Plant Physiol. (1971) 47, 230–235

Chlorophyll Synthesis in *Chlorella*[1]

REGULATION BY DEGREE OF LIGHT LIMITATION OF GROWTH

Received for publication August 11, 1970

SAMUEL I. BEALE[2] AND DAVID APPLEMAN
Department of Botanical Sciences, University of California, Los Angeles, California 90024

ABSTRACT

The degree of light limitation of growth is the primary controlling factor of chlorophyll synthesis during photoautotrophic growth of *Chlorella*. The chlorophyll content of the cells increases when light is limiting for growth as occurs in dense cultures, or in cultures under low incident light, or when the light is used less efficiently through partial inhibition of photosynthesis by 3-(*p*-chlorophenyl)-1,1-dimethylurea. The chlorophyll content decreases when light is not limiting for growth, as occurs in cells in high light intensity and in dilute suspensions. The initial lag in rate of chlorophyll synthesis in a freshly inoculated culture can be attributed to light at first not being growth limiting, and then becoming growth limiting as the cell suspension becomes denser. Continuous culture experiments support the above conclusions by showing that under steady state conditions the chlorophyll content is inversely related to the relative available light.

Most algae, *Chlorella* included, do not require light in order to carry out chlorophyll synthesis, and therefore, the regulation of chlorophyll synthesis in algae appears to be quite different from that involved in the greening of etiolated higher plants. There are, however, some common features of chlorophyll synthesis in algae and higher plants: δ-aminolevulinic acid synthetase, the putative first enzyme of chlorophyll synthesis, has not been detected in extracts of any green plant, including algae (3); chlorophyll synthesis in *Chlorella* appears to be dependent upon protein synthesis (2), as it is in higher plants (4, 5); mutants of *Chlorella* exist which behave like higher plants with respect to a light requirement for protochlorophyllide reduction and chlorophyll formation (7, 8).

Sargent (14) and Myers (10, 11) reported that *Chlorella* produced more chlorophyll under low light conditions than under high light conditions. This is perhaps related to the observations of Šesták (15) and others, as discussed by Rabinowitch (13), that shaded leaves contain more chlorophyll than leaves exposed to full sunlight.

A detailed investigation of some of the physiological parameters related to variations in the chlorophyll content of *Chlorella* was conducted by Shugarman and Appleman (16–19). They showed that the formation of chlorophyll was specifically suppressed when dilute cultures of *Chlorella* were exposed to high light intensity, the effect being designated the lag phase of chlorophyll synthesis. The duration of the lag phase could be altered by changing the incident light intensity on the culture or the initial cell population density, and by the presence of certain sugars in the culture medium.

A number of possible explanations were put forward concerning the physiological basis of the lag phase. First, the lag could be due to some intrinsic induction period required before the enzymes necessary for chlorophyll biosynthesis are produced within the cells. When a newly inoculated culture is placed in the light, perhaps some component of the chlorophyll biosynthetic system is destroyed and then resynthesized during the induction period. Second, perhaps light suppresses chlorophyll synthesis directly. Then the lag phase might reflect the time required for the cells to overcome the light-induced suppression of chorophyll synthesis, *i.e.*, to adapt to the new light environment. Third, the lag phase might represent a feedback control exerted by a product of photosynthesis over another process, chlorophyll biosynthesis, which provides a key component of the photosynthetic apparatus, namely, chlorophyll. The cells might adjust their chlorophyll content in response to the light available for carrying out photosynthesis. Fourth, inasmuch as growth rate and chlorophyll biosynthetic rate appear to be inversely related, perhaps there is a competition for a common precursor of chlorophyll and other cellular components.

The experiments described in this paper were designed to indicate which of the above explanations most accurately accounts for the lag phase of chlorophyll synthesis.

MATERIALS AND METHODS

Chlorella vulgaris Beijerinck was obtained originally from S. Granick (6). The methods of culturing the cells, the culture medium, and the measurement of packed cell volume and chlorophyll content are basically similar to those of Shugarman and Appleman (17).

Cells were grown in a defined mineral medium, the composition of which is given in Table I. Culture flasks were 500-ml Erlenmeyer flasks, each fitted with a bubbler tube extending down into the culture. Two hundred milliliters of culture medium were placed into each flask. Then gauze-covered cotton plugs were placed in the mouths of the flasks, and cotton was forced into the top ends of the bubbler tubes. The flasks were autoclaved and then allowed to cool to room temperature before inoculation with an axenic suspension of cells. A filtered and humidified mixture of 5% CO_2 in air was passed through each bubbler tube at a total rate of 100 ml/min.

The inoculated flasks were placed on a reciprocal shaker in a temperature-controlled room which maintained the temperature within the cell suspension at 28 to 30 C. Cells were kept

[1] This work was supported in part by a National Institutes of Health Predoctoral Fellowship GM-28419.

[2] Present address: Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

in suspension by a combination of the gas mixture bubbling into the suspension and by the reciprocal shaker.

The culture flasks were illuminated from below by 100-w, clear traffic signal lamps (Ken-Rad). The distance from the top of the lamps to the bottom of the culture flasks was 10 cm.

Light intensity measurements were performed with a Weston Model 603 illumination meter. The intensity of the illuminating lamps was adjusted by controlling the voltage with a variable transformer. An intensity of 8000 lux at the bottoms of the flasks was obtained with approximately 110 volts, and this intensity was used in most of the experiments described.

Packed cell concentration was measured by transferring a sample of cell suspension to a Hopkins vaccine tube and by centrifuging the sample for 5 min at 2500 rpm (full speed) in a tabletop clinical centrifuge (International Equipment Co.).

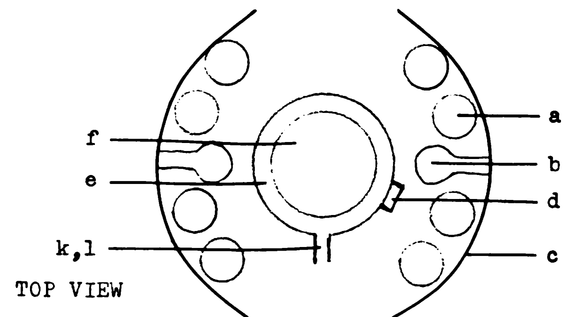
Chlorophyll was extracted from cells with methanol, and the absorbance of the methanol extracts was measured at 665 and 650 nm in a Cary model 14 spectrophotometer. Chlorophyll concentrations were calculated by using the absorption coefficients of MacKinney (9).

For studies of cell populations under steady state conditions, a continuous culture apparatus was designed (Fig. 1). The growth chamber consisted of the space between two concentric glass cylinders, 56 cm high, having an outer diameter of 8.6 cm and a space between the cylinders, or annulus, of 1.45 cm, containing 1100 ml of cell suspension. On the inside of the smaller cylinder, water was circulated at a constant temperature of 25 C. Five percent CO_2 in air was bubbled into an inlet at the bottom of the chamber at a total rate of 250 ml/min. This also served to mix the cell suspension. At the bottom inlet was another tube through which fresh culture medium could be added. A similar tube at the top was connected to a receptacle for spillover and for venting of the CO_2-air mixture. Surrounding the entire growth chamber was a series of fluorescent lamps, together with some incandescent lamps. All of the lamps were attached to reflecting shields which could be moved closer to or away from the growth chamber. The culture medium inlet tube at the bottom of the growth chamber was connected to a large vessel of sterile medium through a solenoid valve. The valve was actuated by an electronic switch connected to a photocell which was attached to one side of the growth chamber and which accepted light passing through the chamber from the other side. The electronic apparatus could be adjusted so that any desired degree of light absorption by the culture could cause the solenoid valve to open and allow fresh culture medium to enter the chamber and dilute the cell suspension within. An equal volume of cell suspension would then be expelled through the tube at the top of the chamber and into the waste receptacle. The bottom of the chamber was equipped with another tube through which samples of the cell suspension could be withdrawn for analysis. The idea for the continuous culture apparatus was obtained from the work of Myers and Clark (12).

3-(*p*-Chlorophenyl)-1,1-dimethylurea[3] was obtained from E. I. DuPont de Nemours and Company, Wilmington, Delaware.

Table I. *Composition of the Liquid Medium for Photoautotrophic Growth of Chlorella*

Component	Concn
	molar
KNO_3	3×10^{-2}
$MgSO_4$	1×10^{-2}
KH_2PO_4	4×10^{-3}
$Ca(NO_3)_2$	1×10^{-3}
FeEDTA	2×10^{-5}
H_3BO_3	2×10^{-5}
$MnCl_2$	5×10^{-6}
$ZnSO_4$	5×10^{-7}
$CuSO_4$	1×10^{-7}
NH_4VO_3	1×10^{-7}
$CoCl_2$	1×10^{-7}
$(NH_4)_6Mo_7O_{24}$	1.5×10^{-8}
Initial pH	5.2



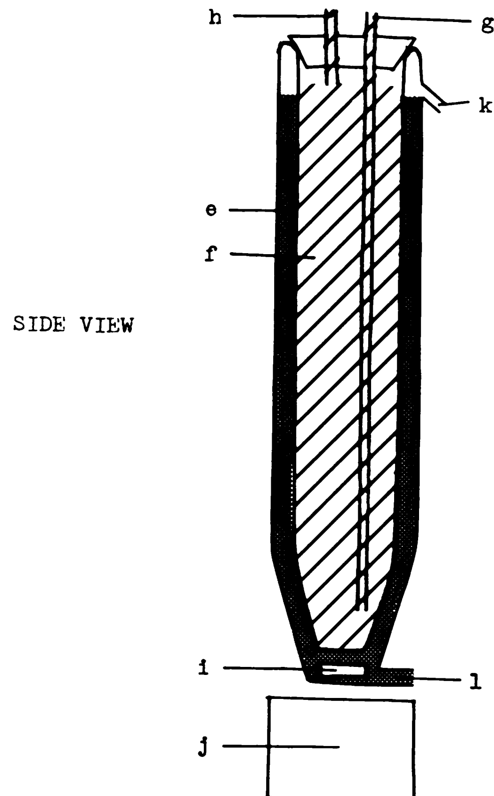


FIG. 1. Continuous culture apparatus. Drawing of the device used to maintain growing cell suspensions of *Chlorella* at constant packed cell and chlorophyll concentrations. a: Fluorescent lamps; b: incandescent lamps; c: light shield and reflector; d: photocell; e: cell suspension; f: cooling water; g: cooling water inlet; h: cooling water outlet; i: magnetic stirring bar; j: magnetic stirring motor; k: overflow and aerating gas outlet; l: inlet for culture medium and aerating gas, and outlet for sampling.

[3] Abbreviations: CMU: 3-(*p*-chlorophenyl)-1,1-dimethylurea; PC: packed cells.

RESULTS AND DISCUSSION

Relation between Chlorophyll Synthesis and Growth Rate. Figure 2 shows the increases in packed cell volume and chlorophyll when a suspension of *C. vulgaris* Beijerinck was inoculated so that the initial packed cell concentration was 0.5 ml/liter of suspension. The logarithms of the packed cell and chlorophyll concentrations are plotted against time so that the initial growth is seen to be exponential and so that the changing rates of both growth and chlorophyll synthesis are clearly visible.

Figure 3 shows the results of a longer term experiment conducted in the same manner as the previous one. This time the data are plotted directly against time, thereby making it clearly seen that after the first 22 hr after inoculation, the growth and chlorophyll synthetic rates were linear. The cell suspension used to inoculate the culture flasks for both experiments was obtained from a culture approximately 72 hr old.

There was very little increase in chlorophyll content during the first, exponential, phase of growth; by the time that growth was at the end of the exponential phase, the amount of chlorophyll per unit of packed cell volume had decreased to about one-third of the initial value. After 24 hr of growth, the rate of chlorophyll synthesis began to increase, and, as the cells continued to grow linearly, the chlorophyll content per unit of packed cell volume increased and, at 72 hr, was about at the same value as at the time of inoculation. At no time did the total amount of chlorophyll decrease absolutely; rather, it increased at varying rates.

Relative Available Light. An estimate of the relative amount of light available to an average cell of a suspension under various culture conditions was obtained as follows. Cell suspensions were prepared containing equal packed cell concentrations but differing known amounts of chlorophyll per unit of packed cell volume. The absorption of light of 680 nm (the red absorption peak) was measured by using 1-cm-square

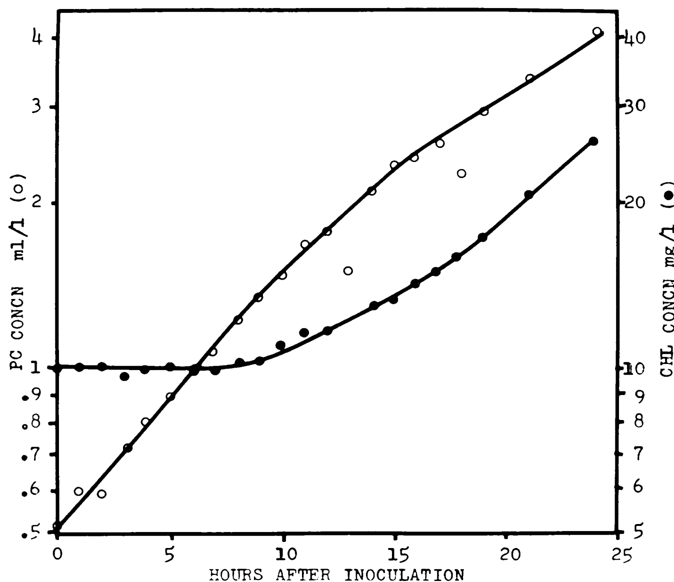


FIG. 2. Early stages of growth and chlorophyll synthesis in *Chlorella*. Photoautotrophic growth and chlorophyll synthesis were measured in a culture of *C. vulgaris* Beijerinck during the first 24 hr following inoculation of a flask with a dilute suspension of cells, taken from a flask in which the packed cell concentration had reached 13 ml/liter of suspension. The logarithms of packed cell (○) and chlorophyll (●) concentrations are plotted against time after inoculation.

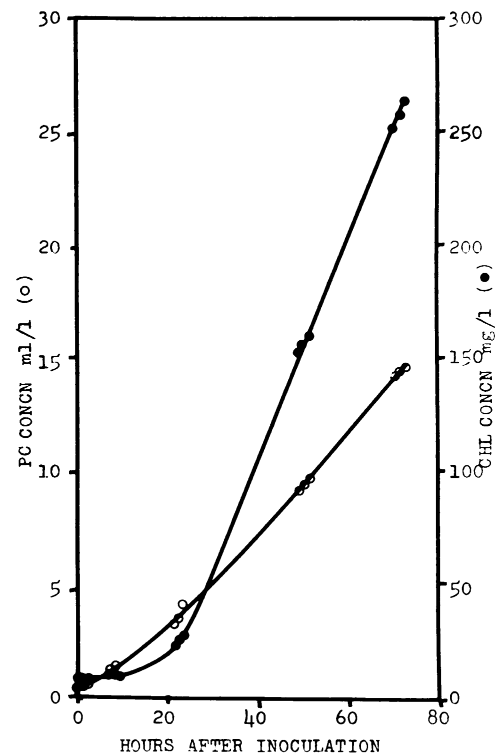


FIG. 3. Late stages of growth and chlorophyll synthesis in *Chlorella*. Photoautotrophic growth and chlorophyll synthesis during 73 hr after inoculation of a culture as described in Figure 2. Packed cell (○) and chlorophyll (●) concentrations are plotted on linear scales.

cuvettes and the conventional optical system of the Cary model 14 spectrophotometer. Assuming that the suspensions scattered light to the same degree, the amount of chlorophyll present within the cells could be related to the light absorption due to that chlorophyll. Two samples, both containing 1 ml of packed cells per liter of suspension and chlorophyll concentrations of 13.45 and 6.43 mg/ml of packed cells had absorbances of 1.034 and 0.845, respectively, at 680 nm. From this data the light absorption due to the chlorophyll within the cells was calculated to be 0.027 absorbance unit/mg of chlorophyll per liter of suspension in a 1-cm light path.

The amount of light available to the average cell in a suspension is approximately the light intensity at a point halfway through the depth of the suspension through which the incident light must pass. The absorption of 680 nm of light at this point is equal to 0.027 times one-half the suspension depth times the chlorophyll concentration in milligrams per liter of suspension. From the definition of absorption

$$\text{absorption} = \log \frac{\text{incident intensity}}{\text{intensity}},$$

the intensity of light available to the average cell in a suspension could thus be found from the chlorophyll concentration and the incident light intensity. Inasmuch as the incident light is expressed in lux, the derived "average available light" is also expressed in lux. It must be emphasized, however, that these calculated light intensities are not absolute numbers, since the spectral distributions would be quite different from that of the black-body radiation of platinum at its melting point, which is used as the photometric reference. Moreover, the spectral distribution may not be the same in any two situations that are compared. However, the average available light has a

useful function in that it provides an approximate way of comparing the amount of light available to an average cell in the suspension under different conditions.

Light as the Limiting Factor for Growth. When a culture of cells is allowed to grow under conditions in which all requirements for growth are in adequate supply, the growth will be exponential; that is, the percentage of increase in mass per unit of time will be constant. If the logarithm of the cell mass is plotted against time, the resulting curve will be a straight line. If, on the other hand, a growth requirement becomes limiting, the growth rate under these conditions will be a reflection of the supply rate of the limiting factor. For example, if the limiting factor were supplied to the culture at a constant rate, then the growth would be linear with time, rather than exponential.

When *Chlorella* is grown photoautotrophically, the source of carbon is carbon dioxide which is supplied by being bubbled into the flask at a constant rate; the only external energy source is the light absorbed by the cells. When the culture is inoculated with a suspension of cells such that the initial packed cell concentration is 0.5 ml/liter of suspension, the increase in cell mass is exponential (Fig. 2). This exponential growth indicates that as the cell mass increases, no growth requirement is depleted to the point where it becomes limiting. After a period of about 12 hr, however, the specific growth rate begins to decline, and after about 20 hr of growth, the increase in cell mass becomes linear with time (Fig. 3). The linear growth suggests that some growth requirement is being supplied to the culture at a constant rate and that this is the limiting factor for growth under these conditions. As has been mentioned above, the only two growth factors which are supplied to the cultures at constant rates are carbon dioxide and light.

The rate of supply of carbon dioxide was 5% in air at a total rate of 100 ml/min, or about 0.013 mole/hr. If this carbon dioxide were all fixed into the cells at the oxidation state of carbohydrate (CH_2O), the increase in mass due to carbon fixed would be 0.39 g/hr. The actual rate of increase of volume of the cell mass in the flasks is 0.072 ml/flask·hr. Because the density of the cells is approximately 1.0, the total rate of mass increase is maximally 0.072 g/hr in each flask. This means that even if all of the mass increase of the cells were due to carbohydrate the rate of fixation of the supplied carbon dioxide would be only one-fifth of the rate at which it is supplied, suggesting that carbon dioxide is not the limiting factor during linear growth.

The chlorophyll concentration of a cell suspension at the time of inoculation at a packed cell concentration of 0.5 ml/liter of suspension is typically 10 mg of chlorophyll per liter (Fig. 2). The 200 ml of suspension have a depth of 3.0 cm in the 500-ml culture flask. The average available light to the cells is 3160 lux when the incident intensity is 8000 lux.

At the time when growth becomes linear, the chlorophyll content of the suspension is approximately 25 mg/liter (Fig. 3). The average available light at this time is 780 lux. This is a 4-fold decrease from the initial conditions, even though the chlorophyll content has increased only 2.5-fold.

In addition to the decrease in light intensity within the cell suspension, there is the dilution of chlorophyll content per unit of packed cells due to the differential rates of increase of cell volume and chlorophyll. Initially the content of chlorophyll is:

$$\frac{\text{10 mg chlorophyll/liter suspension}}{\text{0.5 ml packed cells/liter suspension}} = \text{20 mg of chlorophyll/ml packed cells}$$

At the time when linear growth starts, there are 25 mg of chlorophyll and 3.7 ml of packed cells per liter of suspension (Fig. 3), or 6.75 mg of chlorophyll per ml of packed cells. Hence, there is a 3-fold decrease in the amount of chlorophyll per unit of cell volume as well as a 4-fold decrease in the light intensity at the time of onset of linear growth. It is suggested that the available light may be the limiting factor for growth when the chlorophyll content per unit of packed cell volume is lowered by differential rates of growth and chlorophyll synthesis, and when the light intensity averaged over the depth of the cell suspension in the culture flask is lowered after the first 20 hr of growth. It must now be ascertained that the available light is constant after 20 hr of growth, in order to account for the linear growth.

At 20 hr, the chlorophyll content, as previously stated, is 25 mg/liter of suspension. The total depth of suspension is 3.0 cm, and the absorption of light at 680 nm due to chlorophyll is 0.027 absorbance unit per mg of chlorophyll per liter in a 1-cm light path. The fraction of light which emerges from the culture and, hence, which is not available for photosynthesis, is given by:

$$\text{Absorption} = \log \frac{I_o}{I} = (0.027)(3.0)(25) = 1.35$$

$$\frac{I}{I_o} = \log^{-1}(-1.35) = 0.045$$

Only 4.5% of the incident light passes through the suspension, 95% being absorbed. Thus, essentially all of the light is absorbed at this time, and as the suspension becomes even more concentrated the total amount of light available to the suspension remains essentially constant.

The above discussion shows that the light available to a cell suspension grown under these conditions is constant after the first 20 hr of growth. The data further suggest that after 20 hr light may be the limiting factor for growth and is thus responsible for the linear growth shown in Figure 3.

Chlorophyll Synthesis during Exponential and Light-limited Growth. The results discussed above are consistent with the hypothesis that the lag phase of chlorophyll synthesis is due to the accumulation of a photosynthetic product which somehow prevents chlorophyll synthesis. With conditions under which light is not the limiting factor for growth, photosynthetic reactions can proceed at a greater rate than the photosynthetic products can be utilized. When light becomes limiting, the excess photosynthetic products can be depleted. The onset of rapid chlorophyll synthesis occurs during the time when the growth mode changes from exponential to linear.

Effects on Chlorophyll Synthesis of Rapid Concentration of the Packed Cell Mass. Evidence for lack of an intrinsic lag phase of chlorophyll synthesis was obtained in an experiment designed to produce a chronologically "young" culture with the chlorophyll content of an "older" culture in the stage of rapid chlorophyll synthesis. Six cultures were grown in high light intensity (8000 lux) for 2 hr, starting with 0.5 ml of packed cells per liter. Then, five of the cultures were centrifuged; the cells were pooled in one-fifth of the original volume of medium and then grown in the original incident light intensity. The development of chlorophyll-synthesizing ability in the pooled culture was compared to that in two different control cultures: (*a*) the sixth dilute suspension which was inoculated at the same time as the cells which were pooled, and hence of the same chronological age; and (*b*) a culture which was inoculated 18 hr earlier and which was in the stage of rapid chlorophyll synthesis but which had about the same chlorophyll concentration as the pooled culture. The amount of chlorophyll present in each culture is plotted as a percentage of the values in the samples which were taken immediately after the pooled culture was returned to the light (Fig. 4).

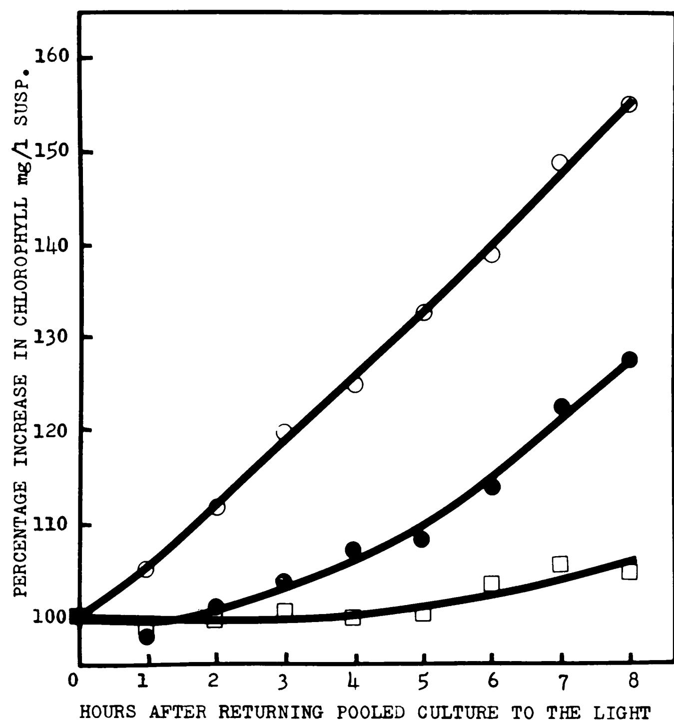


FIG. 4. Effects of concentrating a dilute culture. The relative rates of chlorophyll synthesis were measured in three cultures of *Chlorella*. □: Dilute culture. This was inoculated 2 hr before the first sample was taken. Initial packed cell concentration was 0.5 ml/liter of suspension. ○: Dense culture. Inoculation was 18 hr before the first sample was taken. ●: Pooled culture. Five flasks were inoculated at the same time and to the same packed cell concentration as the dilute culture. At 2 hr after inoculation, the cells were harvested by centrifugation and were resuspended in one-fifth of the original volume of medium, and then were placed back in the light. After pooling, the packed cell and chlorophyll concentrations were similar to those of the dense culture.

During the first 2 hr of illumination after pooling, the rate of chlorophyll synthesis in the pooled culture was as low as that of the dilute culture. After this time the rate rapidly increased until at 6 hr it was as high as that of the older culture in the stage of rapid chlorophyll synthesis.

One possible explanation for the lag phase of chlorophyll synthesis was that the dilute cultures are exposed to high light intensity, with consequent destruction of some light-sensitive component necessary for chlorophyll synthesis. Resynthesis of this component is required during the lag phase before chlorophyll synthesis can resume. The pooled culture experiment indicates that such an explanation cannot account for the entire lag phase. The ability of the pooled culture to synthesize chlorophyll was recovered after 2 hr even though the initial rate of chlorophyll synthesis was as low as that of the dilute culture (Fig. 4). Apparently, continuous high light intensity on the cells is necessary for the suppression of chlorophyll synthesis, and once the cells are concentrated so that the light available to the average cell is low, rapid chlorophyll synthesis proceeds after about 2 hr.

Continuous Culture Experiments. If the lag phase of chlorophyll synthesis represents a control mechanism of the cells such that the chlorophyll content per unit of packed cell volume varies in response to the light which is available to the cells, then it should be possible to dissociate completely the light dependence from the apparent time dependence. In other words, if a cell suspension could be grown in such a way that the packed cell concentration remains constant, then the amount of chlorophyll per unit of packed cells should be a function of the light intensity on the cells. To study this problem, a continuous culture apparatus was developed (Fig. 1).

The data appearing in Table II were obtained as follows. A suspension of cells was allowed to grow in the continuous culture growth chamber at a given incident light intensity and at a given setting of the photocell electronic controller until the samples drawn on 2 consecutive days yielded equal values of packed cell concentration and chlorophyll content per unit of packed cell volume. Then the chamber was sampled on succeeding days until sufficient data were obtained to ensure that the cell suspension was indeed constant with respect to packed cell concentration and chlorophyll content. Next, the incident light intensity was varied, either by moving the light shields containing the lamps or by removing or replacing lamps, keeping the relative proportions of fluorescent to incandescent light constant. The electronic controller was then adjusted, and the packed cell concentration was monitored until the new steady state was reached, after which time samples were again taken for packed cell concentration and chlorophyll content at the new steady state. The data (Table II) in the column marked "relative available light" were calculated as described above.

The hypothesis that the degree of light limitation of growth is the primary controlling factor of chlorophyll synthesis is strengthened by the results of the continuous culture experiments. Under the steady state conditions employed in the

Table II. *Continuous Culture Data*

Condition of constant light absorption by the culture was maintained for each set of observations by automatic dilution of the suspension with fresh medium as the growth proceeded. Samples were taken directly from the growth chamber at 24-hr intervals.

Incident Light Intensity	No. of Samples	Packed Cell Concn	CHL Content	Average Available Light[2]
lux		*ml/l*	*mg/ml packed cells*	*lux*
1,250	4	0.40 ± 0.03	15.00 ± 0.39	950
1,750	8	0.44 ± 0.04	14.76 ± 0.74	1,310
3,000	3	0.55 ± 0.02	12.59 ± 0.87	2,200
15,000	15	3.50 ± 0.70	13.16 ± 1.88	1,890
12,000	3	1.00 ± 0.20	8.14 ± 0.40	8,320
15,000	7	0.42 ± 0.22	4.18 ± 0.41	13,900

[1] CHL: chlorophyll.
[2] See text.

Table III. *Effects of CMU on Growth and Chlorophyll Synthesis during the Lag Phase*

In three separate experiments, cultures were inoculated to an initial packed cell concentration of 0.5 ml/liter of suspension and were allowed to grow for 8 or 9 hr at 8000 lux incident light intensity, in the presence of different concentrations of CMU.

Experiment	Hr between Samples	CMU Concn	ΔPC	ΔCHL		ΔCHL:ΔPC
		μM	*ml/l*	*mg/l*	*% of control*	
1	8	0.00	1.05	1.21		1.16
		0.50	0.60	2.14	177	3.57
		1.00	0.48	2.59	214	5.40
2	8	0.00	0.93	0.65		0.70
		1.25	0.50	1.79	275	3.57
		2.50	0.38	1.64	253	4.33
3	9	0.00	0.80	1.21		1.52
		2.50	0.35	1.80	148	5.16
		5.00	0.15	0.73	60	4.86

continuous culture, the chlorophyll content per unit of packed cell volume is not invariant, but rather, is a function of the incident light intensity and the packed cell concentration. The data suggest that the controlling factor is the average amount of light available for photosynthesis. This average light is approximated by the "average available light." Low values of average available light are associated with high chlorophyll content, and high values, with low chlorophyll content.

It is concluded from the continuous culture experiments that the content of chlorophyll per unit of packed cells is related to the average light intensity on the cells, and not to some time-dependent phenomenon such as an induction phase of chlorophyll synthesis.

Effect of CMU on Chlorophyll Synthesis. The purpose of this experiment was to determine whether the effect of high light on chlorophyll synthesis is a direct photochemical effect or whether it is mediated by some aspect of photosynthesis. If the suppression of chlorophyll synthesis during the lag phase is due to light itself, then the lag phase should occur even when the cells are impaired in their ability to photosynthesize. If, on the other hand, a high rate of photosynthesis is required for suppression of chlorophyll synthesis, and if photosynthesis is inhibited, then chlorophyll synthesis should proceed rapidly even in dilute cell suspensions exposed to high light intensity.

CMU has been shown to be a potent inhibitor of photosynthetic oxygen evolution in living plants, as well as an inhibitor of light-mediated electron transport associated with the production of reducing equivalents, known as photosystem II, in isolated chloroplasts (1). *Chlorella* was inoculated into dilute suspensions (0.5 ml of packed cells per liter) and was placed in 8000 lux of light in the normal manner. The cultures contained varying concentrations of CMU from 0.5 to 5×10^{-6} M. Samples were taken after 8 or 9 hr of growth. Table III shows the effects of CMU on growth and chlorophyll synthesis in three separate experiments. The inhibitor lowered the growth rate, but the chlorophyll biosynthetic rate and the chlorophyll concentration within the cells were both increased, *i.e.*, more chlorophyll was made even though the total packed cell volume was smaller. It thus appears that the effect of light on the suppression of chlorophyll synthesis during the lag phase is due to some aspect of photosynthesis, and when one part of the photosynthetic process—the production of reducing power by photosystem II—is partially inhibited by CMU during the first 8- or 9-hr growth of dilute cell suspensions in high light, the suppression of chlorophyll synthesis is significantly lessened. Under the culture conditions employed in these experiments, the optimal concentration of CMU was about 1 to 2×10^{-6} M, higher concentrations leading to inhibition of both chlorophyll synthesis and growth, as would be expected in the case of more complete inhibition of photosynthesis.

CONCLUSIONS

The results reported here provide certain information concerning the control of chlorophyll biosynthesis and the nature of the lag phase of chlorophyll synthesis in *C. vulgaris* Beijerinck. The following interpretation is suggested.

The chlorophyll content of the cells varies in response to the effective average light intensity on the cells. When light is not limiting for growth, the chlorophyll is present in low concentration in the cells, and when light is limiting for growth, chlorophyll is present at high levels. Light can be made to be limiting for growth in a number of ways. (*a*) The incident light intensity on the cell suspension can be low. (*b*) The cell suspension can be sufficiently concentrated so that the amount of light falling upon the cells, averaged over time and by the thickness of suspension through which the light must pass, is low because of light absorption by the chlorophyll in the cells. (*c*) Photosynthesis can be inhibited by CMU which causes the light falling upon the cells to be used less efficiently for carrying on photosynthesis.

LITERATURE CITED

1. AVRON, M. AND J. NEUMANN. 1968. Photophosphorylation in chloroplasts. Annu. Rev. Plant Physiol. 19: 137–166.
2. BEALE, S. I. 1970. The biosynthesis of δ-aminolevulinic acid in *Chlorella*. Plant Physiol. 45: 504–506.
3. BOGORAD, L. 1966. The biosynthesis of chlorophylls. *In:* L. P. Vernon and G. R. Seely, eds., The Chlorophylls. Academic Press, New York. pp. 481–510.
4. BOGORAD, L. 1967. Biosynthesis and morphogenesis in plastids. *In:* T. W. Goodwin, ed., Biochemistry of Chloroplasts, Vol. II. Academic Press, New York. pp. 615–631.
5. GASSMAN, M. AND L. BOGORAD. 1965. Photocontrol of protochlorophyll synthesis in bean leaves. Plant Physiol. 40: lii.
6. GRANICK, S. 1947. Protoporphyrin 9 as a precursor of chlorophyll. J. Biol. Chem. 172: 717–727.
7. GRANICK, S. 1949. The structural and functional relationships between heme and chlorophyll. Harvey Lect. 44: 220–245.
8. GRANICK, S. 1967. The heme and chlorophyll biosynthetic chain. *In:* T. W. Goodwin, ed., Biochemistry of Chloroplasts, Vol. II. Academic Press, New York. pp. pp. 373–410.
9. MACKINNEY, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140: 315–322.
10. MYERS, J. 1946. Culture conditions and the development of the photosynthetic mechanism. III. Influence of light intensity on cellular characteristics of *Chlorella*. J. Gen. Physiol. 29: 419–427.
11. MYERS, J. 1946. Culture conditions and the development of the photosynthetic mechanism. IV. Influence of light intensity on photosynthetic characteristics of *Chlorella*. J. Gen. Physiol. 29: 429–440.
12. MYERS, J. AND L. B. CLARK. 1944. Culture conditions and the development of the photosynthetic mechanism. II. An apparatus for the continuous culture of *Chlorella*. J. Gen. Physiol. 28: 103–112.
13. RABINOWITCH, E. I. 1945. Photosynthesis and Related Processes. Interscience Publishers, New York.
14. SARGENT, M. C. 1940. Effect of light intensity on the development of the photosynthetic mechanism. Plant Physiol. 15: 275–290.
15. ŠESTÁK, Z. 1963. Changes in the chlorophyll content as related to photosynthetic activity and age of leaves. Photochem. Photobiol. 2: 101–110.
16. SHUGARMAN, P. M. 1966. Studies on chlorophyll formation in *Chlorella*. Ph.D. thesis, University of California, Los Angeles.
17. SHUGARMAN, P. M. AND D. APPLEMAN. 1965. Natural variations of the physiological characteristics of growing *Chlorella* cultures. Plant Physiol. 40: 81–84.
18. SHUGARMAN, P. M. AND D. APPLEMAN. 1966. Chlorophyll synthesis in *Chlorella*. I. Occurrence of a lag phase on initiation of a dilute culture. Plant Physiol. 41: 1695–1700.
19. SHUGARMAN, P. M. AND D. APPLEMAN, 1966. Chlorophyll synthesis in *Chlorella*. II. Effect of glucose and light intensity on the lag phase. Plant Physiol. 41: 1701–1708.