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Spectral Characteristics of *Anacystis* Particles*

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Abstract

Fragments of a blue-green alga, *Anacystis nidulans*, obtained by crushing whole cells in a phosphate buffer (containing digitonin) followed by centrifugation, were examined for their absorption and fluorescence characteristics. Electron micrographs of these fractions are also reported. Two particulate fractions are enriched in chlorophyll *a*. On the basis of the emission spectra (at — 196° C), we speculate that one fraction is enriched in chlorophyll a_2 and the other in chlorophyll a_1 and the lightest fraction has phycocyanin in solution. Some chlorophyll *a* is also present in the lightest fraction. The efficiency of energy transfer from accessory pigments (carotenoids and phycocyanins) to chlorophyll *a* was found to be different in different fractions.

ALLEN et al. (1963) obtained some particles from Chlorella by repeated freezing and grinding followed by sonication and density gradient centrifugation; these particles were enriched in chlorophyll *b* and chlorophyll *a* 673. BUTLER and BAKER (1963) prepared chloroplast fragments from spinach, *Chlorella* and *Euglena* by sonication followed by differential centrifugation; all the fractions contained the chlorophyll pigments in the same ratio as the intact chloroplasts. BOARDMAN and ANDERSON (1964) prepared chloroplast fragments from spinach by solubilization with digitonin followed by differential centrifugation; fractions were obtained which contained different ratios of chlorophyll *b* to chlorophyll *a*. The heavier fractions—enriched in chlorophyll *b* (pigment system II)—seemed to efficiently perform light reaction II (evolution of oxygen with DCPIP[1] as Hill oxidant), and the lighter fractions—enriched in chlorophyll *a* (pigment system I) —light reaction I (reducrion of NADP[2] with reduced DCPIP as H-donor). Several other attempts to physically separate the two pigment systems have been made with some success (BROWN, BRIL and URBACH 1965, BRIL 1965, GROSS, SHEFNER and BECKER 1966, CEDERSTRAND and GOVINDJEE 1966). From the earlier work of DUYSENS (1962) on *Oscillatoria*, it is clear that the two pigment systems in blue-green algae are spectrally well separated. Thus, blue-green algae have been extensively used in the study of two-light reactions in photosynthesis (EMERSON and RABINOWITCH 1960, GOVINDJEE and RABINOWITCH 1960, KOK and GOTT 1960, AMESZ and DUYSENS 1962, JONES and MYERS 1964, GHOSH and GOVINDJEE 1966). Particles from blue-green algae have been used by THOMAS and DE ROVER (1955), PETRACK and LIPMANN (1961), BLACK, FEWSON and GIBBS, 1963, FREDERICKS and JAGENDORF (1964), and SUSOR and KROGMANN (1964) for biochemical investigations. GERHARDT and TREBST (1965) have successfully used lyophilized *Anacystis* for biochemical investigation. Later, PAPAGEORGIOU and GOVINDJEE (1966) described the fluorescence characteristics of such a system and showed that such preparations evolve O_2 with CO_2 as oxidant. FUJITA and MYERS (1966) have succeeded in obtaining a chlorophyll *a* containing fraction from a blue green alga *Anabaena cylindrica* by sonication and differential centrifugation, which has lost activity for the DCPIP-Hill reaction but shows the oxidation of *c* cytochromes.

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1 DCPIP = DiChloro Phenol IndoPhenol.

2 NADP = Nicotinamide Adenine Dinucleotide Phosphate.

114 We attempted to physically separate the pigment complexes in *Anacystis* by the BOARDMAN and ANDERSON method. We have examined some of the spectral characteristics (e.g., absorption spectra, excitation and emission spectra of fluorescence) of the different fractions. Emission spectra at liquid nitrogen temperature show that a partial separation of the pigment complexes was achieved in the present experiment; no biochemical activity was tested.

MATERIALS AND METHODS

Anacystis nidulans, a blue-green alga, was grown in a completely inorganic medium under conditions described by GOVINDJEE and RABINOWITCH (1960). A mixture of 5 % CO_2 and 95 % air was continuously bubbled through the cultures grown in 300 ml modified, Erlenmeyer flasks. The algal suspension was centrifuged to remove the culture medium; the pellet was crushed with sand in a minimum volume of buffer (pH 6.5): 0.15 M Na_2HPO_4, 0.5 M sucrose, 0.6 % digitonin and 0.01 M KCl at 5—10°C. More buffer was then added to obtain dilute suspensions. Sand was removed by appropriate centrifugation. The supernatant was left overnight in complete darkness in the buffer containing digitonin at 4° C. By differential centrifugation in a *Spinco* ultracentrifuge, three fractions were obtained: fraction 1 at 1200× g for 10 minutes, fraction 2 at 10,000× g for 30 minutes, and fraction 3 at 50,000× g for 60 minutes. The last supernatant was fraction 4.

For electron micrographs, *Anacystis* fragments were placed on formvar coated screens, dried and then shadowed with chromium metal at a 30° angle. Pictures were taken with a *Siemens* "I" electron microscope.

Absorption spectra were measured by a *Bausch and Lomb* spectrophotometer (*Spectronic* 505) equipped with an integrating sphere. Fluorescence spectra were made by our recording spectrofluorometer, which is described here for the first time in detail; this instrument—designed by two of us (J. S. and G.)—has been used in our earlier fluorescence work (KREY and GOVINDJEE 1964, GOVINDJEE 1966, GOVINDJEE and YANG 1966, GHOSH et al. 1966, KREY and GOVINDJEE 1966, CEDERSTRAND, RABINOWITCH and GOVINDJEE 1966, CHO, SPENCER and GOVINDJEE 1966, GOVINDJEE, MUNDAY and PAPAGEORGIOU 1966).

Spectrofluorometer: Exciting light (Fig. 1) was obtained from a large *Bausch and Lomb* grating monochromator (focal length = 500 mm; f/5; grating size, 100× ×100 mm; linear dispersion, 3·3 nm/mm of slit opening; blazed at 300 nm). The light source—a tungsten ribbon filament lamp—was operated at 6 volts and 20 amperes by an AC power supply (assembled in the laboratory). The unit described above served as the "exciting" monochromator. The light from this assembly was filtered by appropriate *Corning* and *Schott Jena* colored filters (*Corning* 4—76 for the 400 to 550 nm range; *Corning* 3—69 for the 550 to 750 nm range; and *Schott* RG-10 for the 750 to 800 nm range). The exciting beam impinged at a 30° angle on the bottom (optically clear and flat) of a Dewar flask (*H. S. Martin & Company*, Evanston, Illinois) held vertically. The illuminated area was 1 mm×4 mm. The sample—a thin suspension (optical density at chlorophyll *a* peak = 0·05 for 1 mm path) of *Anacystis* fragments—was pipetted into the Dewar flask to form a 1 mm layer. About 1·5 % of the emitted fluorescence was collected from the bottom surface of the flask by means of a collector lens system. The fluorescent light first passed through complementary filters that remove all traces of scattered light and then through another large *Bausch and Lomb* monochromator (referred to as the "analyzing" monochromator), which had the same characteristics as the "exciting" monochromator, except that its grating is blazed at 750 nm. The light detector was an EMI 9558B photomultiplier tube placed flush with the exit slit of the measuring monochromator. (The EMI photomultiplier was not cooled. Cooling this tube does not increase the signal/noise ratio; it decreases only the dark current.)

The excitation spectra of fluorescence were recorded automatically; the wavelength drum of the "exciting" monochromator was rotated by a synchronous *Hurst* motor (1 RPM). The wavelength range was controlled by two microswitches and

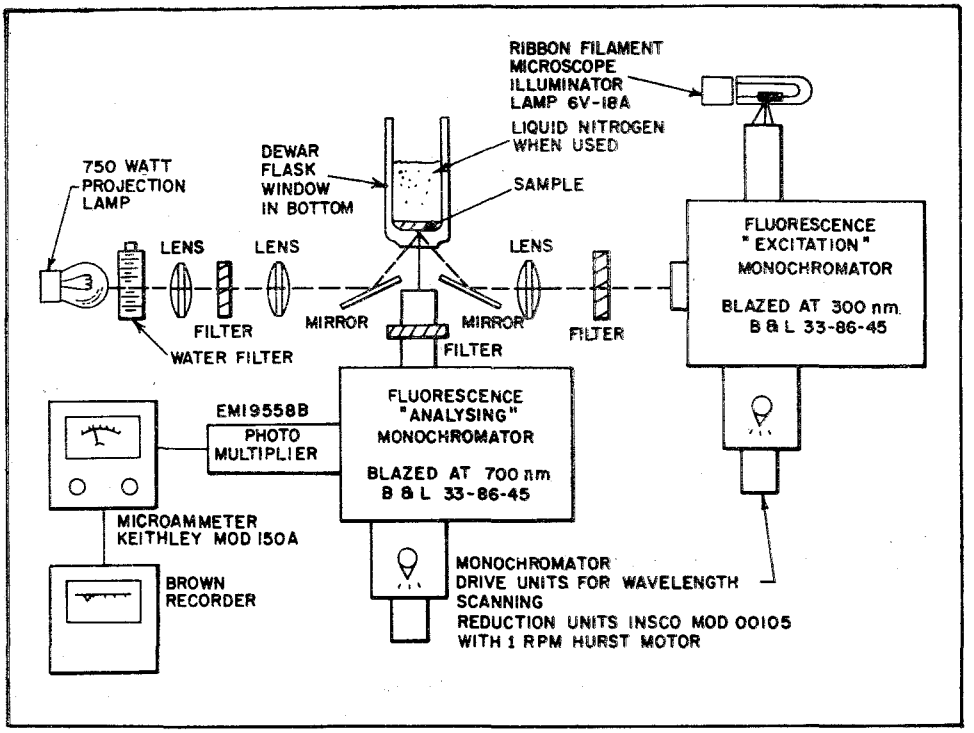

Fig. 1. Block diagram of the spectrofluorometer.

a relay device. The speed of rotation could be varied by means of an *Insco* speed reducer (Model 00105). The signal from the photomultiplier was fed to a *Keithley* microvolt-ammeter (model 150A). This instrument was operated in the 30 mμA to 100 mA range. The signal from the ammeter was then fed to a *Brown* recorder, the speed of which could also be varied with the aid of another *Insco* speed reducer. The excitation spectra were corrected for the variations in the number of incident quanta at different wavelengths. The light intensity at each wavelength was above "compensation" point but far below "saturation" of photosynthesis.

The incident energy was measured with a surface type Bi/Ag thermopile (*Eppley Laboratory*, Inc., Scientific Instruments, Newport, R.I.) which develops a mean emf of 0·040 microvolts per microwatt cm^{-2} of energy incident on the instrument. The thermopile was placed in the light path of the "exciting monochromator"; the emf developed in the thermopile (due to light falling on it) was displayed on the microvolt scale of a *Keithley* microvolt ammeter connected to the two terminals of the thermopile. Microvolt readings for different wavelengths (λ) of excitation were converted to numbers which were directly proportional to the number of incident quanta.

The emission spectra of fluorescence were recorded automatically by scanning the wavelength of the "analyzing" monochromator using a similar set-up as described for the excitation monochromator. Sometimes the instrument was operated manually; measurements were then made on a sensitive *Rubicon* light beam galvanometer ($5{\cdot}8 \times 10^{-4}$ μA/mm; resistance 4720 Ω; period 4 s; C.D. RX 70,000 Ω; equipped with a 2 position range switch; sensitivity: 10^7 photons/mm deflection). The emission

116 spectra were corrected for the variations in the sensitivity of the photomultiplier and the transmission efficiency of the monochromator at different wavelengths. The correction factors were determined by plotting the response of the photomultiplier and the "analyzing" monochromator at different wavelengths to a constant number of incident quanta of different wavelengths.

All the slits of the "exciting" and the "analyzing" monochromators were adjusted to bandwidths of 3·3 nm (or 6·6 nm). Emission spectra were measured by exciting algae with 430 nm and with 605 nm light. Excitation spectra (usually referred to as action spectra) were measured with the "analyzing" monochromator set at 720 nm.

Measurements reported here were made at 20—22° C and at —196° C. For low temperature work, we placed a drop of the suspension on the bottom of the Dewar flask, covered it with a cover slip to obtain a thin film, and then liquid nitrogen was poured to give a 5 cm layer above the sample.

All the experiments reported here have been confirmed six times.

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RESULTS AND DISCUSSION

Electron Micrographs

Fraction 1 contains mainly large pieces of Anacystis cells 2 μm in size (Fig. 2A). They appear to consist of "flat membranes with particles" hiding underneath them (see arrows). These particles seem to range from 50—100 nm in diameter. Often depressions or holes of 50—100 nm in diameter are seen — as if these particles fell out of these places. Fraction 2 contains fragments of membranes and a great number of particles. These particles have the same size (50—100 nm) as the hidden particles in fraction 1. Occasionally, "holes" are seen in the fragments of membranes. Often fraction 2 contains only the 50—100 nm particles. Fraction 3 contains exclusively these particles (Fig. 2B). We do not know whether these particles exist *in vivo* or are artificially formed due to detergent treatment. In sections of untreated algae such particles are also found; it appears as if they are "coming out" of the lamellae. This may be an indication that they appear *in vivo*. Moreover, such particles have also been observed in the plastids of higher plants when leaves are transferred from darkness to light (VON WETTSTEIN 1958, FREY-WYSSLING and MÜHLETHALER 1965, C. SHIMONY, unpublished). However, in our preparations, these particles are more abundant than ever observed *in vivo*. Dr. GANTT (in a personal communication) has pointed out to us that these particles may be artificially formed; for example, vesicles may form by closing of the broken concentric lamellae of *Anacystis* and appear as particles seen in Figure 2.

The blue-green algae are usually considered the most "primitive" of all algae and are perhaps closely related to the photosynthetic bacteria. However, it is doubtful that the "particles" observed in the present study have any relation to the well-known bacterial particles called "chromatophores" since the latter are smaller in size (10—30 nm) in contrast to 50—100 nm particles of *Anacystis*. (For a discussion of the chromatophores in various photosynthetic bacteria, see FULLER, CONTI and MELLIN 1963 and COHEN-BAZIRE 1963.) Recently, GANTT and CONTI (1966) have discovered small granules (approximately 35 nm diameter) attached to the chloroplast lamellae of the red alga *Porphyridium cruentum*; these granules contain phy-

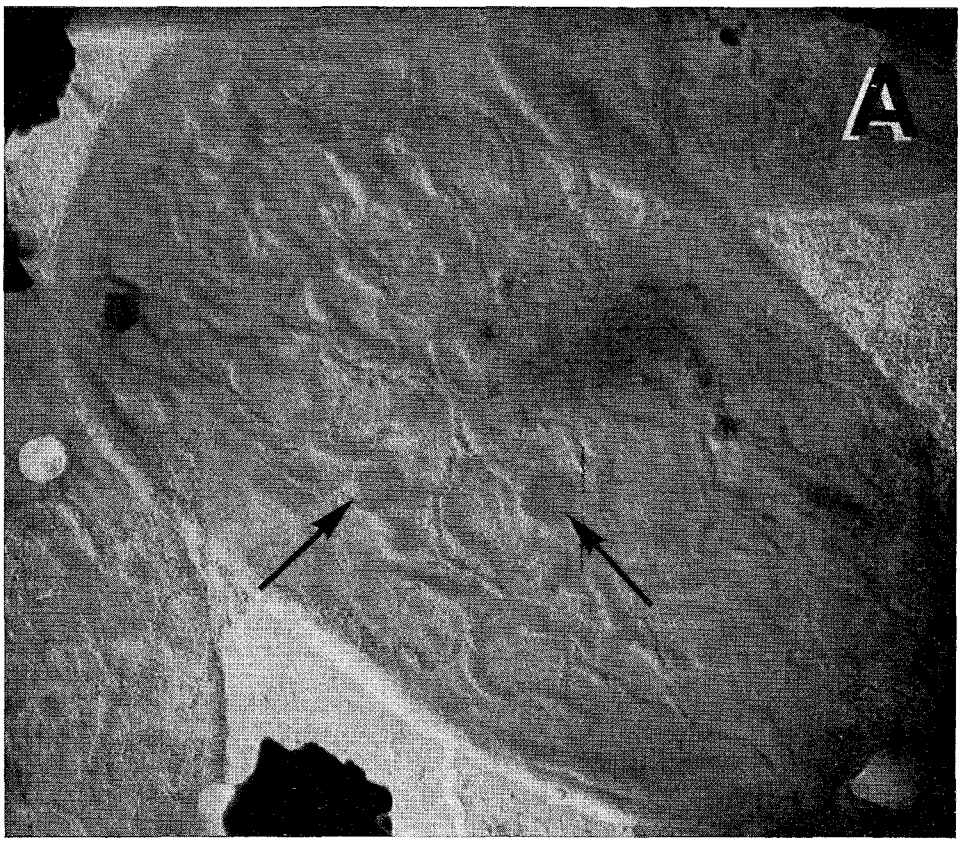

Fig. 2A. Electron micrographs of fraction no. 1 (magnification: 60,000). See text for details.

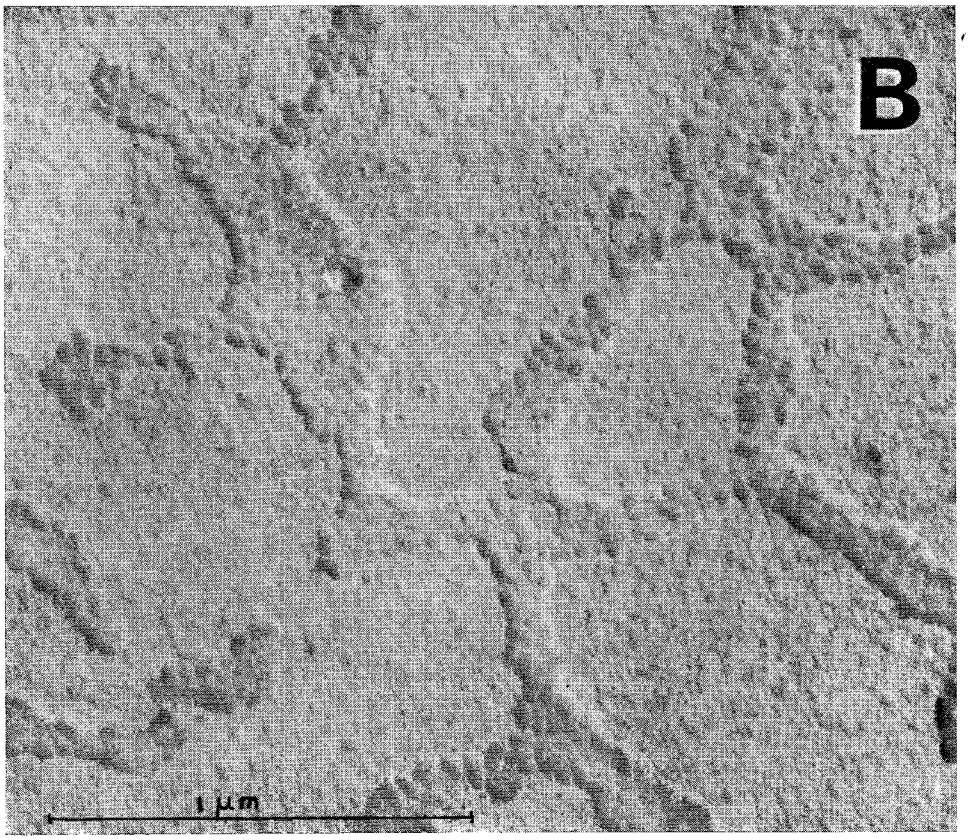

Fig. 2B. Electron micrograph of fraction no. 3 (magnification: 60,000). See text for details.

cobilins and have been named "phycobilisomes." It is likely that the blue-green algae which contain phycobilins also possess "phycobilisomes." However, it is very doubtful that the 50—100 nm particles (shown in Fig. 2) are phycobilisomes since our particles are found in fractions (2 and 3) which are low in phycobilins and enriched in chlorophyll *a* (*vide infra*).

Absorption Spectra

Figure 3 shows the absorption spectra of the 4 fractions for 1 mm pathlength. Table 1 lists the location of peaks and shoulders. The ratio of absorbance (A) at 434 nm, where carotenoids and chlorophyll *a* absorb to that at 671 nm where only chlorophyll *a* absorbs, gives an idea of the ratio of carotenoids to chlorophyll *a*, and the ratio of absorbance at 671 nm to that at 623 nm, gives an indication of the ratio of chlorophyll *a* to phycocyanin. Fraction 2 has lost almost all of its phycocyanin but a good proportion of carotenoids is retained here. Fraction 3 is very similar to fraction 2 (as it was under electron microscope); it contains traces of phycocyanin, but it has a smaller proportion of carotenoids. Fraction 4 contains most of the extracted phycocyanin and some chlorophyll *a* and a fairly large proportion of carotenoids.

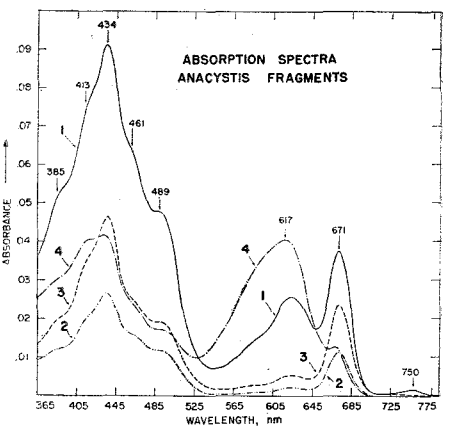

Fig. 3. Absorption spectra of the four fractions of *Anacystis nidulans* (at 22 °C) prepared by breaking the cells while in digitonin followed by differential ultracentrifugation (see text). Path length = 1 mm.

An interesting feature of fraction 4 was the almost equal absorbance (A) at 417 nm and 433 nm ($A_{417} : A_{433} = 0{\cdot}97$). Fraction 1 contains a pigment with a maximum absorption at 750 nm — designated earlier as P750N (GOVINDJEE, CEDERSTRAND and RABINOWITCH 1961, GASSNER 1962 and GOVINDJEE 1963).

Excitation (or Action) Spectra of 720 nm Fluorescence at Room Temperature

Figure 4 shows the general shapes of the action (or excitation) spectra of 720 nm fluorescence of the four fractions. Fraction 4 has a very high fluorescence efficiency since phycocyanin is in solution; there is a large peak at 625 nm due to phycocyanin and shoulders at 671 and 434 nm due to traces of chlorophyll *a*. In fraction 4, the action spectrum shows lower values in the blue than in the red; this may mean that most of the absorption in blue (shown for fraction 4 in Fig. 3) is due to the carotenoids which transfer energy very inefficiently to chlorophyll *a*. Fractions 2 and 3 do not contain much phycocyanin but have clear peaks at 671 and 434 nm since they are enriched in chlorophyll *a*. Fraction 1 is, however, more like the whole cells of *Anacystis* (cf. DUYSENS 1952, GHOSH and GOVINDJEE 1966, PAPAGEORGIOU and GOVINDJEE 1967); it has a high peak due to phycocyanin (625 nm) and shoulders due to chlorophyll *a* at 434 and 671 nm.

Table 1

Absorption Characteristics of Different Fractions.
("A" refers to absorbance or optical density and the number following "A" to the measuring wavelength, in nm.)

Fraction	Peaks (p) and shoulders (s), nm	A_{434}/A_{671}	A_{671}/A_{623}
1	385 (s; chlorophyll *a*), 413 (s; chlorophyll *a*), 434 (p; chlorophyll *a* + carotenoids), 461 (s; carotenoids), 489 (s; carotenoids), 593 (s; phycocyanin) and 671 (p; chlorophyll *a*) and 750 (p; P750N)	2·42	1·45
2	385 (s), 417 (s), 435 (p), 461 (s), 493 (s), 585 (s), 625 (p; chlorophyll *a* + trace of phycocyanin) and 671 (p)	2·36	5·10
3	385 (s), 417 (s), 435 (p), 461 (s), 493 (s), 585 (s), 625 (same as in fraction 2), and 671 (p)	1·98	5·34
4	385 (s), 417 (p), 433 (p), 461 (s), 493 (s), 617 (p; asymmetric band; phycocyanin), and 670 (s; chlorophyll *a*)	3·30	0·31

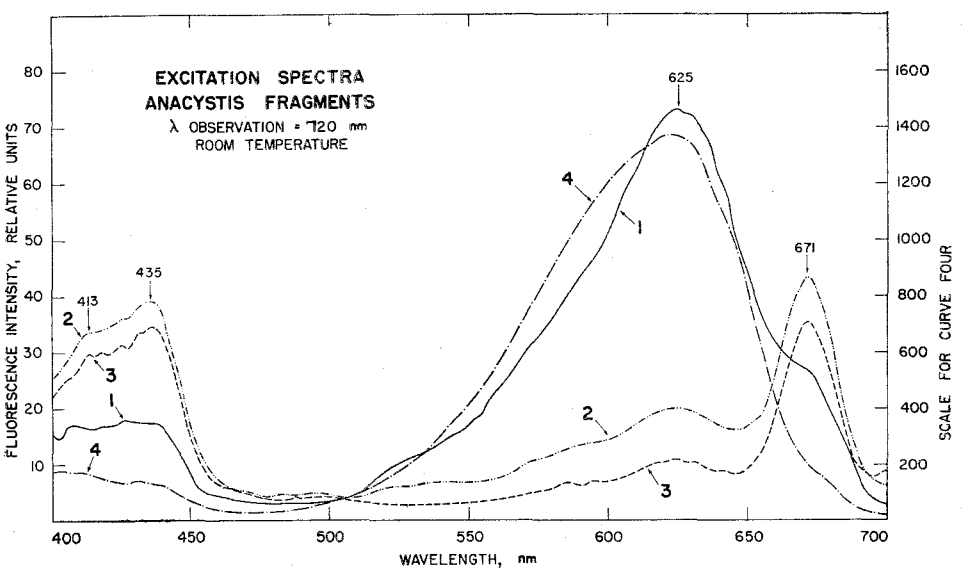

Fig. 4. Excitation (or action) spectra of fluorescence for the four fractions of *Anacystis* (at 22 °C). λ observation = 720 nm. All the curves have been corrected for the number of incident quanta at different wavelengths and they have been divided by the absorbance at 670 nm.

Interpretations dealing with the problem of energy transfer between various pigments are difficult, due to the complexity of 720 nm fluorescence in the blue-green algae. It is composed of at least three components: (i) chlorophyll a_2 (chlorophyll *a*

Table 2

Data from Excitation Spectra: Observation Wavelength, 720 nm; Room Temperature.
E refers to fluorescence intensity (measured at 720 nm); the number that follows "*E*" to the wavelength of exciting light, in nm.

Fraction	Peaks (p) and shoulders (s)*, nm	A E_{625}/E_{670}	B E_{434}/E_{670}	C** E_{625}/A_{625} Relative Units	D E_{670}/A_{670} Relative Units	E E_{434}/A_{434}	F Column C / Column D	G Column D / Column E
1	400—440 (s), 580 (s), 625 (p), 671 (s)	2.65	0·61	2·15	0·55	0·14	3·9	4·0
2	413 (s), 430 (p), 580 (s), 623 (p), 671 (p)	0·46	0·88	1·99	0·84	0·31	2·4	2·7
3	413 (s), 435 (p), 585 (s), 625 (p), 671 (p)	0·30	0·96	0·95	0·70	0·34	1·4	2·0
4	400—440 (s), 585 (s), 622 (p), 680 (s)	5·98	0·53	7·49	3·95	0·61	1·9	6·5

* Positions of shoulders are not very precise; the values are within 5 nm.
** Columns C—E are corrected for different absorbance (*A*) in different samples. [Since *A* for the path-length in the instrument (1 mm) was low (less than 0·05) absorbance values were used instead of percent absorption values for the above correction.

in pigment system II) fluorescence, (ii) chlorophyll a_1 (chlorophyll *a* in pigment system I) fluorescence, and (iii) the tail of phycocyanin fluorescence. Fraction 4 contains phycocyanin in solution and shows a large phycocyanin fluorescence (see section on emission spectra). Therefore, no conclusions concerning energy transfer from phycocyanin to chlorophyll *a* (in fraction 4) is possible. However, one may assume that most of 720 nm fluorescence in the other fractions is due to chlorophyll *a*. Further, one may assume that absolute fluorescence yield of chlorophyll *a* does not change in different fractions since chlorophyll *a* is within particles suspended in the same medium. The ratio of 720 nm fluorescence efficiency excited by 625 nm to that by 670 nm light may then be taken as an index of efficiency of energy transfer from phycocyanin to chlorophyll *a*. In all fractions, the ratio of efficiency for 720 nm fluorescence excited by 625 nm to that by 670 nm is always greater than 1·0 (1·5 to 4·0). That phycocyanin is more effective in causing chlorophyll *a* fluorescence than chlorophyll *a* *in vivo* is known since 1952 (DUYSENS 1952, FRENCH and YOUNG 1952). The transfer efficiency from phycocyanin to chlorophyll *a* is greatest in fraction 1 and decreases in fractions 2—3 (see columns C, D, and F in Table 2).

The ratio of efficiency for 720 nm fluorescence excited by 430 nm to that by 670 nm was always lower than 1·0 (0·5 to 0·15). There are two possibilities: (a) there is greater absorption by phycocyanin at 670 nm than at 430 nm, (b) carotenoids which absorb in the blue but not in the red transfer energy to chlorophyll *a* with very poor efficiency (10—15 %) (see EMERSON and LEWIS 1943 and DUYSENS 1952). The transfer efficiency from carotenoids to chlorophyll *a* appears to be lowest in fraction 1, increasing in other fractions (fraction 2, then fraction 3), if the ratio of 720 nm fluorescence excited by 434 nm to that by 670 nm is taken as an index of the efficiency of energy transfer from the carotenoids to chlorophyll *a*.

Excitation Spectra at Liquid Nitrogen Temperature

The excitation spectra measured at −196 °C (λ observation = 720 nm) are shown in Fig. 5. Since it is difficult to obtain identical thickness of samples at −196 °C, differences in absolute fluorescence intensities cannot be evaluated, and therefore all the curves were adjusted to give a maximum value of 100. The following differences

120 between fractions 2 and 3 were observed: (a) the chlorophyll *a* peak is at 675 nm in fraction 3 and at 672 nm in fraction 2, (b) the ratio of chlorophyll *a* to phycocyanin excited 720 nm fluorescence is lower in fraction 2 than in fraction 3, (c) there are two phycocyanin peaks—one around 596 nm and the other around 630 nm—in both fractions, but the exact shapes of the spectra are very different. It is difficult to decide whether the 3 nm shift, noted in (a), is not simply due to the relatively higher phycocyanin peak. Fraction 4 is relatively enriched in phycocyanin, but the proportion of different forms of phycocyanin is different from that in fraction 1. (The three peaks due to phycocyanin are around 595, 625, and 630.)

Emission Spectra at Room Temperature

Emission spectra obtained upon excitation of the different fractions of *Anacystis* by 430 nm, are shown in Fig. 6 (also see Table 3). Blue (430 nm) light excites mainly chlorophyll *a* and carotenoids. Almost all the fluorescence is from chlorophyll *a* with a maximum around 680—683 nm. Fraction 4, however, has an additional shoulder for phycocyanin fluorescence (around 650 nm). The ratio of fluorescence intensity at 680 nm (F_{680}) to that at 650 nm (F_{650}) is very high (40—50) in fractions 2 and 3, whereas in fractions 1 and 4, this ratio is 17 and 4, respectively, due to larger amount of phycocyanin in them and consequently some absorption by phycocyanin at 430 nm.

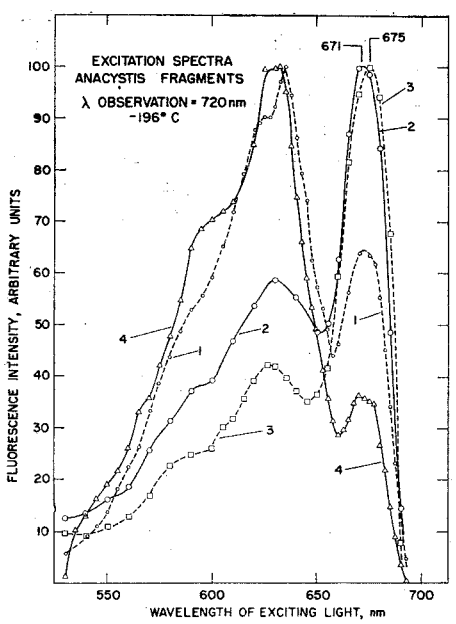

Fig. 5. Excitation spectra of the four fractions of *Anacystis* at —196 °C. λ observation = 720 nm. All the curves have been corrected for the number of incident quanta at different wavelengths and have been adjusted to give the same fluorescence intensity at one of their peaks.

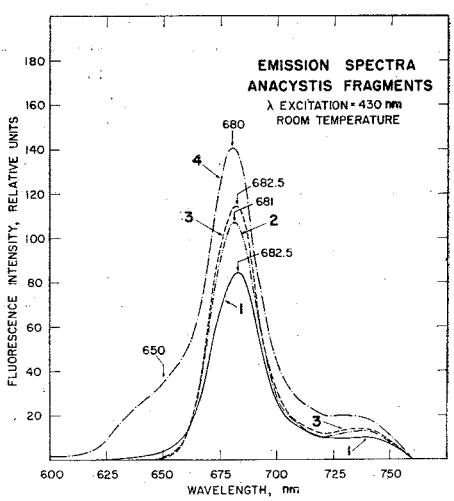

Fig. 6. Emission spectra of the four fractions of *Anacystis* at 22 °C. λ excitation = 430 nm. All the curves have been corrected for the spectral sensitivity of the photomultiplier, the transmission efficiency of the monochromator, and the absorbance at 430 nm.

VREDENBERG (1965) has shown that in *Schizothrix*, fluorescence around 730 nm is due to chlorophyll a_1. OLSON, BUTLER and JENNINGS (1961) have shown that the polarized fluorescence—which is mainly excited by system I—has a maximum in

Table 3

Data from Emission Spectra: Excitation Wavelength, 430 nm, Room Temperature.
("F" refers to fluorescence intensity and the number following it to the wavelength of observation, in nm.)

Fraction	Peaks (p) and shoulders (s), nm	Ratio of Fluorescence Intensities	
		F_{680}/F_{650}	F_{680}/F_{720}
1	683 (p), 735 (s)	16·8	5·9
2	681 (p), 735 (s)	47·5	7·5
3	680 (p), 735 (s)	53·7	7·1
4	650 (s), 680 (p), 735 (s)	3·9	5·8

the far-red (720—730 nm). If one assumes that direct phycocyanin fluorescence at 680 nm and 720 nm is negligible and that a large part of the fluorescence around 720 nm is due to chlorophyll a_1, a ratio of F_{680} to F_{720} may then be taken, in the first approximation, as a ratio of chlorophyll a_2 to chlorophyll a_1 fluorescence. These assumptions are, however, not valid when phycocyanin fluorescence is high as in fractions 1 and 4. However, it may be valid for fractions 2 and 3 since phycocyanin fluorescence is very low. When compared with fraction 3, fraction 2 has slightly more chlorophyll a_2 (F_{680}) than chlorophyll a_1 (F_{720}) fluorescence.

Figure 7 (see also Table 4) shows the emission spectra of the different fractions of *Anacystis* when excited by 605 nm light. Fraction 1 shows peaks for both phycocyanin and chlorophyll *a* fluorescence and fractions 2 and 3 show a dominant chlorophyll *a* peak around 680—683 nm. Fraction 4 shows a dominant phycocyanin peak (655 nm). A high (2·0 to 4·0) ratio of F_{680}/F_{650}—observed in fractions 2 and 3—obviously means a high chlorophyll *a* to phycocyanin fluorescence ratio and a low ratio (1·5)—observed in fraction 1—means a low chlorophyll *a* to phycocyanin fluorescence ratio. The ratio of F_{680} to F_{720} is higher (5·0) in fractions 2 and 3 and lower in fraction 1 (3·8). However, no definite conclusion may be drawn from these ratios since excitation by 605 nm leads to high phycocyanin fluorescence in all fractions except fraction 3.

As expected from the above discussion, F_{650} is found to be several times (30—125) higher when excited by 605 nm than when excited by 430 nm because F_{650} is mainly due to phycocyanin fluorescence and phycocyanin absorbs a great deal more at 605 nm than at 430 nm.

Table 4

Data from Emission Spectra: Excitation Wavelength, 605 nm, Room Temperature.
("*F*" refers to fluorescence intensity and the number following it to the wavelength of observation, in nm.)

Fraction	Peaks (p) and shoulders (s), nm	Ratio of Fluorescence Intensities	
		F_{680}/F_{650}	F_{680}/F_{720}
1	660 (s), 677 (p), 740 (s), 760 (s)	1·5	3·8
2	650 (s), 680 (p), 732 (s)	1·9	5·0
3	650 (s), 681 (p), 730 (s)	3·9	5·3
4	655 (p), 680 (s), 712 (s), 725 (s)	0·5	2·2

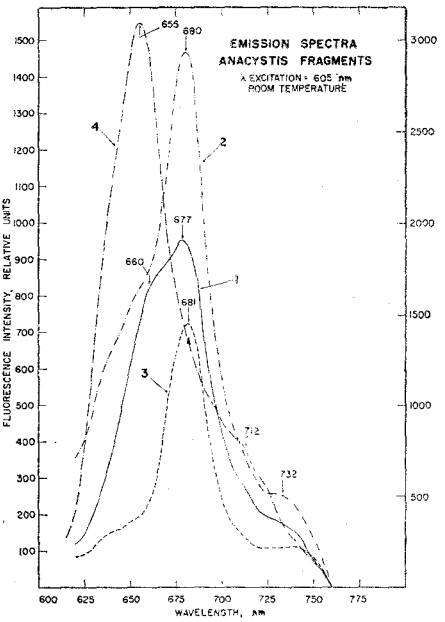

Fig. 7. Emission spectra of the four fractions of *Anacystis* at 22 °C. λ excitation = 605 nm. All the curves have been corrected for the spectral sensitivity of the photomultiplier, the transmission efficiency of the monochromator, and the absorbance at 605 nm. The scale for curve 4 is on the right side.

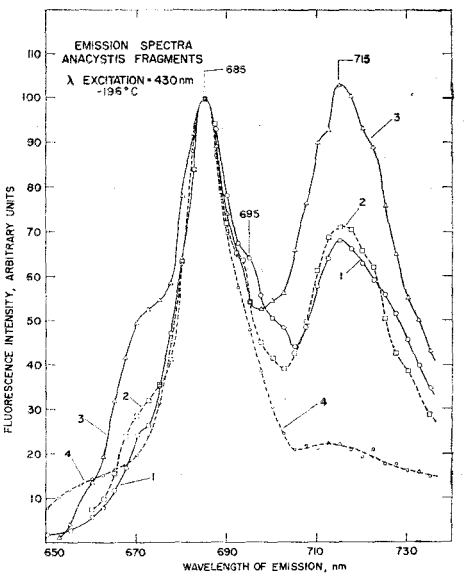

Fig. 8. Emission spectra of the four fractions of *Anacystis* at —196 °C. λ excitation = 430 nm. All the curves have been corrected for the spectral sensitivity of the photomultiplier, the transmission efficiency of the monochromator, and have been adjusted to give the same fluorescence intensity at one of their peaks.

Emission Spectra at Liquid Nitrogen Temperature

Figure 8 shows the emission spectra of *Anacystis* fragments at —196 °C (λ excitation = 430 nm). Fraction 1 has all the three peaks (F_{685}, F_{696}, and F_{720}) as shown earlier by GOVINDJEE (1963) and BERGERON (1963) for whole cells of *Anacystis nidulans*. (The F_{720} was first discovered by S. BRODY (1958) in *Chlorella* and F_{696} by LITVIN, RIKHIREVA and KRASNOVSKY (1960) in bean homogenates.) Since 430 nm light mainly excites chlorophyll *a*, fluorescence is observed from the small amount of chlorophyll *a* in fraction 4; phycocyanin fluorescence is low. It is not clear why F_{720} is almost absent in fraction 4; it is likely that few special chlorophyll *a* molecules that are intimately attached to phycyanin molecules are "dragged" into fraction 4 and these fluoresce with a single peak at 685 nm. The fraction 3 seems to be enriched in chlorophyll a_1 since it has a high F_{720} peak. The ratio of F_{720}/F_{685} is almost 1·0. This conclusion is based on our earlier suggestions that F_{720} is from System I chlorophyll *a* molecules. The fraction 2, however, contains relatively less F_{720} (chlorophyll a_1); F_{720}/F_{685} ratio is much less than 1·0 (0·7).

Emission spectra measured (at —196 °C) when *Anacystis* fragments were excited by 605 nm, in general, confirmed the following general conclusions: (a) in fraction 4 there was a high peak at 660 nm (phycocyanin) and a low peak at 682·5 nm (chlorophyll *a*), (b) in fraction 1 there was a low 660 nm (phycocyanin) peak and a high 685 nm peak (chlorophyll *a*) showing efficient energy transfer from phycocyanin to

chlorophyll *a*, (c) higher long-wave fluorescence (> 700 nm) in fraction 3 (more chlorophyll a_1) than in fraction 2. (Since the fluorescence intensities in fraction 2 and 3 were low, due to the use of extremely dilute suspensions and very poor absorption at 605 nm, the measured spectra were not precise, and thus are not presented in the present paper.)

Our results show that treatment of *Anacystis* with digitonin followed by differential centrifugation leads to a phycocyanin-containing fraction (extracted phycocyanin = fraction 4) and two chlorophyll *a* containing fractions (fractions 2 and 3), and "large pieces of *Anacystis* cells" (fraction 1). Low temperature emission spectra show that fraction 3 is enriched in F_{720} (chlorophyll a_1?) and fraction 2 is poor in F_{720} (chlorophyll a_2?).

These fractions, having different pigment complexes, *may* serve as tools for studying the mechanism of energy transfer in photosynthesis and for finding the ocations of the different pigments on or between the lamellae.

REFERENCES

ALLEN, M. B., MURCHIO, J. C., JEFFREY, S. W., BENDIX, S. A.: Fractionation of the photosynthetic apparatus of *Chlorella pyrenoidosa*. — In: Studies on Microalgae and Photosynthetic Bacteria (ed. by Japanese Soc. Plant Physiol.). Pp. 407—412. Univ. Tokyo Press, Tokyo 1963.

AMESZ, J., DUYSENS, L. N. M.: Action spectrum, kinetics and quantum requirement of phosphopyridine nucleotide reduction and cytochrome oxidation in the blue-green alga *Anacystis nidulans*. — Biochim. Biophys. Acta **64:** 261—278, 1962.

BERGERON, J. A.: Studies of the localization, physicochemical properties and action of phycocyanin in *Anacystis nidulans*.—In: KOK, B., JAGENDORF, A. T. (ed.): Photosynthetic Mechanisms of Green Plants. Pp. 527—536. Nat. Acad. Sci.-Nat. Res. Council, Washington, D.C. 1963.

BLACK, C., FEWSON, C. A., GIBBS, M.: Photochemical reduction of triphosphopyridine nucleotide by cell free extracts of blue-green algae.—Nature **198:** 88, 1963.

BOARDMAN, N. K., ANDERSON, J. M.: Isolation from spinach chloroplasts of particles containing different proportions of chlorophyll *a* and chlorophyll *b* and their possible role in the light reactions of photosynthesis. — Nature **203:** 166—167, 1964.

BRIL, C.: Partial fractionation of natural chlorophylls *a* and *b* in aqueous extracts of certain algae by freeze thawing. — Carnegie Inst. Wash. Year Book **64:** 370—373, 1965.

BRODY, S.: New excited state of chlorophyll. — Science **128:** 838—839, 1958.

BROWN, J., BRIL, C., URBACH, W.: Fractionation of chlorophyll forms from *Euglena* and measurements of light induced absorbance changes. — Plant Physiol. **40:** 1086—1090, 1966.

BUTLER, W. L., BAKER, J. E.: Low-temperature spectra of chloroplast fragments. — Biochim. Biophys. Acta **66:** 206—211 (1963).

CEDERSTRAND, C., GOVINDJEE: Some properties of spinach chloroplast fractions obtained by digitonin solubilization. — Biochim. Biophys. Acta **120:** 177—180, 1966.

CEDERSTRAND, C., RABINOWITCH, E., GOVINDJEE: Absorption and fluorescence spectra of spinach chloroplast fractions obtained by solvent extraction. — Biochim. Biophys. Acta **120:** 247—258, 1966.

CHO, F., SPENCER, J., GOVINDJEE: Emission spectra at very low temperatures (—269° to —196°). — Biochim. Biophys. Acta **126:** 174—176, 1966.

COHEN-BAZIRE, G.: Some observations on the organization of the photosynthetic apparatus in purple and green bacteria. — In: GEST, H., SAN PIETRO, A., VERNON, L. P. (ed.): Bacterial Photosynthesis. Pp. 89—110. The Antioch Press, Yellow Springs, Ohio 1963.

DUYSENS, L. N. M.: Transfer of excitation energy in photosynthesis. — Ph. D. Thesis, U. Utrecht, The Netherlands 1952.

EMERSON, R., LEWIS, C. M.: The photosynthetic efficiency of phycocyanin in *Chroococcus* and the problem of carotenoid participation in photosynthesis. — J. gen. Physiol. **25:** 579—595, 1942.

EMERSON, R., RABINOWITCH, E., Red drop and role of auxiliary pigments in photosynthesis. — Plant Physiol. **35:** 477—485, 1960.

FREDERICKS, W. W., JAGENDORF, A. T.: A soluble component of the Hill reaction in *Anacystis nidulans*. — Arch. Biochem. Biophys. **104:** 39—49, 1964.

FRENCH, C. S., YOUNG, V. M. K.: The fluorescence spectra of red algae and the transfer of energy from phycoerythrin phycocyanin and chlorophyll. — J. gen. Physiol. **35:** 873—890, 1952.

124 FREY-WYSSLING, A., MÜHLETHALER, K.: Ultrastructural Plant Cytology. — Elsevier Publ. Co., Amsterdam 1965.

FUJITA, Y., MYERS, J.: Cytochrome *c* redox reactions induced by photochemical system 1 in sonicated preparations of *Anabaena cylindrica*. — Arch. Biochem. Biophys. **113:** 730—737, 1966.

FULLER, R. C., CONTI, S. F., MELLIN, D. B.: The structure of the photosynthetic apparatus in the green and purple sulfur bacteria. — In: GEST, H., SAN PIETRO, A., VERNON, L. P. (ed.): Bacterial Photosynthesis. Pp. 71—87. The Antioch Press, Yellow Springs, Ohio 1963.

GANTT, E., CONTI, S. F.: Granules associated with the chloroplast lamellae of *Porphyridium cruentum*. — J. Cell Biol. **29:** 423 — 434, 1966.

GASSNER, E.: On the pigment absorbing at 750 mμ occurring in some blue-green algae. — Plant Physiol. **37:** 637—639, 1962.

GERHARDT, B., TREBST, A.: Photosynthetische Reaktionen in lyophilisierten Zellen der Blaualge *Anacystis*.—Z. Naturforsch. **20b:** 879—885, 1965.

GHOSH, A. K., GOVINDJEE: Transfer of the excitation energy in *Anacystis nidulans* grown so as to obtain different pigment ratios.—Biophys. J. **6:** 611—619, 1966.

GHOSH, A. K., GOVINDJEE, CRESPI, H. L., KATZ, J. J.: Fluorescence studies on deuterated *Chlorella vulgaris*.—Biochim. Biophys. Acta **120:** 19 — 22, 1966.

GOVINDJEE: Observations on P750A from *Anacystis nidulans*. — Naturwiss. **23:** 720—721, 1963.

GOVINDJEE: Emerson enhancement effect and two light reactions in photosynthesis. — In: KOK, B., JAGENDORF, A. T. (ed.): Photosynthetic Mechanisms of Green Plants. Pp. 318—334. Nat. Acad. Aci. — Nat. Res. Council, Wgshington, D.C. 1963.

GOVINDJEE: Fluorescence studies on algae, chloroplasts and chloroplast fragments.—In: THOMAS, J. B., GOEDHEER, J. H. C. (ed.): Currents in Photosynthesis. Pp. 93—103. Ad Donker, Rotterdam 1966.

GOVINDJEE, RABINOWITCH, E.: Action spectrum of the second Emerson effect. — Biophys. J. **1:** 73—89, 1960.

GOVINDJEE, YANG, L.: Structure of the red fluorescence band in chloroplasts. — J. gen. Physiol. **49:** 763—780, 1966.

GOVINDJEE, CEDERSTRAND, C., RABINOWITCH, E.: Existence of absorption bands at 730—740 and 750—760 millimicrons in algae of different divisions. — Science **134:** 391—392, 1961.

GOVINDJEE, MUNDAY, J. C., PAPAGEORGIOU, G.: Fluorescence studies with algae: Changes with time and preillumination.—In: OLSON, J. M. (ed.): Energy Conversion by the Photosynthetic Apparatus. Brookhaven Symposia in Biology **19:** 434—445, 1966.

GROSS, J. A. SHEFNER, A. M., BECKER, M. J.: Distribution of chlorophylls in chloroplast fragments. — Nature **209:** 615, 1966.

JONES, L., MYERS, J.: Enhancement in the blue-green alga *Anacystis nidulans*. — Plant Physiol. **39:** 938—946, 1964.

KOK, B., GOTT, W.: Activation spectra of 700 mμ absorption change in photosynthesis. — Plant Physiol. **35:** 802—808, 1960.

KREY, A., GOVINDJEE: Fluorescence changes in *Porphyridium* exposed to green light of different intensity: a new emission band at 693 mμ and its significance to photosynthesis.—Proc. Natl. Acad. Sci. (Washington) **52:** 1568—1572, 1964.

KREY, A., GOVINDJEE: Fluorescence studies on a red alga *Porphyridium cruentum*. — Biochim. Biophys. Acta **120:** 1—18, 1966.

LITVIN, F. F., KRASNOVSKY, A. A., RIKHIREVA, G. T.: Lyuministsentsiya razlichnykh form khlorofilla v listyakh rasteny. [Luminiscence of various forms of chlorophyll in the leaves of plants.]— Dokl. Akad. Nauk SSSR **135:** 1528 — 1531, 1960.

OLSON, R. A., BUTLER, W., JENNINGS, W. H.: The orientation of chlorophyll molecules *in vivo:* Evidence from polarized fluorescence. — Biochim. Biophys. Acta **54:** 615—617, 1961.

PAPAGEORGIOU, G., GOVINDJEE: Oxygen evolution from lyophilized *Anacystis* with carbon dioxide as oxidant. — Biochim. Biophys. Acta **131:** 173—178, 1967.

PETRACK, B., LIPMANN, F.: Photophosphorylation and photohydrolysis in cell free preparations of blue-green algae. — In: MCELROY, W. D., GLASS, B. (ed.): Light and Life. Pp. 621—630. J. Hopkins Press, Baltimore, Md. 1961.

SUSOR, W. A., KROGMANN, D. W.: Hill activity in cell free preparations of a blue green alga. — Biochim. Biophys. Acta **88:** 11—19, 1964.

THOMAS, J. B., DE ROVER, W.: On phycocyanin participation in the Hill reaction of the blue-green alga *Synechococcus cedrorum*. — Biochim. Biophys. Acta **16:** 391—395, 1955.

VREDENBERG, W. J.: Spectrophotometric studies on primary and associated reactions in photosynthesis. — Ph. D. Thesis, U. Leiden, The Netherlands 1965.

VON WETTSTEIN, D.: The formation of plastid structure.—Brookhaven Symposia in Biology **11:** 138—159, 1958.

Ц. Шимоны, Й. Спенсер и Говинджи (Ботанический отдел Университета в Иллинойс, Урбана, Илл., США): **Спектральные характеристики частиц *Anacystis*.** — Photosynthetica 1: 113—125, 1967.

Были исследованы спектры абсорбции и флуоресценции фрагментов сине-зеленой водоросли *Anacystis nidulans*, полученных разрушением целых клеток в фосфатном буфере (содержащем дигитонин) и центрифугированием. В работе приведены также электронные микрофотографии этих фрагментов. Две фракции частиц содержат больше хлорофилла *a*. На основе эмиссионного спектра (при температуре —196 °C) авторы считают, что одна фракция содержит больше хлорофилла a_2 и вторая хлорофилла a_1; самая легкая фракция содержала фикоцианин в растворенном состоянии и также небольшое количество хлорофилла *a*. Эффективность переноса энергии из дополнительных пигментов (каротиноидов и фикоцианинов) на хлорофилл *a* различна в разных фракциях.