chl *a* 680 (38; 30), Chl *a* 685 (7; 7), Chl *a* 693 (0; 3), and Chl *a* 705 (0; 6), the first and the second numbers (within parentheses) representing systems II and I, respectively. The fractions of different forms of Chl *b* are: Chl *b* 640 (26; 33) and Chl *b* 650 (74; 67). These new data would slightly modify the scheme presented here in Fig. 12.12.

Pigments beyond 720nm. The possible role of pigments absorbing extreme red light (>720nm) in photosynthetic systems was first described by Rabinowitch *et al.* (1960) and Govindjee *et al.* (1960). They observed an inhibitory effect of $750 \pm 10\text{nm}$ light on net O_2 evolution by *Porphyridium cruentum* at 700nm. The action spectrum of this effect in *Chlorella pyrenoidosa* and in *Porphyridium cruentum* had a maximum around 740 to 750nm. R. Govindjee (1961) discovered the same effect in the Hill reaction (quinone as oxidant) in *Chlorella* and in

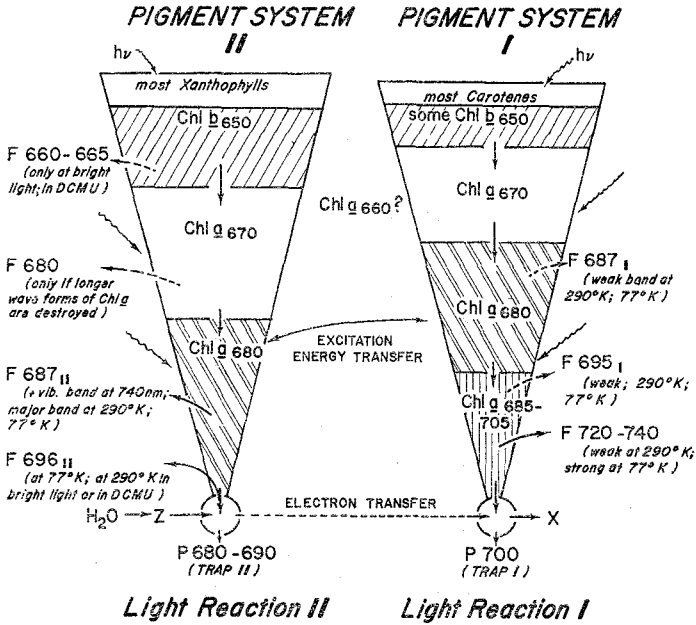


Fig. 12.12. A working hypothesis for the approximate distribution of pigments in the two pigment systems in green algae. Chl *b*, chlorophyll *b*; Chl *a*, chlorophyll *a*; the numbers after Chl *a* indicate the approximate absorption maxima in the red end of the spectrum. (The position of chl *a* 660 is not yet clear to the authors.) The symbol F followed by numbers refers to the suggested fluorescence band, at appropriate wavelengths, in nm. The conditions under which these bands are observed are listed within parentheses. Z, primary electron donor of photosystem II; X, primary electron acceptor of photosystem I; P, pigment (trap). The photosystem I is located on the outer side of the thylakoid membrane and the photosystem II on the inner side such that excitation energy between them is possible. (We cannot, however, discount the possibility that the two systems may be side by side on the thylakoid membrane.) In diatoms and brown algae, Chl *c* replaces Chl *b*. In red and blue-green algae phycobilins replace Chl *b*. The proportion of phycobilins to Chl *a* is high in photosystem I, and low in photosystem II: a large portion of all Chl *a* forms is in photosystem I, and a large portion of phycobilins is in photosystem II. Phycobilins are located in phycobilisomes. The physical arrangement of pigment systems in these algae is not yet clear, and is under active investigation in some laboratories (modified after Govindjee *et al.* 1967).

Table 12.6. Approximate distribution of chlorophyll forms in two fractions, per cent (French *et al.* 1971).

	Chlorophyll <i>b</i>			Chlorophyll <i>a</i>					
	Ch1 <i>b</i> 640	Ch1 <i>b</i> 650	Chlorophyll <i>b</i> as per cent of total	Ch1 <i>a</i> 662	Ch1 <i>a</i> 670	Ch1 <i>a</i> 677	Ch1 <i>a</i> 683	Ch1 <i>a</i> 692	Ch1 <i>a</i> 705
Fraction 1	14	86	20	22	23	29	17	7	3
Fraction 2	14	86	23	25	27	33	8	5	0

pokeweed chloroplasts. However, using narrower band widths and a larger number of wavelengths, she found two peaks at 730 to 740 and 750 to 760nm both in the Hill reaction and in photosynthesis of algae. Govindjee *et al.* (1961) found minor absorption bands in *Chlorella* and *Porphyridium* and a major band at 750nm in *Anacystis*, but the latter alga did not show the inhibitory effect. The initial observations of these absorption bands in green and red algae may have been due to absorption by water or hydroxyl groups as even magnesium oxide suspensions showed similar bands (Govindjee & Cederstrand 1963). However, the fact remains that some absorbing species in algae must be responsible for the inhibitory effect observed by the above mentioned workers.

Lastly carotenoid distribution in the two pigment systems appears to follow a general rule that the oxidized carotenoids (xanthophylls) are mainly found in photosystem II, while the reduced carotenoids (carotenes, especially β -carotene) are identified with photosystem I.

5 LIGHT-INDUCED ABSORPTION CHANGES AND PHOTOSYNTHESIS

Relevant evidence for the two-step mechanism and the electron transport pathway of algae as derived from difference absorption spectroscopy may now be considered. (For information derived from mutants of algae, see Chapter 14, p. 424). What one observes in this type of study is differences between the absorption spectra of algal cells in weak measuring light, that is, with a very low rate of photosynthesis, and in the presence of strong light (when photosynthesis is going on at full speed). These difference absorption spectra reveal reversible changes in several components: active Chl *a* of photosystem II (labelled as P680-P690, has not yet been observed in algae), C550 (a compound absorbing at 550nm has also not yet been seen in algae), plastoquinone (at 254nm), cytochrome b_3 (Cyt b_{559}), cytochrome *f* (Cyt f_{553}), cytochrome b_6 (Cyt b_{564}), plastocyanin (PC, at 660nm), active Chl *a* of photosystem I (P700), P430 (possibly 'X', the primary electron acceptor of photosystem I), NADP (at 340nm), and an absorbance change at 515-520nm. One scheme that includes these intermediates is shown in Fig. 12.13 (where Z is for 'primary' electron donor of photosystem II, Q is for 'primary' electron acceptor of photosystem I, Fd is for ferredoxin, FRS for ferredoxin reducing substance, and R for Fd-NADP reductase).

Levine and Gorman (1966) provided evidence for the existence and participation of Cyt b_{559} in *Chlamydomonas*. Ames (1964a) showed that in *Anacystis* 620nm light (absorbed by phycocyanin) reduces plastoquinone, and 680nm light (absorbed by Chl *a*) oxidizes it. Kouchkovsky and Fork (1964) showed in *Ulva* that plastocyanin was oxidized by photosystem I light and its reduction accelerated by photosystem II light. Duysens and Ames (1962) were the first to show that in *Porphyridium* Cyt *f* was oxidized by photosystem I light (red light, absorbed by Chl *a*) and was reduced by photosystem II light (green light,

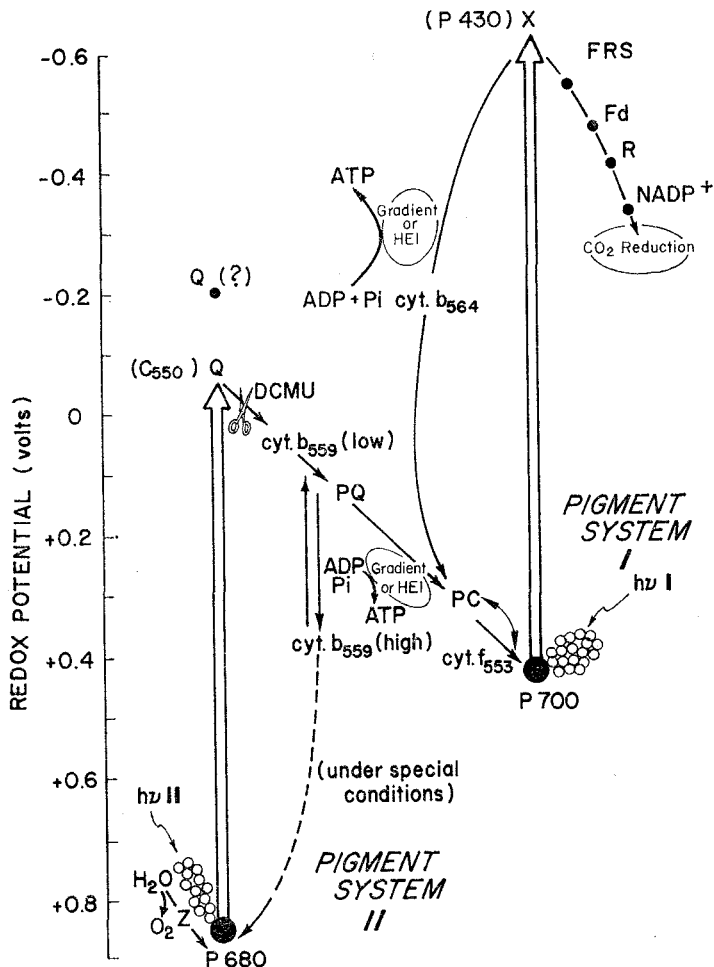


Fig. 12.13. A modified Hill and Bendall scheme of photosynthesis. The two bold vertical arrows represent the two light reactions; all others, dark reactions. Flow of electrons from H_2O to $NADP^+$ is designated as 'non-cyclic' electron flow and from X to the intersystem intermediates as 'cyclic'. A similar cyclic flow of electrons involving only photosystem II shown as dashed line has also been suggested recently. Abbreviations used: Z, the primary donor of photosystem II; P690, the proposed trap of photosystem II; Q (C550), the proposed primary acceptor of photosystem II and the quencher of fluorescence; PQ, plastoquinone; Cyt b_{559} (low and high potential forms) and Cyt b_{564} , cytochromes b ; Cyt f_{553} , cytochrome f ; PC, plastocyanin; P700, the trap for photosystem I; X (P430), the photosystem I electron acceptor; Fd, ferredoxin; R, Fd- $NADP^+$ reductase; $NADP^+$, nicotinamide adenine dinucleotide phosphate; ADP, ATP, adenosine di- and tri-phosphate; Pi, inorganic phosphate; HEI high energy intermediate; DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethyl urea, blocks non-cyclic electron flow (see scissors). Note: the positions of PC and Cyt f may have to be interchanged.

absorbed by phycoerythrin). Amesz and Duysens (1962) demonstrated the same phenomenon in *Anacystis*. This antagonistic effect of light of two different wavelengths is one of the best evidences for the operation of two light reactions in series as presented above. Kok and Gott (1960) (see also Kok & Hoch 1961) showed the crucial role of P700 in *Anacystis* where it was oxidized (bleached) by photosystem I light (Chl *a*) and reduced by photosystem II light (phycocyanin). Finally, Amesz (1964b) showed NADP reduction in intact cells of *Anacystis*. We do not discuss here the role of the complex 515nm change—a part of which may be an index of the membrane potential (Junge *et al.* 1969) and which was discovered by Duysens (1954) in *Chlorella* (see Rubinstein 1964 (*Chlorella*), Govindjee & R. Govindjee 1965 (*Chlorella*), Fork & Amesz 1967 (red and brown algae), Pratt & Bishop 1968 (*Scenedesmus*)). (See also reviews by Fork & Amesz 1969, Levine 1969 and this book Chapter 14, p. 424.)

Recent advances in the study of P690, C550 and P430 have been made mainly with higher plants. We shall briefly discuss the highlights of these studies due to the exciting nature of these discoveries; we suggest that these absorption changes, if they are due to important photosynthetic intermediates, should be looked for in algae.

5.1 P680–P690

From Witt's laboratory in Berlin came several papers on the light-induced absorption changes in chloroplast fragments of higher plants at 435nm and 682–690nm which they feel are due to the reaction centre chlorophyll of photosystem II (Döring *et al.* 1967, 1968, 1969). Using a repetitive flash technique, Döring and co-workers were able to distinguish this absorbance change that has a lifetime of 2×10^{-4} seconds at room temperature, 100 fold shorter than that for P700. The concentration of P680–P690 is of the order of $(0.5-2.0) \times 10^{-3}$ of total chlorophyll. (This makes it doubtful that there is one P690 per photosynthetic unit of photosystem II; perhaps it is only a 'sensitizer' as suggested by Döring and coworkers). The light-induced absorbance changes at 680–690 and at 435nm are present in photosystem II enriched particles, but they cannot be seen in photosystem I fractions (Döring *et al.* 1968). This information, along with the sensitization of P680–690 by pigment system II and the same dependence on intensity as for O₂ evolution has placed it in photosystem II. Likewise, DCMU at appropriate concentrations (acting as a photosystem II inhibitor) blocks this absorbance change, although there is some difficulty in interpreting this information (Döring *et al.* 1969). Floyd *et al.* (1971) have demonstrated that this absorbance change is observable at 77°K which might suggest that in fact it is due to a primary quantum reaction; however to prove this point, one still needs to show that its yield is sufficiently high.

Butler (1972a) has questioned the validity of the assignment of this absorbance change to the photosystem II reaction centre, as it may be due simply to an increase in the fluorescence yield caused by actinic illumination leading to an

apparent decrease in absorption. However, Döring (pers. comm.) has shown that the kinetics of changes at 435nm and 682nm are identical, and that this absorbance decrease is obtained when the photomultiplier is distant (1 metre) from the sample. Under these conditions, the contribution of fluorescence change is negligible. Also, Govindjee *et al.* (1970) have shown that the P680 change occurs in TRIS-washed chloroplasts, where no changes in fluorescence yield occur, and in 'wet' heptane-extracted chloroplasts, where the fluorescence yield changes are drastically reduced. It seems that the P680 change is a real absorption change, but more work is needed before this should be fully accepted. For example, a correlation between its concentration and fluorescence yield changes of photosystem II should be sought as Vredenberg and Duysens (1963) have made for photosynthetic bacteria. Floyd *et al.* (1971), Govindjee and Papageorgiou (1971) and Butler (1972a) considered the possibility that P680 may be an electron donor just as P700 is in photosystem I. Perhaps the available instruments and techniques do not monitor all the P680 change and/or the extinction coefficient of P680 is lower than that of P700!

5.2 C550

A second light-induced absorption change is due to a compound named C550. Discovered by Knaff and Arnon (1969), this absorption change that can be seen at 77°K or at room temperature (when all the cytochromes have been chemically oxidized and are not interfering in this spectral region) has been associated with Q, the 'unknown' primary electron acceptor of photosystem II (Erixon & Butler 1971a). Experiments with DCMU and photosystem II and photosystem I exciting light (Arnon *et al.* 1971) and with photosystem I and photosystem II particles or mutants (Erixon & Butler 1971a) have placed C550 in photosystem II. Erixon and Butler (1971b) found a good correlation between C550 and Q at low light intensities and at liquid nitrogen temperature, but difficulties arise in this correspondence either at room temperature or in far red light (Ben Hayyim & Malkin 1971). Butler (1972b) himself showed that at least one other 'component' (other than the possibility that C550 is indeed the photosystem II primary acceptor), the membrane potential, affects this light-induced absorption change. Whether Q is similarly affected is not yet known. Okayama and Butler (1972) showed that the photoreduction of C550, eliminated by hexane extraction, is restored by the addition of plastoquinone and β -carotene. This may suggest the possibility that C550 change is due to carotenoids! This requires full investigation. In this connexion, we note that Erixon and Butler (1971a) earlier found an increase in absorbance at 543nm associated with the decrease at 550nm; this could imply a shift in the absorbance of a carotenoid type pigment.

5.3 P430

Finally, using flash kinetic spectrophotometry, Hiyama and Ke (1971a,b) have reported an absorption change at 430nm, observable in spinach and blue-green

algal photosystem I particles (as well as spinach sub-chloroplast fractions). This light-induced absorption change, designated as P430, may be the long sought primary electron acceptor of photosystem I. Experiments show that P430 is bleached as fast as P700, the kinetics of the dark recovery of P430 are identical to those of the reduction of artificial electron acceptors that have redox potentials close to that of X (primary acceptor) and the quantum yield and the effective wavelengths for P430 'photoreduction' (?) are the same as for P700 photooxidation. The chemical component that is responsible for this light-induced absorbance change exists only in speculative terms now. However, light-induced EPR absorbance changes of chloroplasts and photosystem I fractions from higher plants have been associated with a bound ferredoxin that has also been suggested to be the primary electron acceptor of photosystem I (Malkin & Bearden 1971, Bearden & Malkin 1972). Lack of 1:1 correspondence, if proven, between the magnitude of EPR signals arising from P700 oxidation and from the reduction of this non-haem iron compound may, however, discount its role as a primary acceptor, but it is interesting to note that ferredoxin bound to a chlorophyll molecule might exhibit an absorption peak at 430nm (Yang & Blumberg 1972).

6 ADAPTATION

The section on absorption spectra did not mean to imply that the pigment composition of algae is static; actually the reverse is true—algae have considerable flexibility in responding to various environmental factors by altering their pigment composition and/or spectral response (see Halldal 1970).

Perhaps the best example of such adaptational strategy is that of the chlorophyll *a*-biliprotein system of the blue-green algae and red algae (see Rabinowitch 1951). The occurrence in Rhodophyceae of such a response to the environment enables the vertical distribution of these algae in nature. Yocum and Blinks (1958) have shown that the photosynthetic efficiency of Chl *a* in *Porphyridium cruentum* is very high in low intensity blue or red light, while in green light, absorbed by phycoerythrin, Chl *a* efficiency is low. Pigment ratio changes and alterations in photosynthetic spectral response have been observed in other members of Bangiophycidae, but the higher red algae, Florideophycidae, do not possess this adaptability. The pigment flexibility of the blue-green alga, *Tolythrix tenuis*, which contains both phycoerythrin and phycocyanin, has been studied by Fujita and Hattori (Fujita & Hattori 1960, 1962, Hattori & Fujita 1959a,b). They find that blue or green light is the most favourable for phycoerythrin formation in the alga, while red light is the least favourable; the opposite is true for phycocyanin formation. However, their study in using only one intensity of light was not complete, as Brody (1958) and Brody and Emerson (1959b) found that for at least *Porphyridium cruentum* the light intensity determined in which way the chromatic adaptation is directed, with complementary chromatic adaptation being more probable at low light intensities than high.

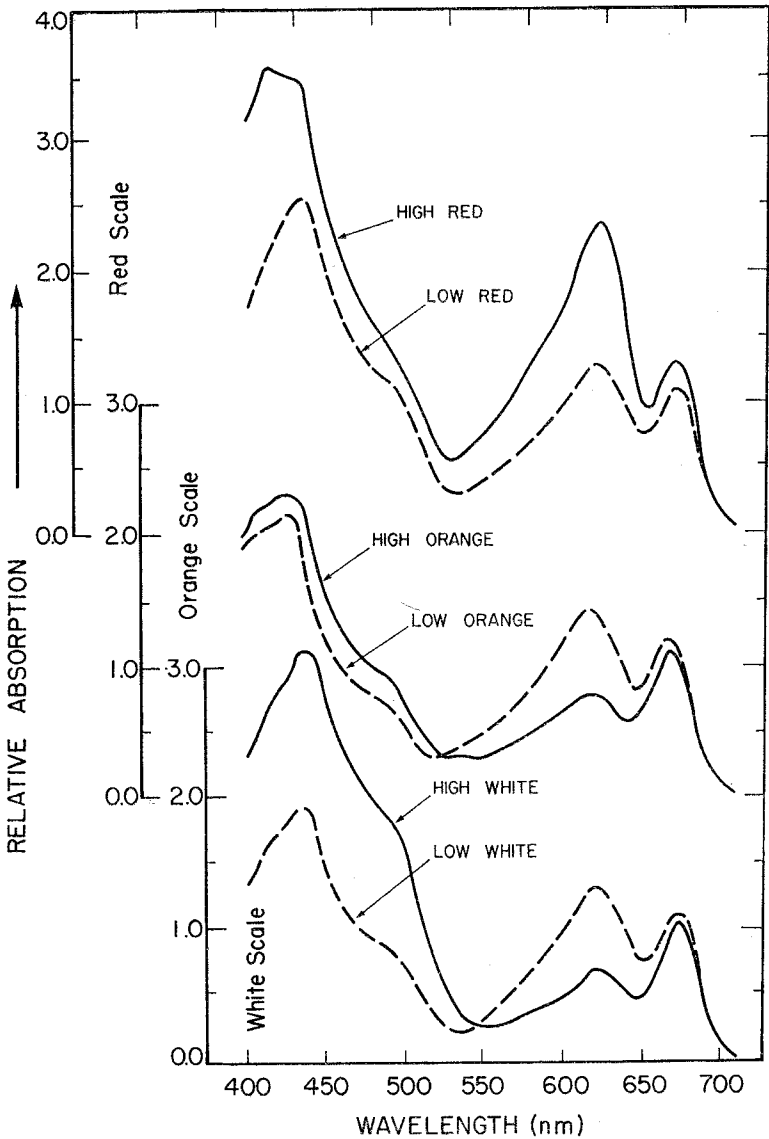


Fig. 12.14. Absorption spectra of different cultures of *Anacystis nidulans* grown in lights of different intensity and color. Spectra of cells grown in high light intensities are shown with solid lines and those in low light intensities with broken lines. The ordinate labelled 'white scale', 'orange scale', and 'red scale' are for cells grown in white, orange and red light, respectively (Ghosh & Govindjee 1966).

Ghosh and Govindjee (1966) showed the same in *Anacystis nidulans* where the pigment which best absorbed the light given during growth is decreased in high intensity light (Fig. 12.14); they also showed that when the ratio of Chl *a* to phycocyanin changed from the usual, the efficiency of energy transfer from phycocyanin to Chl *a*₂ decreased.

Diatoms, dinoflagellates, and brown algae (all with the Chl *a*-Chl *c*-carotenoid system) likewise show environmentally induced pigment changes. The Chl *c*/Chl *a* ratio is greatest in dim light, but there is no drastic difference in the ratio of Chl *a* and Chl *c*/carotenoids with light of varying intensities (Brown & Richardson 1968). Most interesting is the large variability in the ratio of photosynthetically active/inactive carotenoids and action spectra indicate significant flexibility from the violet to green portions of the spectrum (Halldal 1970).

The green algae (Chl *a*-chl *b*-carotenoid system) show several types of variation in pigment composition and spectral response. First, Brown and Richardson (1968) demonstrated in three species of the Chlorophyceae that the Chl *b*/Chl *a* ratio increased in dim light. (It is well known that shade plants have a higher ratio of Chl *b*/Chl *a* than sun plants, see Rabinowitch 1945.) Moreover, the different forms of Chl *a* do vary in concentration and/or spectral response in extreme environmental conditions. The best example is the adaptation of *Ostreobium* to its environment within the coral *Favia pallida* (Halldal 1970). An analysis of the light that penetrates the coral and reaches the layer of algae (Shibata & Haxo 1969) indicates that only light above 680nm contributes significantly to the illumination of the algae; and, to be certain, the photosynthetic rates in the far-red light were very similar to those at 675nm and the absorption spectra showed an increase in the long wavelength forms of Chl *a*. This adaptation could be quickly reverted back to the more expected pattern for green algae on exposure to dim daylight. Oquist (1969) demonstrated a similar pigment flexibility in *Chlorella* exposed to far-red light during growth, although the extent of change was not as pronounced as in *Ostreobium*. Lastly, Halldal and French (1958) and Oquist (1969) showed that green algae grown in light of high intensity characteristically have a high amount of photosynthetically inactive carotenoids, as do Chl *c* containing algae. This can also occur under extreme environmental conditions such as high salt, low temperatures, and low CO₂ concentration (Halldal 1970).

7 REFERENCES

- ALLEN M.B. (1959) Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. *Arch. Mikrobiol.* **32**, 270-7.
- ALLEN M.B. (1961) Evidence for pigments absorbing at 705-10 μ in photosynthetic organisms. In *Light and Life*, eds. McElroy W.D. & Glass B. pp. 479-82. Johns Hopkins Press, Baltimore.
- ALLEN M.B. (1966) Distribution of the chlorophylls. In *The Chlorophylls*, eds. Vernon L.P. & Seely G.R. pp. 511-19. Academic Press, New York & London.

- ALLEN M.B., DOUGHERTY E.C. & McLAUGHLIN J.J.A. (1959) Chromoprotein pigments of some cryptomonad flagellates. *Nature, Lond.* **184**, 1047-9.
- ALLEN M.B., FRENCH C.S. & BROWN J.S. (1960) Native and extractable forms of chlorophyll in various algal groups. In *Comparative Biochemistry of Photoreactive Systems*, ed. Allen M.B. pp. 33-52. Academic Press, New York & London.
- ALLEN M.B., MURCHIO J.C., JEFFREY S.W. & BENDIX S.A. (1963) Fractionation of the photosynthetic apparatus of *Chlorella pyrenoidosa*. In *Studies on Microalgae and Photosynthetic Bacteria*, pp. 407-12. Jap. Soc. Pl. cell Physiol. Univ. of Tokyo Press, Tokyo.
- AMESZ J. (1964a) Spectrophotometric evidence for the participation of quinone in photosynthesis of intact blue-green algae. *Biochim. biophys. Acta*, **79**, 257-65.
- AMESZ J. (1964b) Intracellular reactions of nicotinamide adenine dinucleotide in photosynthetic organisms. Ph.D. Thesis, University of Leiden, The Netherlands.
- AMESZ J. & DUYSSENS L.N.M. (1962) Action spectrum, kinetics and quantum requirement of phosphopyridine nucleotide reduction and cytochrome oxidation in blue-green alga *Anacystis nidulans*. *Biochim. biophys. Acta* **64**, 261-78.
- ARNOLD W. & AZZI J.R. (1968) Chlorophyll energy levels and electron flow in photosynthesis. *Proc. natn. Acad. Sci., U.S.A.* **61**, 29-35.
- ARNOLD W. & MEEK E.S. (1956) The polarization of fluorescence and energy transfer in grana. *Archs. Biochem. Biophys.* **60**, 82-90.
- ARNON D.I., KNAFF D.B., McSWAIN B.D., CHAIN R.K. & TSUJIMOTO H.Y. (1971) Three light reactions and the two photosystems of plant photosynthesis. *Photochem. Photobiol.* **14**, 397-425.
- AVRON M. & NEUMANN J. (1968) Photophosphorylation in chloroplasts. *A. Rev. Pl. Physiol.* **19**, 137-66.
- BEARDEN A. & MALKIN R. (1972) The bound ferredoxin of chloroplasts: a role as the primary electron acceptor of PS I. *Biochem. biophys. Res. Commun.* **46**, 1299-305.
- BENDALL D.S. & HILL R. (1968) Haem-proteins in photosynthesis. *A. Rev. Pl. Physiol.* **19**, 167-86.
- BEN-HAYYIM G. & MALKIN S. (1971) The correlation of C550 change and the fluorescence induction in chloroplasts. Abstr. Proc. 2nd Intern. Conf. on Photosynthesis, Stresa, Italy, p. 1.
- BERGERON J.A. (1963) Studies of the localization, physicochemical properties and action of phycocyanin in *Anacystis nidulans*. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 527-36. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- BISHOP N. (1971) Photosynthesis: the electron transport system of green plants. *A. Rev. Biochem.* **40**, 197-226.
- BLINKS L.R. (1960) Action spectra of chromatic transients and the Emerson effect in marine algae. *Proc. natn. Acad. Sci., U.S.A.* **46**, 327-33.
- BLINKS L.R. (1963) Chromatic transients and enhancement recorded by the glass electrode. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 345-51. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- BOARDMAN N.K. (1970) Physical separation of the photosynthetic photochemical systems. *A. Rev. Plant Physiol.* **21**, 115-40.
- BRIANTAIS J.-M., MERKELO H. & GOVINDJEE (1972) Lifetime of the excited state *in vivo*. III. During fluorescence induction in *Chlorella pyrenoidosa*. *Photosynthetica*, **6**, 133-42.

- BRODY M. (1958) I. The participation of chlorophyll and phycobilins in the photosynthesis of red algae. II. Observations on cellular structures of *Porphyridium cruentum*. Ph.D. Thesis. University of Illinois at Urbana-Champaign.
- BRODY M. & EMERSON R. (1959a) The quantum yield of photosynthesis in *Porphyridium cruentum* and the role of chlorophyll *a* in the photosynthesis of red algae. *J. gen. Physiol.* **43**, 251-64.
- BRODY M. & EMERSON R. (1959b) The effect of wavelength and intensity of light on the proportion of pigments in *Porphyridium cruentum*. *Am. J. Bot.* **46**, 433-40.
- BRODY S.S. & BRODY M. (1959) Induced changes in the efficiency of energy transfer in *Porphyridium cruentum*. I. *Archs. Biochem. Biophys.* **82**, 161-78.
- BRODY S.S. & BRODY M. (1963) Aggregated chlorophyll *in vivo*. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 455-78. Nat. Acad. Sci. Nat. Res. Council, Washington, D.C. **1145**.
- BRODY S.S. & RABINOWITCH E. (1957) Excitation lifetimes of photosynthetic pigments *in vitro* and *in vivo*. *Science N.Y.* **125**, 555.
- BROWN J. (1969) Absorption and fluorescence of chlorophyll *a* in particle fractions from different plants. *Biophys. J.* **9**, 1542-52.
- BROWN J., BRIL C. & URBACH W. (1965) Fractionation of chlorophyll forms from *Euglena* and measurement of light-induced absorbance changes. *Pl. Physiol., Lancaster*, **40**, 1086-90.
- BROWN T.E. & RICHARDSON F.T. (1968) The effect of growth environment on the physiology of algae: light intensity. *J. Phycol.* **4**, 38-54.
- BURNETT J.H. (1965) Functions of carotenoids other than in photosynthesis. In *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin T.W. pp. 381-403. Academic Press, New York & London.
- BUTLER W.L. (1966a) Spectral characteristics of chlorophyll in green plants. In *The Chlorophylls*, eds. Vernon L.P. & Seely G.R. pp. 343-79. Academic Press, New York & London.
- BUTLER W.L. (1966b) Fluorescence yield in photosynthetic systems and its relation to electron transport. In *Current Topics in Bioenergetics*, Vol. I, ed. Sanadi D.R. pp. 49-73. Academic Press, New York & London.
- BUTLER W.L. (1972a) On the relationship between P680 and C550. *Biophys. J.* **12**, 851-7.
- BUTLER W.L. (1972b) The influence of membrane potential on measurements of C550 at room temperature. *FEBS Letters*, **20**, 333-8.
- BUTLER W.L. & HOPKINS D.W. (1970a) Higher derivative analysis of complex absorption spectra. *Photochem. Photobiol.* **12**, 439-50.
- BUTLER W.L. & HOPKINS D.W. (1970b) An analysis of fourth derivative spectra. *Photochem. Photobiol.* **12**, 451-6.
- CALVERT J.G. & PITTS J.N. (1966) *Photochemistry*. John Wiley & Sons, Inc., New York & London.
- CEDERSTRAND C.N. (1965) Spectrophotometric and spectrofluorometric characterization of the two pigment systems in photosynthesis. Ph.D. Thesis. University of Illinois at Urbana-Champaign.
- CEDERSTRAND C.N., RABINOWITCH E. & GOVINDJEE (1966) Analysis of the red absorption band of chlorophyll *a in vivo*. *Biochim. biophys. Acta*, **126**, 1-12.
- CHENIAE G. (1970) Photosystem II and O₂ evolution. *A. Rev. Pl. Physiol.* **21**, 467-98.
- CHO F. (1969) Low temperature spectroscopy of algae. Ph.D. Thesis, University of Illinois at Urbana-Champaign.

- CHO F. & GOVINDJEE (1970a) Fluorescence spectra of *Chlorella* in the 295–77°K range. *Biochim. biophys. Acta*, **205**, 371–8.
- CHO F. & GOVINDJEE (1970b) Low temperature (4–77°K) spectroscopy of *Chlorella*; temperature dependence of energy transfer efficiency. *Biochim. biophys. Acta*, **216**, 139–150.
- CHO F. & GOVINDJEE (1970c) Low temperature (4–77°K) spectroscopy of *Anacystis*; temperature dependence of energy transfer efficiency. *Biochim. biophys. Acta*, **216**, 151–61.
- CHO F., SPENCER J. & GOVINDJEE (1966) Emission spectra of *Chlorella* at very low temperatures (–269°C to –196°C). *Biochim. biophys. Acta*, **126**, 174–6.
- CLAYTON R.K. (1965) *Molecular Physics in Photosynthesis*. Balisdell Publishing Co., New York.
- CLAYTON R.K. (1971) *Light and Living Matter*, vols. I and II. McGraw Hill Book Co., New York.
- DAS M. & GOVINDJEE (1967) A long-wave absorbing form of chlorophyll *a* responsible for the 'red drop' in fluorescence at 298°K and the F723 band at 77°K. *Biochim. biophys. Acta*, **143**, 570–6.
- DAS M., RABINOWITCH E. & SZALAY L. (1968) Red drop in the quantum yield of fluorescence of sonicated algae. *Biophys. J.* **8**, 1131–7.
- DAVIES B.H. (1965) Analysis of carotenoid pigments. In *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin T.W. pp. 489–532. Academic Press, New York & London.
- DÖRING G., BAILEY J.L., WEIKARD J. & WITT H.T. (1968) The action of chlorophyll *a*_{II} in light reaction II of photosynthesis. *Naturwissenschaften*, **55**, 219–24
- DÖRING G., RENGER G., VATER J. & WITT H.T. (1969) Properties of the photoactive chlorophyll *a*_{II} in photosynthesis. *Z. Naturf.* **24b**, 1139–43.
- DÖRING G., STIEHL H.H. & WITT H.T. (1967) A second chlorophyll reaction in the electron chain of photosynthesis—registration by the repetitive excitation techniques. *Z. Naturf.* **22b**, 639–44.
- DUTTON H.J., MANNING W.M. & DUGGAR B.B. (1943) Chlorophyll fluorescence and energy transfer in the diatom *Nitzschia closterium*. *J. phys. Chem. Wash.* **47**, 308–13.
- DUYSENS L.N.M. (1952) Transfer of excitation energy in photosynthesis. Ph.D. Thesis. University of Utrecht, The Netherlands.
- DUYSENS L.N.M. (1954) Reversible changes in the absorbance spectrum of *Chlorella* upon illumination. *Science N.Y.* **120**, 353–4.
- DUYSENS L.N.M. (1963) Studies of primary reactions and hydrogen or electron transport in photosynthesis by means of absorption and fluorescence difference spectrophotometry of intact cells. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf. A.T. pp. 1–17. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- DUYSENS L.N.M. (1964) Photosynthesis. *Progress in Biophysics*, **14**, 1–100.
- DUYSENS L.N.M. & AMESZ J. (1962) Function and identification of two photochemical systems in photosynthesis. *Biochim. biophys. Acta*, **64**, 243–60.
- DUYSENS L.N.M., AMESZ J. & KAMP B.M. (1961) Two photochemical systems in photosynthesis. *Nature, Lond.* **190**, 510–11.
- EMERSON R. (1958) The quantum yield of photosynthesis. *A. Rev. Pl. Physiol.* **9**, 1–24.
- EMERSON R., CHALMERS R. & CEDERSTRAND C. (1957). Some factors influencing the long-wave limit of photosynthesis. *Proc. natn. Acad. Sci., U.S.A.* **43**, 133–43.
- EMERSON R. & LEWIS C.M. (1942) The photosynthetic efficiency of phycocyanin in *Chroococcus* and the problem of carotenoid participation in photosynthesis. *J. gen. Physiol.* **25**, 579–95.

- EMERSON R. & LEWIS C.M. (1943) The dependence of the quantum yield of *Chlorella* photosynthesis on wavelength of light. *Am. J. Bot.* **30**, 165-78.
- EMERSON R. & RABINOWITCH E. (1960) Red drop and role of auxiliary pigments in photosynthesis. *Pl. Physiol.*, Lancaster, **35**, 477-85.
- ERIXON K. & BUTLER W.L. (1971a) Light-induced absorption changes in chloroplasts at -196°C . *Photochem. Photobiol.* **14**, 427-33.
- ERIXON K. & BUTLER W.L. (1971b) The relationships between Q, C550 and cytochrome b559 in photoreactions at -196°C in chloroplasts. *Biochim. biophys. Acta*, **234**, 381-9.
- FISCHER K. & METZNER H. (1969) On chlorophyll and pigment P750 of *Anacystis nidulans*. In *Progress in Photosynthesis Research*, II, ed. Metzner H. pp. 547-51. Laupp Jr. H. Tubingen.
- FLOYD R.A., CHANCE B. & DEVAULT D. (1971) Low temperature photo-induced reactions in green leaves and chloroplasts. *Biochim. biophys. Acta*, **226**, 103-12.
- FORK D.C. (1963) Observations on the function of chlorophyll *a* and accessory pigments in photosynthesis. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 352-61. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- FORK D.C. & AMESZ J. (1967) Light-induced shifts in the absorption spectrum of carotenoids in red and brown algae. *Photochem. Photobiol.* **6**, 913-18.
- FORK D.C. & AMESZ J. (1969) Action spectra and energy transfer in photosynthesis. *A. Rev. Pl. Physiol.* **20**, 305-28.
- FRANCK J. & ROSENBERG J. (1964) A theory of light utilization in plant photosynthesis. *J. Theoret. Biol.* **7**, 276-301.
- FREI Y.F. (1962) The derivative absorption spectra of chlorophyll in algae and leaves at low temperature. *Biochim. biophys. Acta*, **57**, 82-7.
- FRENCH C.S. (1971) The distribution and action in photosynthesis of several forms of chlorophyll. *Proc. natn. Acad. Sci., U.S.A.* **68**, 2893-7.
- FRENCH C.S., BROWN J.S. & LAWRENCE M.C. (1972) Four universal forms of chlorophyll *a*. *Pl. Physiol.*, Lancaster, **49**, 421-9.
- FRENCH C.S., BROWN J.S., WIESSNER W. & LAWRENCE M.C. (1971) Four common forms of chlorophyll *a*. *Carnegie Inst. Wash. Year Book*, **69**, 662-70.
- FRENCH C.S., MYERS J. & McCLEOD G.C. (1960) Automatic recording of photosynthesis action spectra used to measure the Emerson enhancement effect. In *Comparative Biochemistry of Photoreactive Systems*, ed. Allen M.B. pp. 361-5. Academic Press, New York & London.
- FRENCH C.S. & YOUNG V.M.K. (1952) The fluorescence spectra of red algae and the transfer of energy from phycoerythrin to phycocyanin and chlorophyll. *J. gen. Physiol.* **35**, 873-90.
- FUJITA Y. & HATTORI A. (1960) Effect of chromatic light on phycobilin formation in a blue-green alga, *Tolypothrix tenuis*. *Pl. Cell Physiol.*, Tokyo, **1**, 293-303.
- FUJITA Y. & HATTORI A. (1962) Photochemical interconversion between precursors of phycobilin chromoproteins in *Tolypothrix tenuis*. *Pl. Cell Physiol.*, Tokyo, **3**, 209-21.
- GANTT E. & CONTI S.F. (1966) Granules associated with the chloroplast lamellae of *Porphyridium cruentum*. *J. Cell Biol.* **29**, 423-34.
- GASSNER E.S. (1962) On the pigment absorbing at 750 m μ in some blue-green algae. *Pl. Physiol.*, Lancaster, **37**, 637-9.
- GHOSH A.K. & GOVINDJEE (1966) Transfer of excitation energy in *Anacystis nidulans* grown to obtain different pigment ratios. *Biophys. J.* **6**, 611-17.
- GOEDHEER J.H.C. (1966a) Fluorescence polarization and other fluorescence properties of

- chloroplasts and cells in relation to molecular structure. In *Biochemistry of Chloroplasts*, Vol. I, ed. Goodwin T.W. pp. 75-82. Academic Press, New York & London.
- GOEDHEER J.H.C. (1966b) Visible absorption and fluorescence of chlorophyll and its aggregates in solution. In *The Chlorophylls*, eds. Vernon L.P. & Seely G.R. pp. 147-84. Academic Press, New York & London.
- GOEDHEER J.H.C. (1969a) Carotenoids in blue-green and red algae. In *Progress in Photosynthesis Research*, Vol. II, ed. Metzner H. pp. 811-17. Laupp Jr. H., Tubingen.
- GOEDHEER J.H.C. (1969b) Energy transfer from carotenoids to chlorophyll in blue-green, red and green algae and greening bean leaves. *Biochim. biophys. Acta*, **172**, 252-65.
- GOEDHEER J.H.C. (1970) On the pigment system of brown algae. *Photosynthetica*, **4**, 97-106.
- GOODENOUGH V.W. & STAEHELIN L.A. (1971) Structural differentiation of stacked and unstacked chloroplast membranes: freeze-etch electron microscopy of wild-type and mutant strains of *Chlamydomonas*. *J. Cell Biol.* **48**, 594-619.
- GOODWIN T.W. (1965a) Distribution of carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin T.W. pp. 127-42. Academic Press, New York & London.
- GOODWIN T.W. (1965b) The biosynthesis of carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin T.W. pp. 143-73. Academic Press, New York & London.
- GOVINDJEE (1960) Effect of combining two wavelengths of light on the photosynthesis of algae. Ph.D. Thesis. University of Illinois at Urbana-Champaign.
- GOVINDJEE (1963a) Emerson enhancement effect and two light reactions in photosynthesis. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 318-34. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- GOVINDJEE (1963b) Observation on P750 from *Anacystis nidulans*. *Naturwissenschaften*, **50**, 720-1.
- GOVINDJEE & BRIANTAIS J.-M. (1972) Chlorophyll *b* fluorescence and an emission band at 700nm at room temperature in green algae. *FEBS Letters*, **19**, 278-80.
- GOVINDJEE & CEDERSTRAND C.N. (1963) A letter to the editor. *Biophys. J.* **3**, 507-8.
- GOVINDJEE, CEDERSTRAND C.N. & RABINOWITCH (1961) Existence of absorption bands at 730-740 and 750-760 millimicrons in algae of different divisions. *Science N.Y.* **134**, 391-2.
- GOVINDJEE, DÖRING G. & GOVINDJEE R. (1970) The active chlorophyll *a*_{II} in suspensions of lyophilized and Tris-washed chloroplasts. *Biochim. biophys. Acta*, **205**, 303-6.
- GOVINDJEE & GOVINDJEE R. (1965) Action spectra for the appearance of difference absorption bands at 480 and 520 m μ in illuminated *Chlorella* cells and their possible significance to a two step mechanism of photosynthesis. *Photochem. Photobiol.* **4**, 793-801.
- GOVINDJEE & MOHANTY P. (1972) Photochemical aspects of photosynthesis in blue-green algae. In *Biology and Taxonomy of Blue-Green Algae*, ed. Desikachary, T.V. pp. 171-96. U. Madras, Madras (India).
- GOVINDJEE, MUNDAY J.C. & PAPAGEORGIU G. (1967) Fluorescence studies with algae: changes with time and pre-illumination. In *Energy Conversion by the Photosynthetic Apparatus. Brookhaven Symposia in Biology*, **19**, 434-45.
- GOVINDJEE & PAPAGEORGIU G. (1971) Chlorophyll fluorescence and photosynthesis: fluorescence transients. In *Photophysiology*, ed. Giese A.C. pp. 1-46. Academic Press, New York & London.
- GOVINDJEE, PAPAGEORGIU G. & RABINOWITCH E. (1967) Chlorophyll fluorescence and photosynthesis. In *Fluorescence Theory, Instrumentation and Practice*, ed. Guilbault G.G. pp. 511-64. Marcel Dekker, Inc., New York.

- GOVINDJEE & RABINOWITCH E. (1960) Action spectrum of the second Emerson effect. *Biophys. J.* **1**, 73-89.
- GOVINDJEE, RABINOWITCH E. & THOMAS J.B. (1960) Inhibition of photosynthesis in certain algae by extreme red light. *Biophys. J.* **1**, 91-7.
- GOVINDJEE R. (1961) The action spectrum of the Hill reaction in whole algal cells and chloroplast suspensions (red drop, second Emerson effect and inhibition by extreme red light). Ph.D. Thesis. University of Illinois at Urbana-Champaign.
- GOVINDJEE R., GOVINDJEE & HOCH G. (1964) Emerson enhancement effect in chloroplast reactions. *Pl. Physiol., Lancaster*, **39**, 10-14.
- GOVINDJEE R., RABINOWITCH E. & GOVINDJEE (1968) Maximum quantum yield and action spectrum of photosynthesis and fluorescence in *Chlorella*. *Biochim. biophys. Acta*, **162**, 539-44.
- HALLDAL P. (1970) The photosynthetic apparatus of microalgae and its adaptation to environmental factors. In *Photobiology of Microorganisms*, ed. Halldal P. pp. 17-55. John Wiley-Interscience, London, New York, Sydney, Toronto.
- HALLDAL P. & FRENCH C.S. (1958) Algal growth in crossed gradients of light intensity and temperature. *Pl. Physiol., Lancaster*, **33**, 249-52.
- HATTORI A. & FUJITA Y. (1959a) Formation of phycocyanin pigments in a blue-green alga, *Tolypothrix tenuis*, as induced by illumination with colored lights. *J. Biochem.* **46**, 521-4.
- HATTORI A. & FUJITA Y. (1959b) Effect of pre-illumination on the formation of phycobilin pigments in a blue-green alga, *Tolypothrix tenuis*. *J. Biochem.* **46**, 1259-61.
- HATTORI A. & FUJITA Y. (1959c) Spectroscopic studies on the phycobilin pigments obtained from blue-green and red algae. *J. Biochem.* **46**, 903-9.
- HAXO F.T. (1960) The wavelength dependence of photosynthesis and the role of accessory pigments. In *Comparative Biochemistry of Photoreactive Systems*, ed. Allen M.B. pp. 339-60. Academic Press, New York & London.
- HAXO F.T. & BLINKS L.R. (1950) Photosynthetic action spectra of marine algae. *J. gen. Physiol.* **33**, 389-422.
- HILL R. & BENDALL F. (1960) Function of two cytochrome components in chloroplasts: a working hypothesis. *Nature, Lond.* **186**, 136-7.
- HIND G. & OLSON J.M. (1968) Electron transport pathways in photosynthesis. *A. Rev. Pl. Physiol.* **19**, 249-82.
- HIYAMA T. & KE B. (1971a) A new photosynthetic pigment, 'P430': its possible role as the primary electron acceptor of photosystem I. *Proc. natn. Acad. Sci., U.S.A.* **68**, 1010-13.
- HIYAMA T. & KE B. (1971b) A further study of P430: a possible primary electron acceptor of photosystem I. *Archs. Biochem. Biophys.* **147**, 99-108.
- HOCH G. & KOK B. (1961) Photosynthesis. *A. Rev. Pl. Physiol.* **12**, 155-94.
- HOCH G. & KNOX R.S. (1968) Primary processes in photosynthesis. In *Photophysiology*, Vol. III, ed. Giese A.C. pp. 225-51. Academic Press, New York & London.
- HOLT A.S. (1961) Further evidence of the relation between 2-desvinyl-2-formyl chlorophyll *a* and chlorophyll *d*. *Can. J. Bot.* **39**, 327-31.
- HOLT A.S. (1966) Recently characterized chlorophylls. In *The Chlorophylls*, eds. Vernon L.P. & Seely G.R. pp. 111-18. Academic Press, New York & London.
- HOLT A.S. & MORLEY H.V. (1959) A proposed structure for chlorophyll *d*. *Can. J. Chem.* **37**, 507-14.
- JACKSON W.A. & VOLK R.J. (1970) Photorespiration. *A. Rev. Pl. Physiol.* **21**, 385-432.

- JONES L.W. & MYERS J. (1964) Enhancement in the blue-green alga, *Anacystis nidulans*. *Pl. Physiol., Lancaster*, **39**, 938-46.
- JUNGE W., SCHLIEPHAKE W.D. & WITT H.T. (1969) Experimental evidence for the chemiosmotic hypothesis. In *Progress in Photosynthesis Research*, Vol. III, ed. Metzner H. pp. 1383-91. Laupp Jr. H., Tübingen.
- KAUTSKY H., APPEL W. & AMMAN H. (1960) Chlorophyll fluorescenz und kohlenassimilation XII. Mitteilung die fluoreszenzcurve und die photochemie der pflanze. *Biochem. Z.* **332**, 277-92.
- KLEIN R.M. & CRONQUIST A. (1967) A consideration of the evolutionary and taxonomic significance of some biochemical, micromorphological, and physiological characters in the thallophytes. *Q. Rev. Biol.* **42**, 105-296.
- KNAFF D.B. & ARNON D.I. (1969) A concept of three light reactions in photosynthesis by green plants. *Proc. natn. Acad. Sci., U.S.A.* **64**, 715-22.
- KOK B. (1957) Light-induced absorption changes in the photosynthetic organisms. *Acta Bot. Neerl.* **6**, 316-36.
- KOK B. (1959) Light-induced absorption changes in photosynthetic organisms. II. A split-beam difference spectrophotometer. *Pl. Physiol., Lancaster*, **34**, 184-92.
- KOK B. (1961) Partial purification and determination of oxidation reduction potential of the photosynthetic chlorophyll complex absorbing at 700 m μ . *Biochim. biophys. Acta*, **48**, 527-33.
- KOK B. (1963) Fluorescence studies. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 45-55. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- KOK B. & GOTT W. (1960) Activation spectra of 700 m μ absorption change in photosynthesis. *Pl. Physiol., Lancaster*, **35**, 802-8.
- KOK B. & HOCH G. (1961) Spectral change in photosynthesis. In *Light and Life*, eds. McElroy W.D. & Glass B. pp. 397-423. Johns Hopkins Press, Baltimore.
- KOUCHKOVSKY Y. DE & FORK D.C. (1964) A possible functioning *in vivo* of plastocyanin in photosynthesis as revealed by a light-induced absorbance change. *Proc. natn. Acad. Sci., U.S.A.* **52**, 232-339.
- KREY A. & GOVINDJEE (1966) Fluorescence studies on a red alga *Porphyridium cruentum*. *Biochim. biophys. Acta*, **120**, 1-18.
- LATIMER P., BANNISTER T.T. & RABINOWITCH E. (1957) The absolute quantum yields of fluorescence of photosynthetically active pigments. In *Research in Photosynthesis*, eds. Gaffron H., Brown A.H., French C.S., Livingston R., Rabinowitch E., Strehler B.L. & Tolbert N.E. pp. 107-12. Interscience Publ., Inc., New York.
- LEVINE R.P. (1969) Analysis of photosynthesis using mutant strains of algae and higher plants. *A. Rev. Pl. Physiol.* **20**, 523-40.
- LEVINE R.P. & GORMAN D.S. (1966) Photosynthetic electron transport chain of *Chlamydomonas reinhardi*. III: light-induced absorbance changes in chloroplast fragments of the wild type and mutant strains. *Pl. Physiol., Lancaster*, **41**, 1293-300.
- LITVIN F.F. & GULYAEV B.A. (1969) Resolution of the structure of the absorption spectrum of chlorophyll *a* and its bacterial analogs in the cell by measurement of the second derivative at 20° to -196°C. *Dokl. Biophysics*, **189**, 157-60.
- MALKIN R. & BEARDEN A. (1971) Primary reactions of photosynthesis: photoreduction of a bound chloroplast ferredoxin at low temperature as detected by EPR spectroscopy. *Proc. natn. Acad. Sci., U.S.A.* **68**, 16-19.
- MANN J.E. & MYERS J. (1968) On pigments, growth and photosynthesis of *Phaeodactylum tricoratum*. *J. Phycol.* **4**, 349-55.

- MAR T. & GOVINDJEE (1971) Decrease in the degree of polarization of chlorophyll fluorescence upon the addition of DCMU to algae. Abstr. Proc. 2nd Intern. Conf. on Photosynthesis, Stresa, Italy, p. 135.
- MAR T., GOVINDJEE, SINGHAL G.S. & MERKELO H. (1972) Lifetime of the excited state *in vivo* I. Chlorophyll *a* in algae at room and at liquid nitrogen temperature; rate constants of radiationless deactivation and trapping. *Biophys. J.* **12**, 797-808.
- MERKELO H., HARTMAN S., MAR T., SINGHAL G.S. & GOVINDJEE (1969) Mode-locked lasers: measurements of very fast radiative decay in fluorescent systems. *Science N.Y.* **164**, 301-2.
- METZNER H. (1963) Spektralphotometrische messungen an gefriergetrochneten Chlorellazellen. In *Studies on Microalgae and Photosynthetic Bacteria*, pp. 227-37. Jap. Soc. Pl. cell Physiol., University of Tokyo Press, Tokyo.
- MICHEL J.-M. & MICHEL-WOLWERTZ M.-R. (1968) Fractionation of the photosynthetic apparatus from broken spinach chloroplasts by sucrose density-gradient centrifugation. *Carnegie Inst. Wash. Year Book*, **67**, 508-14.
- MOHANTY P. (1972) Regulation of chlorophyll fluorescence during photosynthesis: a study of the factors affecting change in yield and emission of chlorophyll fluorescence in intact algal cells and isolated chloroplasts. Ph.D. Thesis. University of Illinois at Urbana-Champaign.
- MOHANTY P., BRAUN B.Z., GOVINDJEE & THORNER J.P. (1972) Chlorophyll fluorescence characteristics of system I chlorophyll *a*-protein complex and system II particles at room and liquid nitrogen temperature. *Pl. Cell Physiol., Tokyo*, **13**, 81-91.
- MÜLLER A., LUMRY R. & KOKUBUN H. (1965) High performance phase fluorometer constructed from commercial subunits. *Rev. Sci. Instr.* **36**, 1214-26.
- MÜLLER A., LUMRY R. & WALKER M.S. (1969) Light intensity dependence of the *in vivo* fluorescence lifetime of chlorophyll. *Photochem. Photobiol.* **9**, 113-26.
- MURTY N.R. & RABINOWITZ E. (1965) Fluorescence decay studies of chlorophyll *a in vivo*. *Biophys. J.* **5**, 655-61.
- MYERS J. (1963) Enhancement. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 301-17. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- MYERS J. (1971) Enhancement studies in photosynthesis. *A. Rev. Pl. Physiol.* **22**, 289-312.
- NEUSHUL M. (1970) A freeze-etching study of the red alga *Porphyridium*. *Am. J. Bot.* **57**, 1231-9.
- NICHOLSON W.J. & FORTOUL J.I. (1967) Measurement of the fluorescent lifetimes of *Chlorella* and *Porphyridium* in weak light. *Biochim. biophys. Acta*, **143**, 577-82.
- OGAWA T., KANAI R. & SHIBATA K. (1968) Distribution of carotenoids in the two photochemical systems of higher plants and algae. In *Comparative Biochemistry and Biophysics of Photosynthesis*, eds. Shibata K., Takamiya A., Jagendorf A.T. & Fuller R.C. pp. 22-35. University of Tokyo Press, Tokyo.
- OGAWA T. & VERNON L.P. (1969) A fraction from *Anabaena variabilis* enriched in the reaction center chlorophyll P700. *Biochim. biophys. Acta*, **180**, 334-6.
- OGAWA T., VERNON L.P. & MOLLENHAUER H.H. (1969) Properties and structure of fractions prepared from *Anabaena variabilis* by the action of Triton-X-100. *Biochim. biophys. Acta*, **172**, 216-29.
- O'HEOCHA C. (1960) Chemical studies of phycoerythrins and phycocyanins. In *Comparative Biochemistry of Photoreactive Systems*, ed. Allen M.B. pp. 181-203. Academic Press, New York & London.

- O'HEOCHA C. (1962) Phycobilins. In *Physiology and Biochemistry of Algae*, ed. Lewin R.A. pp. 421-35. Academic Press, New York & London.
- O'HEOCHA C. (1965) Phycobilins. In *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin T.W. pp. 175-96. Academic Press, New York & London.
- O'HEOCHA C. (1971) Pigments of the red algae. *Oceanogr. Mar. Biol. Ann. Rev.* **9**, 61-82.
- OKAYAMA S. & BUTLER W.L. (1972) Extraction and reconstitution of photosystem II. *Pl. Physiol., Lancaster*, **49**, 769-74.
- OQUIST G. (1969) Adaptations in pigment composition and photosynthesis by far-red radiation in *Chlorella pyrenoidosa*. *Physiologia Pl.* **22**, 516-28.
- PACKER L., MURAKAMI S. & MEHARD C.W. (1970) Ion transport in chloroplasts and plant mitochondria. *A. Rev. Pl. Physiol.* **21**, 271-304.
- PAPAGEORGIOU G. & GOVINDJEE (1967a) Oxygen evolution from lyophilized *Anacystis* with carbon dioxide as oxidant. *Biochim. biophys. Acta*, **131**, 173-8.
- PAPAGEORGIOU G. & GOVINDJEE (1967b) Changes in intensity and spectral distribution of fluorescence: effect of light intensity on normal and DCMU-poisoned *Anacystis nidulans*. *Biophys. J.* **7**, 375-90.
- PARK R.B. & SANE P.V. (1971) Distribution and function and structure in chloroplast lamellae. *A. Rev. Pl. Physiol.* **22**, 395-430.
- PRAATT L. & BISHOP N.I. (1968) The 520nm light-induced absorbance change in photosynthetic mutants of *Scenedesmus*. *Biochim. biophys. Acta*, **162**, 369-79.
- RABINOWITCH E. (1945) *Photosynthesis and Related Processes*, Vol. I, 399-437, Interscience Pub., Inc., New York.
- RABINOWITCH E. (1951) *Photosynthesis and Related Processes*, Vol. II, 603-828, Interscience Pub., Inc., New York.
- RABINOWITCH E. (1956) *Photosynthesis and Related Processes*, Vol. III, 1375-432; 1793-885, Interscience Pub., Inc., New York.
- RABINOWITCH E. (1957) Photosynthesis and energy transfer. *J. phys. Chem.* **61**, 870-7.
- RABINOWITCH E. (1963) The mechanism of photosynthesis. In *Photosynthetic Mechanisms of Green Plants*, eds. Kok B. & Jagendorf A.T. pp. 112-21. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. **1145**.
- RABINOWITCH E. & GOVINDJEE (1969) *Photosynthesis*. John Wiley & Sons, Inc., New York.
- RABINOWITCH E., GOVINDJEE & THOMAS J.B. (1960) Inhibition of photosynthesis in some algae by extreme-red light. *Science N.Y.* **132**, 422.
- REGER B.J. & KRAUSS R.W. (1970) The photosynthetic response to a shift in the chlorophyll *a* to chlorophyll *b* ratio of *Chlorella*. *Pl. Physiol., Lancaster*, **46**, 568-75.
- ROBINSON G.W. (1964) Quantum processes in photosynthesis. *A. Rev. phys. Chem.* **15**, 311-48.
- RUBINSTEIN D. (1964) A study of absorption changes upon illumination of *Chlorella pyrenoidosa*. Ph.D. Thesis. University of Illinois at Urbana-Champaign.
- RUMBERG B. & WITT H.T. (1964) Analyse der photosynthese mit blitzlicht I. die photooxydation von chlorophyll *a*: 430-703. *Z. Naturf.* **19b**, 693-707.
- RURAINSKI H.J., RANGLES J. & HOCH G. (1971) The relationship between P700 and NADP reduction in chloroplasts. *FEBS Letters*, **13**, 98-100.
- SANE P.V., GOODCHILD D.J. & PARK R.B. (1970) Characterization of chloroplast photosystems I and II separated by a non-detergent method. *Biochim. biophys. Acta*, **216**, 162-78.
- SATO K. (1972) Effect of urea and *o*-phenanthroline on F695 emission in chloroplasts. *Pl. Cell Physiol., Tokyo*, **13**, 23-34.

- SEELY G.R. (1971) Facilitation of energy trapping by different forms of chlorophyll. Abstr. 2nd Intern. Conf. on Photosynthesis, Stresa, Italy, p. 70.
- SHIBATA K. & HAXO F.T. (1969) Light transmission and spectral distribution through epi- and endozoic algal layers in the brain coral, *Favia*. *Biol. Bull.* **136**, 461-8.
- SHIMONY C., SPENCER J. & GOVINDJEE (1967) Spectral characteristics of *Anacystis* particles. *Photosynthetica*, **1**, 113-25.
- SINGHAL G.S. & RABINOWITCH E. (1969) Measurement of the fluorescence lifetime of chlorophyll *a* *in vivo*. *Biophys. J.* **9**, 586-91.
- SINGHAL G.S., WILLIAMS W.P. & RABINOWITCH E. (1968) Fluorescence and absorption studies on chlorophyll *a* *in vitro* at 77°K. *J. phys. Chem.* **72**, 3941-51.
- SMITH J.H.C. & BENITEZ A. (1955) Chlorophylls: analysis in plant materials. In *Modern Methods of Plant Analyses*, Vol. IV, eds. Paech K. & Tracey M.V. pp. 140-96. Springer-Verlag, Berlin.
- SMITH J.H.C. & FRENCH C.S. (1963) The major and accessory pigments in photosynthesis. *A. Rev. Pl. Physiol.* **14**, 181-224.
- STRAIN H. (1951) The pigments of algae. In *Manual of Phycology*, ed. Smith G.M. pp. 243-62. Chronica Botanica Co., Waltham, Mass.
- SZALAY L., RABINOWITCH E., MURTY N.R. & GOVINDJEE (1967) Relationship between the absorption and emission spectra and the red drop in the action spectra of fluorescence *in vivo*. *Biophys. J.* **7**, 137-49.
- TANADA T. (1951) The photosynthetic efficiency of carotenoid pigments in *Navicula minima*. *Am. J. Bot.* **38**, 276-83.
- TEALE F.W.J. (1958) Carotenoid sensitized fluorescence of chlorophyll *in vitro*. *Nature Lond.* **181**, 415-16.
- THOMAS J.B. (1962) Structure of the red absorption band of chlorophyll *a* in *Aspidistra elatior*. *Biochim. biophys. Acta*, **59**, 202-10.
- THOMAS J.B. (1971) The approximate red absorption band of chlorophyll *b* in *Ulva lactuca* at 77°K. *FEBS Letters*, **14**, 61-4.
- THOMAS J.B. & GOVINDJEE (1960) Changes in quantum yield of photosynthesis in the red alga *Porphyridium cruentum* caused by stepwise reduction in the intensity of light preferentially absorbed by the phycobilins. *Biophys. J.* **1**, 63-72.
- THORNER J.P. (1971) Chlorophyll *a*-protein complex of blue-green algae. In *Methods in Enzymology*, Vol. XXIII, eds. Colowick S.P. & Kaplan N.O. pp. 682-7. Academic Press, New York & London.
- THORNER J.P. & OLSON J.M. (1971) Chlorophyll-proteins and reaction center preparations from photosynthetic bacteria, algae and higher plants. *Photochem. Photobiol.* **14**, 329-41.
- TOMITA G. & RABINOWITCH E. (1962) Excitation energy transfer between pigments in photosynthetic cells. *Biophys. J.* **2**, 483-99.
- VERNON L.P. & AVRON M. (1965) Photosynthesis. *A. Rev. Biochem.* **34**, 269-96.
- VREDENBERG W.J. & DUYSSENS L.N.M. (1963) Transfer of energy from bacteriochlorophyll to a reaction center during bacterial photosynthesis. *Nature, Lond.* **197**, 355-7.
- WASSINK E.C. & KERSTEN J.A.H. (1946) Observations sûr le spectre d'absorption et sûr le rôle des caroténoïdes dans la photosynthèse des diatomés. *Enzymologia*, **12**, 3-32.
- WEAVER E.C. (1968) EPR studies of free radicals in photosynthetic systems. *A. Rev. Pl. Physiol.* **19**, 283-94.
- WEBER G. (1960) Fluorescence parameters and photosynthesis. In *Comparative Biochemistry of Photoreactive Systems*, ed. Allen M.B. pp. 395-411. Academic Press, New York & London.

- WEEDON B.C.L. (1965) Chemistry of the carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin T.W. pp. 75-125. Academic Press, New York & London.
- WILLIAMS W.P., MURTY N.R. & RABINOWITCH E. (1969) The complexity of the fluorescence of *Chlorella pyrenoidosa*. *Photochem. Photobiol.* **9**, 455-69.
- WITT H.T. (1967) On the analysis of photosynthesis by pulse techniques in the 10^{-1} to 10^{-8} second range. Nobel Symposium, Almqvist and Wiksell, Stockholm, **5**, 261-316.
- WITT H.T., MÜLLER A. & RUMBERG B. (1961) Experimental evidence for the mechanism of photosynthesis. *Nature, Lond.* **191**, 194-5.
- YANG C.S. & BLUMBERG W.E. (1972) Quantitative studies on the EPR signals of PS I and ferredoxin. *Biochem. biophys. Res. Commun.* **46**, 422-8.
- YOCUM C.S. & BLINKS L.R. (1958) Light-induced efficiency and pigment alterations in red algae. *J. gen. Physiol.* **41**, 1113-17.