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Pigment Formation and Growth in Blue-Green Algae in Crossed Gradients of Light Intensity and Temperature

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The blue-green algae contain chlorophyll *a*, phycocyanin, phycoerythrin, and a number of yellow pigments, mainly carotenoids. The color of the blue-green algae may vary greatly within the same species when grown under different conditions. These color variations are due to changes in the pigment ratios in the algae. Light is supposed to be a factor affecting these changes; both its intensity and its spectral composition have been assumed to be important in this respect. Oltmanns (1892) showed that the blue-green algae assumed different colors when grown at high and low light intensity, and Engelmann and Gaidukov (1902) reported that the color of some blue-green algae depended upon the spectral composition of the light, and that the resulting color was complementary to that of the light to which they were illuminated. Since that time a number of observations have been gathered on the effect of light on the color of algae. Two theories, founded upon entirely different principles arose, namely: (1) the color that the algae assume is a *complementary chromatic adaptation* depending upon the spectral composition of the light; *e.g.*, in the sea the red algae at greater depth have a clear red color which is complementary to the blue-green light extending to this depth; (2) the algae at low light intensity will form more accessory pigments effective in photosynthesis so they catch a high percentage of the

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light; *e.g.*, in the sea at greater depth, the red algae are shadow plants with a high amount of accessory pigments effective in photosynthesis (*light intensity adaptation*). At present there is no general agreement as to which of the two theories above is right (for review of the literature on this subject see Rabinowitch, 1945 p. 424).

Factors other than the light intensity most certainly are also involved in the processes affecting the pigment ratios in plants. The temperature under which they are grown is one.

Recently an apparatus has been built in which algae, grown on agar, are exposed simultaneously to crossed gradients of light intensity and temperature (Halldal and French, 1956). This apparatus gives the opportunity for study of the growth of the organism and of the formation and destruction of pigments under different light intensity and temperature conditions, and also for direct study of the interaction of these two factors.

Anacystis nidulans Drouet

When the blue-green alga *Anacystis nidulans* Drouet (Kratz and Allen's strain; see Kratz and Myers, 1955) was grown in the apparatus, there was a striking difference in color in different parts of the growth area. Figure 1 shows a series of photographs taken at 12 hour intervals during an experiment. At the same time a record of the changes in appearance was made with color film.

After 12 hours above 45°C at 1,000 foot-candles and above 50°C at 25 foot-candles, the alga was killed and completely bleached. It had grown significantly from 33 to 44°C at 1,000 foot-candles, and from 28 to 45°C at 25 foot-candles. The color within these limits was fresh green. At lower temperatures at all intensities the inoculum showed little change, if any.

After 24 hours a striking difference in the color in different parts of the growth area started to develop. The alga was now completely killed at temperatures above 44°C at 1,000 foot-candles, and above 45°C at 25 foot-candles. At high temperature close to the killing boundary, and at light intensities from 400 to 1,000 foot-candles, the color was bluish-green, presumably owing to greater phycocyanin formation under these conditions. The blue-green color was most striking from 500 to 800 foot-candles. At light intensities from 500 to 1,000 foot-candles, and at temperatures from 30 to 42°C, the color was changed from green to yellow, mainly due to destruction of phycocyanin (see p. 412). At light intensities from 25 to 500 foot-candles, and at temperatures between 25 and 45°C the color was somewhat deeper green, presumably due to high chlorophyll content. At lower temperatures and light intensities, from 13 to 25°C, and from 25 to 400 foot-candles, the

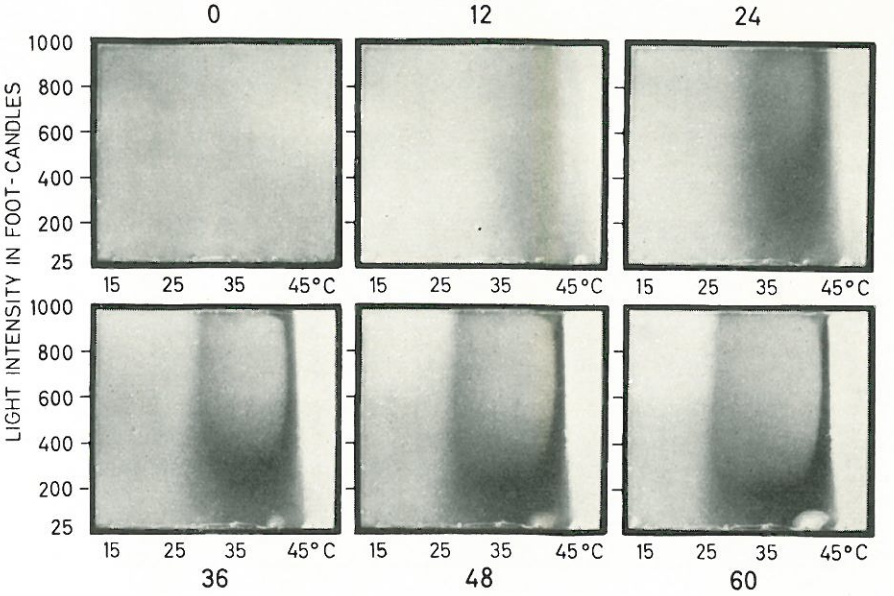

Figure 1. *Photographs taken at 12 hours intervals showing the growth pattern of Anacystis nidulans when exposed to crossed gradients of light intensity and temperature.* The figures at each picture show numbers of hours after the gradients were set up. For color description see text.

inoculum remained unchanged, while the alga at these same low temperatures, but at higher light intensities, started to bleach out in 24 hours.

During the rest of the experiment this pattern showed only small changes, but the boundaries between the different zones developed more sharply, and the zones themselves became more strikingly different. However, one of the boundaries in particular was not stationary; namely the one at 25 to 32°C parallel to the light intensity scale. This boundary moved slowly during the experiment toward lower temperatures. After 24 hours it was found at 32°C, and, after 60 hours, at the end of the experiment it was at 25°C. Several experiments with this alga show that this is the pattern that develops under normal conditions. However, the amount of inoculum will change the time for development. If a very light inoculum is used, the pattern of 12 hours in the above description will not occur. On the other hand, a heavy inoculum will keep the condition of 12 hours in Figure 1 for some time, from one to three days, but the final pattern under normal conditions will be like that described above.

In order to analyze the pigments formed under different conditions, pieces of agar with the alga were cut off the plate and placed upon a piece of opal glass. The absorption of the samples was measured in a Beckman DK-2

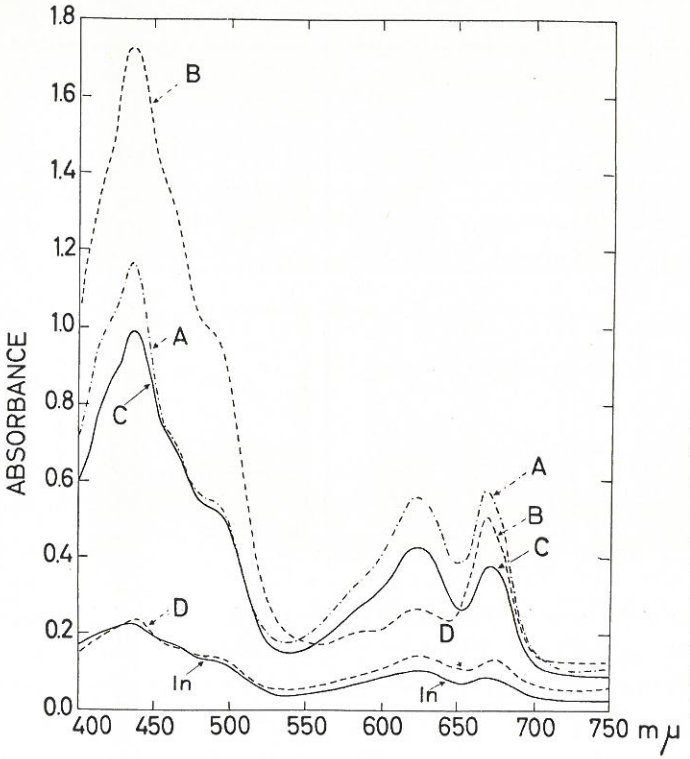

Figure 2. *Absorption curves of the inoculum* (In), *and of four samples at the end of an experiment showing variations in the relative and absolute pigmentation of different samples.* A: sample from 30°C, 100 f.c.; B: 38°C, 900 f.c.; C: 45°C, 250 f.c.; D: 15°C, 100 f.c.

spectrophotometer. A piece of agar not containing the alga, also attached to opal glass was used as a blank. This procedure is based upon the diffusing plate method of Shibata *et al.* (1954) and was suggested for this purpose by Shibata (pers. comm.). In the experiment that is described below, a rather heavy inoculum was used. The samples were taken after two days of growth, but the pattern was somewhat similar to that for 24 hours in Figure 1.

In Figure 2 are shown absorption curves of the inoculum used and of four samples taken from different parts of the growth area. Phycocyanin has an absorption maximum around 620 mμ, and the red absorption maximum of chlorophyll-*a in vivo* is around 670 to 680 mμ. The relative heights of the absorption curves at these wavelengths will thus give information about the relative amount of phycocyanin and chlorophyll *a* present in the sample. It is evident that the relative amount of phycocyanin and chlorophyll *a* varies greatly with growth conditions. There is also a significant difference in the shape as well as in the wavelength position of the red absorption peak of chlorophyll *a*. Furthermore the amount of yellow pigments formed at different parts of the growth area was highly variable, as is shown by the change

in shape and height of the curves in the blue part of the spectrum. Also in this part of the spectrum a slight wavelength shift of the maximum is indicated.

By using the curve analyzer of French *et al.* (1954) a quantitative analysis of the observed spectral differences has been attempted. In the first place the *in vivo* absorption characteristics of the principle individual pigments present in Anacystis can be estimated. From these characteristics it is then possible to estimate the quantitative relations between the various pigments in the original samples, and thus to obtain pertinent information on the interaction of light and temperature in pigment synthesis.

Phycocyanin

As mentioned above, phycocyanin has an absorption maximum in solution around 620 mμ (Svedberg and Katsurai, 1929). In order to derive the phycocyanin absorption curve for the intact pigment in Anacystis, two absorption spectra were chosen which were very similar in shape except for the spectral region from 550 to 660 mμ, where differences were evidently caused by different phycocyanin contents. The absorption spectra of two samples, one taken from 37°C and 600 foot-candles, and the other from 33°C and 600 foot-candles, had these characteristics (Figure 3). The curves were adjusted for equal chlorophyll content by making them coincide in height at the chlorophyll *a* red absorption peak at 670 mμ, and the 33°, 600 f.c. curve was then subtracted from the 37°, 600 f.c. curve. The resulting difference curve had a maximum at 625 mμ, and a shape very similar to the phycocyanin absorption spectrum of Svedberg and Katsurai (*l.c.*), and also to the curve obtained by Latimer (1956) for phycocyanin. There is, however, a significant difference in the peak position. Svedberg and Katsurai found the maximum at 616 mμ, and Latimer at 620 mμ. The position of the phycocyanin peak may, however, be different in solution and in living algae, and different treatments during the extraction may also possibly influence the wavelength position of the peak. When different samples were used in our analysis, the resulting phycocyanin absorption curves revealed some variations, both in peak position and in shape. The peak position in our analysis is expected to shift if absorption curves of some of the carotenoids have long "tails" extending into this part of the spectrum, and the amount of these carotenoids varies. The differences might also possibly be caused by minor pigments absorbing around 500 to 650 mμ; *e.g.*, phycoerythrin. The presence of phycoerythrin, which some blue-green algae contain in small quantities, was, however, not revealed in the absorption spectra of Anacystis.

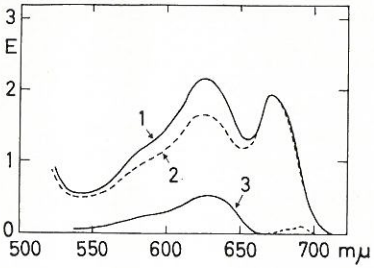

Figure 3. *The deviation of the phycocyanin* (3) *absorption curve.* (1) sample from 37°C, 600 f.c.; (2) 33°C, 600 f.c. E = Absorbance (Arbitrary units).

The difference curve of Figure 3 has been chosen as our phycocyanin absorption curve because it was derived from absorption spectra of Anacystis which at wavelengths other than from 550 to 600 mμ had the greatest similarity; and because it had a great similarity to the absorption curve of phycocyanin in solution, except for its peak position.

Chlorophyll a

Chlorophyll *a* is the only known chlorophyll in blue-green algae. Anacystis therefore gave a good opportunity to study the spectrum of this pigment *in vivo*. Above 550 mμ, the absorption of Anacystis is predominantly caused by two pigments: chlorophyll *a* and phycocyanin. Subtracting the right amount of phycocyanin from an absorption spectrum of Anacystis should give the absorption of chlorophyll *a* in this region of the spectrum. In order to decide on the proper amount of phycocyanin to subtract, certain assumptions had to be made.

From absorption spectra of other plants containing no chlorophyll *b*, and such small amounts of other chlorophylls that their presence was not revealed in the absorption spectra of the living plants, it was deduced that the distance between the red absorption peak of chlorophyll *a* and the nearest valley at shorter wavelength is 28 mμ. From the red absorption peak to the next maximum at shorter wavelength the distance is 48 mμ. These figures came from Shibata (1958) for Neottia, and Halldal (1958) for Dinophyceae. About the same distances are found in organic solvents also; 26 and 46 mμ respectively (for reviews see Rabinowitch, 1951, and Smith and Benitez, 1955). If the phycocyanin curve of Figure 3 is subtracted from the absorption spectrum of Anacystis, the distances from the red absorption peak in the difference curve to the nearest valley and the nearest peak at shorter wavelength will vary with the assumed relative proportion of the two pigments. In constructing Figure 4, this proportion was chosen such as to give the above mentioned numerical values of these two spectral distances.

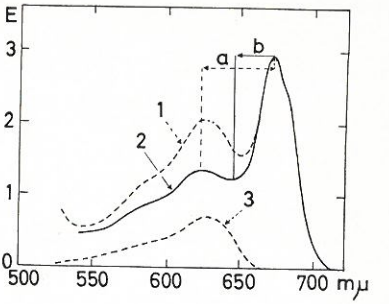

Figure 4. *The deviation of the combined chlorophyll a* (2) *absorption curve in the red part of the spectrum.* Phycocyanin (3) from Figure 3. (1) sample from 31°C, 600 f.c. a = 48 mμ; b = 28 mμ.

Chlorophyll a, 670 mμ, and Chlorophyll a, 682 mμ

When absorption curves of samples originating from different parts of the growth area were compared, it was evident that the position of the red absorption peak shifted (see Figure 2). In most cases it was at 670 mμ, but at temperatures around 24 to 25°C, it was at 675 mμ. Corresponding to this shift, there was also a small change in the wavelength position of the blue absorption peak from 435 mμ to slightly below 440 mμ. Another feature of the curves is a shoulder around 680 mμ. This shoulder was more or less pronounced in various samples. It was most clearly visible in samples from very low temperature and at extremely high temperatures close to the killing boundary. Very low light intensity also made this shoulder show up. This suggested that two components contribute to the red absorption peak of chlorophyll *a*.

In Figure 5, an absorption curve from a sample taken from 37°C and 900 foot-candles is subtracted from one taken at 24°C and 250 foot-candles. The resulting difference has a great resemblance to the absorption curve of chlorophyll *a*, with a maximum at 682 mμ. It was therefore assumed that two different forms of chlorophyll *a* contributed to the absorption by Anacystis; one with maximum absorption in red light at 670 mμ, and the other with maximum absorption at 682 mμ. Variations in the relative amounts of these two components explain the change in peak position and in the shape of the curves.

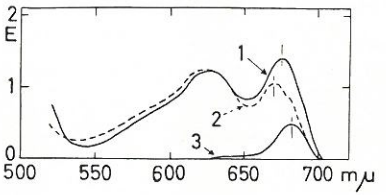

Figure 5. *The deviation of the chlorophyll a 682 mμ* (3) *absorption curve in the red part of the spectrum.* (1) sample from 24°C, 250 f.c.; (2) 37°C, 900 f.c.

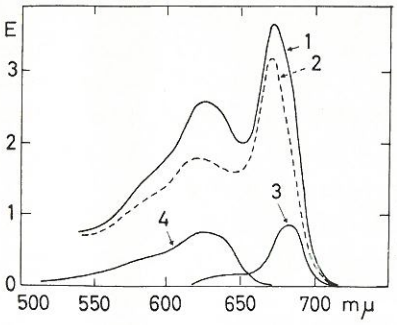

Figure 6. *The deviation of the chlorophyll a 670 mμ (2) absorption curve in the red part of the spectrum.* Phycocyanin (4) from Figure 3. Chlorophyll *a* 682 (3) from Figure 5. (1) Sample from 31°C, 600 f.c.

Figure 6 shows how the spectrum of chlorophyll *a*, 670 mμ was derived from the absorption spectrum of the sample illustrated in Figure 4 by subtraction of (1) the phycocyanin spectrum (Figure 3), and (2) the spectrum of chlorophyll *a*, 682 mμ (Figure 5), in such relative proportions that the shape of the difference curve at wavelengths longer than 670 mμ is similar to that of chlorophyll *a* in solution at wavelengths longer than its red absorption maximum.

The phycocyanin curve of Figure 3, the chlorophyll *a*, 670 mμ curve, and a curve similar in shape to the chlorophyll *a*, 670 mμ curve, but with a maximum at 682 mμ (chlorophyll *a*, 682 mμ), were all traced and added together in the curve analyzer of French *et al.* (1954). It was then possible, by choosing the proper values of the "absorbance" scales of these curves, to reproduce the absorption curve from 600 mμ to longer wavelengths of any sample of Anacystis. The fit was closer than 10 per cent.

Yellow Pigments

The absorption of Anacystis in the blue part of the spectrum is caused by a number of different pigments. Chlorophyll *a* has a high absorption in this region, and the carotenoids absorb mainly at these wavelengths. There are other yellow pigments present in the algae also. For example, Forrest *et al.* (1957) have analyzed the formation of pteridines in Anacystis, and they have shown that their formation was intimately concerned with the photosynthetic activity of the alga.

From curve analysis it was evident that the relative amount of the different yellow pigments varied over a wide range. It was therefore not possible to derive a single curve for the sum of these pigments. However, as some of the curves have a very similar shape, but a different magnitude in this region, their main difference must be of a quantitative nature. By the use of such curves it should be possible to derive the absorption spectrum of chlorophyll *a* in the blue region.

Blue Absorption Spectrum of Chlorophyll a

The absorption spectrum of a sample at 27°C and 900 foot-candles, and one from 33°C and 600 foot-candles were very similar in shape in the blue and green region of the spectrum. In order to make the chlorophyll content of these equal, they were made to coincide at their red absorption peak, and the curve of 33°C, 600 foot-candles was subtracted from that of 27°C, 900 foot-candles, thus giving a curve representing the yellow pigments in these two samples (Figure 7). This curve was then used to subtract the yellow pigments from the curve of sample 33°C, 600 foot-candles. Some assumptions had to be made in deciding on the appropriate factor to be applied to the curve before subtraction.

It is known from solutions that the ratio blue absorption peak/red absorption peak of chlorophyll *a* is about 1.30, somewhat depending upon the solution (from 1.25 to 1.40); see Rabinowitch (1951) and Smith and Benitez (1955). It was assumed that the ratio between these two chlorophyll *a* peaks *in vivo* was about the same. Further, it is known that the absorption of chlorophyll *a* in solution is very low, practically zero, around 475 mμ. It was therefore assumed that chlorophyll-*a* *in vivo* has a very low absorption somewhere between 475 and 500 mμ. The absorption factor for the yellow pigment curve was then computed to meet these requirements (Figure 8).

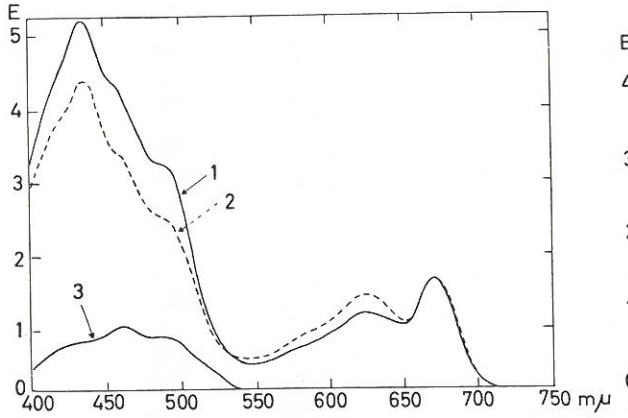

Fig. 7.

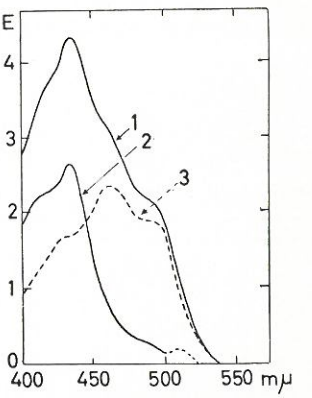

Fig. 8.

Figure 7. *The deviation of the absorption curve representing the yellow pigments* (3) *in samples taken from 27°C, 900 f.c.* (1) *and from 35°C, 600 f.c.* (2).

Figure 8. *The deviation of the combined chlorophyll a* (2) *absorption curve in the blue part of the spectrum.* (1) sample from 35°C, 600 f.c. Yellow pigments (3) from Figure 7.

The resulting curve is very similar in shape to the absorption curve of chlorophyll *a* in the blue part of the spectrum.

It was further assumed that the blue absorption of chlorophyll-*a in vivo* is caused by two different components, as is the case in the red region of the spectrum. Since the chlorophyll *a* of the sample 33°C, 600 foot-candles was found to consist mainly of the 670 mμ form, it was assumed that the blue absorption peak of chlorophyll *a*, 670 mμ is where the difference curve of Figure 8 has a maximum, namely at 435 mμ.

The slight shift in the blue absorption peak mentioned on page 407 is the only indication of the presence of two chlorophyll *a* forms in the blue part of the spectrum. The difference between these two forms in the red part of the spectrum was found to be 12 mμ. As the difference in the peak position at different wavelengths is on a frequency basis, the peaks would be only 8 mμ apart at wavelengths around 440 mμ. Due to the many substances absorbing in the blue part of the spectrum, a clear distinction between two peaks in such a position would be very difficult to observe.

The Final Curves in the Visible Region

Based upon the above analysis, the curves of Figure 9 were drawn. At wavelengths longer than 600 mμ, it has been possible to reproduce any absorption spectrum from Anacystis, by adding the appropriate amount of each of the curves in Figure 9. In our analysis there has been only one minor detail indicating the presence of two chlorophyll *a* forms in the blue region of the spectrum, and our analysis did not reveal the small bands in the absorption curves of chlorophyll *a* around 500 to 600 mμ, which are known from solutions. In order to indicate these uncertainties, the absorption curves of chlorophyll *a* have been dotted at wavelengths shorter than 600 mμ.

Phycocyanin has a slight increase in absorption from 500 mμ toward shorter wavelengths in solution (Svedberg and Katsurai, 1929; Latimer, 1956). The shape of the phycocyanin curve from 525 mμ to shorter wavelengths is based upon this.

Figure 10 shows as an example how the absorption spectrum of a sample from 37°C and 600 foot-candles can be decomposed into a number of individual pigment spectra. The curve marked yellow pigments is here derived from the original absorption curve by subtraction of the two chlorophyll curves and the phycocyanin curve.

Pigments Formed at Different Locations in the Growth Area

As any curve could be separated into its main components: chlorophyll *a*, 670; chlorophyll *a*, 682; phycocyanin; and yellow pigments; this gave oppor-

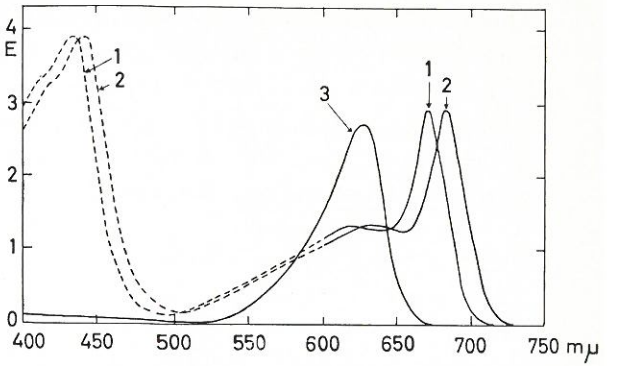

Figure 9. *The absorption curves of: phycocyanin* (3), *chlorophyll a 670 mμ* (1), *and chlorophyll a 682 mμ* (2). The uncertainty of a few assumptions in the yellow to violet region of the spectrum has been indicated by making the lines of the chlorophyll *a* curves dotted.

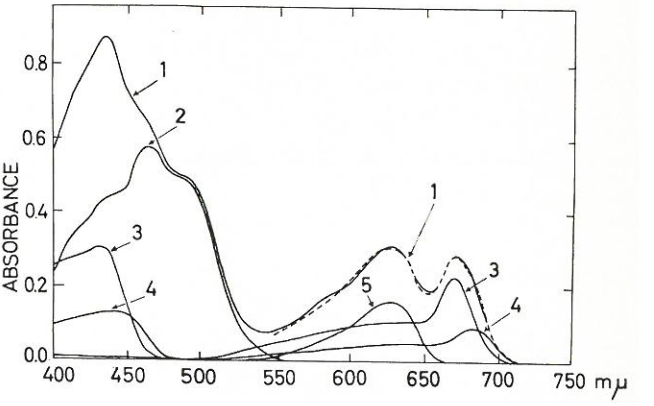

Figure 10. *The absorption curve from a sample grown at 37°C. 600 f.c.* (1) *separated into its phycocyanin* (5), *chlorophyll a 670 mμ* (3), *chlorophyll a 682 mμ* (4), *and yellow pigment components* (2). The dotted line is the sum of chlorophylls and phycocyanin.

tunities for the study of the formation of these different pigments at different temperatures and light intensities. The values necessary for this study can be obtained by calculations from simple equations based upon the absorbance at 625 mμ (phycocyanin); 670 mμ (chlorophyll *a*, 670); 682 mμ (chlorophyll *a*, 682); and 465 mμ (yellow pigments) more easily than by subtracting curves.

Figure 11 shows a three-dimensional representation of the relative amount of phycocyanin; chlorophyll *a*; and yellow pigments at different places in the growth area. This represents the growth pattern before the yellow region in the center started to develop, in this experiment 48 hours (cp. Fig. 1, 24 hours). A few irregularities in the pattern were corrected by interpolation.

The chlorophyll content was highest around 300 foot-candles between 30 and 40°C, but it was also high over a rather wide temperature range around this light intensity, and over a wide light intensity around 30°C.

For phycocyanin the formation was highest at high light intensity and temperature, with a decrease toward extreme light intensity, and a very sharp

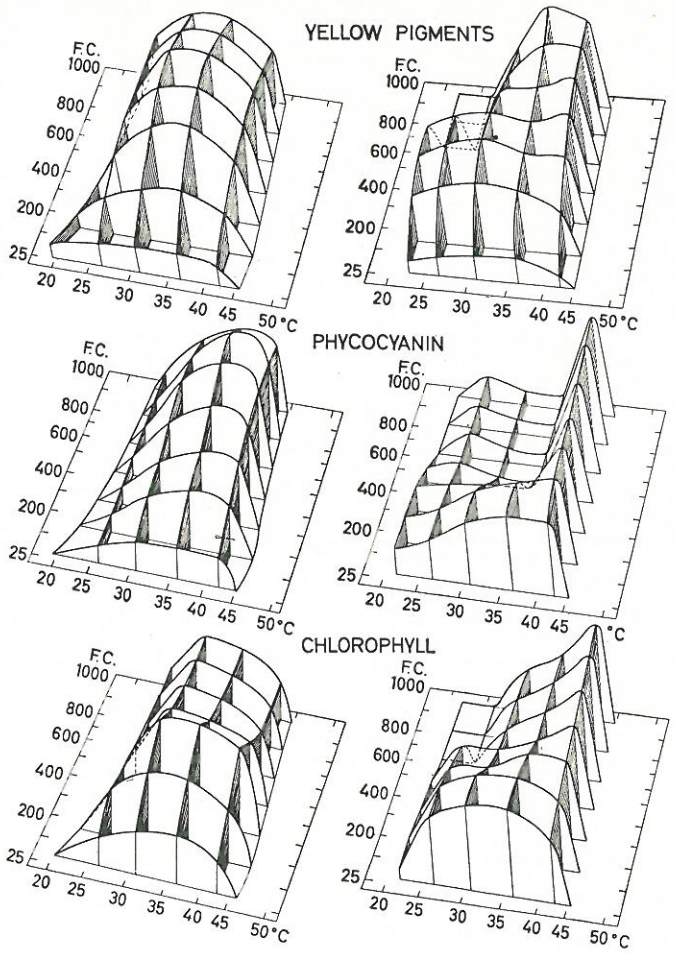

Fig. 11. Fig. 12.

Figure 11. *Three-dimentional representation of total chlorophyll a, phycocyanin, and yellow pigments at an intermediate stage* (48 hours in this experiment) *in the development of the growth pattern.*

Figure 12. *Three-dimentional representation of total chlorophyll a, phycocyanin, and yellow pigments after the final growth pattern was developed* (60 hours in this experiment).

drop at extreme temperature. The phycocyanin content lessened both toward lower light intensity and temperature.

The formation of yellow pigments was greatest between 400 and 800 foot-candles, and from 30 to 35°C. Thus the formation of yellow pigments had a maximum between those of chlorophyll and phycocyanin.

The situation described above was not stationary, but an intermediate stage

leading into the striking pattern illustrated in the latter part of the experiment of Figure 1. A three-dimensional representation of the pigment composition within the pattern of Figure 1, 48 hours, is given in Figure 12 (in the present experiment this pattern was actually attained after 60 hours).

The chlorophyll content was highest at the highest temperature and light intensity within the growth area, but a high chlorophyll content was found at all light intensities, and increasing with increasing light intensity, within the narrow stripe from 43 to 45°C. Also at low light intensity, around 100 foot-candles, over a wide range af temperature from 26 to 45°C, the chlorophyll content was very high with a maximum at 35°C. Both from the narrow high temperature stripe and the wider area in the low intensity region, the content decreased evenly toward the middle of the growth area. A very sharp drop was found at 26°C at all light intensities.

The narrow stripe at high temperature was set apart from the central part of the growth area mainly by its high phycocyanin content, which lessened rapidly with decreasing temperature, and which also decreased slightly with decreasing light intensity within this narrow stripe. A rather high phycocyanin content was also recorded at low light intensity over a wide range of temperature from 26 to 45°C. Both from the narrow high temperature stripe and the wider area in the low intensity region the phycocyanin content decreased rapidly toward the middle of the growth area where a very low phycocyanin content was recorded. At light intensities above 400 foot-candles there was a slight increase in phycocyanin content from the central area toward lower temperature with a maximum at 26°C followed by a rapid decrease.

The content of yellow pigments was found to be highest in the central area from 35 to 42°C, and from 500 to 1,000 foot-candles. It gradually decreased toward both low light intensity and temperature, and decreased rapidly toward the killing boundary at high temperature.

The ratio chlorophyll *a* 670/chlorophyll *a*, 682 of a growth pattern similar to that of Figure 1, 48 hours is given in Table I.

Table 1. *The ratio chlorophyll a, 670*/chlorophyll *a*, 682 at different light intensities and temperatures.

Light Intensity in Foot-candles	Temperature in °C			
	15	30	38	44
900	—	2.64	2.86	2.20
400	2.60	3.72	3.40	2.62
200	1.22	2.55	2.36	1.90
75	1.38	2.40	2.24	1.40

The ratio was highest at 400 foot-candles and 30 to 38°C, and from this area it decreased both toward lower and higher light intensity and temperature. It was particularly low when low light intensity was combined with either a high or a low temperature.

Anabaena sp.

Figure 13 shows the growth pattern at the end of an experiment with Anabaena sp. (obtained from M.B. Allen). This alga did not develop the striking zones that Anacystis did. However, near the low temperature boundary a yellow stripe developed, which by spectrographic analysis was shown to be caused mainly by a destruction of phycocyanin. The killing boundary at high temperature of this species was 44°C at 1,000 foot-candles, and 45°C at 25 foot-candles. At lower temperature the growth boundary was at 28°C at 1,000 foot-candles and at 25°C at 25 foot-candles. The latter boundary, however, did move slowly toward lower temperatures during the experiment, and this species presumably grows fairly well at temperatures below 25°C, particularly at low light intensity.

Figure 14 shows three absorption spectra of Anabaena measured from samples all taken at 38°C, but at different light intensities. The curves were made to coincide at the red absorption peak of chlorophyll. At 600 foot-candles the phycocyanin content was somewhat higher than at 950 foot-candles, while the relative amount of yellow pigments had gone down considerably. Finally, at 75 foot-candles, the relative amount of phycocyanin was still higher than in the two preceding samples, and this sample had an extremely low amount of yellow pigments. By the maximum at 575 mμ, the latter curve revealed the presence of phycoerythrin which was not indicated in the two other curves.

The absorption spectra recorded from other samples show that the shape of the curves was very much the same in samples taken from the same light

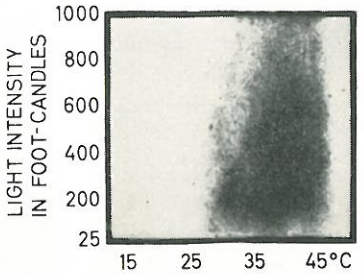

Figure 13. *The growth pattern of Anabaena sp. after 50 hours growth in crossed gradients of light intensity and temperature.*

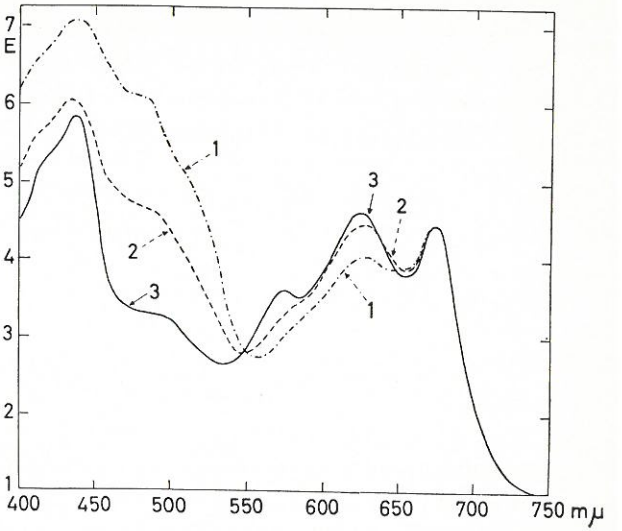

Figure 14. *Absorption curves of three samples of Anabaena sp. taken from the same temperature at 38°C, but at different light intensities after 50 hours exposure to crossed gradients of light intensity and temperature of Figure 13.* (1) sample from 950 f.c.; (2) sample from 600 f.c.; (3) sample from 75 f.c.

intensity. However, the phycocyanin content was highest at intermediate temperatures around 38°C and lessened somewhat toward both higher and lower temperatures. The maximum around 575 mμ was recorded in all samples taken at low light intensity.

Thus, the relative amount of the different pigments formed in Anabaena sp. was particularly dependent upon the light intensity. At high light intensity a very large amount of yellow pigments was formed, but little phycocyanin, and phycoerythrin in such small amounts that it was not revealed in the absorption spectra of living cells. When grown under lower light intensity, Anabaena formed much less yellow pigments, but more phycocyanin, and at very low light intensity phycoerythrin was formed in such amount that it was revealed in the absorption spectra of living cells.

Discussion

Liquid Cultures and Agar Growth

It may be suspected that the different characteristic areas which developed under different light and temperature conditions as described in connection with Figure 1, are specific for the growth of Anacystis on agar, and that lack of nutrients, for instance, may be one of the reasons for the change that occurred at 30 to 43°C, 400 to 500 foot-candles, where the color changed from fresh green to yellow. However, our experience with liquid cultures strongly suggests that this is a direct temperature-light intensity dependent

effect. If a liquid culture of Anacystis is kept at low light intensity, it can be grown over a wide range of temperatures. However, if the light intensity is high, Anacystis is very sensitive to minor changes in temperature. In the case of a somewhat low temperature, the culture will turn yellow after a short time; if the temperature is kept within a very narrow range in the higher region, the alga can be maintained in growth with a blue-green color. A slight increase of this temperature will completely bleach the alga evidently due to killing. So far our experiences from liquid cultures are consistent with those from growth on agar.

Chlorophyll a, 670 mμ, and Chlorophyll a, 682 mμ

Above we presented evidence for the existence of two spectroscopically different forms of chlorophyll *a* in live Anacystis. Curves which have a similar shift in the position of the red absorption maximum, and which also have an indication of a shoulder around 680 mμ have been recorded by French and Halldal (unpublished results) for *Chlorella pyrenoidosa* (Emerson's strain) and for *Chlorella* sp. (TX 71105 of Sorokin and Myers). French (1957) analyzed the first derivative of absorption curves of *Chlorella pyrenoidosa*, grown at different temperatures and light intensities. The curves had features around 670 to 680 mμ which indicated that the absorption in this region of the spectrum is caused by two components. When he integrated the first derivative curve, it coincided well with the absorption curve measured for the same sample in the Beckman spectrophotometer.

The existence of two spectroscopically different types of chlorophyll-*a in vivo* has been suggested by Duysens (1952); Yocum and Blinks (1954); and Vorob'eva and Krasnovskii (1956). Vorob'eva and Krasnovskii (*l.c.*) reported a shift in the wavelength of the red absorption peak of leaves grown under different light intensities and temperatures, and suggested that there is an active form with maximum absorption in the red at 670 mμ, which is transformed by strong illumination to an inactive form with a maximum at 678 mμ.

Recently, Shibata (1957), in studies of the protochlorophyll-chlorophyll transformation in intact leaves, showed that protochlorophyll first is transformed, in the light, to a chlorophyll *a* form with maximum absorption in the red at 684 mμ. Then, in the dark, the maximum changes to 673 mμ; this was in turn succeeded by a final shift to 677 mμ. The latter change may possibly be caused by a relative increase in the 684 mμ form.

French and Young (1956) calculated the curves for the active and inactive pigments of the red algae *Delesseria decipiens* and *Porphyra naiadum* from data of Haxo and Blinks (1950). They found an absorption curve for inactive

pigments of these algae with a reasonable approximation to the *in vivo* absorption spectrum of chlorophyll *a*, and according to their Figures 6—8 (d), with a maximum around 680 mμ, while the absorption of the active pigment has its maximum around 670 mμ in the red part of the spectrum.

Thus, a number of observations suggest the existence of two chlorophyll *a* forms. Only one form of chlorophyll *a* is known in solutions. It is therefore assumed that the two different forms which seem to be present *in vivo* are due to different bindings of chlorophyll to other substances, presumably proteins.

Lately, Latimer (1958) showed that selective scattering will influence many measurements of the absorption spectra of turbid suspensions. By use of a diffusing plate according to the method of Shibata *et al.* (1954) this distortion is greatly diminished. If it is not completely eliminated this may be the reason for the changes that occurred in our measurements. However, as the optical system was the same in all our measurements, and as there seems to be a correlation between change in shape of the curves and conditions on the growth area, the existence of the two chlorophyll *a* forms is probably real.

Complementary Chromatic Adaptation—Intensity Adaptation

In the introduction two of the theories concerning the effect of light on the pigment ratios in algae were summarized: (1) the *complementary chromatic adaptation* theory that the algae assume a color complementary to that of the incident light. This effect has not been subject to analysis in the present investigation; (2) the *light intensity adaptation* theory that the pigments formed are determined by the intensity of the light irrespective of its spectral composition. The present investigation shows that the intensity of "white light" is highly decisive for the pigments formed in both Anacystis and in Anabaena.

The pigment ratios in Anacystis are rather complex and also variable with time (see Figures 11 and 12). It is evident, however, that at low light intensity at all temperatures within the growth area the contents of both chlorophyll and phycocyanin were high, and that it lessened toward higher light intensity except in the narrow stripe around 42 to 45°C where it increased with increasing light intensity. Within the growth area the relative amount of yellow pigments was consistently high where the relative amount of chlorophyll and phycocyanin was low and *vice versa*. This suggests that the photosynthetic activity was low in the center of the growth area where the color changed from fresh green to yellow. The function of the yellow pigments under these conditions might be to act as a light screen, thus

protecting the photosynthetic apparatus from too strong light. The narrow blue-green stripe at high temperature around 42 to 45°C at all light intensities suggests that the photosynthetic apparatus is more effective here than at lower temperatures and high light intensity. The answer to this can only be given by maintaining Anacystis under these conditions and then measuring the photosynthetic activity of the alga.

In the experiment with Anabaena the pigment pattern was more simple. At low light intensity, the contents of both phycocyanin and phycoerythrin were very high, while the amount of yellow pigments was extremely low. It is well established that the phycobilins are accessory pigments in photosynthesis and it is equally clear that light absorbed by the yellow pigments is less effectively utilized (for review see Blinks, 1954). Lately, it has been shown that β-carotene presumably can actively participate in photosynthesis (Lynch and French, 1957).

The results from the Anabaena experiment suggest that two types of adaptation take place: (1) at low intensity accessory pigments such as phycobilins and possibly β-carotene in its active state (suggested by the shoulder at 490 mμ) are formed and give high photosynthetic capacity; (2) with increasing light intensity the relative amount of these pigments was reduced, but more yellow pigments other than that (or those) causing the shoulder at 490 mμ were formed, which may well be yellow pigments inactive in photosynthesis. These yellow pigments possibly act as a light screen in a similar way as β-carotene in oil drops in Trentepolia and Dunaliella (see Blinks, 1954), thus protecting the photosynthetic apparatus of the alga from high light intensity, as was also suggested for Anacystis above.

The experience from laboratory growth of blue-green algae is that they must be kept at a very low light intensity. These algae are in many cases in nature exposed to direct sunlight of the highest possible intensity when floating on tropical water; along the sunny sides of the sea shore; and on desert soil. In the laboratory they will ordinarily die when exposed to only a fraction of natural sunshine. The causes for this may be many. Experiments with Anacystis and Anabaena showed that both the growth of these algae and the relative amount of pigments formed, varied from one light intensity — temperature combination to the other. It may be assumed that the conditions in nature favor the formation of some photosynthetically inactive pigments at high light intensity which act as a light screen.

Summary

The blue-green algae *Anacystis nidulans* and *Anabaena* sp. (obtained from M. B. Allen) have been grown on agar in crossed gradients of light intensity

and temperature. A striking, well-defined pattern developed for Anacystis, which had great variations from one temperature — light intensity combination to another. Anabaena had a more uniform pattern.

Based upon curve analysis of absorption spectra from samples of Anacystis taken from different parts of the growth area, the *in vivo* absorption spectra of two chlorophyll *a*, phycocyanin, and yellow pigments have been derived.

A three-dimensional representation of the different pigments formed in Anacystis at different light intensities and temperatures at two stages of the development is given.

Phycoerythrin was not revealed in the absorption spectra of Anacystis, while the absorption spectra of Anabaena from samples taken at low light intensity had a clear maximum at 575 mμ, indicating the presence of this pigment.

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