THE RELATION BETWEEN CHLOROPHYLL CONTENT AND RATE OF PHOTOSYNTHESIS

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I

INTRODUCTION

This work was undertaken in an attempt to discover whether a quantitative ratio exists between chlorophyll content and the rate of photosynthesis.

Piester (1912), working with yellow, light green, and normal varieties of a number of plants, found no direct correlation between the chlorophyll content and the rate of photosynthesis. Willstätter and Stoll (1928), working with excised leaves of yellow, etiolated, chlorotic, and normal plants, likewise found no direct relation between the two. In neither case were measurements of stomatal apertures made. It is therefore possible that in the excised leaves the stomates were not wide open and that the rate of photosynthesis was limited by a deficient supply of carbon dioxide.

Likewise, even in chlorotic leaves, the intensity of incident light is greatly diminished by passing through a leaf. Consequently all chloroplasts may not be adequately illuminated and light may become a limiting factor. In normal or dark green leaves this source of error is even greater because the chloroplasts which are furthest from the surface of incident light may receive very little light. This results in a high rate of photosynthesis for the surface chloroplasts and a low rate for the more deeply buried ones. The experimentally measured rate of photosynthesis can then be only an average of the two. From this it is evident that the work of both Piester (1912) and Willstätter and Stoll (1928) is open to question on the ground of limiting light and possibly of limiting carbon dioxide.

Chlorotic leaves are often produced on unhealthy plants having an abnormal metabolism. The photosynthetic rate of chloroplasts in such leaves is probably different than the rate of chloroplasts in healthy leaves. Emerson (1929), to avoid as much as possible an abnormal cell metabolism, grew *Chlorella vulgaris* in nutrient solutions lacking iron but containing 1.5 per cent glucose. The cells thus produced were chlorotic, but by obtaining sugar from the culture medium they grew fairly well. Using graded amounts of iron in a series of cultures he was able to produce varying amounts of chlorophyll. In any particular series he...
found a definite relationship between the chlorophyll content and the rate of photosynthesis. His conclusions are based upon two parallel curves, one containing five points and the other four points. Since so few data were given in his paper and since different series showed such great differences when compared one with another, and since iron may have affected the cell metabolism, this work was undertaken to see if the same relationship existed between chlorophyll content and the rate of photosynthesis when the chlorophyll was varied by other elements such as magnesium and nitrogen. Likewise it was thought desirable to find out if all iron series curves were parallel or whether some were and others were not, and whether there was a definite relationship existing when all curves were plotted on the same sheet.

If no other factor is limiting, and if there is some relation between chlorophyll content and rate of photosynthesis, it seems likely that equal amounts of chlorophyll should cause equal rates of photosynthesis.

Deficient iron undoubtedly affects the cell metabolism, other than through its effect on chlorophyll. In this work therefore, series were run in which the chlorophyll content was varied by furnishing graded amounts of magnesium and nitrogen as well as iron. In this manner an element not in the chlorophyll molecule, and metallic and non-metallic elements in the chlorophyll molecule were used to control the chlorophyll content. If a direct ratio were found for the iron, magnesium, and nitrogen series, then it would appear as if there were a definite relationship between the chlorophyll content and the rate of photosynthesis.

In the correction for respiration, Emerson (1929) states that fluctuations were so slight that a uniform correction of 8.0 mm. of oxygen per hour for 10 mm. of cells could be applied. Working at a temperature of 25°C., which was 5° higher than Emerson used, we found that for the same time and volume of cells the respiration varied from 8.0 to 22.0 mm. of oxygen. The respiration readings of cultures in the same series varied less than those in different series. Emerson (1929) gives a set of respiration readings for a given series which varies from 6.7 to 11.0 mm. of oxygen. Possibly if he had run respiration determinations on all series, his photosynthetic curves might have been closer together.

In this work no correlation between the chlorophyll content and the rate of respiration was found. In some cases the more chlorotic cells had the higher rate and in other cases the greener cells had the higher
rate. Since the rate of respiration varied over such a wide range, it was thought that the most accurate means of correcting for it was to make a separate determination with each culture.

The organism used by Emerson (1929) was \textit{Chlorella vulgaris}, while in this work a pure strain of \textit{Chlorella} of undetermined species was used. This culture was obtained through the courtesy of Dr. E. F. Hopkins of the Laboratory of Plant Physiology at Cornell University, and is of the same strain as that used by Hopkins and Wann (1926), and by Hopkins (1930).

\section*{II}

\textbf{Method of Varying Chlorophyll Content in Chlorella Cells}

A number of elements are cited in the literature as affecting the formation or disappearance of chlorophyll. Various workers have shown that chlorosis may be produced by deficiencies of nitrogen, potassium, phosphorus, calcium, magnesium, sulfur, iron, and titanium, or by excesses of potassium or chlorine.

The stock cultures of \textit{Chlorella} were carried along on dextrose potato-agar slants in test tubes. To inoculate a series, a loopful of cells from such a slant was suspended in sterile, distilled water and a definite volume of the suspension added to 100 cc. of sterile nutrient solution in a 250 cc. Erlenmeyer flask with a sterile pipette. A 200 watt Mazda unfrosted bulb was used as the source of light. The culture flasks were arranged in a circle around the bulb at a radius of 21 cm. The bulb was elevated 25 cm. above the center of this circle, so that the flasks of culture solutions were about 32 cm. from the source of light. A dull white reflector, 42 cm. in diameter, was placed above the source of light to diffuse the light more evenly over the cultures. This arrangement gave satisfactory light conditions for the growth of \textit{Chlorella}.

Determinations of photosynthesis and chlorophyll content were made after 3 to 6 days of growth, at the end of which time the cells had obtained a good gradation of chlorophyll content. This method produced pure cultures of \textit{Chlorella}, which is a necessary prerequisite for a significant determination of photosynthesis. The gradation in chlorophyll was produced by varying the amounts of iron, magnesium, or nitrogen added to the culture medium.

The nutrient solution was that of Emerson (1929) somewhat modified. It had the following composition:

\textbf{1. Chlorophyll Gradation Produced by Varying the Iron Concentration}

\begin{align*}
2 \text{NaC}_{6}\text{H}_{12}\text{O}_{7} \cdot \text{H}_{2}\text{O}\text{ (Baker)} & : 1.00 \text{ gm.} \\
\text{KNO}_{3}\text{ (Baker)} & : 1.26 \text{ gm.} \\
\text{MgSO}_{4}\text{ (Baker)} & : 2.46 \text{ gm.} \\
\text{KOHPO}_{4}\text{ (Kahlbaum)} & : 1.22 \text{ gm.} \\
\text{Glucose \text{ (Kahlbaum)}} & : 15.00 \text{ gm.} \\
\text{Distilled water} & : 1000.00 \text{ cc.}
\end{align*}
This medium contains a slight amount of iron as an impurity but the cultures low in iron gave a sufficiently low chlorophyll content for the purpose of this work. Varying amounts of a standard iron solution were added to cultures of the above composition, so as to give iron concentrations varying from zero to two parts per million. The standard iron solution was prepared by dissolving 1.0018 gm. of iron wire of 99.84 per cent purity in 10 per cent hydrochloric acid by volume, using heat. Chlorine gas was prepared by adding concentrated hydrochloric acid to crystals of potassium permanganate. This gas was bubbled through the solution in which the iron wire had been dissolved. After the solution had been saturated with chlorine gas, it was evaporated to dryness on a water bath. The residue was taken up in 15 cc. of concentrated hydrochloric acid and made up to 1 liter with distilled water. This made a convenient standard solution containing 1 mg. of iron per cubic centimeter.

2. Chlorophyll Gradation Produced by Varying the Magnesium Concentration

The same nutrient solution was used as for the iron experiments except that sodium sulfate (Baker) was substituted for magnesium sulfate in the ratio of 1.42 gm. for 2.46 gm.; and that 10 mg. of iron per liter were added. This solution contained such a slight amount of magnesium as an impurity, that white cells could be produced in the cultures low in magnesium. A standard solution of magnesium was prepared by dissolving magnesium chloride in distilled water, so that the concentration was 1 mg. of magnesium per cc. Varying amounts of this standard solution were added to the cultures so as to give concentrations ranging from 0.02 to 2.0 parts per million.

3. Chlorophyll Gradation Produced by Varying the Nitrogen Concentration

The nutrient solution was the same as for the iron experiments, except that potassium chloride (Baker) was substituted for potassium nitrate in the ratio of 0.94 gm. for 1.26 gm., and that 10 mg. of iron per liter were added. As with the cultures low in magnesium, very low amounts of chlorophyll could be obtained with the cultures low in nitrogen. The standard solution of nitrogen was prepared by dissolving potassium nitrate in distilled water so that the concentration was 10 mg. of nitrogen per cc. Varying amounts of this solution were added to the cultures so as to give nitrogen concentrations ranging from 10 to 80 parts per million.

Determination of the Rate of Photosynthesis

The manometric method used for measuring the rate of photosynthesis and of respiration was developed by Warburg (1919) using the simple or blood-gas manom-
eter of Barcroft and Haldane (1902). Warburg (1919, 1924, 1931) and Emerson (1929) used this method in their photosynthetic work with Chlorella. Descriptions of the method as given by Warburg and Emerson are summarized in the author’s thesis (Fleischer, 1933).

In this work the Chlorella cells were centrifuged out of the culture medium and suspended in Warburg’s carbonate mixture 9.1 cc. of this suspension, containing a known amount of cells, usually from 8 to 15 mm.³, and 6 cc. of the carbonate mixture were placed in the vessel, submerged in the thermostat, illuminated, and shaken for an adjustment period of 10 minutes. During this period the stop-cocks must be closed to allow the water vapor in the gas space to come to equilibrium with the carbonate mixture. At the end of the adjustment period the stop-cocks are opened momentarily while the liquid levels in the manometer arms are being leveled, and then closed during a run. If the stop-cocks are left open during the adjustment period and closed at the beginning of a run, then the water vapor equilibrium will not have been attained and the vapor pressure of the carbonate mixture will cause an increase in pressure in the manometer, thereby making the rate of photosynthesis appear greater than it really is.

During a run the amount of gas evolved for 30 minutes in the light was determined and added to the amount absorbed in the dark during the next 30 minutes, thereby giving the amount of oxygen evolved in photosynthesis.

To show that the environmental conditions under which photosynthesis was measured were not toxic to the Chlorella cells, determinations were made from several different cultures in which the rate was measured every 15 minutes, for at least 75 minutes.

<table>
<thead>
<tr>
<th>TIME</th>
<th>mm.³ of oxygen produced</th>
<th>mm.³ of oxygen produced</th>
<th>mm.³ of oxygen produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per 8.0 mm.³ of cells</td>
<td>Per 9.2 mm.³ of cells</td>
<td>Per 9.2 mm.³ of cells</td>
</tr>
<tr>
<td>min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>21.8</td>
<td>28.0</td>
<td>21.1</td>
</tr>
<tr>
<td>15</td>
<td>24.9</td>
<td>31.8</td>
<td>21.3</td>
</tr>
<tr>
<td>15</td>
<td>26.6</td>
<td>34.0</td>
<td>22.6</td>
</tr>
<tr>
<td>15</td>
<td>25.7</td>
<td>32.8</td>
<td>23.9</td>
</tr>
<tr>
<td>15</td>
<td>25.9</td>
<td>33.1</td>
<td>23.5</td>
</tr>
<tr>
<td>15</td>
<td>26.1</td>
<td>33.4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>25.5</td>
<td>32.6</td>
<td></td>
</tr>
</tbody>
</table>

1 Warburg’s carbonate mixture Number 9 is composed of 15 cc. of 0.10 mol sodium carbonate plus 85 cc. of 0.10 mol sodium bicarbonate.
These data are plotted on the accompanying graph. Since the curves are straight lines they indicate that the rate is constant with time and that no significant toxic effect is evident at the end of 60 minutes.

To show that the intensity of light used in photosynthesis was non-limiting, determinations of photosynthesis were made with various dilutions of the same culture. If the weaker dilutions gave a proportionately higher rate of photosynthesis, it would indicate that in the more concentrated suspensions, the cells nearest the light were shading those furthest from the light. But if all of the dilutions gave proportionately the same ratio within the experimental error, then light would not be limiting. The data below are plotted in the accompanying graph and indicate clearly that the intensity of light was great enough to be non-limiting for the process of photosynthesis, since all of the dilutions gave the same proportionate rate of photosynthesis.

TABLE II

Effect of Light upon the Rate of Photosynthesis using Various Dilutions of the Same Culture

<table>
<thead>
<tr>
<th>Time</th>
<th>mm.³ oxygen produced per cell volume indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.2 mm.³</td>
</tr>
<tr>
<td>min.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>21.1</td>
</tr>
<tr>
<td>15</td>
<td>21.3</td>
</tr>
<tr>
<td>15</td>
<td>22.6</td>
</tr>
<tr>
<td>15</td>
<td>23.9</td>
</tr>
<tr>
<td>15</td>
<td>23.5</td>
</tr>
</tbody>
</table>

mm.³ O₂/hr. per 9.2 mm.³ of cells

89.9 | 85.4 | 85.6

The light from a 500 watt Mazda projection bulb, concentrated and made parallel by a plano-convex lens was thrown on the cell suspension through the glass side of the thermostat. The light intensity incident on the Warburg vessels was 75,000 lux as measured by a Weston illumination meter, model 603.

The thermostat used was a DeKhotinsky constant temperature bath which maintained the temperature constant to within 0.002°C. as checked by a Beckmann thermometer. To correct for changes in atmospheric pressure, a control vessel was filled with 7 cc. of carbonate mixture, immersed, and shaken in the water bath, while a photosynthetic run was being made with the other vessels.
Fig. 1. Graph of Table I

Fig. 2. Graph of Table II
containing *Chlorella* cells. Warburg (1919) used an empty vessel as a control and later (1924) used a vessel containing Ringer's solution. The change in pressure in the control vessel was applied as a correction to the pressure readings of the other vessels to eliminate the effect of changes in temperature or barometric pressure.

The volume of gas absorbed in the dark was added to that evolved in the light during an equal period of time, thereby correcting for respiration. The gas volumes were expressed as mm.$^3$ of oxygen per 10 mm.$^3$ of cell volume or per 50 sq. cm. of cell surface and plotted against their respective chlorophyll content. The latter were expressed respectively as gm. of chlorophyll per 10 mm.$^3$ of cell volume or per 50 sq. cm. of cell surface.

Cell volume and cell surface were measured as follows: The diameters of 200 cells were measured with an ocular micrometer under an oil immersion lens. The volume and surface of a single average cell were calculated from the diameter. The average number of cells per cubic centimeter for five samples of cell suspension was determined with an American standard haemacytometer. The product of the volume of an average cell and the number of cells per cubic centimeter gave the total volume of cells per cubic centimeter of cell suspension. Similarly the total cell surface per cubic centimeter of cell suspension was obtained. The probable error of the method was calculated from the formula:

$$\text{Probable error of a single observation is equal to:}$$

$$0.6745 \sqrt{\frac{V^2 - nM^2}{n - 1}}$$

The probable error as expressed as per cent of the mean was 3.03 per cent.

The cell volumes used for determination of photosynthesis were between 8.0 and 15.0 mm.$^3$ of cells as stated above. Since the same type of Warburg glass vessels were used as those employed by both Warburg and Emerson, the cell constants are of the same order of magnitude. In our work the cell constants ranged from 0.49 to 0.60. The manometric readings ranged from 20 to 400 mm. showing that enough oxygen was evolved to give a significant reading. Any experimental determination of less than 20 mm. was discarded since experimental errors might affect such low readings.

### IV

**Determination of Chlorophyll Content**

The various methods of chlorophyll determination as given in the literature are summarized below:

- **Gravimetric.**—Willstätter and Stoll (1928).
- **Colorimetric.**—Maiwald (1923), Schertz (1928), Deuber (1928), Oserkowsky (1932), Guthrie (1928), Sprague and Troxler (1930), Harriman (1930).
Spectrometric.—Schertz (1928).
Spectrophotometric.—Wurmser and Duclaux (1921), van den Honert (1929), Emerson (1929), Emerson and Arnold (1932).
Spectrographic.—Lewkowitsch (1928), Dastur and Buhariwalla (1928), Dastur and Desai (1933).
Spectrocolorimetric.—Monteverde and Lubimenko (1913), Hubbenet (1925), Zaitseva (1928, 1929), Lubimenko and Hubbenet (1932).

Since this work dealt with chlorotic cells, the amount of chlorophyll to be extracted was too small to be measured gravimetrically. Colorimetric, spectrophotometric, and spectrocolorimetric readings may be influenced by substances dissolved out of the cells by the extracting solvent. Therefore the per cent of error would increase as the chlorophyll content decreased and at very low ranges the determination of chlorophyll would be unsatisfactory. Likewise since the amounts of chlorophyll to be extracted are so small, it would be extremely difficult to separate quantitatively the green pigments from the rest of the plant extract.

The spectrographic method was tried using a Bausch and Lomb quartz spectrograph. However, the edges of the absorption bands at chlorophyll ranges near the extinction point are not as clear and definite as the results of Dastur and Buhariwalla (1928) would seem to indicate. Consequently the position of the curve and its apex point could not be determined sharply.

In an attempt to eliminate these sources of error an apparatus was devised whereby the extinction point of an absorption band was determined spectrocolorimetrically. A spectroscope was mounted over a single colorimeter tube in which the depth of chlorophyll solution could be varied.

Front and side views of the apparatus are shown in Fig. 3. SP is the spectroscope with E the eyepiece and S the slit end. In all of the work a slit opening of 100μ was used. G is a Corning filter of heat resisting signal glass, No. 243 and 3.12 mm. thick. This filter absorbs all of the visible spectrum except a band in the red from 6160 Å to 6960 Å. The absorption band of chlorophyll used lies in the center of this visible band let through by the glass filter. This elimination of the rest of the spectrum allows the observer's eye to concentrate better on the absorption band and to detect with greater ease the presence or absence of the absorption band near the extinction point. Since the absorption band is con-
trasted with the strips of the spectrum adjacent to the band, the diffusion of light by dissolved or suspended substances does not matter. The absorption band from 6520 Å to 6700 Å used in this work lies in the region of maximum absorption of chlorophyll a and b and outside of the range of absorption of carotin and xanthophyll. Emerson (1929) used this same band in his work.

C is the glass colorimeter tube in which the depth of chlorophyll solution may be varied by raising or lowering the plunger P in the tube T. O is the three-way stopcock for draining the system at the end of a determination. M is a mirror which reflects light from L, a 25 watt internally frosted Mazda bulb, up through the glass bottom of the colorimeter tube.

![Diagram of apparatus for chlorophyll determination](image)

**FIG. 3. Apparatus for chlorophyll determination**

A standard chlorophyll solution was prepared from a sample of pure crystal chlorophyll (a + b), obtained through the courtesy of Dr. Frank Schertz of the Bureau of Plant Industry, U. S. Department of Agriculture. This standard solution contained 0.0144 gm. of chlorophyll per liter of methyl alcohol. Known dilutions were made, placed in the colorimeter tube, and the depth of solution at which the absorption band just faded out was determined. After several weeks of running such determinations the human eye becomes very sensitive in the detection of this extinction point and fairly good checks could be obtained. In each case at least five depth readings were made and averaged.
A set of readings is given below to show the amount of variation. The concentrations of chlorophyll are given in percentages of the standard solution and the depths in millimeters at which extinction of the absorption band occurred.

**TABLE III**

*Amount of Variation in Determination of Extinction Points*

<table>
<thead>
<tr>
<th>Depths at which extinction occurred</th>
<th>Standard solution of chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 per cent</td>
</tr>
<tr>
<td></td>
<td>mm.</td>
</tr>
<tr>
<td>23.1</td>
<td>15.1</td>
</tr>
<tr>
<td>23.8</td>
<td>13.6</td>
</tr>
<tr>
<td>23.9</td>
<td>14.6</td>
</tr>
<tr>
<td>24.6</td>
<td>13.4</td>
</tr>
<tr>
<td>23.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Average</td>
<td>23.7 mm.</td>
</tr>
</tbody>
</table>

For purposes of calculation, a column of chlorophyll solution 1 sq. cm. in cross-section was used as a basis. To show that the amount of absorption was directly proportional to the concentration of the solution, extinction readings were made for a series of dilutions in the same range as that used in the experimental work.

**TABLE IV**

*Relation between Absorption of Light and Amount of Chlorophyll*

<table>
<thead>
<tr>
<th>Standard solution</th>
<th>Depth at which extinction occurred</th>
<th>Vol. of solution in tube 1 sq. cm. in area when extinction occurred</th>
<th>Chlorophyll per cc. of solution</th>
<th>Chlorophyll in tube 1 sq. cm. in area</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>23.7</td>
<td>2.37</td>
<td>X 0.000000086 = 0.00000205</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>14.2</td>
<td>1.42</td>
<td>X 0.00000144 = 0.00000204</td>
<td></td>
</tr>
<tr>
<td>13.0</td>
<td>11.0</td>
<td>1.10</td>
<td>X 0.00000187 = 0.00000206</td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>9.6</td>
<td>0.96</td>
<td>X 0.00000216 = 0.00000207</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.00000206</td>
<td></td>
</tr>
</tbody>
</table>

Hence when looking through a column of solution 1 sq. cm. in cross-section, the absorption band just fades out when 0.00000206 gm. of chlorophyll are present in that column of solution, regardless of the volume of the solvent. To illustrate:

Assume that at a depth of 20 mm. the absorption band has just faded out.
Therefore in a column of that solution, 1 sq. cm. in area, there would be 2 cc. containing 0.00000206 gm. of chlorophyll. If there were 50 cc. of the original solution, then there would be a total of (0.00000206 × 50) = 0.0000103 gm. of chlorophyll in that solution. In this manner the chlorophyll content of any solution may be determined.

In Table III the variation between any two settings does not exceed 10 per cent. However, when the average of five determinations is compared with any other average as in Table IV, the variation drops to 1.5 per cent. After the preliminary work, an average of ten settings was used so that the variation would be still less. This new method of visual determination of the extinction of an absorption band is therefore quite accurate for chlorophyll measurement.

To facilitate the computation of the chlorophyll content, the accompanying graph (Fig. 4) was constructed. The depth in centimeters at which the extinction of the absorption band occurred is plotted against the grams of chlorophyll present.
in 50 cc. of solution at the extinction point. The extinction depth having been determined in the colorimeter, the corresponding amount of chlorophyll may be read off from the graph.

Emerson (1929) extracted the pigments from *Chlorella* cells with methyl alcohol but he does not state the technique used. Emerson and Arnold (1932) washed the *Chlorella* cells with distilled water and then poured boiling water over them and let them stand for 2 minutes. The cells were then centrifuged out of the water and extracted with methanol until white. In our work a known amount of cells was boiled in methyl alcohol for 2 minutes, the cells centrifuged to the bottom of the tube, and the clear supernatant chlorophyll solution poured off. The cell residue was suspended in methyl alcohol, again centrifuged, and the supernatant liquid added to the first extract. This solution was made up to a volume of 50 cc. with methyl alcohol and the chlorophyll content determined as described above.

This method produced pure white cell residues and for completeness and ease of extraction was found to be superior to any of the following:

1. Repeated grinding of the cells with a mortar and pestle in 80 per cent or 100 per cent acetone, 95 per cent ethyl alcohol, ethyl ether, or methyl alcohol.
2. Shaking the cells in an International bottle shaking machine for an hour with any of the above solvents.
3. Emerson and Arnold's method (1932) as described above.
4. Boiling for 5 minutes in any of the above solvents except methyl alcohol.
5. A combination of any of the above except boiling in methyl alcohol.

To find out whether boiling in methyl alcohol decomposed the chlorophyll, readings were made on three samples of a 15 per cent standard solution of chlorophyll. One sample was left unboiled, the second was boiled for 1 minute, and the third was boiled for 5 minutes. The data below show that boiling in methyl alcohol for as long as 5 minutes caused no significant decomposition.

**TABLE V**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Depth at which extinction occurred</th>
<th>Vol. of solution in tube 1 sq. cm. in area when extinction occurred</th>
<th>Chlorophyll in cc. of solution</th>
<th>Chlorophyll in tube 1 sq. cm. in area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unboiled</td>
<td>9.6</td>
<td>0.96</td>
<td>X 0.00000216 = 0.00000207</td>
<td>X 0.00000216 = 0.00000210</td>
</tr>
<tr>
<td>Boiled 1 min. in methyl alcohol</td>
<td>9.7</td>
<td>0.97</td>
<td>X 0.00000216 = 0.00000210</td>
<td>X 0.00000216 = 0.00000210</td>
</tr>
<tr>
<td>Boiled 5 min. in methyl alcohol</td>
<td>9.7</td>
<td>0.97</td>
<td>X 0.00000216 = 0.00000210</td>
<td>X 0.00000216 = 0.00000210</td>
</tr>
</tbody>
</table>
CHLOROPHYLL CONTENT AND RATE OF PHOTOSYNTHESIS

The differences are within the experimental error of the determination, indicating that boiling in methyl alcohol for 2 minutes, as done in the experimental work, caused no significant decomposition of the chlorophyll.

The absorption band used was that within the limits of 6520 Å and 670 Å, when a depth of 43.6 mm. of a 13 per cent standard solution was used. This band was then 180 Å wide when light passed through a column of solution 1 sq. cm. in cross-section and containing 0.00000815 gm. of chlorophyll. This solution was considerably stronger than the extinction concentration in order to determine more sharply the edges of the absorption band. At the extinction concentration the band is somewhat narrower and the edges are not so distinct.

V
DISCUSSION OF RESULTS

Since all of the series belonging to any one group, for example all of those deficient in iron, were treated identically except for the amount of iron added, it is not necessary to plot each individual series separately. If they are plotted separately, some curves are parallel and others are not, whereas if all are plotted together on the same sheet, a definite linear relationship between chlorophyll content and amount of photosynthesis is evident. This illustrates the danger of using too few data as done by Emerson who has only two curves and nine determinations. In some cases his curves differ by 100 per cent which he does not regard as significant. In our work a total of 104 determinations were made upon which the conclusions are based.

A. Experiments with Chlorophyll Gradation Produced by Varied Iron Concentration

The deficiency of an element may alter the relation between cell volume and chlorophyll content or between cell surface and chlorophyll content and thereby influence the rate of photosynthesis. Therefore the data were calculated so that the results were expressed on a cell volume basis, on a cell surface basis, and on a basis independent of surface or of volume. If the curves are similar, regardless of the manner of expression, then it indicates that the element has not affected the rate of photosynthesis by any effect upon the cell surface or cell volume.
It will be apparent that while surface and volume curves might be similar, they could not be identical with only a change in scale. This latter result is due to variations in cell diameter. *Chlorella* cells which have been grown under identical conditions of light, temperature, and nutrient solution, will exhibit a normal variation in average cell diameter of as much as 5 microns. An example will illustrate why these variations prevent surface and volume curves from being identical with only change of scale. 10 mm.\(^3\) of cells with an average cell diameter of 10 microns have a total cell surface of 60.0 sq. cm., whereas the same volume of cells with an average cell diameter of 15 microns have a total cell surface of 39.9 sq. cm. Although the volumes are equal, the surfaces differ considerably. Therefore surface and volume curves are not identical with only change in scale, and furthermore, they could not be, unless the average cell diameter were equal.

The three different types of graphs, surface, volume, and independent, were made in order to determine whether surface or volume somehow had a specific influence upon the rate of photosynthesis. For example, if there existed a better proportionality between chlorophyll content and rate of photosynthesis in the surface plotting than in the volume plotting, we might expect that diffusion of oxygen out of or carbon dioxide into the cells was a controlling factor. But since all three types of curves were similar, it shows that neither surface nor volume play any special rôle in controlling the rate of photosynthesis.

The data for the three cases are plotted on the accompanying graphs. In each case there is a linear relationship between the rate of photosynthesis and the chlorophyll content, indicating that the results are not appreciably influenced by the manner of expression. In each case the curve passes through the origin, showing that the rate of photosynthesis does not drop off sharply as the chlorophyll content decreases, but that there is a continuous and steady decrease in both quantities as the origin is approached.

In the results of the series given in his paper, Emerson (1929) obtained two curves, parallel but somewhat separated. In neither case did his curves approach the origin. They also flattened out asymptotically at the higher chlorophyll ranges. In contrast to his
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![Graph showing relationship between chlorophyll content and oxygen evolution.](image)

**Fig. 5.** Iron volume plotting

![Graph showing relationship between chlorophyll content and oxygen evolution.](image)

**Fig. 6.** Iron surface plotting
curves, the data here presented form a straight line curve passing through the origin. In this work there were three cultures in a series and when curves of different series were compared, some were parallel and others were not. But when the results of all of the series were plotted on the same sheet, a linear relationship was evident. Possibly if Emerson had run more series he might have found the same situation existing.

In order to have a complete range of chlorophyll content, determinations were made with cells grown in a full nutrient solution. These results are plotted on each of the graphs for iron, nitrogen, and magnesium and the points are indicated by “x” instead of by circles. In each of the iron graphs, the full nutrient points lie close to the curve, indicating that even at this high chlorophyll content, chlorophyll is limiting the process of photosynthesis.

In none of the results was any correlation found between chlorophyll content and the rate of respiration.

The results of the twelve iron series, thirty-five cultures in all, given in this paper support Emerson’s conclusion that the rate of
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Fig. 8. Nitrogen volume plotting

Fig. 9. Nitrogen surface plotting
Fig. 10. Nitrogen independent plotting

Fig. 11. Magnesium volume plotting
photosynthesis is proportional to the chlorophyll content when the latter is varied by varying the iron concentration. However, the curves given here differ from those of Emerson as explained above and appear to substantiate his conclusion better than his own data.
do, since his curves do not form a straight line passing through the origin.

B. Experiments with Chlorophyll Gradation Produced by Varied Nitrogen Concentration

The data for this set of experiments were treated the same as the data for the iron experiments and the results plotted on a surface basis, on a volume basis, and on an independent basis in the accompanying graphs. In each case there is a linear relationship between the rate of photosynthesis and the chlorophyll content. Each curve is a straight line intersecting the abscissa slightly to the right of the origin. Since low nitrogen results in low vegetative growth, this offset may be due to the retardation of photosynthesis by the accumulation of carbohydrates.

The full nutrient determination also lies close to these curves, indicating that chlorophyll is still limiting the process of photosynthesis at this high chlorophyll content.

The results of the seven nitrogen series, twenty-one cultures in all, given in this paper indicate that the rate of photosynthesis is proportional to the chlorophyll content, when the latter is varied by varying the nitrogen concentration.

C. Experiments with Chlorophyll Content Varied by Varying the Magnesium Concentration

The data for this set of experiments were treated the same as the data for the iron and nitrogen experiments and the results plotted on a volume basis, on a surface basis, and on an independent basis in the accompanying graphs. It was noted that cells, chlorotic through deficient magnesium, were larger than cells which were green and abundantly supplied with magnesium. However, the surface, volume, and independent curves are similar, showing that the greater size of chlorotic cells did not significantly affect the rate of photosynthesis.

Although the magnesium curves are similar to each other, they differ from the nitrogen curves and the iron curves. Therefore it appears that the magnesium concentration has an effect upon the rate of photosynthesis separate from its effect through varying chlorophyll content. The shape of the curves indicates that magnesium becomes
limiting for photosynthesis before it becomes limiting for chlorophyll production. These curves do not approach the origin, which favors the hypothesis that magnesium is doubly involved in the process of photosynthesis.

At low concentrations of magnesium the rate of photosynthesis is relatively independent of the chlorophyll content. As the magnesium concentration is increased, the rate of photosynthesis rises rapidly and during the rise is relatively independent of the chlorophyll content. Eventually the rate of photosynthesis reaches the value indicated by the full nutrient determinations and at that point the relation between the rate of photosynthesis and the chlorophyll content is comparable to the relation existing in the iron and nitrogen graphs for similar values.

The above data indicate therefore, that the presence of magnesium is necessary for the process of photosynthesis in addition to its necessity for chlorophyll formation. Two possible explanations are offered:

1. Magnesium is directly concerned chemically or photochemically in the process of photosynthesis.
2. Magnesium affects an internal factor other than chlorophyll, this internal factor being concerned in the process of photosynthesis.

André (1916) found that the period of greatest photosynthetic activity (April, May, June) was correlated with the greatest amounts of magnesium both organic and inorganic, and also with the greatest organic magnesium/inorganic magnesium ratio. Since his data are given as total amounts rather than as percentages of dry weight, it seems likely that the rapid leaf growth occurring at that time of year would account for the increase in total magnesium. Therefore his data do not prove conclusively that the amount of magnesium present influences the rate of photosynthesis.

Since magnesium has a pronounced catalytic effect upon some enzymatic reactions, such as those of phosphotases, it is possible that it may in some similar way catalytically affect the rate of photosynthesis.

* Full nutrient determinations are indicated by "x" on the graphs.
VI

CONCLUSIONS AND SUMMARY

1. Data are presented which support the conclusion of Emerson (1929) that the rate of photosynthesis is proportional to the chlorophyll content when the latter is varied by varying the iron supply. These data give a straight line passing through the origin, which is not true of Emerson's results.

2. Similar data are presented which show that a similar relation exists when nitrogen controls the chlorophyll content.

3. Evidence is given which indicates that magnesium plays a part in the process of photosynthesis in addition to its effect upon the chlorophyll content.

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