STUDIES ON PHOTOSYNTHESIS

Some Effects of Light of High Intensity on Chlorella*

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INTRODUCTION

It is the purpose of this report to present quantitative data on some effects of high light intensities on *Chlorella*. No comparable data have as yet been published, although a number of workers, using various techniques, have studied the effects of intense light on the green plant. It has been generally demonstrated that after long exposure of a plant to strong light there may be a disappearance of the products of photosynthesis (solarization), a decrease in the apparent rate of photosynthesis, or microscopically observable injury to the tissue. In this paper the term solarization will be used to indicate a reduction in photosynthetic rate due to prolonged exposure to light.

The literature has been reviewed in the more recent work of Emerson (1935), Fockler (1938), Holman (1930), and Stålfelt (1939) and need not be considered in detail here. Most of the work has dealt qualitatively either with the ecological aspects of the problem or with the mechanism by which the observed effects might be brought about. The data to be presented in this paper describe the solarization effect as a function of light intensity and time.

EXPERIMENTAL

As experimental material there were used cultures of *Chlorella vulgaris*,[1] of *Protococcus sp.*[1, 2] and of *Chlorella pyrenoidosa*.[3] These have been grown in a ⅛ Detmer solution

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[1] Identified by Professor Felix Mainx of the German University at Prague. Obtained through the courtesy of Dr. C. E. Skinner by whom they had been isolated from soil.

[2] *Protococcus* was used only to support data obtained with *Chlorella*. A comparatively low rate of photosynthesis makes this organism more difficult to work with.

[3] Obtained through the courtesy of Dr. Robert Emerson.

as recommended by Miss Meier (1934), with and without the addition of glucose. Cultures were grown in 500 cc. Erlenmeyer flasks in darkness and in light with either air or 5 per cent CO_2 in air bubbled through. To obtain darkness flasks were wrapped in photographic light-proof paper and kept in covered iron pails. Cultures grown in light were placed uniformly around the 5.5 cm. water jacket surrounding a 300 watt bulb. The intensity at the illuminated side of the flasks was approximately 450 foot-candles.

Sterile precautions were observed only when glucose was added to the nutrient solution, although microscopic tests for contamination were made in all cases.

Measurements were made by the familiar Warburg technique, using one illuminated experimental vessel with flat bottom and a non-illuminated thermobarometric control. The volume of the experimental flask was 14.22 cc. to the level of Brodie fluid as calibrated with mercury. When used as described below 1 mm. increase in pressure corresponds to an oxygen evolution of 0.56 c.mm.

The constant temperature bath was held at 26°C. $\pm 0.01°$ as checked by a Beckmann thermometer. High light intensities were obtained by an optical system mounted horizontally beneath the bath.[4] The light of a projection bulb was condensed by two planoconvex lenses each of 6 inches diameter and 7 inches focal length. The horizontal beam was then reflected vertically upward through the glass bottom of the bath by a concave mirror. The area of the light beam in cross-section at the level of the experimental flask was large enough so that the flask was always illuminated during the 3 cm. amplitude of its shaking cycle. The light thus passed through 8 inches of water before reaching the flask and most of the infrared was removed. Various intensities were obtained by the use of three different projection bulbs and a series of screens. For higher intensities, a Westinghouse or General Electric 1000 watt projection bulb with a C13D filament was used. For lower intensities, projection bulbs of 250 or 500 watts were sufficient. Light intensities of over 30,000 foot-candles could be obtained with new 1000 watt projection bulbs. Unfortunately these bulbs have a rather rapid decay and a short average life.

Light intensities were measured by a Weston photronic cell, rigidly mounted immediately over the bath and in the light beam above the experimental flask. This was wired to a calibrated micro-ammeter. The light intensity falling on the bottom of the experimental flask was found to be 1.7 times as great as the intensity of the photronic cell in its fixed position at which all readings were taken. This factor was therefore used as a constant correction. Readings were taken at the beginning and end of each run, when the experimental flask was not in the light beam, and the average value considered representative. Changes in line voltage never caused more than 5 per cent variation in the two readings.

Most of the experiments to be considered were carried out in a potassium carbonate-bicarbonate buffer corresponding to the sodium carbonate-bicarbonate buffer No. 9 of Warburg (1919) (0.015 M K_2CO_3, 0.085 M $KHCO_3$). According to the calculations of Smith (1937) from the data of MacInnes and Belcher (1933) this gives a CO_2 concentration of 78.7×10^{-6} M. In one experiment buffer No. 11 was used (0.005 M K_2CO_3, 0.095 M $KHCO_3$ = 290×10^{-1} M CO_2). The buffer method was used because of its simplicity and higher degree of accuracy. Pressure changes are due entirely to oxygen

[4] Essentially similar to that described by Smith (1937).

uptake or evolution. No harmful effects of the buffer have ever been observed, even though suspensions have been kept in it in the icebox for as long as 24 hours.

In several check experiments the cells were suspended in ⅓ Detmer solution saturated with 5 per cent CO_2 and the gas space swept out with 5 per cent CO_2. The use of nutrient solution depends on the differences in solubilities of carbon dioxide and oxygen. Interpretation of such results requires a knowledge of the photosynthetic quotient, which would be difficult to obtain with the limited cell volumes used. Hence only a small number of these runs were made.

Immediately before an experiment the cells were centrifuged out of the nutrient solution, taken up in the buffer, and centrifuged out again in a graduated tube. To the packed cells fresh buffer was added to give a suspension in which each 1 cc. contained 0.01 cc. of cells. The suspension was then kept in the dark except when aliquot portions were withdrawn. When the cells were grown in darkness, all further operations except the reading of cell volume were performed in the dark. Aliquot portions were then withdrawn in the dark by an automatic pipette. When nutrient solution was used in place of the buffer, the stock suspension was made up in ⅓ Detmer solution.

1 cc. of the cell suspension (containing 0.01 of cells) was added to 7.2 cc. of buffer in the experimental flask. The flask and manometer were placed in position without illumination and about 5 minutes allowed for adjustment to equilibrium. The "zero" reading was then taken in the dark. The light was immediately turned on and succeeding readings taken every 5 minutes. A jump observed between the 0 and 5 minute readings (and in fact the pressure change accompanying any change in light intensity) is therefore largely an instrumental error due to readjustment of temperature equilibrium. The above procedure was adopted, in spite of the inherent error in the first two readings, because of its reproducibility.

The temperature increase within the flask caused by light absorption as referred to above is negligible in its effect on the physiological processes involved. The temperature increase produced by 28,000 f.-c. in a shaken flask containing eight times the usual number of algal cells was only about 0.4°C. as measured by a mercury thermometer. The Warburg instrument must always be considered as a very sensitive gas thermometer.

The authors consider of utmost importance the fact that only very dilute cell suspensions were used. This reduces shading of cells to a minimum not obtainable with any other combination of materials and methods, with the possible exception of that of van den Honert (1930) and van der Paauw (1932) who used a single layer of *Hormidium* cells. More dilute suspensions cannot be made without great reduction in the accuracy of measurements, which is already somewhat limited. On the other hand, tripling the thickness of the suspension reduces the solarization effect and prolongs the time of each observation.

RESULTS

The course of gas exchange in *Chlorella vulgaris* under very high light intensities is described by Fig. 1. (Unless otherwise indicated, all data apply to measurements made on *Chlorella vulgaris* in buffer No. 9.) Oxygen evolution or uptake, indicated as millimeters of pressure of manometric fluid, is plotted against time of illumination. The curves have been shifted vertically so that their 5 minute readings coincide. (The error

involved in the 5 minute, and at this very high intensity probably also in the 10 minute, readings has been already pointed out.) At these intensities the initial rate of oxygen evolution soon falls off. There follows an oxygen uptake which increases to, and for some time remains at, a fairly constant rate. A comparison shows that this constant rate of oxygen uptake is much greater than the rate of oxygen uptake indicated by the curve for dark respiration which has been included.

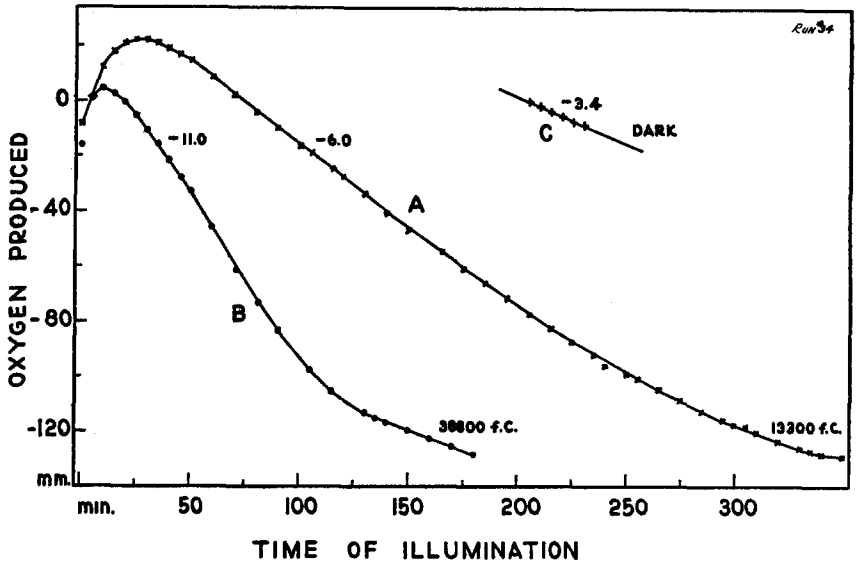

FIG. 1. Course of gas exchange in *Chlorella vulgaris* exposed to 13,300 f.-c. and 38,800 f.-c. of light. Oxygen produced or consumed is expressed in millimeters of pressure on the manometer. The rate above each curve is millimeters per 10 minutes.

After the consumption of a certain volume of oxygen (which occurs earlier at higher intensities) the slope (rate) begins to decrease. Runs carried out for even longer periods than those illustrated here have shown that the rate continues to decrease approaching zero. At about the time the die-away begins there is observable to the eye a distinct bleaching of the cells which continues until they are completely colorless, both microscopically and macroscopically.

It should be noted that the effects shown in Fig. 1 cannot be associated with a change in the CO_2 concentration of the buffer. A change of 100 mm. manometer pressure (56 c.mm. O_2) causes a change of only about 3.4 per cent in the CO_2 concentration provided by buffer No. 9. The highest rate ever noted in this buffer (35 mm./10 minutes, Fig. 10) was maintained for 70 minutes without any evidence of inhibition.

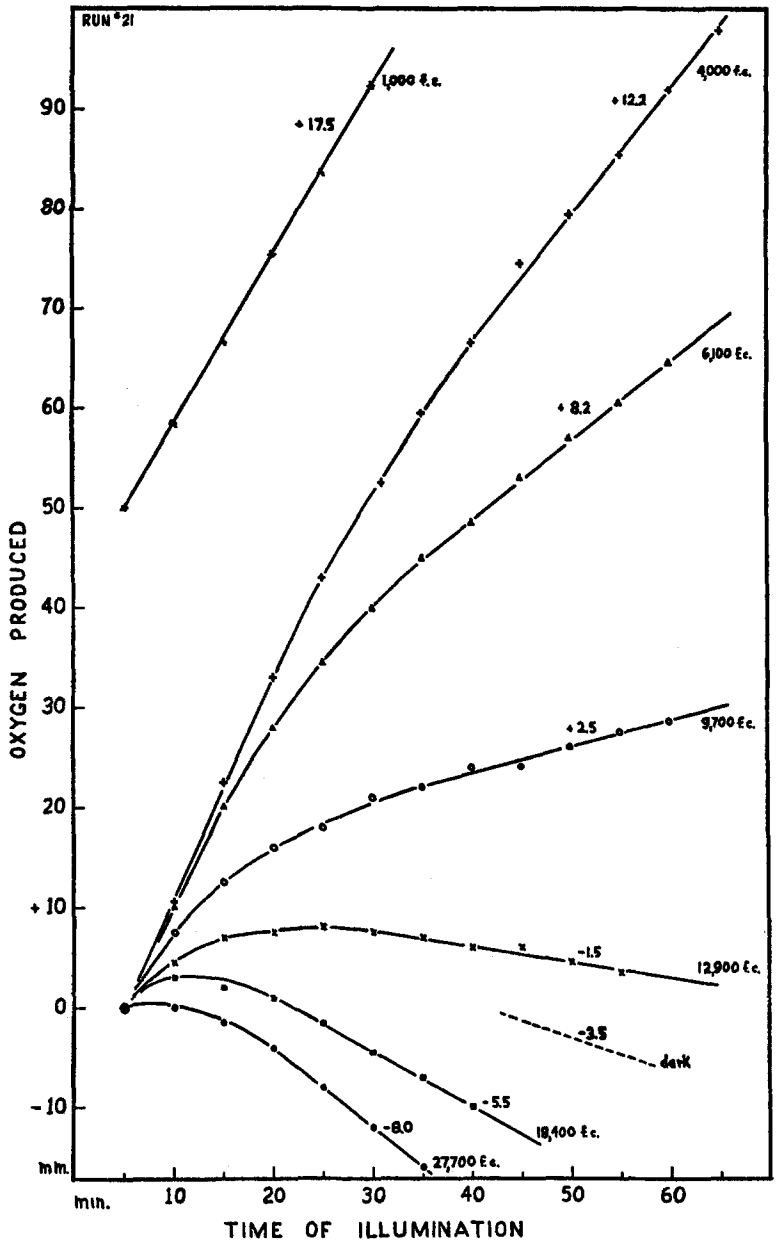

FIG. 2. Final steady rates of oxygen exchange depend on the incident light intensity. The rates are expressed as millimeters pressure change per 10 minutes and are shown by the numbers just above each curve.

In Fig. 2 is a family of curves for different light intensities, each obtained on a separate aliquot portion of a single batch of cells. At lower light intensities the final rate remains positive showing only partial inhibition of photosynthesis. When curves like those of Fig. 2 are run at still lower intensities a point is reached at which no decrease in rate can be observed. In fact at these lower intensities (in this case 1,000 f.-c. or less) there is often a slight increase in rate during a long run. It is of interest that the initial rates at 4,000 and 6,100 f.-c. are nearly identical and some-

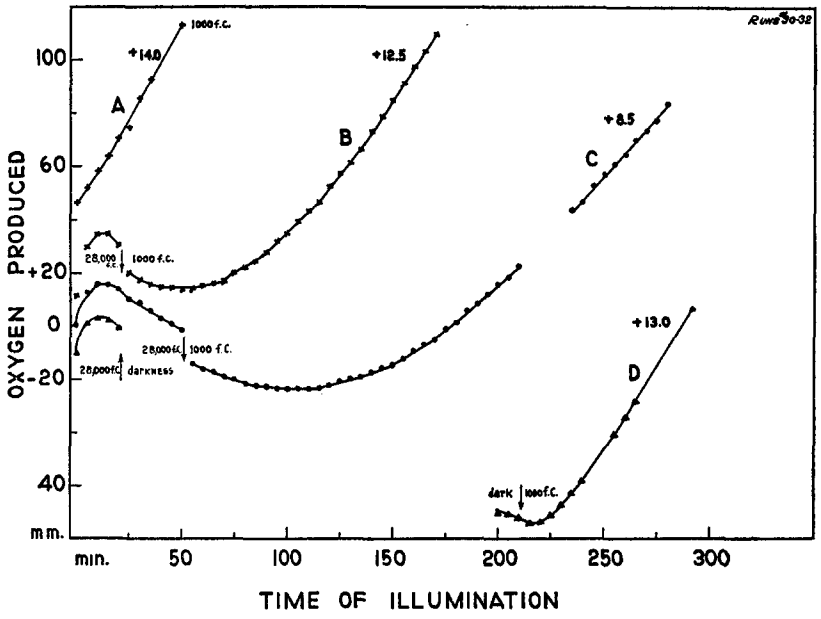

FIG. 3. Progressive injury of cells by 28,000 f.-c. of light and recovery in darkness (D) and in 1,000 f.-c. (B and C).

what higher than the rate at 1,000 f.-c. At some intensity between 1,000 and 4,000 f.-c. light saturation is reached and injury becomes apparent only after a considerable period of illumination.

Recovery experiments indicate that, at least at the higher intensities, there is an injury which becomes more severe with time. Several such experiments are illustrated in Fig. 3. Curve A shows photosynthesis in 1,000 f.-c., an intensity somewhat below the so called light saturation and at which no depression in rate has ever been observed for these cells. Curves B and C show the recovery of cells exposed to 28,000 f.-c. for 20 and 50 minutes respectively, and then changed to 1,000 f.-c. intensity. Curve D shows the recovery of cells exposed for 20 minutes to 28,000 f.-c.,

given a 175 minute rest in darkness, and then exposed to 1,000 f.-c. The numbers adjacent to the curves are rates of oxygen evolution, expressed

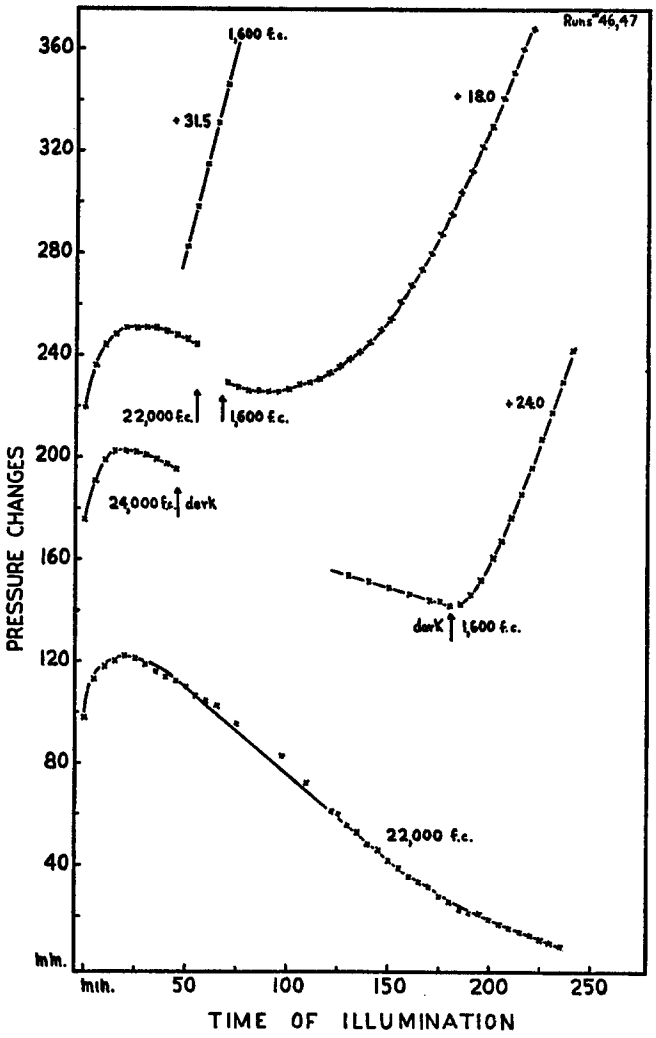

FIG. 4. Recovery curves of *Chlorella* in nutrient solution saturated with 5 per cent CO_2.

as increase in millimeter pressure per 10 minutes, after the curve has become a straight line.

It is apparent that on the constant downward slope of a curve such as either of those of Fig. 1 a progressive injury is taking place. As compared

to B (Fig. 3), recovery after the long exposure of C is much slower and less complete (though a still longer recovery time might have resulted in a further increase in rate). Curve D, as compared with B, indicates that recovery takes place in darkness as well as in 1,000 f.-c. But if exposure to strong light is prolonged as in either of the curves of Fig. 1, no recovery can be demonstrated, even after 12 hours of darkness.

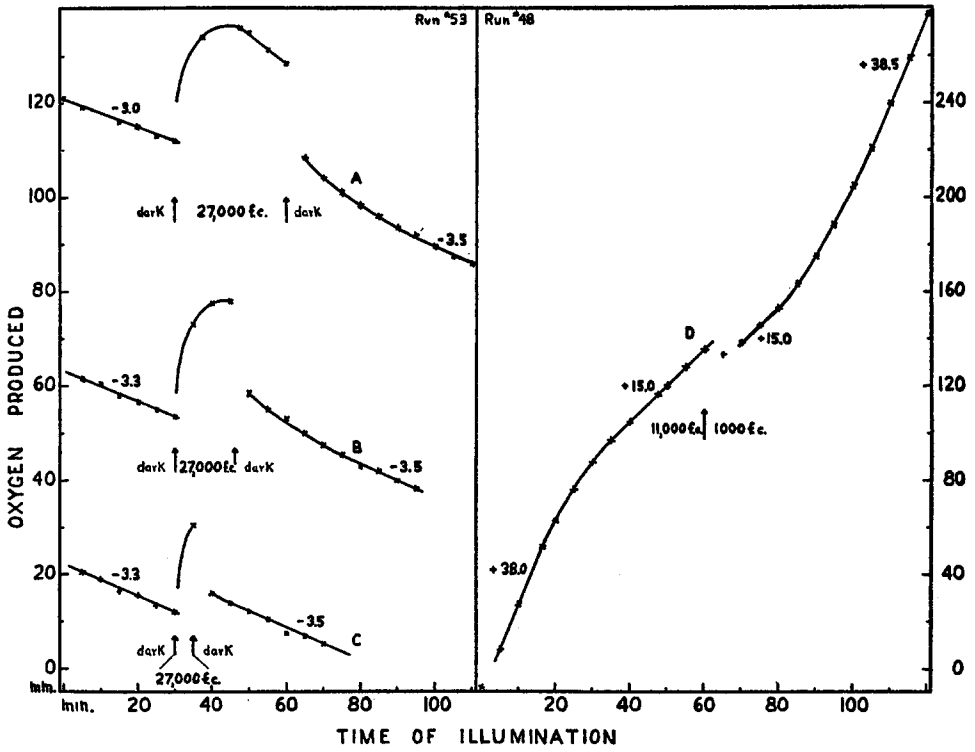

FIG. 5. Recovery in darkness shows stimulated respiration after exposure to bright light (A, B, C). There is complete and rapid recovery under 1,000 f.-c. (D).

The recovery experiments described above as performed in K_2CO_3-$KHCO_3$ buffer have been repeated using nutrient solution saturated with 5 per cent CO_2. Several of the curves thus obtained are shown in Fig. 4. The results are essentially similar.

It, therefore, seems reasonable to suppose that along the constant downward slope of the curves such as those of Fig. 1, there occurs with time a progressive injury which becomes irreversible when the die-away is reached.

Two other types of recovery experiments are illustrated in Fig. 5. Curves A, B, and C demonstrate the return to normal respiration in the dark after

30, 15, and 5 minutes exposure, respectively, to 27,000 f.-c. intensity. It is seen that there is a carry-over of the increased oxygen uptake after the light is turned off. And the return toward the normal rate of respiration is slower after longer exposure to the intense light.

Curve D of Fig. 5 shows recovery in 1000 f.-c. after exposure to 11,000 f.-c. 10 minutes after the reduction in intensity the rate is identical with that obtained under 11,000 f.-c. Recovery then proceeds rapidly.

In Fig. 6 there is presented evidence as to the time relations involved in the process producing injury under very high light intensity. If instead of 1,000 or 1,600 f.-c. illumination during recovery, an intermediate intensity is used (in this case 7,500 f.-c.), there takes place a rather rapid readjustment to a new constant rate. And as seen in this family of curves, the final rate depends upon the time of previous exposure to 23,000 f.-c. The final rate is thus a measure of the combined effects of 7,500 f.-c. and the preliminary exposure to 23,000 f.-c. If there is made the simplifying assumption that after 35 minutes exposure to 23,000 f.-c. the cells exhibit no photosynthesis (rate of O_2 exchange $= -6.0$ under 7,500 f.-c.) and further that this level of oxygen uptake prevails for all other curves, then the addition of 6.0 to the final rate for each curve will give the "true rate of photosynthesis." The rates of photosynthesis so obtained are plotted in Fig. 7 against time of previous exposure to 23,000 f.-c. (figures in parentheses at the end of each curve of Fig. 6). The shape of this curve is, of course, independent of our assumption that a zero photosynthesis is reached. A very similar curve obtains if the approximate initial rates under 7,500 f.-c. are plotted instead of final rates. Obviously a very great reduction in photosynthesis takes place within the first few minutes exposure to 23,000 f.-c. (in this case 50 per cent inhibition in 4 minutes).

It has been found useful to examine the constant slopes (see Fig. 1) as a function of light intensity. Occasionally there occurs a slight break in the constant slope. (This takes place at the 50 minute point in the curve of Fig. 1 for 38,000 f.-c. which was especially selected as a demonstration.) Such a break has shown up but rarely, although most of the curves have been followed only for about 50–70 minutes. For consistency, then, in the following discussion reference will be made only to the first constant slope, which will be called the "final rate of oxygen evolution" or simply the "final rate."

A number of families of curves similar to that of Fig. 2 have been obtained for cells grown under various conditions. Cells were grown in darkness and in light, in 1 per cent and 0 per cent glucose, with air and with 5 per cent CO_2 in air bubbled through. There are six different combinations

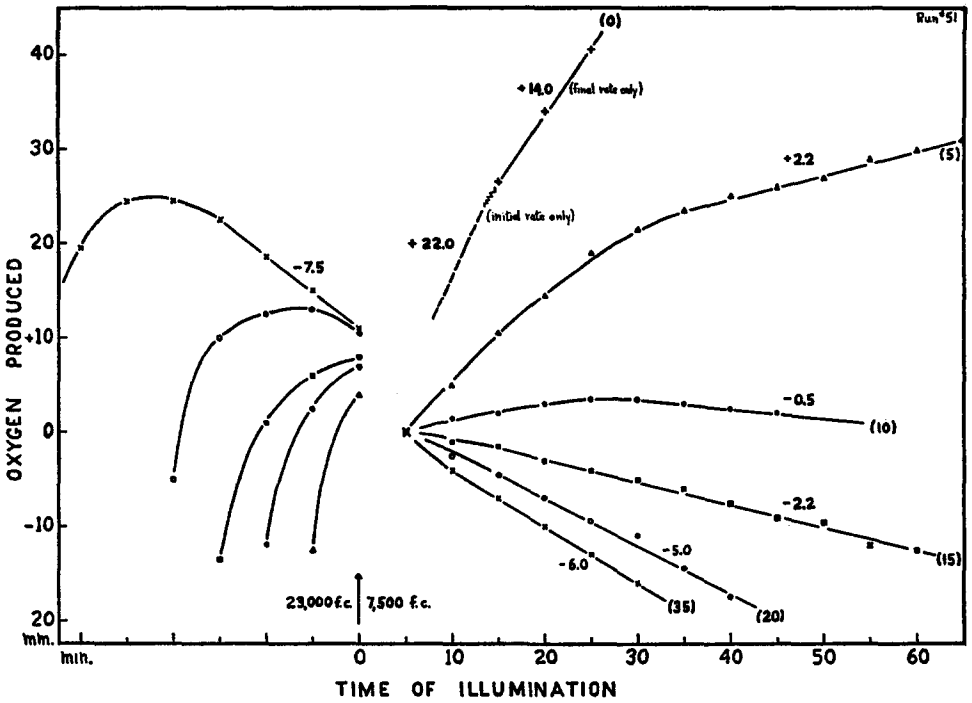

FIG. 6. Readjustment under 7,500 f.-c. after exposures of 5 to 35 minutes to 23,000 f.-c. The time of exposure to the intense light is given at the end of each curve (top curve no exposure, bottom curve 35 minutes before turning on 7,500 f.-c.).

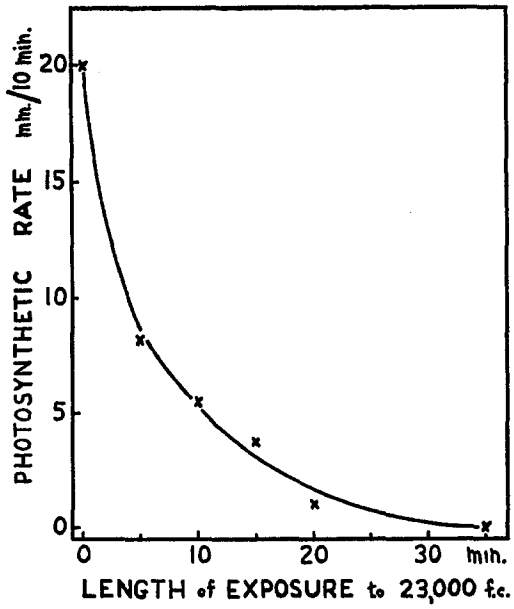

FIG. 7. Residual photosynthetic activity after varying exposures to 23,000 f.-c. A rate of 20 equals 100 per cent for this batch of cells under 7,500 f.-c.

of these factors under which algae can be grown. In Figs. 8, 9, and 10 the final rates obtained from each family of curves are presented as a function of light intensity. Final rates at higher intensities were determined as indicated in Fig. 2. Points at lower intensities were determined by shorter runs. Rates at these lower intensities are plotted in the insets with intensity on an expanded scale.

Inspection of the six curves reveals four points of interest:

1. Above a certain critical light intensity, which depends upon the previous history of the cells, the rate of oxygen evolution falls off in a continuous and predictable manner with increasing light intensity, finally becoming negative.

2. Cells grown in darkness show a depression in rate of oxygen evolution at lower intensities than comparable cells grown in light; *i.e.*, they are more sensitive to light.

3. Cells grown in high CO_2 in light show a depression in rate of oxygen evolution only at much higher light intensities than comparable cells grown in low CO_2; *i.e.*, they are less sensitive to light. This is exhibited in the curves as a broad plateau at which oxygen evolution is independent of light intensity.

4. Regardless of previous history all curves approach a maximum rate of oxygen uptake at high light intensities. This rate is about two to four times as great as the rate of oxygen uptake in dark respiration before exposure.

The data of Fig. 11 (as well as other data not here illustrated) obtained with *Protococcus* demonstrate that the qualitative nature of the effect described for our strain of *Chlorella vulgaris* is not a species peculiarity. Similar data have also been obtained for *Chlorella pyrenoidosa.*

The plateau effect illustrated in Figs. 9 and 10 has been studied further in the experiment shown in Fig. 12. Cells grown in 5 per cent CO_2 and light were studied at high intensities, using both buffer No. 9 and also buffer No. 11 which has over $3\frac{1}{2}$ times the CO_2 concentration of No. 9. The dotted portions of the curves for the final rates are plotted with some uncertainty. Even so, the curves are of importance in two respects. It is demonstrated that the solarization effect cannot be due simply to inadequate CO_2 provision. (This point is also borne out by the experiments of Fig. 4 where nutrient solution saturated with 5 per cent CO_2 was used.) At intensities greater than 12,000 f.-c., cells receiving $3\frac{1}{2}$ times the CO_2 concentration show very little increase in the final rate. On the other hand, there is a considerable increase in rate in the plateau region.

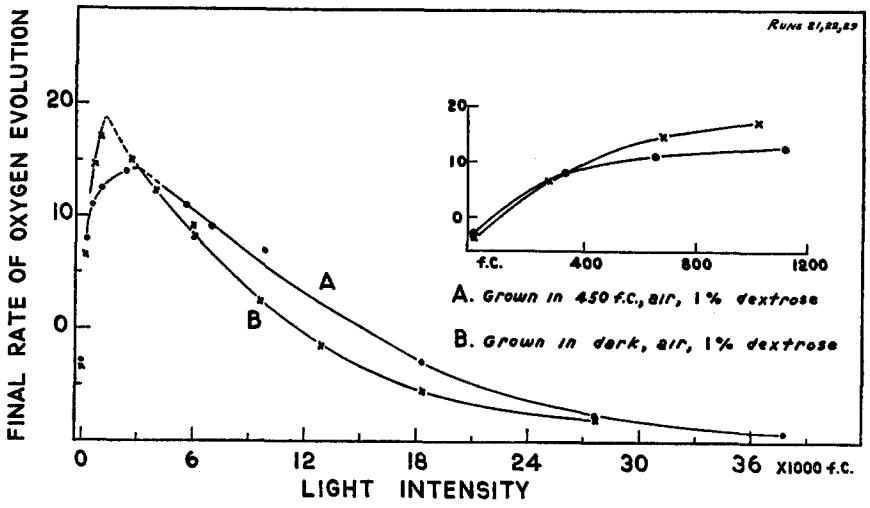

FIG. 8

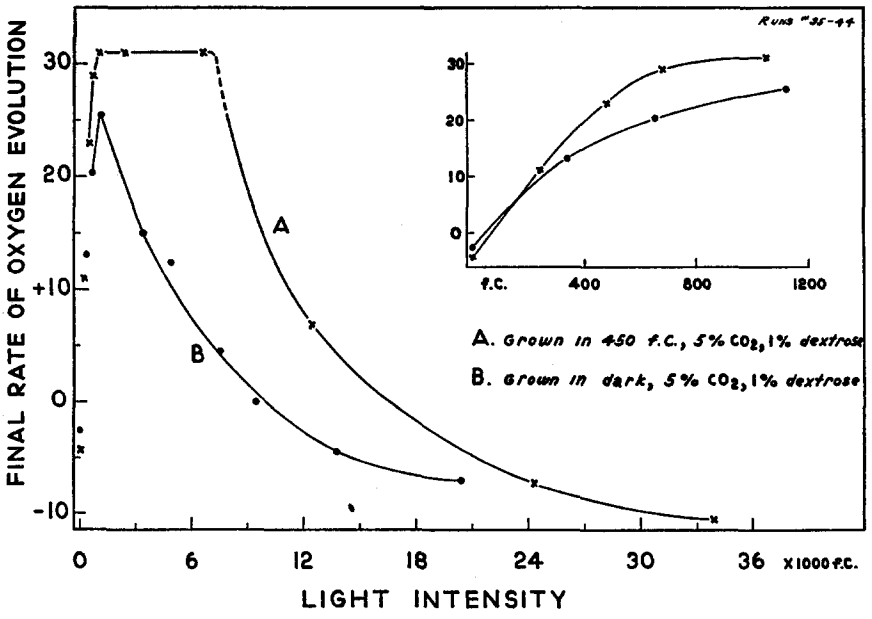

FIG. 9

FIGS. 8, 9, and 10. Showing the effect of conditions of culture on response to light.

It would be highly desirable to locate within the cell those mechanisms by which the above effects might be brought about. The use of HCN and of narcotics such as the urethanes might be expected to throw some light on this question. Data on the influence of narcotics have not as yet been obtained. A few experiments have been carried out on the influence of HCN. One of these is illustrated in Fig. 13. It was found by preliminary

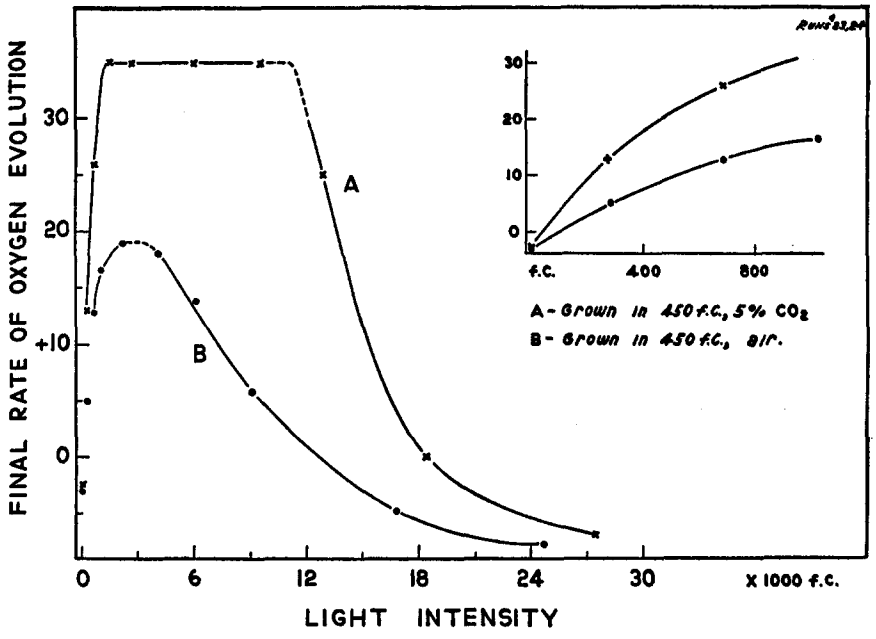

FIG. 10

experiments that for cells grown in light with 5 per cent CO_2, a 0.01 M KCN solution in the buffer mixture would prevent any oxygen evolution above the compensation point in the intensity range of about 200 to 1,000 f.-c. This is a somewhat higher concentration than has commonly been used in the study of photosynthesis. Warburg's (1920) data in one of his experiments indicate that at about 1,800 f.-c. he was approaching a compensation point with 0.005 M KCN. A lower concentration such as 0.005 M KCN still allows for our cells an appreciable oxygen evolution in this intensity range. Both of these concentrations stimulate the rate of dark respiration.

In Fig. 13 the upper curve was interpolated from the five experimental points by analogy to the upper curve of Fig. 10 for cells grown under identi-

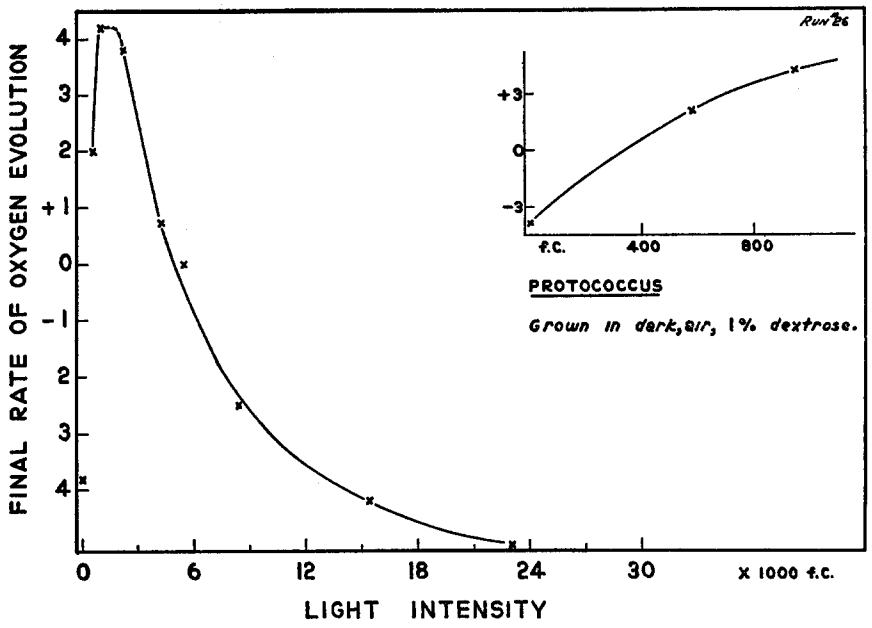

FIG. 11. Rate-intensity curve for *Protococcus*, illustrating qualitative similarity to *Chlorella*.

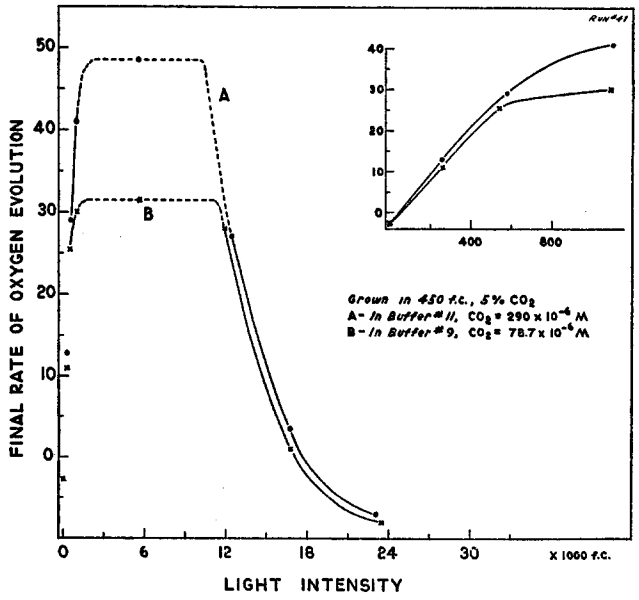

FIG. 12. Effect of CO_2 tension on the light response. High CO_2 (A) increases the maximum rate but does not prevent injury at high intensities.

cal conditions. The lower curve of Fig. 13 was obtained when 0.01 M KCN was added to the buffer mixture. Both curves seem to be approaching the same limiting value, though there are too few points to establish this with certainty. Evidently a cyanide concentration which will prevent any net photosynthesis as measured by oxygen evolution does not block the mechanism responsible for the oxygen uptake at high intensities.

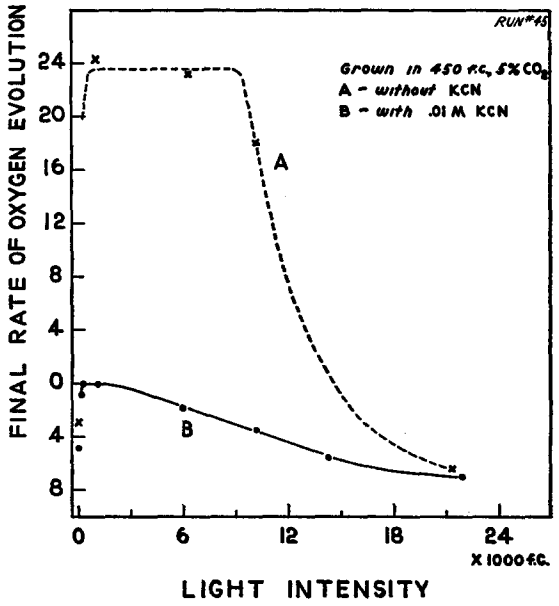

FIG. 13. Effect of KCN on response to light

DISCUSSION

It is rather surprising that the phenomena noted here have not already been adequately described. Probably no previous workers have used so great a range of light intensity. However, a number have used intensities ranging up to about 10,000 f.-c. And Smith (1937) has described certain of the kinetic properties of photosynthesis on the basis of observations made at intensities up to 282,000 lux using the water plant *Cabomba*. At the higher intensities he noted a "small decrease in rate to take place after three or four hours." He obviated this by using a red filter which probably also cut the total intensity in half.

Only two previous papers give results directly comparable with ours. Emerson (1935), using a similar technique, found an injurious effect of 4 or 16 hours exposure to 4,500 f.-c. on *Chlorella*, but only when the CO_2 supply was either inadequate or lacking. Injury was noted by a considerable decrease in the photosynthetic rate after as compared to the rate before exposure. He did not consider the accompanying chlorophyll destruction great enough to account for the decline in photosynthesis. For instance, a 70 per cent decrease in rate was accompanied by only a 20 per cent loss of chlorophyll.

This effect found by Emerson is suggestive of, but not directly com-

parable with, our experience that cells grown in low CO_2 (0.03 per cent) are more susceptible to a depressing effect of light on the rate of oxygen evolution. We are probably dealing in different ways with the same phenomenon. However, in the same paper[5] is the statement: "Never in the course of hundreds of experiments has the writer found a decline in rate attributable to high light intensities, though this effect has often been looked for, and light intensities up to about 100,000 meter candles have been used." If the algae were grown either in bubbling 5 per cent CO_2 as in his earlier work (1929), or in the 6.7 per cent CO_2 used in this experiment, then his cells were probably comparable to those described by the upper curve of Fig. 10; *i.e.*, more resistant to light injury.

A second paper offering direct comparison is that of Fockler (1938) in which he described the effect of high light intensity on the shade fern *Trichomanes radicans*. His light intensities (expressed by him only in relative units) probably were not as high as ours. But his use of a thin-leaf plant (fronds only about 1 cell thick) should have reduced shading of cells to a minimum.

Fockler is not explicit about his experimental method. He apparently measured by the Winkler method the oxygen dissolved in the water circulated over the submerged frond. He does not refer to Emerson's (1935) paper and may not have provided adequate CO_2. His rates were measured only at hourly intervals. However, after 1 hour's exposure to sunlight he got no apparent photosynthesis but an oxygen uptake which increased in rate until the 3rd hour. Fronds exposed for 1 or 2 hours showed partial recovery of normal photosynthetic activity in 5 days, full recovery in 14 days of moderate light. Fronds exposed for 4 hours had not yet fully recovered in 14 days. A similar though less pronounced effect was obtained with *Laminaria digitata*. Fockler was interested in the effect of light on respiration. Much of his data are on colorless plant tissue. On such material he noted at an hour's exposure an increase in respiration up to 100 per cent. With longer illumination this gradually fell off, approaching its original value. Because of this he believes that his experiments with *Trichomanes* show the result of two processes: an increased respiration and an inactivation of photosynthesis, both of which probably result from "eine Störung im kolloidalen System des Protoplasmas."[6]

The recent work of Stålfelt (1939) (which has come to our attention since the experimental work was done) seems also to have some bearing

[5] Emerson, R., Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1935, **3**, 130.

[6] Fockler, H., *Jahrb. wissensch. Bot.*, 1938, **87**, 89.

on the problem. Of particular interest are his experiments on two species of lichens. In one experiment over a period of 6 days (10 hours light of 16,000 lux, 14 hours dark) there occurred daily a light-inhibition of photosynthesis which amounted to about 26 per cent of the average rate. In each 14 hour period recovery was about 23 per cent complete. This reversible light-inhibition was further shown to be proportional to light intensity from 4,000 to 48,000 lux and independent of temperature up to 20°C., the optimum temperature for photosynthesis in these plants. Above 20° an additional temperature-inhibition took place which was not reversible in darkness. In fact an inhibition of photosynthesis could be obtained by exposure to higher temperatures (20–28°) in the dark.

Stålfelt's work indicates that the light-inhibition occurring in lichens is probably closely related to the similar effects reported here for *Chlorella* and suggests that the phenomena observed for *Chlorella* may profitably be studied also as a function of temperature. But his observations are of no immediate aid in the interpretation of our data.

The authors realize full well the difficulties involved in reaching a full explanation of the observed phenomena. The inadequacy of our data is paralleled by the lack of complete or directly comparable data in the literature. The task of this discussion is, therefore, to arrive at some working hypothesis which will account for the data so far obtained.

It should be emphasized that all the data apply merely to the uptake or evolution of oxygen by the algal cells. Nothing at all is known about the total CO_2-O_2 exchange.

The evidence from our data is of two kinds, that from the "time" curves (Figs. 1–7) and that from the "intensity" curves (Figs. 8–13).

In very high light intensities the rate of oxygen uptake is much greater than the rate of dark respiration. For want of a better name, this excess oxygen uptake is called "photo-oxidation," at the same time recognizing that it may not be the simple photochemical reaction which the name implies. That photo-oxidations sensitized by chlorophyll can take place has been shown by the work of Gaffron (1933) and of Kautsky and Hormuth (1937).

The problem, then, is to relate the three processes of respiration, photosynthesis, and photo-oxidation in such a way as to account for the observed O_2 exchange. In introducing the term photo-oxidation it is assumed that respiration is constant and independent of light. The O_2 exchange now depends on the balance between photosynthesis and photo-oxidation. In regard to the photosynthesis two alternate hypotheses are suggested: (1) it

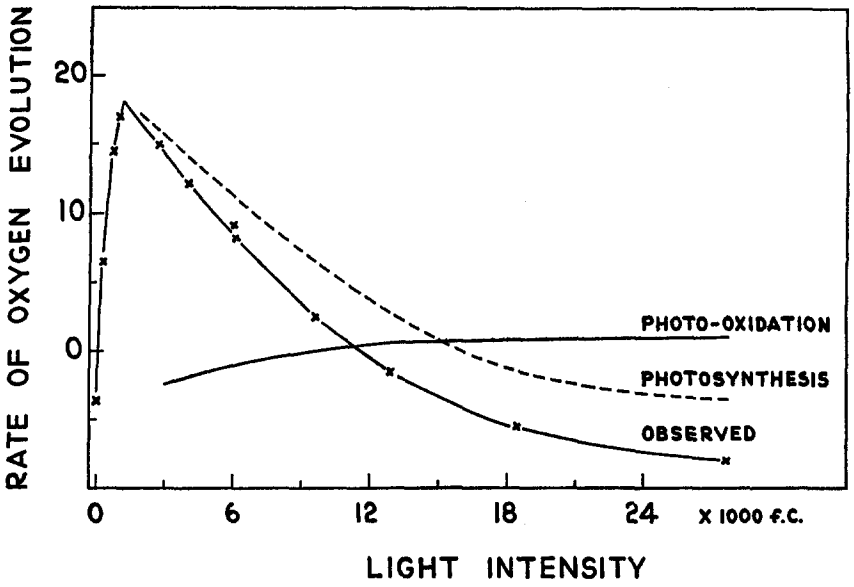

FIG. 14. Hypothetical intensity curve for photo-oxidation. Photosynthesis inhibited.

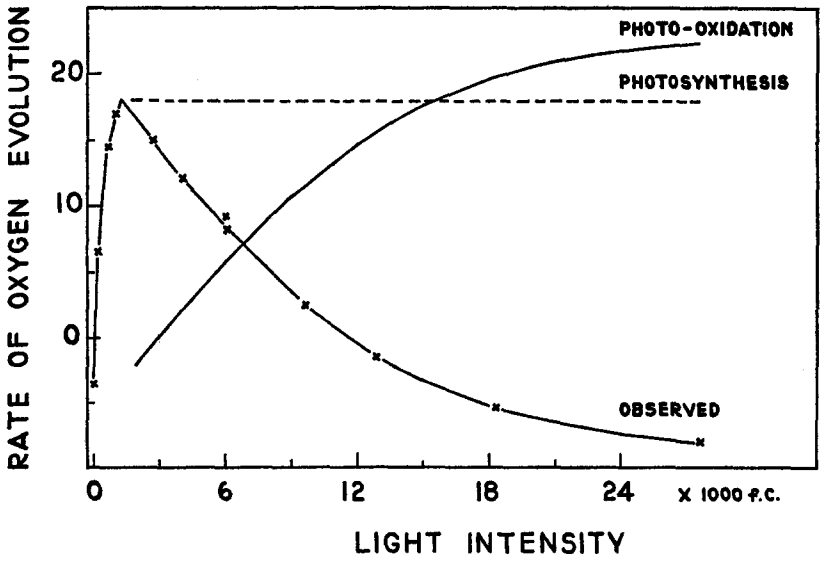

FIG. 15. Hypothetical intensity curve for photo-oxidation. Photosynthesis constant at high intensities.

is progressively inhibited by increasing light intensities; or (2) it continues at a maximum value at all higher intensities.

These two hypotheses are illustrated graphically in Figs. 14 and 15 based on the data obtained from the family of curves of Fig. 2 and curve B of Fig. 8.

In Fig. 14 the assumption is followed that zero photosynthesis is reached within the range of intensities studied. The "photosynthesis" curve is drawn quite arbitrarily except that it fulfills this condition. The observed final rate of O_2-evolution must be the net effect of photosynthesis and photo-oxidation (plus respiration). The hypothetical photo-oxidation curve is thus obtained by point-by-point subtraction of the "observed" from the photosynthesis curve. It, of course, represents a negative evolution of oxygen. Like the photosynthesis curve it is plotted with its base line at the level of dark respiration. Thus, at the intensity at which the curves of photosynthesis and "photo-oxidation" cross, the net O_2-evolution is the -3.5 (mm./10 min.) of dark respiration. As here drawn the photo-oxidation approaches a maximum value, although this is not at all certain since the photosynthesis curve has been drawn with a good deal of uncertainty.

Fig. 15 results from the second hypothesis that photosynthesis continues at a maximum value at all higher intensities. The maximum rate of photosynthesis is taken as the value approached at intensities of about 1,000 f.-c. (where photo-oxidation must be small).

An essential difference in the consequences of the two hypotheses is that the first requires a much smaller rate of photo-oxidation, approaching the magnitude of dark respiration. This is in accord with other observations in the literature on the effects of light on respiration. The second hypothesis, on the other hand, requires tremendously higher rates of photo-oxidation. We can find no evidence for such high rates. In fact, the complete lack of response to 1,000 f.-c. after 20 and 50 minutes in 28,000 f.-c. (curves B and C, Fig. 3) indicates that the photosynthetic mechanism is inactivated in both cases. The lack of response to a $3\frac{1}{2}$ times increase in CO_2 concentration at high intensities (Fig. 12) also makes the assumption of a maximum photosynthesis at these high intensities highly unlikely. And it is impossible to account for the constant downward slopes (as those of Fig. 1) and the character of the recovery curves (Fig. 3) by the assumptions involved in the second hypothesis. However, the second hypothesis has been considered since it represents a contrast to the first.

Assuming, therefore, that photosynthesis is progressively inhibited by increasing intensities, the curves of Fig. 3 indicate that under 28,000 f.-c.

intensity the cells suffer a progressive injury with increasing exposure, from which they recover less completely and more slowly. However, the complete lack of response to 1,000 f.-c. after 20 and 50 minutes in 28,000 f.-c. (B and C) seems to show that the photosynthetic mechanism is fully inactivated in both cases. This fact explains the constant rates of oxygen uptake in Fig. 1 during a period in which progressive injury is taking place. There must be two distinct phenomena involved: (1) the complete inactivation of the photosynthetic mechanism within the first few (20–30) minutes, followed by (2) a progressive destruction of some cellular material which eventually goes to completion and stops photo-oxidation (the total O_2-uptake approaches a limit). When the second process has gone so far that the photo-oxidation rate begins to decrease, the cells are completely bleached and can no longer recover. It may well be, therefore, that photo-oxidation depends on chlorophyll absorption. But the inactivation of the photosynthetic mechanism is here due to another effect and there need be no direct relation between chlorophyll content and depression of photosynthesis. The maximum absorption of oxygen takes place when the photosynthetic mechanism has been inactivated and there is still much chlorophyll present.

Two characteristics of the phenomenon involved in the process of photosynthesis inactivation are described by Figs. 5 and 7. Fig. 7 indicates that the process is extremely rapid, and a comparison of Figs. 5 and 7 makes it clear that the 5 minutes of bright light which greatly depress photosynthesis do not appreciably affect the rate of oxygen uptake (as indicated by the "carry-over" into the dark).

Thus the rapid process required to reach the final steady rate at a given light intensity is considered a destruction of some factor in the photosynthetic mechanism. This factor is reduced to a concentration at which it is maintained at a steady state for any given intensity. The concentration of this factor would then limit the photosynthetic rate and, along with photo-oxidation, determine the final rate of oxygen evolution. Such a conception would apply equally well to all of the curves of Fig. 2. The maintenance of the steady downward slope of curves A and B of Fig. 1 is now easily explained. The progressive injury to photosynthesis does not affect the rate since photosynthesis has already been stopped by the destruction of some photosynthetic factor during the first few minutes of irradiation. The die-away is explained as a decrease in photo-oxidation when its substrate or sensitizer is almost used up.

At lower light intensities (4,000–12,000 f.-c., Fig. 2) only partial inhibition of photosynthesis takes place, and a steady but reduced rate is observed. This does not result in permanent injury during the time of ob-

servation. Recovery in 1,000 f.-c. from such a condition is rapid (see Fig. 5, curve D).

As already pointed out, the intensity curves in Figs. 8, 9, and 10 show that with increasing light intensities the final rate of O_2-uptake approaches a common limiting value apparently independent of the history of the cells. On the other hand, the maximum possible rate of photosynthesis varies greatly with the previous history of the cells. Again, this points to the first hypothesis that all photosynthesis has been stopped by the very high light intensities. Otherwise it would be necessary to assume that the same differences in previous history which favor high photosynthetic rates also favor proportionately high photo-oxidation rates.

Further mention should be made of the two types of intensity curves such as A and B of Fig. 10. In both cases measurements were made in the No. 9 buffer ($CO_2 = 78.7 \times 10^{-6}$ M). For curve A this is only about $\frac{1}{17}$ as great a CO_2 concentration as the 5 per cent CO_2 in which the cells were grown. Most measurements of intensity curves for algae have been made under similar conditions. For the cells of curve B the buffer provides CO_2 concentration seven times greater than that in which they were grown. In this case the rate of photosynthesis seems to be limited by some internal factor, probably the same one attacked during the first few minutes of exposure to high light intensities. In regard to curve A the suggestion is here proposed that cultures in high CO_2 and light develop a photosynthetic mechanism of high rate capacity. Buffer No. 9 in which the runs were made furnishes too little CO_2 for the cells to reach their maximum rates; and so over a range of several thousand foot-candles intensity CO_2 is the limiting factor. It is possible that through this range of increasing light intensity, inactivation of the photosynthetic mechanism has taken place to the same extent as seen in the lower curves (Figs. 9 B and 10 B). But the original capacity for photosynthesis is so great that CO_2 remains the limiting factor over a considerable range. This is supported by the results shown in Fig. 12. It seems likely that if the CO_2 concentration were increased enough, curve A would rise to a very high value. The plateau would then disappear and the curve would show the effects of partial inactivation of the photosynthetic mechanism at intensities much less than 12,000 f.-c.

If Fig. 14 be accepted as picturing the relation between photo-oxidation and photosynthesis which results in the observed curves, then in all cases the sharp downward breaks in the intensity curves are largely due to inactivation of the photosynthetic mechanism rather than to photo-oxidation which increases but slowly with increasing light intensity.

Unfortunately the data on cyanide inhibition are not complete enough

to be of much help. The points at 300 and 1,200 f.-c. (curve B, Fig. 13) and the data of Warburg (1920) seem to indicate that in this intensity range internal photosynthesis is not blocked. If this also holds for higher intensities, then interpretation is difficult since it is not certain to what extent the internal photosynthesis compensates for photo-oxidation. It is true that the cyanide curve (B, Fig. 13) looks like an inverted form of the hypothetical photo-oxidation curve of Fig. 14. However, lack of knowledge of the specific effect of cyanide at these higher intensities allows little weight to be placed on this similarity.

Obviously, more data must be assembled before the solarization effect of high light intensities can be completely explained. Some oxidation in excess of dark respiration takes place under very high light intensity. We have tentatively called this "photo-oxidation." We have examined two alternative hypotheses for the behavior of photosynthesis. The assumption of a maximum photosynthesis continuing under very high light intensities has been shown to be untenable. On the other hand, all of our data are consistent with the view that with increasing intensities photosynthesis is progressively inhibited while photo-oxidation is progressively increased at a much lower rate.

SUMMARY

1. The effect on oxygen evolution of *Chlorella vulgaris* produced by light intensities up to about 40,000 f.-c. has been studied by the use of the Warburg technique.

2. Above a certain critical intensity, which is determined by the previous history of the cells, the rate of oxygen evolution decreases from the maximum to another constant rate. This depression is at first a completely reversible effect.

3. With a sufficiently high intensity this constant rate represents an oxygen uptake greater than the rate of dark respiration. During such a constant rate of oxygen uptake a progressive injury to the photosynthetic mechanism takes place. After a given oxygen consumption the rate falls off, approaching zero, and the cells are irreversibly injured.

4. The constant rate of oxygen evolution (2 and 3) decreases in a continuous manner with increasing light intensities, approaching a value which is approximately constant for all lots of cells regardless of previous history.

5. Two alternative hypotheses have been presented to explain the observed phenomena. The more acceptable of these proposes quick inactivation of the photosynthetic mechanism, the extent of inhibition depending on the light intensity.

6. In *Chlorella vulgaris* solarization is influenced by the previous history of the cells.

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