# CULTURE CONDITIONS AND THE DEVELOPMENT OF THE PHOTOSYNTHETIC MECHANISM

## IV. Influence of Light Intensity on Photosynthetic Characteristics of Chlorella*

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Numerous data in the literature indicate that the photosynthetic characteristics of an organism depend upon previous conditions of culture (*e.g.* Aufdemgarten, 1939; Emerson, Green, and Webb, 1940; McAlister and Myers, 1940; Myers and Burr, 1940; Sargent, 1940). The apparatus for the continuous culture of unicellular algae described by Myers and Clark (1944) permits the systematic study of the effects of culture conditions by examination of a single variable at a time. The present paper considers the effect of light intensity of culture on the subsequent photosynthetic behavior of *Chlorella pyrenoidosa*. Supporting data on parallel effects of light intensity on cellular characteristics are presented in the preceding paper of the series (Myers, 1946).

### EXPERIMENTAL

*Chlorella pyrenoidosa* was grown at 25°C. with 4.4 per cent carbon dioxide in Knop's solution in three units of the continuous-culture apparatus as described in the preceding papers (1944, 1946). The data to be reported were obtained in two series of experiments. Series I was done before and Series II in parallel with the work of the preceding paper.

*Series I.*—Photosynthetic characteristics were studied manometrically by the Warburg technique using a bath with glass bottom thermostated at 25 ± 0.05°C. Some measurements were make in a Knop's solution (pH 4.45) saturated with 4.4 per cent carbon dioxide. An aliquot of cells was centrifuged out, washed in Knop's, and then taken up in a measured volume of fresh Knop's solution. Equal amounts of this final suspension were pipetted into each of a pair of vessels and an additional measured volume of Knop's added to one of the vessels to obtain different liquid:gas ratios. The rates of $O_2$-evolution and $CO_2$ uptake could then be calculated. Other measurements of photosynthesis were made in the Warburg No. 11 buffer (0.005 M $K_2CO_3$ + 0.095 M $KHCO_3$) using a suspension prepared by centrifuging and washing an aliquot of cells in water and in buffer and then taking up in a measured volume of buffer. In all cases the rate in terms of millimeters of pressure change per hour was determined graphically by taking 5 minute readings over a period of about 1 hour. The period of illumination was followed by a period of about an hour in darkness for measurement of respiration.

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Illumination for the measurements was provided by a bank of seventeen 60 watt Mazda lamps closely arranged in two rows 27 cm. below the reaction vessels. Lamps rated at 120 volts were operated at 112 ± 1 volt as provided by a voltage stabilizer and variable transformer. About 600 foot-candles of illumination are provided at the positions of the reaction vessels. In determination of the light intensity curves, the rates in five vessels were measured simultaneously. Intensity was then varied by the use of Jena NG-series neutral filters attached to the bottoms of the vessels by holders which also masked out all stray light. The net transmission of the filters was estimated from the spectral absorption curves obtained on a General Electric recording spectrophotometer. The incident intensity upon the five filter vessel combinations was not uniform but was measured each day by a calibrated barrier type photocell enclosed in an Erlenmeyer flask and immersed in the bath. By a slide and carriage arrangement the photocell could be moved to the mean position of each vessel.

In practice the culture suspension was harvested at daily intervals, leaving as an inoculum for the next interval a constant amount as indicated by a mark at an arbitrary height on the chamber. The relation between the amount of inoculum and the amount of the daily sample is an index of rate of growth. A culture was maintained at a given light intensity until the rate of growth and the rate of photosynthesis became constant. Thereafter, the light intensity curves in No. 11 buffer and measurements under 4 per cent $CO_2$ were made as described above.

The population densities (cubic millimeters of cells per milliliter of suspension) were determined on duplicate aliquots of suspension by centrifuging in Bauer and Schenk tubes as described in the second paper of this series (Myers and Clark, 1944). Densities of population were arbitrarily adjusted to about 1.0 c.mm. per ml. (actually ranging from 0.90 to 1.64 in different cultures). In each experiment several determinations of pH were made on the freshly harvested suspension by means of a Coleman glass electrode.

*Series II.*—Data obtained in the first series (see below) indicated that the assimilatory quotient does not vary systematically with light intensity of culture and averages about −0.92. The manometric technique was therefore modified by making all measurements in Knop's solution saturated with 4.4 per cent carbon dioxide, using single flasks for each determination, and assuming an assimilatory quotient of −0.90. Light intensity curves were obtained as a routine in five flasks as otherwise described in Series I. Cell volumes were determined on duplicate aliquots by centrifuging in Van Allen thrombocytocrit tubes as described in the preceding paper (1946). Population densities were adjusted to lower values (0.60 to 0.70 c.mm. cells/ml.) since other data (yet to be published) indicate that at low light intensities rate of growth is somewhat higher at lower population densities at which there is less mutual shading.

## RESULTS

Light intensity curves of Series I obtained with cells immersed in the No. 11 carbonate-bicarbonate buffer are presented in Figs. 1, 2, and 3. Abscissas represent light intensity in foot-candles during the measurements. Ordinates

represent rate of $O_2$ evolution (in c. mm. $O_2$/hour/c.mm. cells) corrected for respiration. Although respiration rates were not determined with very great precision they seemed to depend somewhat on the light intensity of culture.

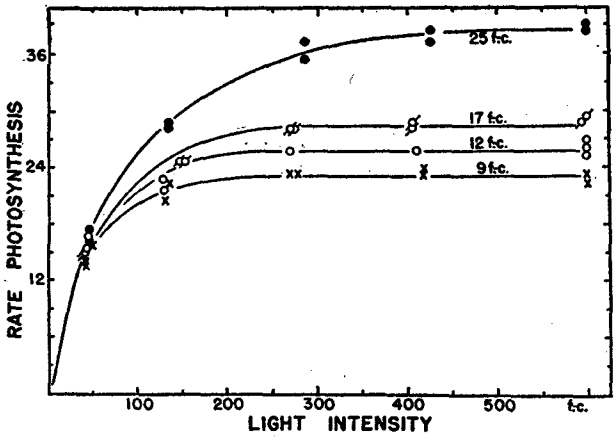


FIG. 1. Light intensity curves for cells cultured at the light intensity indicated on each curve. Rate = c.mm. $O_2$/hour/c.mm. cells measured in No. 11 buffer. Series I.

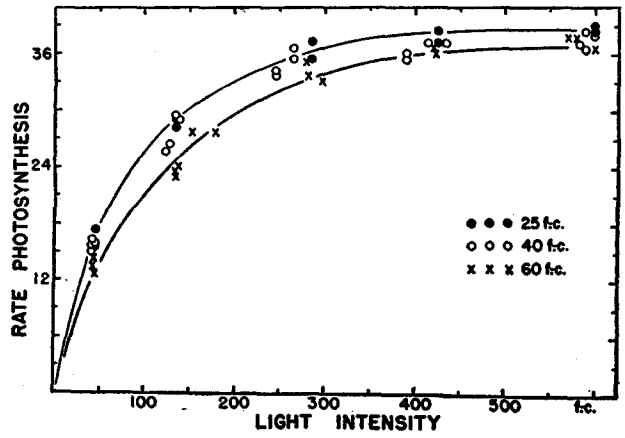


FIG. 2. Light intensity curves for cells cultured at the light intensity indicated for the various points. Rate = c.mm. $O_2$/hour/c.mm. cells measured in No. 11 buffer. Series I.

Cultures grown at higher intensities (Fig. 3) gave respiratory rates in the range of 1.8 to 2.4 c.mm. $O_2$ /hour/c.mm. cells; at the lower intensities (Fig. 1), 1.2 to 1.8.

Each of the curves of Figs. 1, 2, and 3 characterizes cells grown at the indicated light intensity. Each curve is drawn as a best fit for experimental points

obtained on at least two different samples from the same culture. (For purposes of comparison the curves for cells grown at 25 and 60 f.-c. are repeated in different figures.) In terms of their response to light intensity cells grown at different intensities differ in two important respects: (1) the maximum rate of photosynthesis attained at high intensities and (2) the shape of the light intensity curve at lower intensities.

The maximum rates of photosynthesis attained for each of the curves of Figs. 1, 2, and 3 are plotted against the light intensity of culture in Fig. 4 *b*. Three regions of light intensity, AB, BC, CD, can be recognized in Fig. 4 *b* corresponding to the groupings of the curves in Figs. 1, 2, and 3, respectively. Maximum capacity for photosynthesis is attained by cells grown at intensities in the range of 25 to 60 f.-c. The exact nature of the region BC (whether tilted, or flat as drawn) cannot be decided. As indicated by Fig. 2, the precision of the measurements is hardly good enough to distinguish between 25, 40, or 60 f.-c. Below a light intensity of growth of about 25 f.-c. the maximum capacity for photosynthesis falls off rapidly (BA). Above about 60 f.-c. the maximum capacity for photosynthesis falls off slowly (CD).

Measurements of rates of photosynthesis in Knop's solution + 4 per cent $CO_2$ under 600 f.-c. (saturating light and $CO_2$) yielded the data plotted in Fig. 4 *a*. No determinations of respiratory rates were made; the respiration corrections used (1.2 to 2.4 c.mm. $O_2$/hour/c.mm. cells) were obtained from the measurements in the carbonate-bicarbonate buffer. Mean values of the assimilatory quotient for cells grown at the different light intensities are presented as the last column of Table I. Values of the quotient did not vary significantly with the possible exception of the lower value of $-0.86$ obtained for cells grown under 3.25 f.-c. The shape of the curve is similar to that of curve 4 *b*. It is of interest that for cells cultured at intensities above 25 f.-c. the rate of photosynthesis measured in Knop's + 4 per cent $CO_2$ is roughly 15 to 20 per cent higher than the rate obtainable in the No. 11 buffer (*cf.* Fig. 4 *a* and 4 *b*). Only for cells cultured at very low intensities do the two methods yield equivalent values.

That the curves 4 *a* and 4 *b* are not a chance result of the order of experiments or the particular population density or pH of each experiment can be seen by inspection of the first four columns of Table I. The light intensities used are listed in chronological order for each culture.

Presented in the fifth column of Table I are the relative growth rates for each light intensity.[1] Fig. 4 *c* shows the data on growth rate plotted against the

[1] In the continuous-culture apparatus, which insures a perfectly logarithmic growth, the relative growth rate, $k$, is defined by the equation $\log \frac{N}{N_0} = k\,t$. In applying the equation, $N_0$ is expressed in milliliters of inoculum at the beginning of each interval and $N$ in terms of total milliliters of suspension ($N_0$ + milliliters sample) at the end of a time interval, $t$, of 1 day.

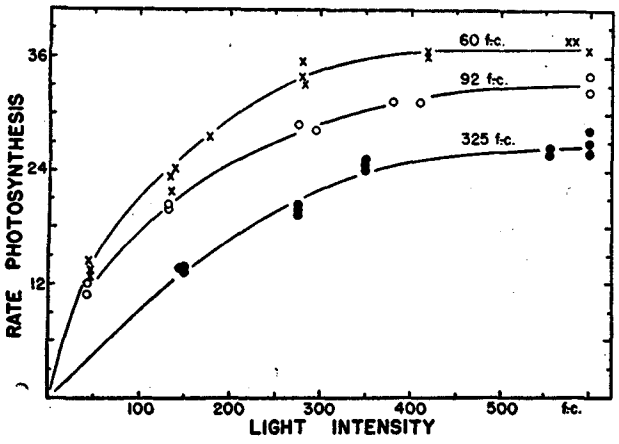


FIG. 3. Light intensity curves for cells cultured at the light intensity indicated on each curve. Rate = c.mm. $O_2$/hour/c.mm. cells measured in No. 11 buffer. Series I.

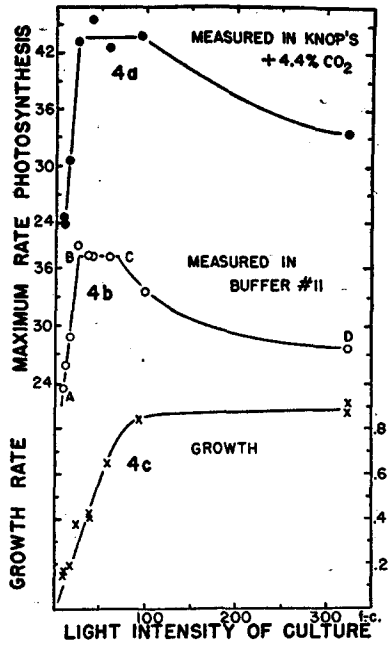


FIG. 4. (*a*) The maximum rate of photosynthesis (c.mm. $O_2$/hour/c.mm. cells) measured at 625 f.-c. in Knop's + 4.4 per cent $CO_2$ as a function of the light intensity of culture. (*b*) The maximum rate of photosynthesis (c.mm. $O_2$/hour/c.mm. cells) measured 625 f.-c. in the Warburg No. 11 buffer as a function of the light intensity of culture. (*c*) Relative growth rate as a function of the light intensity of culture. Data of Series I.

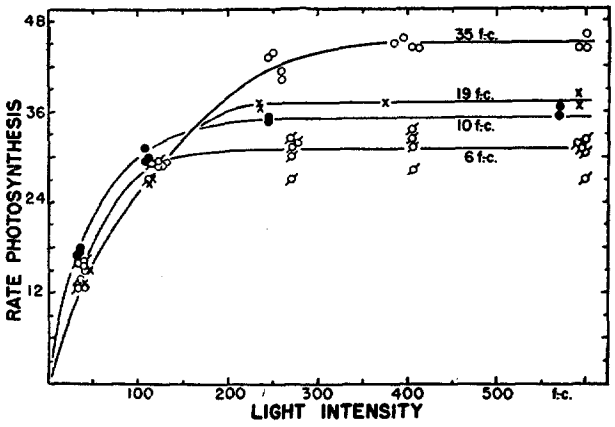


FIG. 5. Light intensity curves for cells cultured at the light intensity indicated on each curve. Rate = c.mm. $O_2$/hour/c.mm. cells measured in Knop's + 4.4 per cent $CO_2$. Series II.

TABLE I

*Culture Data*

| Culture No. | Culture intensity | Population density | ph | Growth rate (see text) | Assimilatory quotient (see text) |
|---|---|---|---|---|---|
| | *f.-c.* | *c.mm./cc.* | | | |
| 38 | 325 | 1.34 | 5.8 | 0.910 | — |
| 40 | 325 | 1.56–1.64 | 5.8 | 0.865 | 0.86 |
| | 95 | 0.92–1.08 | 5.8 | 0.840 | 0.91 |
| | 40 | 1.24 | 5.8 | 0.432 | — |
| | 60 | 1.16–1.24 | 5.9 | 0.648 | 0.89 |
| 41 | 40 | 0.94–1.02 | 5.8 | 0.408 | 0.94 |
| | 12 | 1.06–1.14 | 5.7 | 0.168 | 0.93 |
| 42 | 25 | 0.90–0.94 | 5.8 | 0.384 | 0.93 |
| | 9 | 1.18–1.20 | 6.0 | 0.144 | 0.93 |
| 43 | 17 | 1.18–1.22 | 5.9 | 0.192 | 0.94 |

light intensity of culture. At low intensities ( < 60 f.-c.) growth rate is proportional to light intensity. At high intensities ( > 100 f.-c.) growth is nearly independent of light intensity. A similar type of curve was obtained by Bristol-Roach (1928) from a study of rates of growth of *Scenedesmus*.

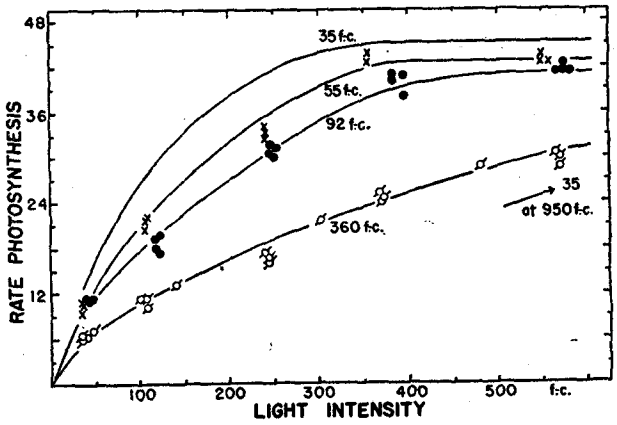


FIG. 6. Light intensity curves for cells cultured at the light intensity indicated on each curve. Rate = c.mm. $O_2$/hour/c.mm. cells measured in Knop's + 4.4 per cent $CO_2$. Series II.

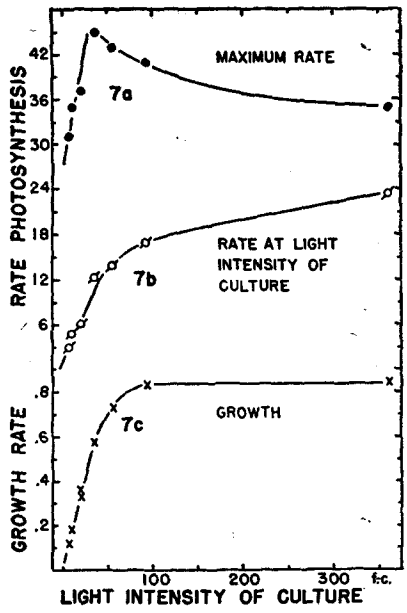


FIG. 7. (*a*) The maximum rate of photosynthesis (c.mm. $O_2$/hour/c.mm. cells) measured at 625 f.-c. in Knop's + 4.4 per cent $CO_2$ as a function of light intensity of culture. (*b*) The rate of photosynthesis (c.mm. $O_2$/hour/c.mm. cells) prevailing in the cultures for each light intensity of culture estimated from Figs. 5 and 6. (*c*) Relative growth rate as a function of light intensity of culture. Data of Series II.

The data of Series II experiments are presented in Figs. 5, 6, and 7. Light intensity curves obtained in Knop's solution + 4.4 per cent carbon dioxide follow the same general trend as those of the first series obtained in the carbonate-bicarbonate buffer. One difference appears in the actual crossing of the curves in Fig. 5 indicating for cells grown at the lower light intensities an enhanced photosynthetic ability measured at low intensities. This is a phenomenon commonly recorded in the literature of "sun" and "shade" plants (*cf.* Boysen-Jensen and Müller, 1929) but not observed by Sargent (1940) in his study on *Chlorella*. The appearance of the phenomenon here is not entirely consistent and it is difficult to see why a similar effect is not apparent in Fig. 1 of the first series. Another possible difference lies in the lack of any plateau in Fig. 7 *a* as compared to Fig. 4 *a*, although this hinges upon the significance of a single point of each curve at 92 to 95 f.-c.

The light intensity curve of growth (Fig. 7 *b*) of the second series is somewhat steeper than that of the first series (Fig. 4 *c*), probably reflecting the decreased population densities and better light distribution maintained in the second series. Both growth curves appear to extrapolate toward zero light intensity although this is obviously impossible since some energy must be required to maintain the basal metabolism of the culture.

## DISCUSSION

Cells cultured within the range of light intensities reported here always develop a capacity for a maximum rate of photosynthesis[2] much higher than they ever experience during growth. This is indicated in Fig. 7 *b* which is drawn from data interpolated from the curves of Figs. 5 and 6 in order to express the rate actually attained in the culture chambers. At higher intensities of culture (> 35 f.-c.) the capacity for photosynthesis (Fig. 7 *a*) decreases although the rate actually attained during culture (Fig. 7 *b*) increases. Presumably at still higher intensities or culture (> 360 f.-c.) cells might be produced with a capacity for photosynthesis no greater than actually experienced during growth. (Like conclusions obtain from a similar analysis of the curves of Figs. 1, 2, and 3.)

The above argument rests on the assumption that the rates of oxygen evolution measured in the Warburg flasks approximate the rates actually experienced

[2] As used herein *capacity for photosynthesis* or *maximum rate of photosynthesis* describes the rate of photosynthesis, in terms of oxygen evolution, which obtains under conditions of light and carbon dioxide saturation at 25°C. According to classical interpretation it is determined by the rate of the *dark* or enzymatic or Blackman reactions. Actually any such measured rate is merely a rate of oxygen evolution and includes the effects of any processes opposing photosynthesis (*e.g.* photooxidations) over and above the dark respiration.

in the culture chambers. Several experiments on cells grown at higher light intensities have confirmed this assumption. Duplicate samples, one taken directly from the culture suspension, and the other with cells washed and resuspended in fresh Knop's solution of the same pH (5.8), have been compared in Warburg vessels of approximately the same volume. Retention of carbon dioxide by the fluid occurs at this pH so that rates of oxygen exchange cannot easily be calculated, but equal rates in vessels of the same volume and liquid: gas ratios must mean equal rates of oxygen evolution. Comparisons have also been made of cells washed and resuspended in fresh Knop's at pH 4.5 with cells in the original suspension adjusted to pH 4.5. Rates obtained for cells in the original suspension were generally slightly but not significantly higher than those obtained for washed cells in fresh medium. Fig. 7 *b* therefore seems a justifiable estimate of rate of photosynthesis attained in the culture chambers.

From Fig. 7 *a* it appears that at a rather narrow optimum range of light intensity of culture *C. pyrenoidosa* develops a maximum capacity for photosynthesis in terms of oxygen evolution per unit volume of cells. Above about 50 f.-c. there occurs a falling off in photosynthetic capacity with increasing intensity of culture. This appears to be correlated with the transition region between the light-limited and light-saturated rate of growth (Fig. 7 *c*).

The limitation of growth rate is in itself an interesting problem. Certainly the factor causing the limitation is not located within the photosynthetic mechanism since none of our light intensity curves of photosynthesis show complete light saturation in the cultures themselves. This is in accord with the data of Bristol-Roach (1928) who studied the effects of glucose and light intensity on rate of growth of *Scenedesmus*. At low light intensities the addition of 1 per cent glucose more than doubled the rate of growth; at high intensities it had practically no effect.

The data suggest the hypothesis that at light intensities above those which limit growth ( $\sim$ 50 f.-c.) the cells develop mechanisms to dispose of the excess photosynthetic products above those used in the usual dissimilatory processes, One such mechanism might easily depend upon photooxidation processes which are known to occur (Franck and French, 1941; Myers and Burr, 1940), or upon "oxidation processes inside the assimilatory mechanism" in the sense of Gaffron (1939, 1940; *cf.* also Franck and Gaffron, 1941). In terms of such processes the falling off of curve 7 *a* and the bending over of curve 7 *b* above 50 f.-c. may be reasonably explained. An important implication of this line of reasoning is that (for cells as usually cultured) any photosynthesis-saturating light intensity is "unphysiological" in the sense that photosynthetic products must be accumulating faster than they can be handled by the usual cellular mechanisms that lead to growth. In long time experiments "effects of light on respiration" and other anomalies would seem to be expected.

Still another explanation must be found for the phenomenon illustrated by comparison of curves 7*b* and 7*c*. In changing from a light intensity of culture of 92 to 360 f.-c. the rate of growth is not increased; yet the net rate of oxygen evolution in the culture increases appreciably. At 360 f.-c. photosynthetic products are produced faster and must result in proportionately greater storage material or must diffuse out of the cells into the culture medium. Probably both of these effects occur. From the data of the preceding paper (1946)

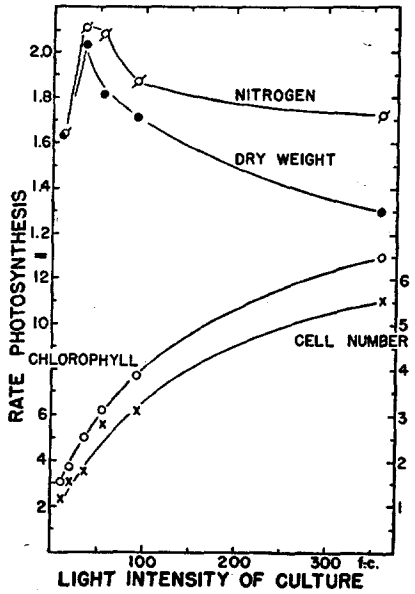


FIG. 8. Maximum rate of photosynthesis (c.mm. $O_2$/hour/unit cellular quantity) as a function of light intensity of culture for various indices of cell quantity. Data of Fig. 7*a* recalculated in terms of the data of Table I of the preceding paper (Myers, 1946). Units of cellular quantity are micrograms nitrogen, $10^{-5}$ gm. dry weight, arbitrary units chlorophyll, $10^6$ cells for cell number.

cells grown at 360 f.-c. have a 10 per cent greater dry weight than at 92 f.-c. At the same time the algae evidently secrete some organic material into the media since bacterial growth always develops in cultures (even without citrate) if sterile precautions are not maintained.

The falling off in capacity for photosynthesis at culture intensities below 25 f.-c. (Figs. 4*a*, 4*b*, and 7*a*) finds no apparent explanation. Inspection of the light intensity curves suggests that the effect observed here involves a quite different factor in the photosynthetic mechanism than that which operates at a high culture intensity (*cf.* Fig. 1 with Fig. 3 and Fig. 5 with Fig. 6). The

light intensity curves are of further interest in relation to those of previous workers. Cells cultured at the lowest intensities (Fig. 1) give curves approaching the Blackman type with a relatively short transition between the light-limiting and light-saturating portions of the curve. Unfortunately the true slopes of the curves under the light-limiting range of intensities cannot be estimated accurately from the present data although this is a matter of considerable importance.

The data of Fig. 7*a* have been replotted in Fig. 8 in terms of other indices of cell quantity obtained from Table I of the preceding paper (1946). The curve of Fig. 7*a* in terms of unit cell volume is not changed in shape if plotted in terms of unit dry weight or unit nitrogen. In each case maximum capacity for photosynthesis is attained at a light intensity of culture of about 35 f.-c. In terms of rate per cell or rate per unit chlorophyll, however, this effect is entirely masked by the pronounced increase in cell size and decrease in chlorophyll concentration with increasing light intensity of culture. In terms of unit chlorophyll or unit cell number the maximum capacity for photosynthesis increases continuously with increasing light intensities of culture.

As a means of varying the capacity for photosynthesis the control of light intensity as used here affords a possible variation of 30 to 45 per cent from the maximum value, which is approximately that obtained by Sargent (1940). It is much less than the 85 per cent variation recorded by Emerson and Arnold (1932) on cells grown under neon and mercury lamps. However, they acknowledged that they did not attain a maximum light-saturated rate for cells poor in chlorophyll which would make their range of variation appear greater than it actually was.

## SUMMARY

1. *Chlorella pyrenoidosa* has been grown in a continuous-culture apparatus under various light intensities provided by incandescent lamps, other conditions of culture being maintained constant. Light intensity curves for cells immersed in the No. 11 Warburg buffer and in Knop's solution + 4.4 per cent $CO_2$ at a saturating light intensity were determined as characteristics of the photosynthetic mechanism. These characteristics were referred to the centrifuged cell volume as an index of quantity of cellular material.

2. Cells grown at intensities in the range of about 35 f.-c. develop a capacity for a high rate of photosynthesis (c.mm. $O_2$/hour/c.mm. cells). At culture intensities above or below this range the cells produced have a lower capacity for photosynthesis. A similar effect is observed for rate of photosynthesis per unit dry weight or rate per unit cell nitrogen.

3. The rate of photosynthesis per cell or rate per unit chlorophyll shows no maximum at any light intensity of culture but increases continuously throughout the range of light intensities studied.

4. Maximum rate of growth is attained at a light intensity of about 100 f.-c. The hypothesis is advanced that at culture intensities above that needed to give maximum rate of growth (100 f.-c.) a mechanism is developed which opposes the photosynthetic process and removes the photosynthetic products.

5. The low capacity for photosynthesis shown by cells grown at culture intensities below 35 f.-c. finds no immediate explanation.

6. The shape of the light intensity curve is markedly affected by the light intensity at which the cells have been cultured. Cells grown at lower intensities give light intensity curves approaching the Blackman type with a short transitional region between light limitation and light saturation.

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