ASPECTS OF THE PHYSIOLOGY OF BLUE-GREEN ALGAE

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In a study of the process of photosynthesis in blue-green algae two possibilities are uppermost in the investigator's mind. One of these is that the assimilatory process may differ from that found in green plants because of the presence of the accessory blue pigment. This has been the opinion of many investigators since Engelmann submitted evidence on the point fifty years ago. It is even possible that the blue pigment has a function similar to that of chlorophyll. The other possibility is that the assimilatory process in blue-green algae may be peculiar because in the cells of this group, the pigments are distributed (or aggregated) otherwise than in green plants. If this is the case, a sufficiently refined study of the process should yield information about photosynthesis in general. Inextricably bound up with photosynthesis in this class of algae is the subject of color changes, changes in the kinds and amounts of pigments in the cells under different conditions.

Studies of the physiology of the blue-green algae have hitherto been hampered by (1.) the difficulty of isolating species favorable for experimentation, (2.) the difficulty of finding a medium for their growth which will assure an abundance of healthy uniform material and (3.)

the lack of an adequate technique of experimentation. The last need has been abundantly supplied by the technique worked out by Warburg. An account of the solution of the other two problems will be the subject of the next chapter.

For a proper understanding of the results of this research and of the difficulties in the way of attaining them, a description of some features of the biology of blue-green algae will be necessary. This will be given in the next section. Previous work on those aspects of the physiology of these plants with which this paper principally deals will be discussed in later chapters where it can be compared with the original work described.

General Biology of the Blue-green Algae

I.

The blue-green algae seldom occur as solitary cells. A majority of the species are filamentous, and of the remainder, nearly all grow in more or less loose colonies held together by extensive gelatinous sheaths. In Gloeocapsa the sheath may have twenty-five times the volume of all the cells enclosed. In the exposed situations on rocks and other bare surfaces in which these organisms grow more profusely than any other algae, the function of the sheaths in preventing water-loss is easy to understand. Cells growing in submerged habitats often have thinner sheaths. This opens the tempting possibility that under

appropriate conditions of cultivation, the organisms might be grown with very thin sheaths or none, but so far these conditions have not been realized. By mechanical means, the large aggregates which arise under all conditions favorable for growth, may be broken up without injury to the individual cells. Thus it is possible to obtain suspensions suitable for pipetting, diluting, and plating.

The cell of a blue-green alga is enclosed by a thin cell-wall of cellulose (Klein 1915). The cytoplasm consists of an outer colored portion, the cortex, and an inner colorless portion, the central body. Whether or not there is a nucleus present is a subject of dispute (Gardner 1906, Haupt 1923) but nobody claims to have found a fully organized nucleus. The structure of the cortex is also in debate. Whether it should be called a chromatophore is a battle of words. The cell pigments are restricted to the cortex and within it may be either distributed in discrete bodies so small as to be barely resolved by the microscope (Hegler 1901, Kohl 1903, cited by ~~in~~ Haupt) or diffused uniformly (Gardner, Haupt). In view of the lack of agreement among the authorities, and of the uncertainty of the criterion on which the lack of agreement is based, it appears to me that the dispute can not be settled by direct visual examination of the cells. In any case there is a difference in arrangement of pigments between the Cyanophyceae and other green plants which may be of physiological importance.

At least four pigments occur in the blue-green algae (Geitler 1925 p.3), chlorophyll, carotin, phycocyanin, and sometimes phycoerythrin. Of the first two nothing need be said; their characteristics are not a singularity of this research. The last two were first observed by Kützing in 1843 (Czapek 1922 p.598) and have been principally studied by Molisch, Kylin, and Boresch. Phycocyanin is a blue pigment with a wine-red fluorescence occurring in all blue-green and some red algae. Phycoerythrin is a red pigment with an orange fluorescence occurring in many blue-green and all red algae. As the species with which I work have never exhibited reddish tinges, I believe them to lack phycoerythrin. Hence I shall discuss only phycocyanin although the two pigments are so similar in all observed characteristics except color that a description of one fits the other closely. Phycocyanin can be obtained from many algae by extraction with water and purified by precipitation with ammonium sulphate (Kylin 1910). Several modifications have been observed differing slightly in color (Kylin 1931, Boresch 1921). It is a conjugated protein with a molecular weight of about 200,000 (Svedberg and Lewis 1928, Svedberg and Katsurai 1929) whose colored portion can be split off by peptic digestion (Kylin 1910). The principal constituent of its ash is calcium, and traces of magnesium and iron are also present (Lemberg 1928). The absorption spectrum is well known with its maximum at

about 615 mμ, and occasionally a second maximum at about 575 or 550 mμ. While it has been shown that chlorophyll and other fluorescent pigments can effect oxygen transfer when illuminated in vitro (Gaffron 1927), no such photochemical phenomenon has been found with phycocyanin (Gaffron, unpublished work).

II.

Many species of blue-green algae, especially among the Oscillatoriaceae, exhibit movements. These may be of a locomotive nature, taking place in the direction of the axis of the filament, or may be twisting movements, or wavings of the end of the filament. The velocity of the movement is a function of temperature and the direction is determined by photo- and chemotaxis (Pieper 1915). If a mass of filaments is placed on an agar plate, the filaments at the periphery of the clump spread out radially and the most active crawl away from the main group. Filaments will even bore through the agar toward the lower surface if this is oriented toward a source of light. These phenomena have been utilized in obtaining pure cultures of filamentous Cyanophyceae. Although explanations have been offered for the mechanism of the movement, none is supported by convincing evidence. (Krenner 1925).

The gelatinous or mucilaginous sheaths characteristic of blue-green algae are an unusually favorable medium for

bacteria. Except when the algae are growing themselves as symbionts in other plants (e.g. Anabena Cycadeae in roots of Cycas), they probably always support a population of bacteria in their sheaths. The bacteria can easily be demonstrated by staining in any blue-green algae collected at random. The relationship is probably not obligate, as an identification of the bacteria shows that they are common heterotrophic soil bacteria customarily existing saprophytically. The fact that blue-green algae will develop in media free of fixed nitrogen has led many to ascribe to them the ability to carry on this process but the case must be considered as not proved. Nobody has yet studied this point with a pure culture of blue-green algae, and several forms of nitrogen-fixing bacteria have been cultured from the sheaths of various species of blue-greens. (Jones 1930).

Isolation and Cultivation of Blue-green algae

I.

Principally to Winogradsky and Beijerinck who first developed extensively the methods of isolating and cultivating microorganisms and to E.G.Pringsheim who first worked with blue-green algae, is due the knowledge of the subject now at hand. In the literature may be found references to species-pure cultures of several species of blue-green algae obtained by various writers, all using the technique of Pringsheim. In Mainx's list (Junk 1929) of pure strains of algae described in the literature as isolated in pure or species-pure culture, out of over 200 species, 30 are Cyanophyceae. Two points are to be noted. First, nearly all the species mentioned are filamentous. Second, only 10 are in absolutely pure culture and these are all motile forms.

The mineral requirements of blue-green algae are similar to those of other green plants. Potassium, iron, magnesium, phosphorus, sulphur and perhaps calcium are necessary. So far as is known the organisms can not assimilate elementary nitrogen. They will grow in inorganic media only in the presence of carbon dioxide and light. Although some species will grow at high temperatures (up to 75°C, Smith 1933 p.41) there is no reason to believe that most species of the class can subsist at temperatures conspicuously higher than the usual biological range.

Most of these algae grow fastest when submerged, although many species will grow if merely kept in a moist atmosphere.

It is possible, then, to cultivate blue-green algae on strictly inorganic media which will discourage the growth of heterotrophic organisms. Such media are (a.) liquid media made of salts dissolved in pure water which may or may not be imbibed in sand, gypsum plates, and the like and (b.) solid media made up in silica gel. Agar may be allowed to stand for a week or more in several changes of tap-water and then of distilled water until all of the carbon compounds except insoluble carbohydrates of high molecular weight have been oxidized by bacterial action and washed away, whereupon it becomes a very unfavorable medium for heterotrophic organisms, but retains the convenient characteristics of ordinary agar.

The peculiar difficulties of isolating blue-green algae are not avoided by the use of these media. Even when the colonies of a coccus-form are broken up by shaking with sand, microscopic examination shows that most of the resulting entities are small colonies rather than individual cells. When a suspension so treated is plated on agar or pipetted on silica gel, the probability of picking off a colony which has grown from a single cell is negligable. Moreover, with each cell, in its gelatinous sheath, goes its accompanying host of bacteria. The bacteria can not ordinarily be washed or scraped off by any method

harmless to the alga. Pure cultures of several motile forms have been obtained (Pringsheim 1913, Schramm 1913) by inducing trichomes to crawl through a mass of agar toward a source of light. Not only is this method restricted to motile forms but it can be used only when the culture conditions are very favorable so that the organism retains its motility.

II.

For the study of photosynthesis by the Warburg method, a coccus-form alga is essential. Filamentous and other colonial algae do not distribute themselves uniformly in the medium, but adhere to each other in clumps. The gradient in light intensity within the vessel which already introduces an error into the experiment, under the best conditions, now becomes exaggerated in each clump and there may be added a sharp gradient in gas-tension from the outside to the inside. Besides, such algae can not easily be manipulated in measurable quantities. So, with the requirements of this technique in mind, I set out to isolate a coccus-form blue-green alga.

A suitable organism later identified as a Gloeocapsa or Gloeothece sp. (hereafter referred to as Gloeocapsa) was growing in the water of an aquarium in the botanical laboratory at Harvard. A few drops of water from the aquarium were streaked on agar slants containing a medium

devised by Beijerinck, of the following composition:

NH_4NO_3	0.5 g./l
$MgSO_4.7H_2O$	0.2
KH_2PO_4	0.2
$CaCl_2$	0.1
$Fe_2(SO_4)_3$	trace

By repeated streaking on agar slants, colonies of the alga were obtained free of other algae.

Many attempts were made to cultivate Gloeocapsa on silica gel plates in the hope of freeing them from bacteria. At first silica gel was made from Na_2SiO_3 and HCl, but later from K_2SiO_3 and H_3PO_4 with the idea that the residue of K_3PO_4 after dialyzing would be less toxic than NaCl. But no growth was obtained on this substrate, whatever the medium with which it was imbibed.

Meanwhile, a species of Oscillatoria was isolated from the edge of a colony on an agar plate and used for culture experiments to obtain an indication of a liquid medium suitable for Cyanophyceae. The best growth was obtained in media similar to that given above, made up in water redistilled from a Pyrex still, but containing K_2HPO_4 instead of KH_2PO_4, and containing excess of $CaCO_3$. Faster growth was obtained at 30^o than at higher and lower temperatures. When the medium was aerated with a mixture of 5% CO_2 in compressed air much better growth occurred than in a stagnant medium.

III.

On the basis of this experience, colonies of Gloeocapsa were inoculated into the following medium and grew very well.

NH_4NO_3	0.5 g./l
$MgSO_4.7H_2O$	0.2
K_2HPO_4	0.95
KH_2PO_4	0.05
$Fe_2(SO_4)_3$	0.002
$CaCO_3$	excess

The cultures were aerated and kept at 25°- 30°.

For NH_4NO_3could be substituted KNO_3 without changing the suitability of the medium. If commercial distilled water was used instead of redistilled water, no growth occurred. The toxic effect of ordinary distilled water has been noted previously (Pringsheim 1913).

As the cells could not be freed from the fine precipitate of $CaCO_3$ in the medium, they could not be used for manometric and other experiments. All attempts to make a medium without precipitate in distilled water failed. Any medium sufficiently alkaline to permit growth precipitated a calcium salt. If calcium was omitted or added originally as Iceland spar, coarsely broken up in a mortar, no growth occurred. Powdered sulphur was added to some cultures and washed sand to others, to simulate the habitat furnished by a layer of precipitate, but no growth occurred in cultures lacking calcium. But a medium made up in Pasadena

tap-water, without $CaCO_3$, was found to meet the requirement and to give better growth than the other media. Although a heavy precipitate formed in this medium during auto/claving, it disappeared rapidly during aeration with 5% CO_2 in air. The composition of the medium finally used was as follows:

KNO_3	0.5 g./l
$MgSO_4.7H_2O$	0.2
K_2HPO_4	0.2
$Fe_2(SO_4)_3$	0.002

The alkalinity of the tap-water makes possible the reduction in amount of K_2HPO_4 and the hardness makes it unnecessary to add a calcium salt. The pH of this medium after autoclaving and after equilibrium is attained with 5% CO_2 in air, is between 7.5 and 8.0. Although there is enough iron in the tap-water to permit rapid growth, the cells have a deeper color if iron is added.

Another coccus form was isolated by the same method from water collected in Tujunga Canyon. It has been identified provisionally as a Chroococcus species. This organism has been cultivated successfully in a liquid medium, but only when the medium is made up in tap-water from Pacific Grove, California. Commercial distilled water is toxic to it, and no medium so far devised permits growth in redistilled water. Gloeocapsa will not grow in a medium of the composition given above, if made up in Pacific Grove tap-water. As these differences in the suitability of differ-

ent natural waters depend, presumably, on the presence or absence of very small quantities of substances, I consider that an experimental elucidation of them lies outside my field.

Both organisms grow best in light of low intensity. More will be said of the relation of Gloeocapsa to light in a later section. Gloeocapsa grows very well at 30° or 35° but Chroococcus will not grow rapidly at temperatures above 25°.

Both of these organisms grow well on slants of washed agar on which heterotrophic organisms multiply very slowly. In this way stock cultures are kept in a cool room by a north window.

The cells are grown in culture vessels made by sealing into the closed-off top of a 300 cc. Erlenmyer flask an outlet tube and inlet tube reaching nearly to the bottom to permit the passage of a stream of gas. The flasks are filled with 225 cc. of medium, the inlet and outlet tubes plugged with cotton, and the whole sterilized. Each flask is inoculated under sterile conditions with 5 cc. of dense suspension from an old culture. The flasks stand in a glass-bottomed water-bath kept at an approximately uniform temperature by regulating the rate of flow of water through it. Below the bath is the source of light which may be one or more incandescent bulbs or a neon or mercury glow-tube.

In the tap-water medium specified above, Gloeocapsa

may increase fifteen-fold in three weeks. This does not approach the rate of reproduction of blue-green algae at a time of waterbloom, when the upper layers of a pond will be filled with a dense suspension of algae in a few days. From my experiments it appears that the conditions for good growth are different for different blue-green algae, and that the differences can not be referred to variations in a single condition (e.g. pH). The conditions for the extremely rapid growth of water-bloom will probably be worked out some day. Meanwhile, the growth obtained with Gloeocapsa is very satisfactory for experimental purposes. Moreover, it is much faster than any reported for other blue-green algae in pure culture. As growth occurs at a fairly rapid rate in a medium of strictly known composition (p. 12) it can be said that the conditions for growth of Gloeocapsa are far better worked out than they have been at any time in the past for any blue-green alga.

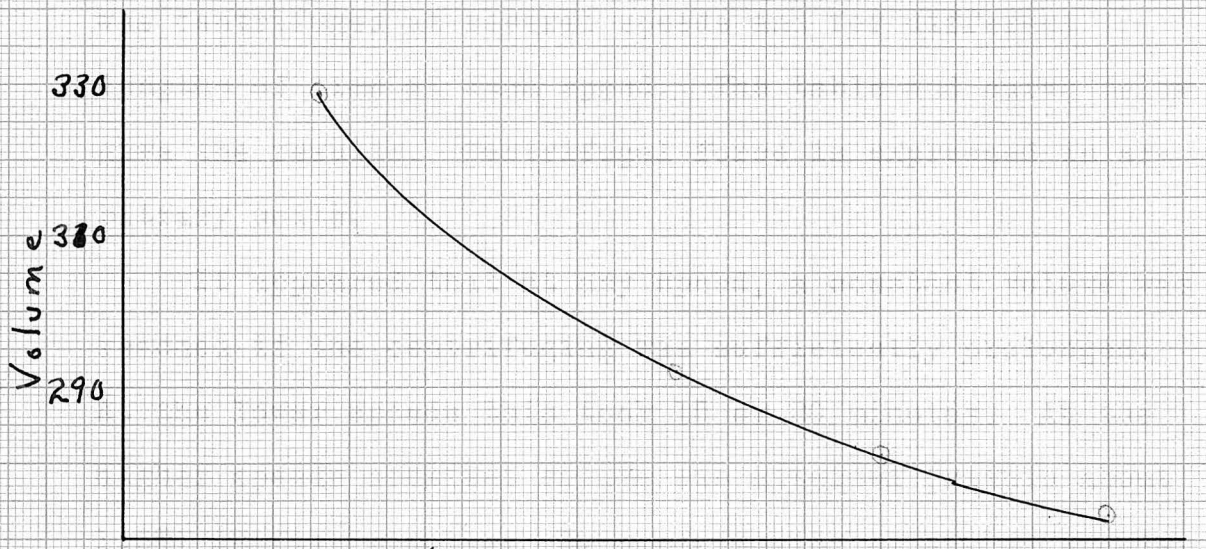
IV.

An experiment was performed to determine the accuracy with which cell-samples could be transferred by pipetting measured samples of suspension. Six 5 cc. samples of a suspension of Gloeocapsa were centrifuged in a haematocrit. The haematocrit could be read to within 2 $mm.^3$ and within these limits, the six samples had exactly the same volume.

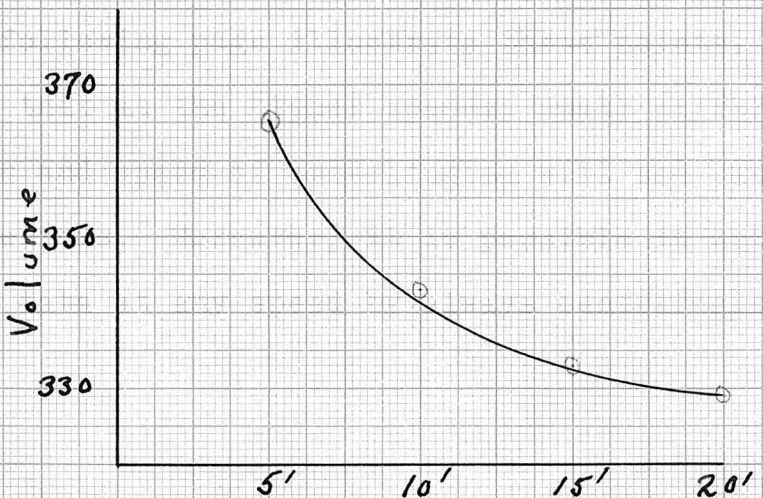
In view of the possibility of squeezing water out of

Follows page 14

Volume determinations on centrifuge



Centrifugal force in arbitrary units
Equilibrium at different velocities



Duration of centrifuging
Equilibrium at lowest speed

the gelatinous sheaths by pressure (Gerard and Rubinstein 1934), an experiment was performed to see whether the cells could be centrifuged to constant volume. A sample was centrifuged at several speeds and for different lengths of time at each speed. The table and curve below give the results. Centrifugal force is expressed in arbitrary units.

It appeared that at any speed, a constant volume was reached if the centrifuging was sufficiently prolonged. At the lower speeds this took 15 or 20 minutes. At the higher speeds constant volume was attained (within about 1.5%) in five minutes. By centrifuging at higher speeds the volume could be made smaller, but at the highest speeds of the centrifuge, a difference in centrifugal force of 20% decreased the volume less than 3%. Curves on the following page illustrate these points. It is evident that at the higher speeds, small differences in duration and speed of centrifuging will not cause appreciable errors. Moreover, it is possible to obtain comparable data at any speed by always centrifuging long enough.

V

A word of description of the organisms used in these experiments is in place. As two distinguished taxonomists could not agree even on the genus to which one of the organisms belonged, I shall not labor to classify them.

The organism referred to as Gloeocapsa is an ellipsoid with dimensions of about 8.5μ by 5.0 μ. Under the microscope it appears granular but no structure can be discerned. The cells hardly ever occur singly. Colonies from a vigorously shaken suspension contain from two to at least sixty cells. The cells are separated by the laminated sheaths which surround each group containing 2^n cells. The colony, including the sheath has a volume of from twice to twenty-five times the total volume of cells. There is no relation between the ratio $\frac{\text{volume of colony}}{\text{volume of cells}}$ and the number of cells in a colony. The cells may be blue-green, olive-green, gray-green, yellow-green, lemon-yellow or rust-brown.

The organism referred to as Chroococcus is a sphere with a diameter of about 12.5 μ. It does not form colonies. I have been unable to observe a sheath, but as the cells stick to each other and to the walls of a flask I suspect its presence. The cells are invariably of a light blue-green color when healthy.

Experiments on Assimilation

I.

Methods

The metabolism of Gloeocapsa and Chroococcus was studied by the technique developed by Warburg (1924). The pressure change caused by the evolution and consumption of carbon dioxide and oxygen by a suspension of cells in a small vessel was measured manometrically under the assumption that only oxygen and carbon dioxide were exchanged. The results obtained by this method can not in a rigorous sense be accepted unless the assumption is tested by gas analysis experiments. However, as the results obtained with blue-green algae do not differ essentially from those with green algae (e.g. the assimilatory quotient, $\frac{\Delta CO_2}{\Delta O_2}$, is equal to -1.00 in both forms) which have been tested by gas analysis, it is unlikely that in the kind of experiment reported here, an error has been introduced in the assumption. From the pressure change in millimeters of manometer fluid could be calculated the actual gas-exchange in cubic millimeters according to the following method. A full discussion can be found in Warburg (1924) or Gaffron (1929).

If in a Haldane-Barcroft vessel, containing V_f mm.^{3} of liquid and V_g mm.^{3} of air-space, an evolution or consumption of a single gas, x mm.^{3}, occurs without change of volume (V_g) then the level of fluid in a connected mano-

meter will change h mm. The quantities x and h are related by the equation

$$x = h \frac{V_g \frac{273}{T} - V_f \alpha}{P}$$

when T is the absolute temperature, α is the Bunsen absorption coefficient for the gas in question, and P is normal pressure expressed in millimeters of the manometer fluid (P = 10,000 for Brodies s solution, used in these experiments). The expression in parentheses is characteristic of (1.) the temperature and (2.) the gas in question. For any one gas at one temperature it can be calculated with the help of a set of tables. It is called the vessel constant and is represented by k_{o_2}, k_{co_2}, etc.

In experiments on photosynthesis, pressure change of two gases occurs simultaneously. Then

$$h = h_{o_2} + h_{co_2}$$

$$x_{o_2} = h_{o_2} k_{o_2}$$

and $$x_{co_2} = h_{co_2} k_{co_2}$$

In order to determine x_{o_2} and x_{co_2}, two vessels of different volumes are used. In each vessel is put the same number of cells, but in the small vessel is put a large volume of fluid and into the large vessel a small one. If the quantities relating to the former vessel are written majuscule and the quantities relating to the latter are

written minuscule then the following equations hold:

$$x_{o_2} = h_{o_2}\, k_{o_2} \qquad X_{o_2} = H_{o_2}\, K_{o_2}$$

$$x_{co_2} = h_{co_2}\, k_{co_2} \qquad X_{co_2} = H_{co_2} K_{co_2}$$

$$h = h_{o_2} + h_{co_2} \qquad H = H_{o_2} + H_{co_2}$$

$$x_{o_2} = X_{o_2}$$

$$x_{co_2} = X_{co_2}$$

From these, the following equations can be derived algebraically:

$$x_{o_2} = \frac{H\, K_{co_2} - h\, k_{co_2}}{\frac{K_{co_2}}{K_{o_2}} - \frac{k_{co_2}}{k_{o_2}}}$$

$$x_{co_2} = \frac{H\, K_{o_2} - h\, k_{o_2}}{\frac{K_{o_2}}{K_{co_2}} - \frac{k_{o_2}}{k_{co_2}}}$$

The quantities h and H can be observed, the vessel constants can be calculated, and the volumes of CO_2 and O_2 evolved or consumed are determined.

These equations can be used only for solutions not buffered for carbon dioxide. In solutions buffered for carbon dioxide such as Warburg's carbonate-bicarbonate mixtures (see below) no changes in pressure result from changes in the concentration of carbon dioxide, within cer-

tain limits. Then

$$x_{o_2} = k_{o_2} h$$

As respiration is assumed to go on in the light at approximately the same rate as in the dark, removing oxygen and evolving carbon dioxide, it is necessary to correct readings obtained in the light by adding (with changed sign) the readings for equal periods in the dark. That respiration is greater after a period of illumination than after a long period in the dark has been demonstrated by a number of writers (Warburg and Negelein 1922). However, although this increase may be large relative to the rate of respiration (perhaps as much as 100%, van der Paauw 1932), as the rate of respiration is only about 3% of the rate of photosynthesis, the error introduced is small in experiments done at light and carbon dioxide saturation. Moreover, if the dark readings are always taken soon after the light readings, a more nearly correct value for the respiration is obtained. This was the procedure in all experiments reported here. In the absence of any method for measuring the two processes separately when they occur simultaneously, this is the most economical assumption.

Usually 8 cc. of cell suspension was used in a rectangular vessel with a capacity of about 13 cc. The cells were centrifuged out of the culture medium, washed in the medium in which they were to be studied and suspended in a fresh lot of the medium. A large number of media were

tried and most discarded. Media containing tap-water were found unsuitable because they were partially bufferred for carbon dioxide so that pressure changes were not related in a calculable manner with changes in the concentration of the gas. Media containing commercial distilled water were found to be very toxic, causing a rapid fall in photosynthesis. A medium containing KH_2PO_4, $MgSO_4$, and KNO_3 in the same amounts as the culture medium, made up in redistilled water and saturated with 5% CO_2 in air was very satisfactory from the point of view of the cells. However, such a medium is cumbersome compared with Warburg's carbonate-bicarbonate mixtures, especially because it prolongs greatly the necessary time of preparation for each experiment. Another objection to it will be pointed out later. Mixtures of $\frac{M}{10}$ carbonate and bicarbonate of potassium made up in redistilled water caused a slow fall in photosynthesis. However when $\frac{M}{20}$ instead of $\frac{M}{10}$ mixtures were used, and $MgSO_4$ and KNO_3 were added to the medium, photosynthesis usually remained constant, or increased a little for several hours. In the more basic mixtures, and occasionally in the others, a slow fall occurred after the first quarter hour. Evidently this medium may be considered as barely meeting the requirements of the cells, and very slight variations of an unknown nature are sufficient to cause damage. Curves illustrate the points above.

Nearly all of the work was done at 30° C in a thermostat which kept the temperature within 0.05°C. The ther-

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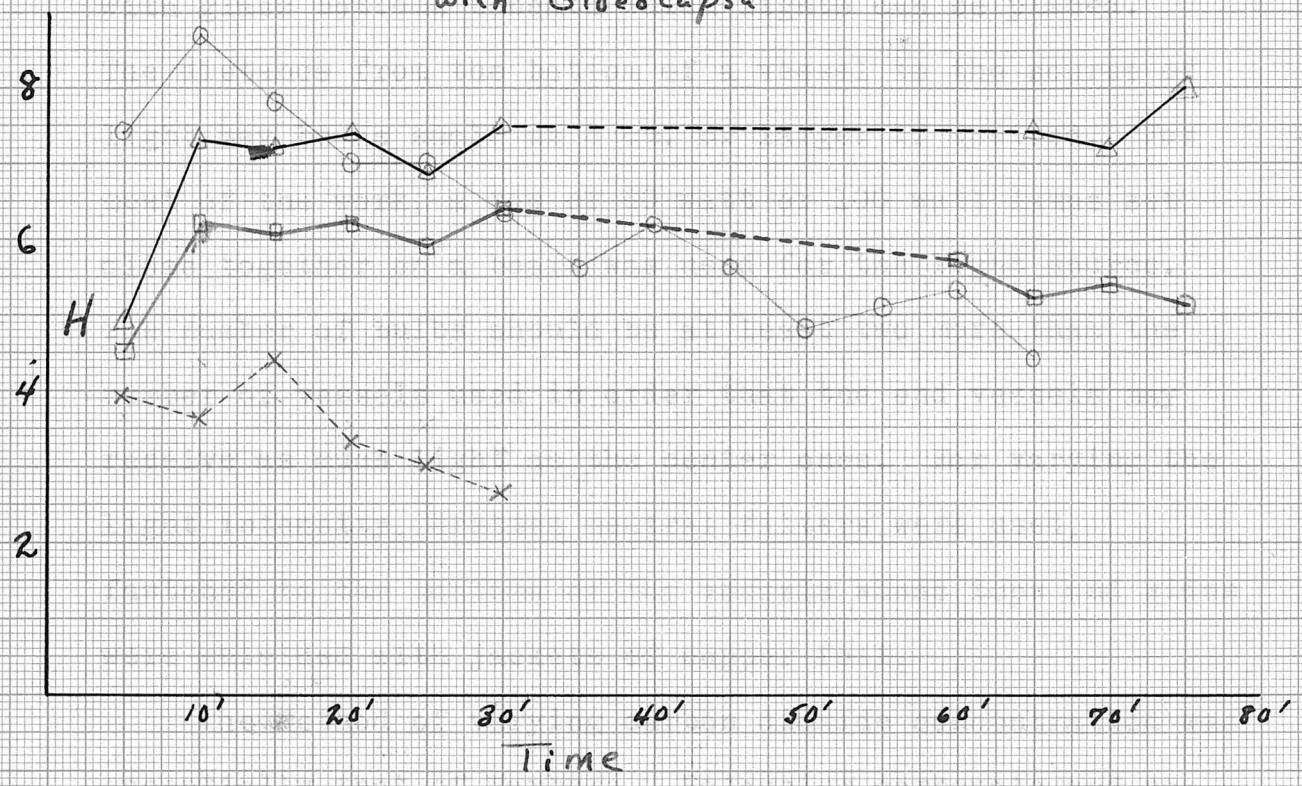
Suitability of media

for manometric experiments

with Gloeocapsa



——— 0.03M $KHCO_3$ 95%, K_2CO_3 5% + KNO_3+$MgSO_4$ in commercial distilled water
——— same in redistilled water
——— 0.1 M $KHCO_3$ + K_2CO_3 + KNO_3+$MgSO_4$ in redistilled water
- - - - 0.05 M $KHCO_3$ 25%, K_2CO_3 75% + KNO_3 + $MgSO_4$

Dotted ink lines indicate lights on but no readings taken

mostat is constructed with overhanging shoulders provided with glass bottoms beneath which lights can be placed. The distance from the bottom of a vessel to the source of light may be as small as 8 cm. or larger if desired. For most of the work, a row of 60 watt or 100 watt lamps set close together on a board was used for the light source. The number of bulbs should be at least two more than the number of vessels used in order that the end vessels may receive as much light as the center ones. For varying the light intensity, Wratten neutral filters were used, fastened to the bottoms of the vessels whose tops and sides were shielded with jackets of copper foil.

The method of varying carbon dioxide concentration was essentially that of Warburg. Potassium carbonate and potassium bicarbonate in $\frac{M}{20}$ solution were mixed in different proportions. The dissociation of these compounds furnishes dissolved carbon dioxide in different concentrations, depending on the proportion of carbonate and bicarbonate present. From the equations for the first and second dissociation constants of carbonic acid can be derived the equation:

$$[CO_2] = K \frac{\alpha^2}{\beta} \frac{[KHCO_3]}{[K_2CO_3]}^2$$ where

α is the degree of dissociation of the bicarbonate and β is the degree of dissociation of the carbonate, that is

$$\alpha = \frac{[HCO_3^-]}{[KHCO_3]} \quad \text{and} \quad \beta = \frac{[CO_3^=]}{[K_2CO_3]}$$

The ~~constant~~ quantity $K\frac{\alpha^2}{\beta}$ varies with the total concentration of potassium and the temperature. While Warburg (1919) gives values for the quantity $K\frac{\alpha^2}{\beta}$ for different mixtures (of sodium carbonate and bicarbonate) and different temperatures, the values of this quantity are in question. In recent years other methods of calculating the concentration of carbon dioxide in such mixtures have been developed which depend on more accurately known constants. The values for carbon dioxide concentration differ from Warburg's. Since these differences are due, in effect, to differences in the value of $K\frac{\alpha^2}{\beta}$, the relative concentrations as given by Warburg are likely to be correct.

Besides the advantage of easy manipulation, the carbonate mixtures possess the characteristic of being buffered for carbon dioxide. When one molecule of carbon dioxide is used up two molecules of bicarbonate decompose to form carbonate and carbonic acid.

$$2KHCO_3 \rightleftharpoons K_2CO_3 + H_2O + CO_2$$

Within limits which can be calculated (Warburg 1919), the only changes in pressure which occur are due to consumption and evolution of oxygen. This approximately doubles the size of the manometer readings and hence doubles the accuracy of the experiment. Moreover, it makes it possible to work at low carbon dioxide concentrations with the assur-

ance that the concentration will remain approximately constant for a period long enough to permit a series of readings. A small sample of a neutral solution saturated with carbon dioxide at a low partial pressure will lose all of the gas in a few minutes.

Objection is made to the use of these mixtures (van den Honert 1930) on the ground that in the different mixtures the cells are exposed to high and varying alkalinities. Experiments done in this laboratory have shown that this is a minor effect in all the organisms tested and can be neglected, at least in the range of carbon dioxide saturation.

II.

Experiments

In the study of photosynthesis, as of many other vital processes, the most useful tool is a determination of the variations in rate with changes in each of a number of conditions. The principal external factors controlling the rate of assimilation are (1.) light intensity, (2.) temperature and (3.) partial pressure (or concentration) of carbon dioxide. The only internal factor on which we can lay our hands at present is chlorophyll concentration.

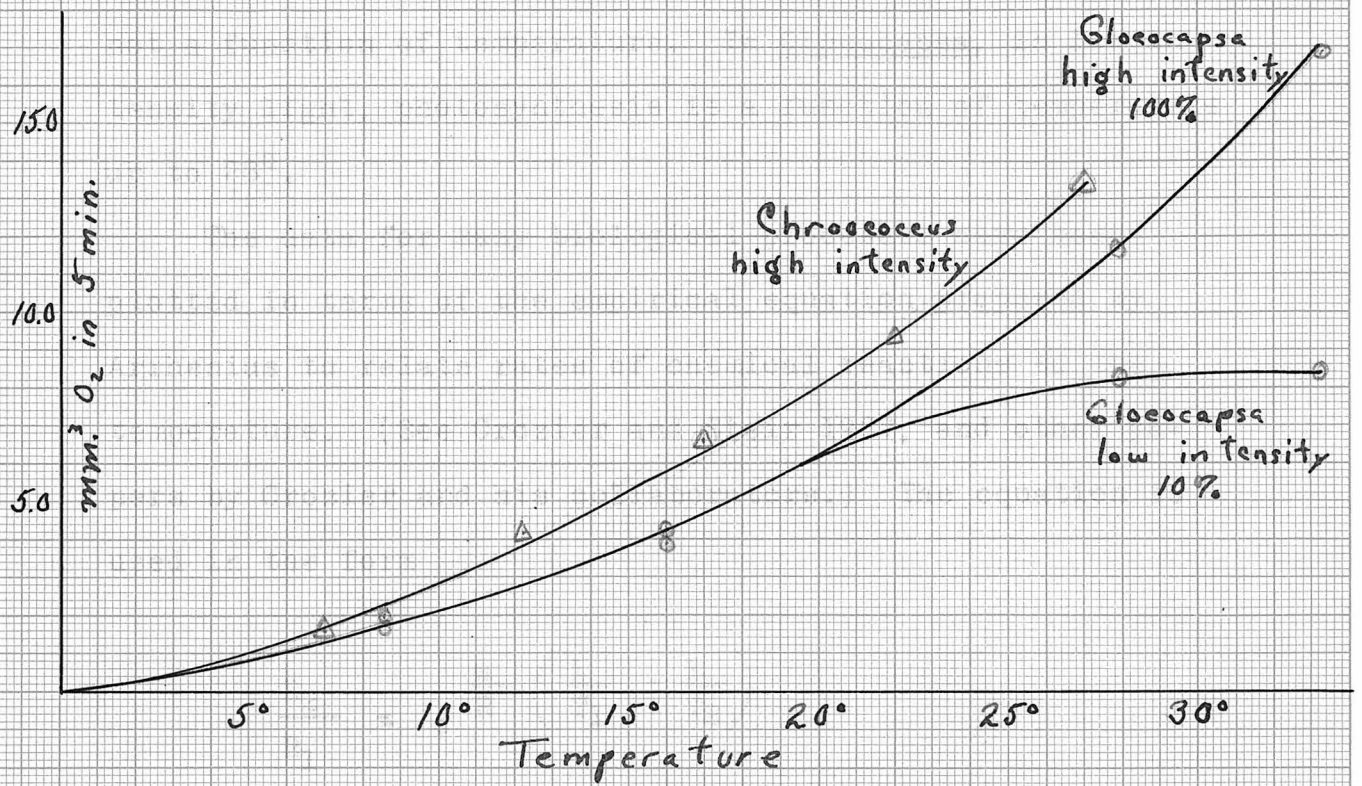
When a leaf or a suspension of algae is illuminated, at a reasonably high temperature and in the presence of plenty of carbon dioxide, with different light intensities, it is found that the rate of assimilation is proportional to the intensity at low intensities, but becomes constant at high intensities. When, now, the temperature is varied as well, it appears that photosynthesis is a function of temperature at high light intensities but at low light intensities is independent of temperature. The meaning of this set of facts was recognized by Blackman but most clearly stated by Warburg (1925). Namely, in the reduction of carbon dioxide by a green plant, at least two processes occur, one following the other. One is a photochemical process, dependent on the light intensity but having, as most such processes have, a low temperature coefficient. The other is a chemical process, sensitive ~~to~~

to temperature but independent of light. At the present time a wealth of evidence of varied sorts has accumulated to support this interpretation. This is the touchstone by which we will first test the nature of the assimilatory process in blue-green algae.

On the ~~opposite~~ following page are the curves relating photosynthesis in Gloeocapsa to temperature at two light intensities. It differs in some respects from the typical curve for leaves of cherry laurel as found by Blackman and Matthaei (1905, cited ~~in~~ by Stiles) and for Chlorella as found by Emerson (1929). These authors found an S-shaped curve at high light intensities, while the curve for Gloeocapsa shows no sign of becoming flat at the top. However, as it has been found lately that Gloeocapsa will grow well at 40°, it is possible that if the curve had been determined above 33°, it would have flattened out. The curve for Chroococcus plotted on the same page has the ~~typical S-shape~~ same shape. Chroococcus will not grow well at temperatures much above 25°. The most remarkable feature of the curves for Gloeocapsa is the small difference between them. The light intensity called 100% was found in another experiment to be barely saturating and the ratio between photosynthesis in 100% intensity and in 10% intensity at 30° was about the same as in this experiment, namely $\frac{2}{1}$. An experiment with Chlorella (Emerson, unpublished work) in which these quantities had about the same values showed

Follows page 26

Rate of assimilation at different temperatures and different light intensities



Gloeocapsa

Illumination: 4 100-watt bulbs
low intensity = 10% of this

Medium: tapwater
360 mm.³ cells in each vessel

$$M = \frac{K_{CO_2} \cdot K_{O_2}}{K_{CO_2} - K_{O_2}}$$

Chroococcus

Illumination 5 60-watt bulbs

Medium: $\frac{M}{10} KHCO_3$ 85%
$\frac{M}{10} K_2CO_3$ 15%
2.5 mm³ cells

Temperature	100% I M	100% I H	100% I X_{O_2}	10% I M	10% I H	10% I X_{O_2}
8.6°	1.07	1.9	2.0	1.09	1.6	1.7
16.0	1.15	3.4	3.9	1.17	3.7	4.3
27.9	1.27	9.2	11.7	1.30	6.3	8.2
33.3	1.32	12.8	16.9	1.35	6.2	8.4

Temp.	K_{O_2}	H	X_{O_2}
7.0°	0.856	1.9	1.6
12.3	0.891	4.7	4.2
17.0	0.915	7.2	6.6
22.0	0.961	9.9	9.5
27.0	1.040	12.9	13.4

that above 12°, photosynthesis in low intensity light was not a function of temperature. In Gloeocapsa, in low intensity light, photosynthesis is a function of temperature up to 28°.

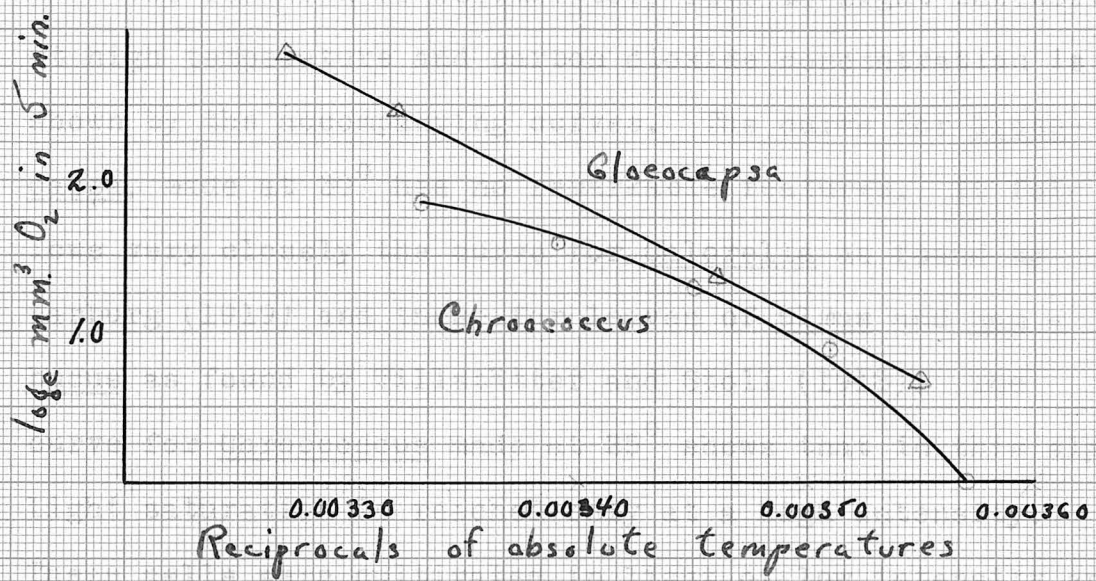
The data for many biological processes have been plotted in terms of the empirical equation proposed by Arrhenius to relate rates of chemical reactions to the temperature. (See Crozier and Steir 1927, and other papers by Crozier and his collaborators.) The equation is used in the form

$$\frac{R_2}{R_1} = e^{\frac{\mu}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where R_1 and R_2 are the rates at the absolute temperatures T_1 and T_2, R is the gas constant in calories per degree, and μ is, for chemical reactions, the heat of activation. The data for Chroococcus and for the curve at high light intensity for Gloeocapsa have been plotted in these terms. The Chroococcus curve is similar to the curves found for Chlorella (Emerson 1929). The value of μ drops from 29,000 at the higher temperatures to 8,300 at the lower temperatures. The curve for Gloeocapsa, however, although determined over a greater range, is approximately a straight line with a slope of about 14,700. It should be noted again, that if the curve had been extended above 33°, a sharper curvature might be expected at the upper end.

Follows p. 27

Temperature characteristic of photosynthesis



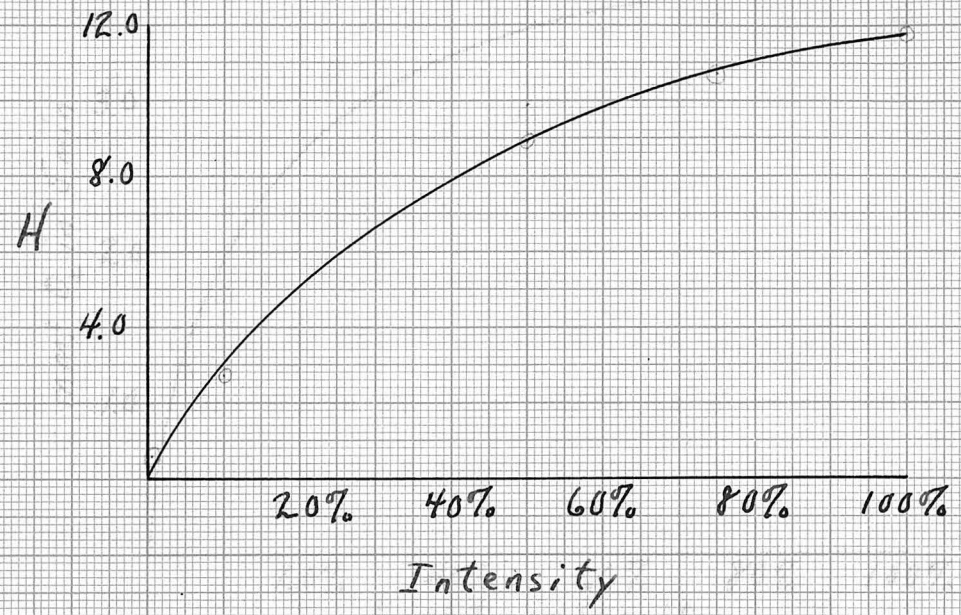
Data on previous sheet

The relation of assimilatory rate to light intensity at high temperature and carbon dioxide concentration is shown by the accompanying curves. The curve for Gloeocapsa, made at 30° has no remarkable features. It follows very closely the curve for Chlorella as found by Warburg (1919) and those for leaves of Ulmus and Sambucus as found by Willstätter and Stoll (1918). The curve for Chroococcus made at 25° shows that in this alga light saturation is reached only at a high intensity. These cells were cultivated in light of low intensity and contained a great deal of pigment. Judging by experiments on Gloeocapsa (described below), increases in chlorophyll are paralleled by increases in phycocyanin. If phycocyanin takes no part in the process of assimilation and is present in large quantities in the cell, this is the shape of curve that would result. Some curves made for Gloeocapsa have suggested the same effect, but as there are reasons for doubting their accuracy, I do not include them. However, this is a point which will bear further investigation. If time had permitted, I should have investigated the curve for assimilation in different intensities of white and of blue light, for blue and yellow cells of Gloeocapsa (concerning these cells, more will be said in the next chapter.). If the blue pigment takes no part in photosynthesis we should expect that light saturation would be reached only at a high value in white light, but at a lower value in blue light which is little

Follows page 28

Rate of assimilation at different light intensities

Chroococcus



$5.4\ mm^3$ cells Temperature: 25.3°

Illumination: 3 100-watt bulbs. Variations effected by use of Wratten neutral filters.

Medium: KNO_3, KH_2PO_4, each 250 mg. / liter

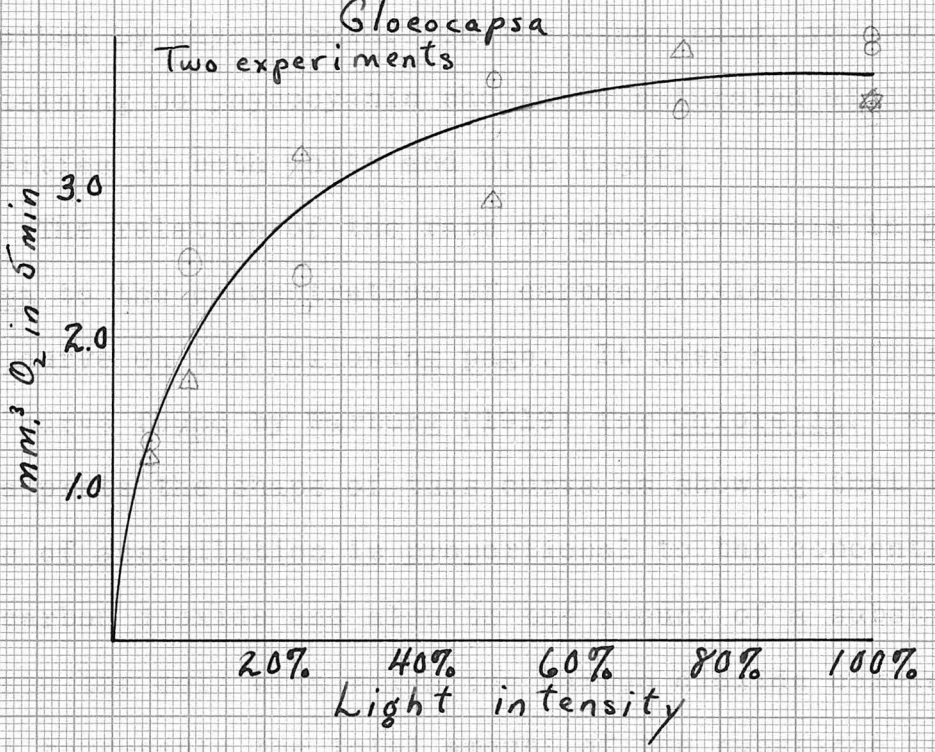
$MgSO_4 \cdot 7H_2O$ 500 mg. / liter

Saturated with 5% CO_2 in air

Illumination	H
0.5%	11.8
10%	10.7
50%	8.9
75%	2.7
100%	0.6

Follows page 28

Rate of assimilation at different light intensities



△ 26 April ○ 18 January

Temperature: 30°C

Illumination: 4. 100-watt bulbs. Variations effected by use of Wratten neutral filters

Media: 18 January KNO_3, KH_2PO_4 each 250 mg. / liter
$MgSO_4 \cdot 7H_2O$ 500 mg. / liter
saturated with 5% CO_2 in air

26 April $\frac{M}{20}$ $KHCO_3$ 80%, $\frac{M}{20}$ K_2CO_3 20%
KNO_3, $MgSO_4$ in same amounts

Intensity	26 April H	26 April K_{O_2}	26 April X_{O_2}	18 January H	18 January X_{O_2}
5%	2.4	0.405	0.9	1.5	1.3
10	2.5	0.405	1.3	2.9	2.5
25	4.7	0.515	2.4	2.5	2.4
50	5.6	0.515	2.3	3.6	3.7
75	5.7	0.515	2.9	3.4	3.5
100	5.2	0.405	2.7	4.0	3.9

absorbed by phycocyanin. On the other hand, yellow cells containing no phycocyanin should be saturated at low intensities in both white and blue light.

The relation of the rate of photosynthesis in Gloeocapsa to the concentration of carbon dioxide is shown by the curve on the following page. It does not differ from the curve found by Warburg (1919) for Chlorella. Warburg interprets the shape of this curve as showing that the rate of assimilation is proportional to the concentration of carbon dioxide and also to the amount of a substance within the cell with which the carbon dioxide combines. If A represents the total amount of the substance in the cell, x represents the amount uncombined, and A-x represents the amount combined with carbon dioxide, then by the mass action law,

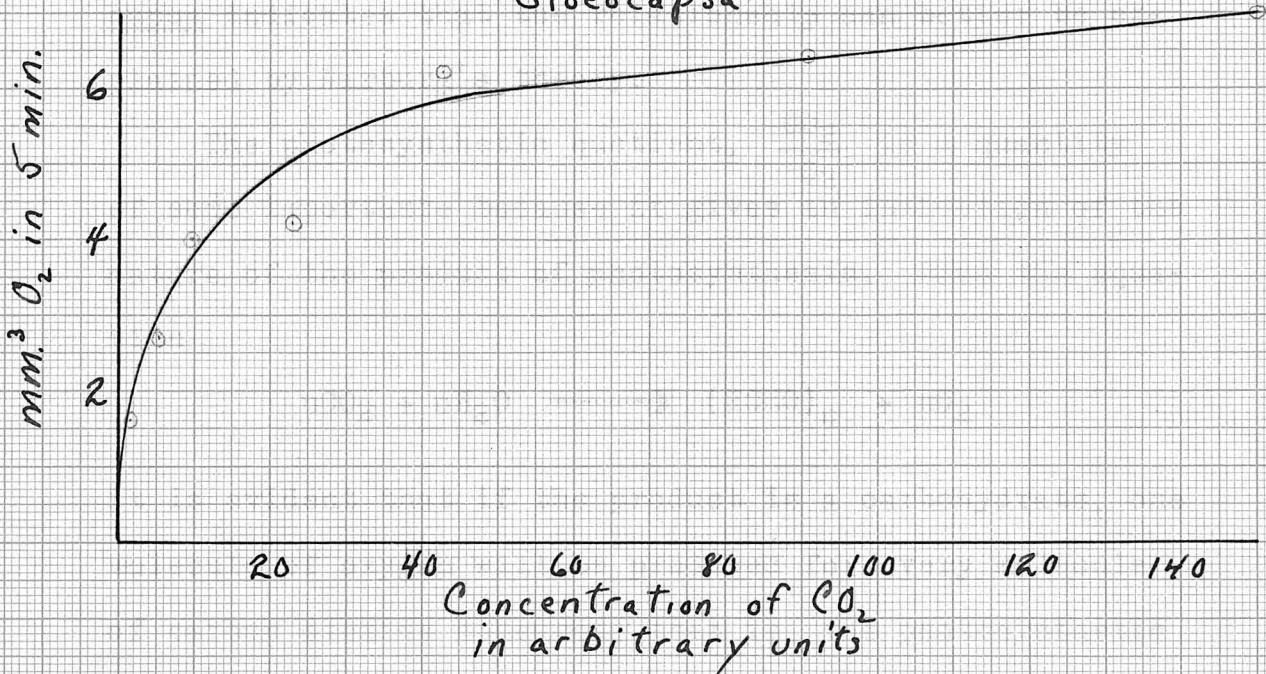
$$\frac{[CO_2] \cdot x}{A - x} = K$$

The curves found by van den Honert and van der Paauw differ from this. They show photosynthesis as a linear function of carbon dioxide concentration until saturation is almost reached. From this the authors argue that the process by participation in which, carbon dioxide becomes a limiting factor, is diffusion. This phenomenon may be an artifact depending on their technique which did not include the precaution of saturating the medium with gas at the beginning of the experiment. The curve for Gloeo-

Follows page 29

Rate of assimilation at different CO_2 concentrations

Gloeocapsa



Temperature: 30°C Illumination: 4 100-watt bulbs

68 mm.³ cells per vessel

Medium: $\frac{M}{20}$ $KHCO_3$ plus $\frac{M}{20}$ K_2CO_3 in proportions as described by Warburg (1919)

KNO_3 250 mg./liter $MgSO_4 \cdot 7H_2O$ 500 mg./liter

Mixture	K_{O_2}	H	X_{O_2}
#3	0.405	4.0	1.6
5	0.405	6.7	2.7
6	0.493	8.0	4.0
7	0.515	8.1	4.2
8	0.493	12.5	6.2
9	0.515	12.4	6.4
10	0.512	13.7	7.0

capsa indicates that the relationship can better be explained by Warburg's theory.

The photosynthetic quotient, $\frac{CO_2}{O_2}$ is a quantity of some importance because it gives an indication of the nature of the product of photosynthesis. From the equation

$$nCO_2 + nH_2O \longrightarrow (HCHO)_n + nO_2$$

it is evident that if the product is a carbohydrate, one molecule of oxygen will be produced for every molecule of carbon dioxide consumed; that is, the photosynthetic quotient will be -1. Likewise, if a fat is the product, the quotient will ~~be greater than one~~ lie between 0 and -1. The work of Bonnier and Mangin (1892, cited ~~in~~ by Spoehr) and of Willstätter and Stoll (1918) has shown that for a large number of plants the quotient is -1. For Gloeocapsa, at different times the following values have been obtained:

-0.95 , -1.06 , -1.00

For Chroococcus, all the determinations gave larger values:

-1.09 , -1.12, -1.16 , -1.17

As the errors of the determination by the Warburg method are large, variations are not surprising, but variations all in one direction demand inspection. In the case of Chroococcus, at least, the values should be checked by gas analysis. It has been found by microchemical tests

(Gardner 1906) that the cells of Cyanophyceae contain glycogen instead of starch. From this we might expect the photosynthetic quotient to be -1. No information is available on the presence of sugar in these organisms.

Another quantity of interest, is the rate of photosynthesis by a unit volume of cells in unit time. This can be defined as the number of cubic millimeters of oxygen evolved by ten cubic millimeters of cells in one hour. The photosynthetic activity varies greatly from culture to culture depending on the amount of chlorophyll in the cells. The difficulty of obtaining cultures of Gloeocapsa with the same amount of chlorophyll per cell will be explained in the next chapter. For dark cells, containing a large amount of chlorophyll, values of 16.5 and 19.6 have been obtained. These values are about one-tenth and one-sixth as large as values given by Emerson (1929) for Chlorella containing large amounts of chlorophyll. However, as noted above the total volume of cells is only a small fraction of the total volume of a colony, when its sheath is included. The latter is probably the volume measured in centrifuging. Hence the actual volume of living material is much smaller than the experimental value indicates, and the photosynthetic activity per unit volume of living material is correspondingly larger.

III.

From these experiments it appears that the photosynthetic process in Gloeocapsa and Chroococcus is fundamentally like that in other green plants. The existence of a photochemical reaction and a Blackman reaction is demonstrated. From the temperature curves one can predict that, relatively to the photochemical reaction, the Blackman reaction is slower in Gloeocapsa than in, for example, Chlorella. Or to put it the other way, relatively to the Blackman reaction, the photochemical reaction is faster in Gloeocapsa than in Chlorella. This may be a result of the different arrangement of chlorophyll, but the relation is not made plain by a single experiment. The thick gelatinous sheaths, by increasing the length of the path along which carbon dioxide must diffuse, might raise this process to the status of a limiting factor, but the curve relating assimilation to carbon dioxide concentration indicates that this is not so. The intensity curve for Chroococcus and some unpublished curves for Gloeocapsa indicate that light saturation occurs only at high intensities. This would be the case if the light reaction were slow relatively to the Blackman reaction. ~~Which~~ This, apparently, is not true. I offer as explanation that the blue pigment, when present in large amounts, can accomplish this. If it does, it can not be said that is has a function in photosynthesis.

The Colors of Blue-green Algae

I.

An ancient question which perhaps has an answer is, "Why is grass green?" It seems likely that some progress may be made toward answering it by finding out why some plants are not green. The different classes of algae have characteristic colors and in two of the classes, the Rhodophyceae and the Cyanophyceae, the colors of members of the same species (or even individuals) vary considerably under different environmental conditions. Three principal theories have been proposed to account for the colors and color changes of algae.

Engelmann (1883) observed the rate of assimilation of several kinds of algae in light from the different parts of the spectrum. He concluded that they assimilated fastest in light of a color complementary to their own and suggested that this effect controlled their distribution in nature. Gaidukov (1902) cultivated Cyanophyceae in light from different parts of the spectrum and found that they assumed a color approximately complementary to that of the light in which they were grown. This important amendment to the idea of Engelmann he called "complementary chromatic adaptation." According to Engelmann's theory, red algae grow better than other algae in deep water, because they assimilate faster in the blue light which alone penetrates the sea to a considerable depth.

According to Gaidukov's theory, a cyanophycean transplanted to deep water will turn red (or its offspring will be red). At the present day, the principal supporter of the theory is Harder (1922, 1923) who, for red and blue Cyanophyceae, confirms the findings of both Engelmann and Gaidukov.

Oltmanns (1893) grew marine algae in light of different colors and light of different intensities and concluded that the differences in the color of the algae depended principally on differences in the intensity of the light. Nadson (whose papers are, unfortunately, unavailable; cited in Harder 1923, Schindler 1913, and elsewhere) observed Cyanophyceae growing under natural conditions and came to the same conclusion. The assimilation of red and green algae in lights of different colors and intensities led v. Richter (1912) to the conclusion that the pigments other than chlorophyll took no part in photosynthesis and hence, the theory of Engelmann was false. At the present time, the theory of Oltmanns has no distinguished supporters. It is either omitted or mentioned slightingly in all of the standard textbooks.

A third proposal was made by Schindler (1913) on the basis of experiments on the cultivation of Cyanophyceae. He found that in light of any color, old cultures began to fade and that renewal of the medium, or mere addition of a

nitrate restored the original color. He pointed out that the light intensity influenced the rate of growth of the organism and by thus changing the rate of depletion of the medium, hastened the initiation of fading. He found that light color was without influence. Boresch (1921) found that the concentration of both nitrate and iron in the medium influenced the color of Cyanophyceae. This theory which first appeared in a paper by Magnus and Schindler (1912) is generally accorded credence, but the denial of an influence of color is usually discredited.

The methods of these experimenters will bear inspection. In the first place, the culture experiments of Gaidukov, Harder (1922), and Schindler lasted for months. During such periods, Schindler alone obtained considerable growth. Gaidukov performed his experiments on microscope slides where one would expect little growth. The majority of Harder's colonies died during the experiments. Boresch completed his experiments in periods ranging from three days to three weeks. During this time he obtained considerable growth, except when he started with chlorotic colonies. It is immediately apparent that those authors who ascribe the important role in color changes to nutrient factors are the ones who have mastered the technique of cultivating the organisms.

The light sources used for culturing, with or without color filters, were full sunlight, a Nernst lamp, and

diffuse daylight. Some of Harder's experiments were conducted in black boxes open at one end. This end was covered with a color filter and directed toward a north window. In such exceedingly weak light an intensity gradient is probably not important. Schindler, Oltmanns, and Nadson obtained an intensity gradient by using full sunlight and diffuse light. Gaidukov took no account of the different intensity of light emitted at different wavelengths by all ordinary light sources.

The experiments on the assimilation of colored algae in colored lights are at present outside my province.

II.

While cultivating Gloeocapsa in the fashion described earlier in this paper, I observed that the cultures varied considerably in color. When grown in bright light, they were light green and when grown in dim light they were dark green or blue-green. In addition, old cultures often became yellow-green or yellow. In an attempt to hasten the growth of a culture, I put it in a bath about 10 cm. from a 100-watt incandescent bulb. In ten days it had turned yellow. Replaced in the usual culture bath at a distance of 25 cm. from a 40-watt bulb it became blue-green in 48 hours. The process was repeated several times with the same and other cultures. Cultures have since been

grown at a distance of 8 cm. from a 200-watt bulb, in which case they became yellow-brown (or buff-colored) in three days.

As the spectral distribution of light differs somewhat in incandescent bulbs of different brightness, it is desirable to distinguish between the possible effects of color and intensity. For this purpose, several additional sources of light were used. A mercury glow-tube operated by a 15,000 volt, 30 milliampere neon sign transformer furnished low intensity blue light. A hot-cathode mercury glow-tube operated by a 600 volt 1 ampere transformer furnished high intensity blue light. A neon glow-tube operated by the 15,000 volt transformer ~~neon light~~ furnished high intensity red light and a similar tube screened by several layers of filter paper furnished low intensity red ~~blue~~ light. These are by no means sources of monochromatic light, but the neon tubes emit no light of wave-length less than 500 mμ while practically all of the energy of the mercury tubes is emitted at wave-lengths below 580 mμ. Neither is the total energy emitted equal in the different sources of bright light or in the different sources of dim light. It appeared that these differences were without significance for the chief results of the experiments.

In light of all colors, the results were similar; in bright light the cultures became yellow or yellow green and

in dim light they were dark blue-green. There was no indication of complementary chromatic adaptation. Centrifuged masses of cells always had the same color as the original suspensions, so no question arises of the relation of the color of a suspension to its density. In the absence of a satisfactory standard color scale, a verbal description of the colors must suffice.

As Boresch (1921) has given data on the amounts of blue, green, and yellow pigments in cells of Phormidium grown on different concentrations of iron, it would be desirable to give the same data for these cells. Unfortunately, no method described in the literature for extracting phycocyanin has been found to succeed with Gloeocapsa. A determination of the chlorophyll content was made at the end of each experiment. A measured volume of cells was washed with distilled water and extracted completely with methyl alcohol. The extract was made up to a standard volume and the extinction coefficient determined spectrophotometrically in light of wave-length 659 mμ from a neon tube. Work done in this laboratory (Emerson and Arnold 1932) makes it possible to calculate the concentration of chlorophyll in methyl alcoholic solution from this extinction coefficient. The table below gives the results expressed as moles of chlorophyll in 1 $mm.^3$ of cells:

Illumination		Moles chlorophyll in 1 $mm.^3$ cells
White	high	0.656×10^{-10}
	low	2.76×10^{-10}
Blue	high	1.14×10^{-10} 1.28×10^{-10}
	low	1.90×10^{-10} 2.06×10^{-10}
Red	high	0.676×10^{-10}
	low	1.73×10^{-10}

It is evident that in every case there is less chlorophyll in the cells grown in bright light. The order of magnitude of the experimental error is indicated by the figures for two separate experiments in blue light.

Extraordinary precautions were found necessary in making and handling methyl alcoholic extracts of Gloeocapsa. If a measurement was made immediately after extraction and another several hours later the extinction coefficient was found to have fallen. After twenty-four hours, it had fallen to one half its original value. If the extract was kept in the refrigerator, the extinction coefficient fell only a little in twenty-four hours. Boiling the extract, and keeping it in a full flask tightly stoppered to prevent the entrance of oxygen, did not prevent fading. For practical purposes, the problem was solved by three experiments. The extinction coefficient of a fresh extract was determined and the extract divided into three samples. One was left on the laboratory bench. One was put in a tightly

covered can in the laboratory. One was put in the cold room at 0.5°, close to a 25 watt lamp. After twenty-four hours, the samples exposed to light at room temperature and at 0.5° had faded considerably. The sample kept in the dark at room temperature had the same extinction coefficient as on the previous day. Photochemical destruction of chlorophyll in crude alcoholic extract is a well-known phenomenon (Willstätter and Stoll) but such a rapid destruction is not reported in the literature. In making extractions after this, all extracts and fractions were kept in the dark as much as possible.

When methyl alcohol was added to blue-green cells, they became greenish brown in a few seconds. This also occurred with ethyl alcohol and acetone. After the extraction was complete (as shown by the lack of color of the alcohol used for the final washing) the cells which had been blue-green were left purple while the cells which had been yellow were white. I consider this color to be an indication of the phycocyanin originally in the cells. I have not found in the literature any mention of a reaction between pure phycocyanin and alcohol.

To determine whether temperature had an effect, cultures were grown at 20° and 30° in low intensity blue light. No difference in color was apparent. Cultures have since been grown at 40° with no evident change. A slight difference in chlorophyll concentration was found,

probably lying outside the experimental error.

Temperature	Moles chlorophyll in 1 mm^3 cells
30°	2.49×10^{-10}
20°	2.06×10^{-10}

In view of the work of Schindler and Boresch, it was necessary to determine whether differences in the composition of the medium had an effect. Cultures were made up containing different amounts of iron. Low concentrations of iron prevented the formation of pigment, and made the cells lighter (yellower, or less blue) than cells in normal medium. The changes in chlorophyll concentration were marked.

Experimental Conditions		Moles Chlorophyll in $1mm^3$ cells
High Intensity White light	Fe 2.8 mg./l.	0.446×10^{-10}
	Fe trace	0.0744×10^{-10}
High Intensity Blue light	Fe 2.8 mg./l.	1.28×10^{-10}
	Fe trace	0.194×10^{-10}

However, it is not to be though that an impoverishment of the medium occurred in the other experiments. In bright light, the number of cells doubled in about three days. The experiments were usually concluded at this time. A three weeks culture in dim light contained fifteen times the number of cells inoculated into it, and was still dark blue-green. I have no figures on the con-

tents of older cultures, but similar cultures under the same conditions usually grow for at least five weeks before they begin to fade, or become less active.

From these experiments it seems clear that no such phenomenon as complementary chromatic adaptation occurs in Gloeocapsa. Although this alga probably does not contain phycoerythrin as do all the algae on which similar experiments have previously been done, it seems probable that the laws governing it, govern also the other algae. The conditions chiefly controlling the concentration of pigment in Gloeocapsa are the composition of the medium and the intensity of incident light. Temperature may have an effect and so may the color of the incident light, but these effects must be minor.

III.

The theory of Engelmann and Gaidukov is accepted as true by the majority of textbooks, and so I judge, it is commonly accepted by botanists. Now that the record of my experiments casts serious doubt on the theory, I wish to attack it on theoretical grounds. This attack is a simple and reasonable one but in the extensive literature on the relation of plant color to light, it has nowhere appeared.

If there is in a plant a photoactive pigment, or pigment-complex, which has a high absorption in, let us say, blue but not in yellow, then when this plant is exposed to blue light it will grow rapidly but when exposed to yellow light it will grow slowly. The production of the pigment or pigment-complex may itself be a photochemical process or a chemical process, but in either case it is evident that the faster the cell grows, the lower will be the concentration of the pigment or pigment-complex. Then when the plant is exposed to light rich in the rays which its pigment or pigment complex absorbs strongly, it will be poorly pigmented but when it is exposed to light of color not complementary to its own it will be richly pigmented. To this extent we can expect a specific effect of wave-length on plant-color, and it is in exactly the opposite direction from complementary chromatic adaptation. The process described is analogous to the processes occurring in sun and shade plants. Plants do not develop chlorophyll richly when given plenty of light, but when given little. As a corollary we may add that as the differences in the extinction coefficients of plant pigments are small over the whole range of visible light, compared with the differences in intensity under which a plant will grow, we should expect plant pigmentation to depend little on color and much on intensity.

The argument as I present it is perhaps too much simplified. The problem is complex and not all of its complexities are understood. Never the less, I believe that a kernel of truth is here.

Although the subject of plant colors does not attract much attention at present, it should be subjected to the same scrutiny which is applied to more fashionable subjects. The Engelmann-Gaidukov theory is a kind of magic and should be cast out of the body of science.

Summary

Two coccus-form species of blue-green algae have been isolated in species-pure culture and cultivated on a number of solid and liquid media. For one species, a medium of strictly-known composition has been developed.

The principal characteristics of the process of assimilation in these algae have been studied by the manometric method. The process is essentially like that in other green plants. The shape of the curve relating assimilation to light intensity gives an indication that the blue-pigment of the Cyanophyceae does not take part in photosynthesis.

A study of the relation of the color of a blue-green alga to environmental conditions shows that the color depends principally on the chemical constitution of the medium and the intensity of incident light. Temperature and light color may have minor effects.

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