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By E. D. McALISTER and JACK MYERS[1]
Division of Radiation and Organisms, Smithsonian Institution

The bulk of our knowledge of chlorophyll photosynthesis has come from observations on carbon dioxide assimilation and oxygen production during the process in a living plant. Recently, fluorescence studies have also contributed to the development of theories for the kinetics of photosynthesis (cf. Franck and Herzfeld, 1937, Kautsky and Hormuth, 1937, Ornstein et al., 1938). Unfortunately, there is little agreement between the experimental data of these various groups of workers, which have been obtained under different conditions and on various plant materials. Their contributions have been of great value in suggesting that changes in intensity of fluorescence are related to changes in the rate of photosynthesis during the induction period, and consequently that there must be a relationship between the two phenomena.[2] However, the lack of other than inferential information on the exact state of photosynthesis corresponding to a particular state of fluorescence leaves the relationship on insecure ground. These workers have not been unaware of this but have lacked facilities for following changes in carbon dioxide assimilation or oxygen production with a rapidity at all comparable to that of their fluorescence observations.

[1] National Research Fellow.

[2] The concept of a relationship between intensity of fluorescence and rate of photosynthesis does not require that fluorescence enter into the process of photosynthesis in any way. The chlorophyll in the plant, after absorbing radiation, can dispose of this energy in various ways. It is either used in photosynthesis or some other photochemical process, is lost as heat, or is emitted as fluorescence radiation of longer wave length (red). The distribution of energy among these three outlets must change during the induction period, as shown by all the fluorescence and photosynthesis induction curves reported in the literature. In other words, it is believed possible that changes in fluorescence can tell us something about the photochemical processes; but it is not held that fluorescence enters in any way into the process of photosynthesis. The amount of energy appearing as fluorescence is very small compared to that used in photosynthesis. According to the measurements of Vermeulen et al. (1937), only 0.1 to 0.2 percent of the energy absorbed by *Chlorella* is re-emitted as fluorescence.

The rapid spectrographic method of carbon dioxide measurement developed in this laboratory fulfills this need. It is the purpose of this preliminary report to present simultaneous records of induction in fluorescence and photosynthesis obtained on two different types of plants and over a wide range of experimental conditions. To the writers' knowledge this is the first time that these two phenomena have been observed simultaneously during the induction period. Such an attack gives promise of clarifying the interpretation of both phenomena. As will be seen below, it has been possible to distinguish two different processes involved in the induction period. In one of these intensity of fluorescence and rate of carbon dioxide uptake are related inversely; in the other a direct relationship exists.

The only observations on fluorescence and photosynthesis during the steady state appearing in the literature are those of Wassink et al. (1938, 1939), using *Chlorella*. The present work also includes further measurements during the steady state in wheat.

The authors take pleasure in acknowledging their indebtedness to Dr. C. G. Abbot and to Dr. E. S. Johnston for their interest in, and support of, this work.

EXPERIMENTAL PROCEDURE

Two types of plant material have been used. Wheat (variety, *Marquis*) [3] was grown for 4 to 8 days on cloth netting over running tap water and with 300 to 400 foot-candle illumination from an unfiltered tungsten lamp. Measurements were made on eight or nine young plants in a cylindrical, water-jacketed chamber of about 100 cc. volume similar to that previously described by McAlister (1937).

Chlorella pyrenoidosa [4] was grown in 250-cc. Erlenmeyers held in a mechanical shaker in a water bath thermostated at 23° C., and with either air or air containing 4 percent carbon dioxide bubbled through. Light intensity of 300 to 400 foot-candles was provided by a tungsten lamp placed beneath the glass bottom of the bath and filtered through 2 cm. of water. This intensity corresponds to about 3×10^4 ergs/cm.^{2}/sec. of visible radiation. Knop's nutrient solution was used with the addition of 0.1 cc. per liter of "solution A" of Hoagland and Arnon (1938), as recommended by Emerson and Lewis (1939). The addition of the microelements of "solution A" (boron,

[3] Seed kindly supplied by H. H. McKinney, of the U. S. Department of Agriculture.

[4] From a culture originally obtained through the courtesy of Dr. Robert Emerson.

manganese, zinc, copper, molybdenum) gave a noticeably more rapid growth than occurred in their absence. Cultures containing about 0.3 cc. of cells per 100 cc. were harvested by centrifuging, and the cells resuspended in fresh nutrient of the same composition for the experimental work. Forty cc. of an algal suspension containing 0.2 to 0.4 cc. of packed cells was placed in a glass chamber measuring $0.6 \times 10 \times 20$ cm. This density of cells absorbed about 50 percent of the incident beam. An air stream of up to 50 cc./sec. was forced through the suspension, entering at the bottom through fine holes in a glass tube. This arrangement broke up the air stream into fine bubbles, providing a maximum area of contact between liquid and gas. Constant temperature of 24° C. was maintained by immersion in a small water bath.

Instead of the closed system and recirculation method previously used (McAlister, 1937, 1939), a constant flow of gas was passed through the plant chambers without recirculation. A spectrographic analysis of the effluent gas from the plant chamber then gives a direct measure of the rate of carbon dioxide uptake or production by the plants. The constant-flow method has an additional advantage in that the plants are kept under a constant environment. Before reaching the plant chamber the gas flow was divided into two portions, "A" and "B." Part "A" continued into the plant chamber at a constant rate of flow of up to 100 cc./sec. as measured by a Venturi gauge. By means of a two-way stopcock part "B" could be either discarded or passed into the optical absorption cell of the spectrograph, as desired. By means of another stopcock the effluent gas from the plant chamber could be either passed into the absorption cell or discarded. This arrangement permitted an easy comparison of the effluent and influent air of the plant chamber. A water-cooled condenser, through which the air passed before entering the absorption cell, removed enough water vapor from the air to prevent condensation on the windows of the cell. Condensation on the optical parts of this cell was further avoided by maintaining it 3° or 4° C. above the temperature of the air stream.

The spectrographic apparatus previously used has been modified in several respects in order to adapt it to the present work. The optical path of the absorption cell has been shortened to about 20 cm. to permit analysis of higher concentrations of carbon dioxide. Its volume is about 70 cc. Because of the small changes in carbon dioxide to be measured, it has been necessary to amplify the indications of the galvanometer used for reading the thermocouple current. A well-defined and uniformly intense rectangle of light is reflected by

the mirror of the primary galvanometer so that it moves across an opaque straight-edge partly covering a Weston photronic cell. Current from the photronic cell actuates a secondary galvanometer. The amplification thus obtained can be varied from 1 to 500 times by changing the intensity of the rectangle of light falling on the photocell. An amplification of 50× was usually sufficient. The amplification factor was measured and found to be constant well within the other experimental errors involved, particularly that involved in the zero drift.

Continuous recording has been substituted for the alternate 30-second readings previously taken. A light spot from the secondary galvanometer makes a continuous record on a sheet of photographic paper held on a drum which is rotated at constant speed by a synchronous motor. This procedure does not permit alternate "zero" readings to be taken and hence makes it impossible to eliminate the slow "zero drifts" which are apparent on many of our records.

Illumination was provided by two 1,000-watt projection bulbs. A uniformly intense spot of light was focused on the plant chamber with spherical reflectors (15-inch diameter, 9-inch focal length). This light was filtered through 13 cm. of water and 6 cm. of a solution of copper nitrate of such concentration that the plants received no light of wave length longer than 6400 A. Incident intensities were varied by use of a series of screens and were measured by means of a vacuum thermocouple and microammeter. No voltage regulator was available; the variation in incident intensity caused by fluctuations in line voltage never exceeded 5 percent during the short periods of illumination used. The "high intensity" commonly used was about 60×10^4 ergs/cm.^{2}/sec. "Low intensity" was a fifth of this unless stated otherwise.

Fluorescence intensity was measured by a circular, barrier-type photocell ("Electrocell") of 44 mm. effective diameter connected to a Moll galvanometer of 1.2-second period. The photocell disk was covered by two filters, a Corning H. R. red (3.6 mm.) and a blue-purple Corex A (4.2 mm.). These filters allowed only light of wave length longer than 6500 A to reach the photocell. The red filter itself was found to fluoresce strongly when illuminated. This feature was reduced to an unobjectionable minimum by placing the purple filter in front of the red so as to absorb most of the radiation causing the fluorescence.

In studies on wheat the photocell was placed at one side of the plant chamber facing the plants and just out of the incident beam;

in the *Chlorella* experiments it was placed immediately behind the rectangular chamber.

With the photocell placed so that it received directly the full intensity of the light incident on the plants, the galvanometer deflection was less than 2 percent of that produced by the steady-state fluorescence of plant material. This galvanometer also records on the same photographic paper on the rotating drum. Perfect time alignment of the "fluorescence" and "carbon dioxide" galvanometer indications is provided by a narrow slit parallel to the axis of the drum, through which both spots must pass.

RESULTS

A number of induction curves, selected from the several hundred which have been obtained, are presented on the following pages. The curves have been so selected as to include representative data illustrating the induction behavior under a wide range of experimental conditions. Figures 1 to 11 are photographic reproductions of the original galvanometer tracings. In each pair the upper curve is the recording of the fluorescence intensity; the lower, the recording of the rate of carbon dioxide uptake. In some cases the tracings have been darkened with India ink to insure their reproduction. Small horizontal arrows have frequently been added to mark the first appearance of the fluorescence tracing on the original record.

The procedure used in obtaining these induction curves was as follows: The recording drum was started about a minute before the beginning of illumination and continued for a minute or so after the plant was darkened. The period of illumination was usually 4 minutes. Vertical lines have been drawn on the records to indicate the times of transition from dark to light and from light to dark. Dotted lines have been added to show the probable course of the "dark" reading (respiration rate and "zero" of fluorescence), interpolated through the light period. One-minute intervals are indicated by vertical dashes above the fluorescence zero line.

In a number of cases, and particularly with suspensions of algae, it has been desirable to observe the time over which an instantaneous plant response is spread by our instruments. This has been accomplished by entirely stopping the gas flow for a short time (15 seconds) during a dark period, allowing the respired carbon dioxide to accumulate. The resumption of gas flow washes the accumulated carbon dioxide through the system and produces a wedge-shaped dip in the carbon dioxide curve (see curves 2, 5, and 8 of fig. 8). The

width of this wedge approximates the time response of the instruments to an instantaneous change in rate of the same magnitude. For wheat at a flow rate of 100 cc./sec. this time is about 6 seconds, and for *Chlorella* at 50 cc./sec. it is 15 seconds. The proportionately longer time in the algal suspension is apparently due to the liquid-gas diffusion lag. Thus in the recording of the rate of carbon dioxide uptake there appears both a "smearing out" of the plant response (as discussed above) and a lateral shift due to the time of transit of gas from the plant chamber to the absorption cell. The lag in fluorescence recording is merely the 1.2-second period of the galvanometer.

In order to obtain a comparison of respiration and photosynthesis, the influent gas stream has been occasionally by-passed directly into the absorption cell, usually for 30- or 45-second periods (as described above, p. 3). This is seen in the curves as a short horizontal shelf which gives a somewhat discontinuous appearance to the curves but establishes the level of respiration.

Figure 1 illustrates the behavior of wheat in normal air. At high intensities (40 to 60×10^4 ergs/cm.^{2}/sec.) and after a long dark rest the fluorescence intensity rises rapidly to a maximum and then falls off more slowly toward a final steady value. During this time the rate of carbon dioxide uptake has risen to a steady value. Comparison of curves 1 and 2 indicates that both photosynthesis and fluorescence are influenced by the length of the preceding dark rests. Furthermore, when the dark rest is not sufficiently long, the preceding light treatment is also of importance, as shown by curves 3 and 4. Under low light (4 to 10×10^4 ergs/cm.^{2}/sec.) (curve 5) or short dark rests (curves 6 and 7) both photosynthesis and fluorescence approach a rectilinear course in their attainment of the final steady state.

Figure 2 demonstrates the effects of low oxygen and low carbon dioxide pressures. Behavior of wheat in "tank" nitrogen [5] is shown in curve 1. The fluorescence curve has the same general features as the fluorescence curve in air, except that the maximum is reached more slowly and the decay is more gradual. The rate of respiration in tank nitrogen is about one-half that in air (see curves 1 and 2 of figures

[5] A good grade of "water-pumped" compressed nitrogen. While no oxygen analyses on this are available, it is reasonably certain that the oxygen content was less than ½ percent. Complete removal of oxygen was impossible at the high rate of flow used. This is referred to throughout the paper as tank nitrogen, low oxygen, or ½ percent oxygen.

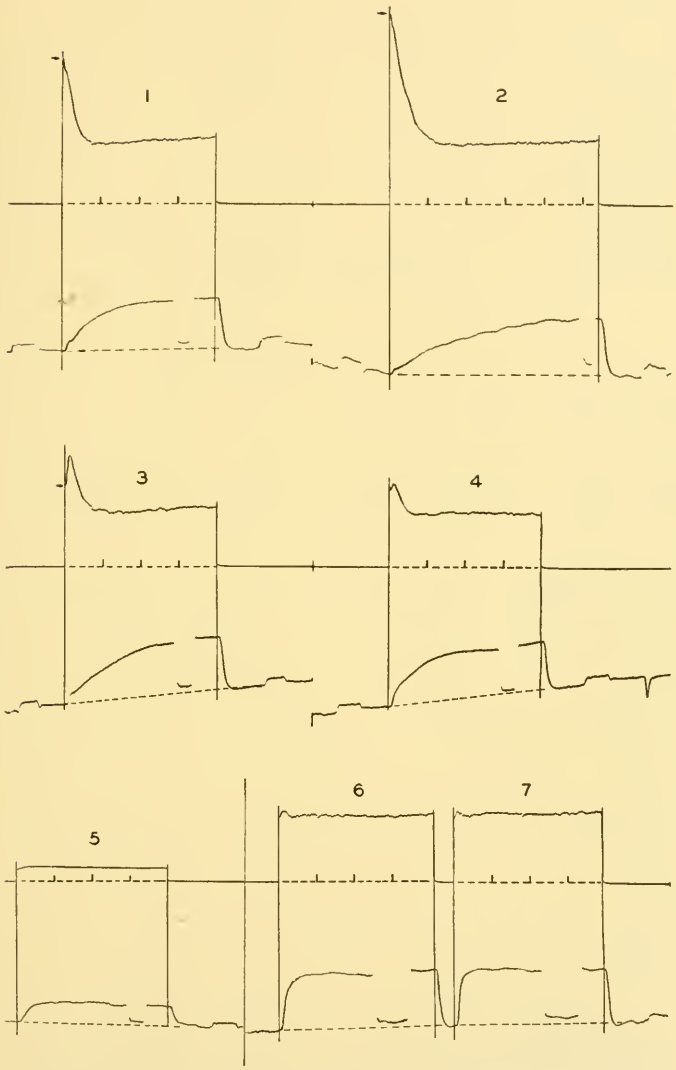

FIG. 1.—The induction behavior of wheat in normal air. The effects of the preceding light and dark treatment.

1. In high light after 10 minutes light, 20 minutes dark rest.
2. In high light after 10 minutes light, 60 minutes dark rest.
3. In high light after 40 minutes light, 12 minutes dark rest.
4. In high light after 4 minutes light, 12 minutes dark rest.
5. In low light after 10 minutes dark rest.
6. In high light after 2 minutes dark rest.
7. In high light after ½ minute dark rest.

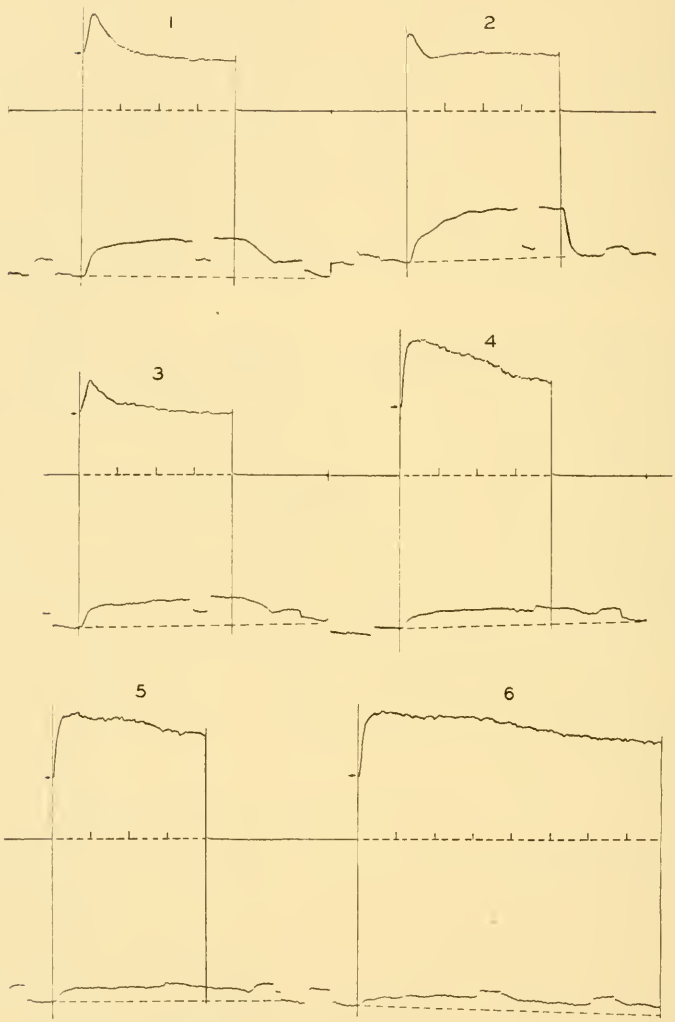

FIG. 2.—The induction behavior of wheat in low oxygen pressure. The effects of varying carbon dioxide concentrations. All curves taken in high light, after 10 minutes in high light and normal air, 10 minutes dark rest with specified carbon dioxide concentration in commercial nitrogen.

1. 0.006 percent carbon dioxide in nitrogen.
2. In normal air.
3. 0.004 percent carbon dioxide in nitrogen.
4. 0.0015 percent carbon dioxide in nitrogen.
5. 0.0006 percent carbon dioxide in nitrogen.
6. Zero carbon dioxide in nitrogen.

3 or 6). The apparently higher respiration rate in the nitrogen than in air (curve 2) is due to the increased sensitivity of our instruments at the lower carbon dioxide concentration of the tank nitrogen (about 0.006 percent). In examination of all the curves it must be remembered that the sensitivity of the spectrographic method is higher at lower concentrations of carbon dioxide. If the carbon dioxide concentration is still further reduced by shunting a measured portion of the gas through soda-lime, the series of curves 3, 4, 5, and 6 is obtained for different carbon dioxide levels. It is apparent that carbon dioxide has a (direct or indirect) quenching effect on the fluorescence. The "dark pick-up" exhibited by these and other curves for low carbon dioxide concentrations will be discussed below.

The effect of decreased oxygen pressure is seen in comparing curves 1 and 2 of figure 3. In 20 percent oxygen the induction in carbon dioxide uptake is longer, although the fall in fluorescence is much more rapid than in low oxygen. In normal air, apparently owing to changes within the plant (curves 3 and 5), the fluorescence decay is even more rapid and actually produces a minimum in the fluorescence curve. When all carbon dioxide is removed from air or from tank oxygen, curves such as 4 and 6 are obtained. The rate of carbon dioxide production is at first decreased but later builds up again to approximately the rate of dark respiration. In no case is there a fluorescence minimum in the absence of carbon dioxide.

Figure 4 shows the peculiar effects of high carbon dioxide on the induction in fluorescence and carbon dioxide uptake in wheat. As seen in curves 1 and 2 the fluorescence falls rapidly to a minimum, followed by a rise to a second maximum and then a decrease to the level of the steady value. Simultaneously the rate of carbon dioxide uptake goes through a series of convolutions which seem to be correlated with the fluorescence changes. This behavior occurs also under low oxygen pressures as shown by curves 3 and 4. The carbon dioxide records here are very irregular, owing to incomplete premixing of the carbon dioxide added to the tank nitrogen.

The curves of figure 5 and curves 5 and 6 of figure 4 show the transition in behavior from air to higher carbon dioxide concentrations.

Figure 6 illustrates the induction behavior accompanying changes in light intensity or in carbon dioxide concentration. A sequence of light intensities (without intervening dark periods) is shown in curve 1 for 0.03 percent carbon dioxide in air, and in curve 2 for 0.03 percent carbon dioxide in nitrogen. The induction effects seem to be somewhat more pronounced in air than under low oxygen pressure.

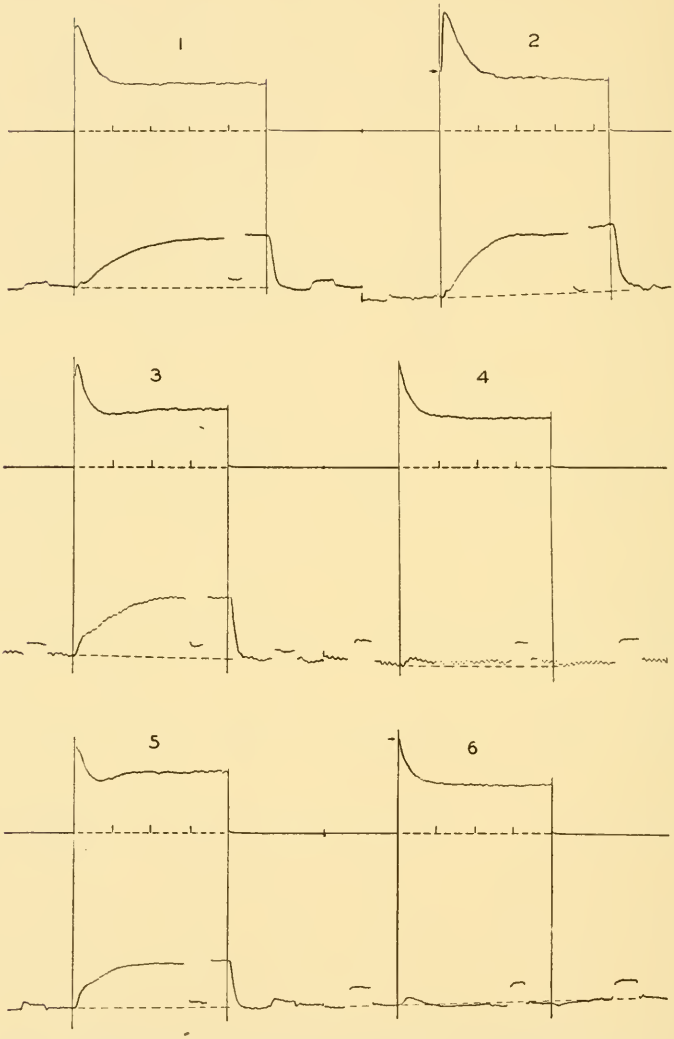

FIG. 3.—The induction behavior of wheat at high light intensities. The effects of oxygen and carbon dioxide pressures.

1. In 0.03 percent carbon dioxide, 20 percent oxygen; after 15 minutes light, 30 minutes dark rest.
2. In 0.03 percent carbon dioxide, low oxygen; after 15 minutes light, 30 minutes dark rest.
3. In 0.03 percent carbon dioxide, 20 percent oxygen; after 4 minutes light, 10 minutes dark rest.
4. In zero carbon dioxide, 20 percent oxygen; after 4 minutes light, 10 minutes dark rest.
5. In 0.03 percent carbon dioxide, 20 percent oxygen; after 15 minutes light, 10 minutes dark rest.
6. In zero carbon dioxide, "tank" oxygen, after 15 minutes light, 10 minutes dark rest.

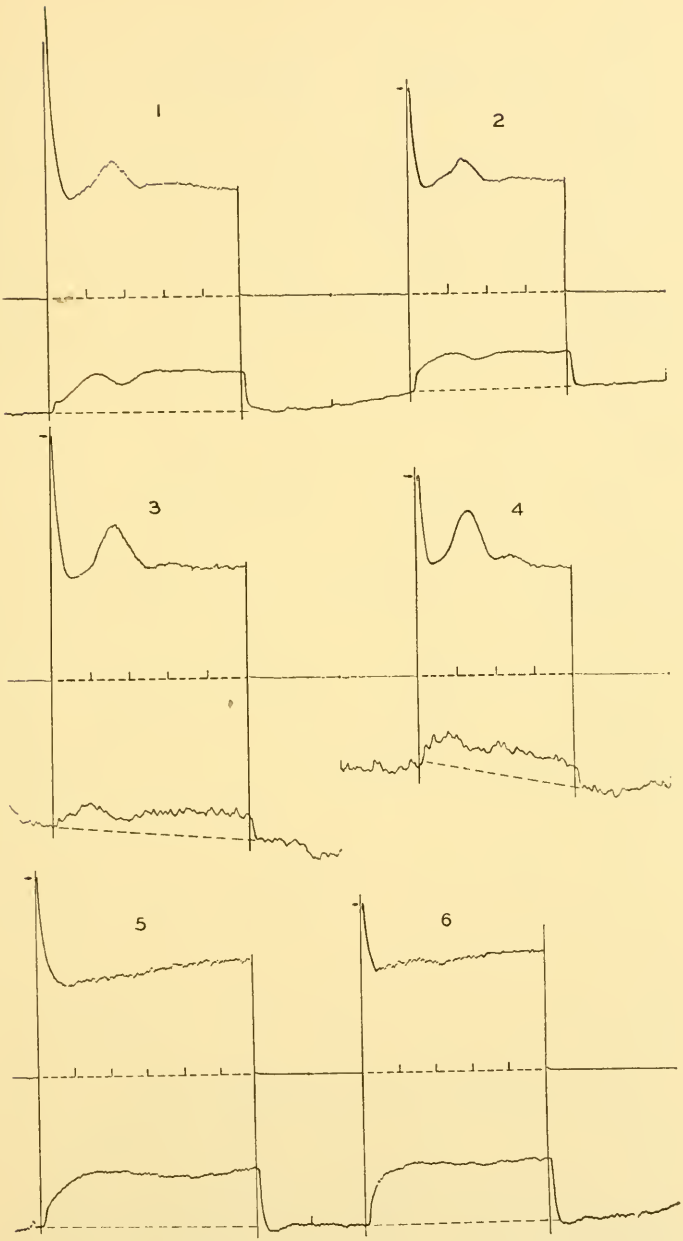

FIG. 4.—The induction behavior of wheat in high light intensities and high carbon dioxide pressures.

1. In 0.36 percent carbon dioxide in air, after 10 minutes light, 10 minutes dark rest.
2. In 0.36 percent carbon dioxide in air, after 5 minutes light, 5 minutes dark rest.
3. In 0.36 percent carbon dioxide in nitrogen, after 10 minutes light, 20 minutes dark rest.
4. In 0.36 percent carbon dioxide in nitrogen, after 5 minutes light, 5 minutes dark rest.
5. In normal air, after 10 minutes light, 10 minutes dark rest.
6. In normal air, after 6 minutes light, 5 minutes dark rest.

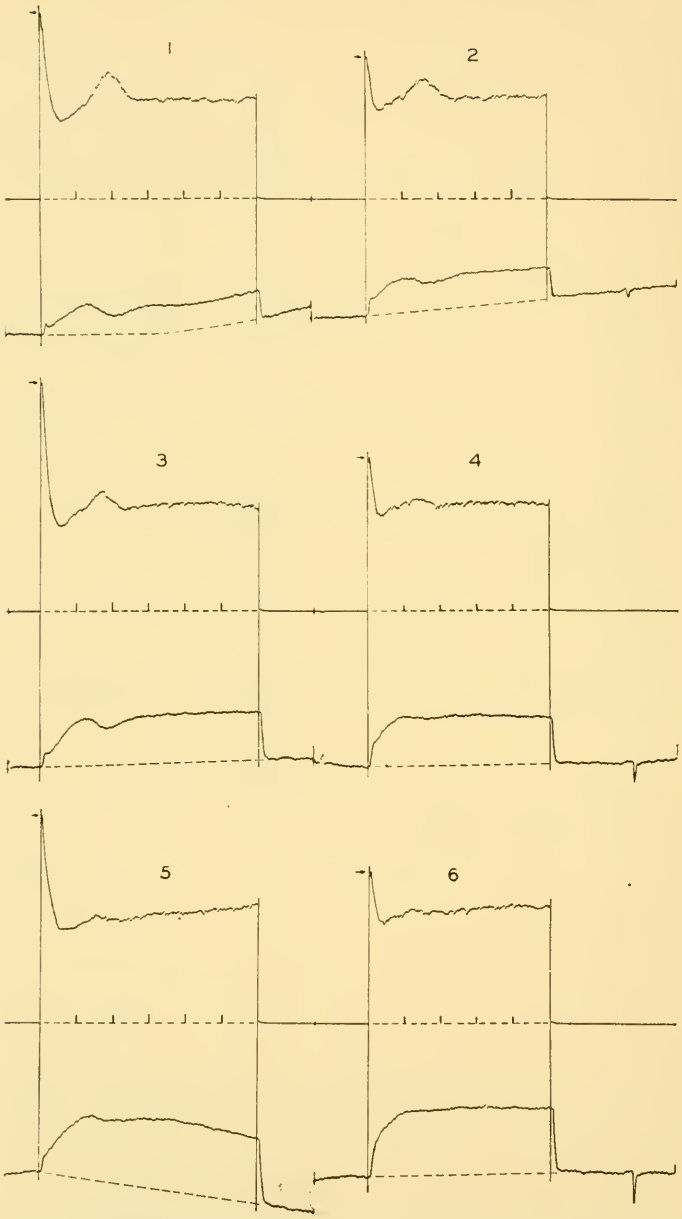

FIG. 5.—The induction behavior of wheat in high light intensities and high carbon dioxide pressures (in air). The effect of carbon dioxide pressure.

1. In 0.24 percent carbon dioxide, after 10 minutes light, 10 minutes dark rest.
2. In 0.24 percent carbon dioxide, after 6 minutes light, 5 minutes dark rest.
3. In 0.12 percent carbon dioxide, after 10 minutes light, 10 minutes dark rest.
4. In 0.12 percent carbon dioxide, after 6 minutes light, 5 minutes dark rest.
5. In 0.07 percent carbon dioxide, after 10 minutes light, 10 minutes dark rest.
6. In 0.07 percent carbon dioxide, after 6 minutes light, 5 minutes dark rest.

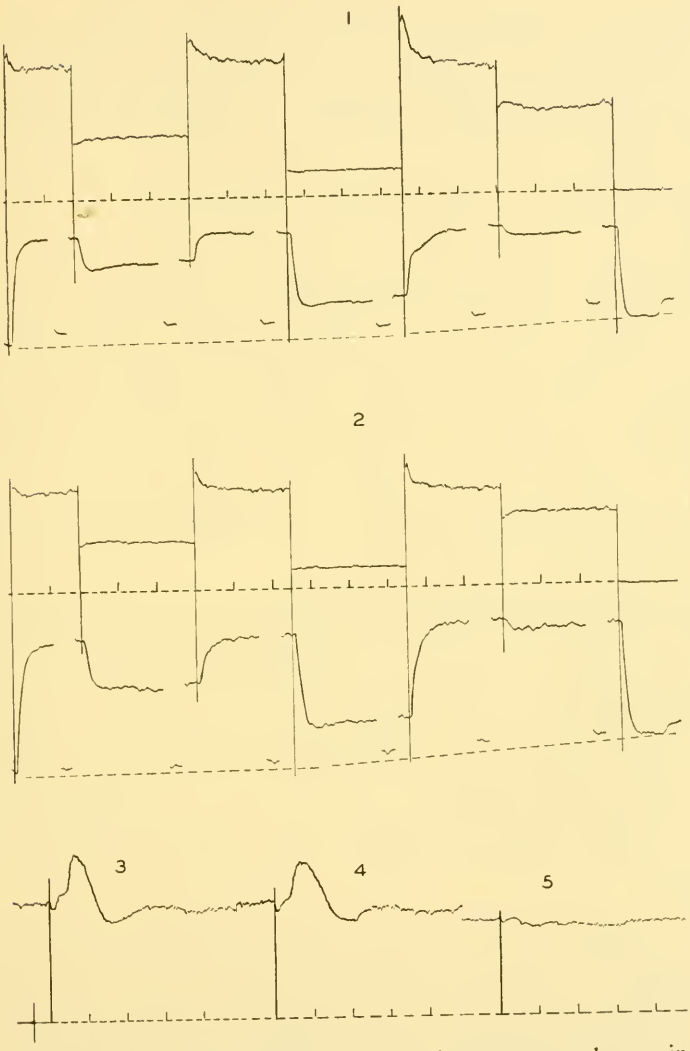

FIG. 6.—The induction behavior of wheat in response to changes in light and carbon dioxide pressure.

1. A sequence of intensities: (in relative units) 100, 49, 100, 21, 100, 74, zero. In normal air.
2. A sequence of intensities: (in relative units) 100, 49, 100, 21, 100, 74, zero. In 0.03 percent carbon dioxide in nitrogen.
3. Fluorescence only, in high light throughout. Carbon dioxide changed from 0.03 to 0.4 percent.
4. Fluorescence only, in high light throughout. Carbon dioxide changed from 0.03 to 4.0 percent.
5. Fluorescence only, in high light throughout. Carbon dioxide changed from 0.4 to 4.0 percent.

(For 3, 4, 5 vertical lines represent time of change in carbon dioxide concentration.)

Curves 3, 4, and 5 show the course of intensity of fluorescence following sudden changes in carbon dioxide concentration.

Unfortunately, our apparatus does not permit us to follow changes in the rate of carbon dioxide uptake accompanying such changes in carbon dioxide pressure, or to measure the final rate of photosynthesis in 4 percent carbon dioxide. However, we have been able to establish from steady-state measurements that at the high light intensity used (about 60×10^4 ergs/cm.^{2}/sec.) this wheat was under a carbon dioxide limitation at 0.03 percent carbon dioxide and not far from a light limitation at 0.4 percent carbon dioxide. The increase in the steady rate of photosynthesis from 0.03 to 0.4 percent carbon dioxide is about threefold; in comparison the change from 0.4 to 4 percent must have been small. It is highly probable here that the "burst" of fluorescence is associated with an increase in rate of photosynthesis. It is to be noted that the reverse case (not illustrated) of changing from 0.4 to 0.03 percent, and from 4 to 0.03 percent gave rise to changes in fluorescence so small as to be negligible compared to those of curves 3 and 4 of figure 6.

A number of experiments have been made on the steady-state behavior of photosynthesis and fluorescence in wheat. The technique used is illustrated in figure 7. The plants were exposed to a given light intensity for 10 minutes. The recording drum was then started and a record taken for the succeeding 3-minute period. A reading on the influent air was made during each interval. This procedure was repeated for each light intensity studied. Thermocouple readings of the incident light intensity were taken every 15 seconds during the recording in order to average out the effects of varying line voltage.

The induction behavior of *Chlorella* is illustrated in figures 8, 9, and 10. The behavior of cells cultured in 4 percent carbon dioxide resembles that of wheat, as shown by curves 1 and 2 of figure 8. Cells grown in air behave very differently. As shown in curves 3 to 8, the rate of carbon dioxide uptake follows a course similar to that found by Aufdemgarten (1939a) for *Stichococcus*. In comparison to the final value the early fluorescence maximum is not nearly as high as in wheat and is followed by a rapid decline to a pronounced minimum. A correlation between the minima in fluorescence intensity and in rate of carbon dioxide uptake is evident from these curves. A similar course is followed at higher carbon dioxide concentrations, as shown in curves 1, 2, and 3 of figure 9. The effect of light intensity is demonstrated in curves 4 to 10 of figure 9.

Figure 10 shows the behavior of *Chlorella* under various low carbon dioxide concentrations. As the carbon dioxide concentration is decreased the minima in both fluorescence and carbon dioxide uptake tend to disappear. (The peculiar appearance of the carbon

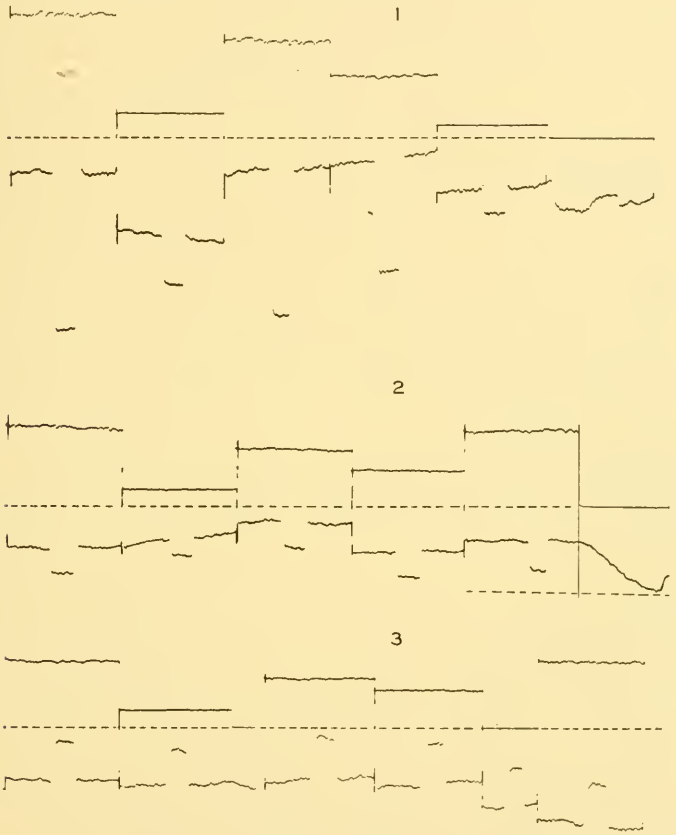

FIG. 7.—The steady-state behavior of wheat in response to light intensity. Record taken only from the 10th to 13th minute of illumination at each intensity. Highest intensity about 60 × 10^4 ergs/cm.^{2}/sec. in each series.

1. In 0.03 percent carbon dioxide in nitrogen. Average relative light intensities in sequence: 99.8, 21.6, 79.2, 50.0, 11.6, zero.
2. In about 0.006 percent carbon dioxide in nitrogen. Average relative light intensities in sequence: 65, 14.4, 48.6, 30.7, 64.4, zero.
3. In zero carbon dioxide in air. Average relative light intensities in sequence: 60.9, 13.8, 46.7, 32.3, zero, 62.3.

dioxide record of curve 1 is due to the fact that the zero drift of the primary galvanometer shifted the light spot entirely away from the exposed area of the amplifier photocell.) It is apparent in figure 10 that for *Chlorella* the rate of respiration measured 90 seconds after

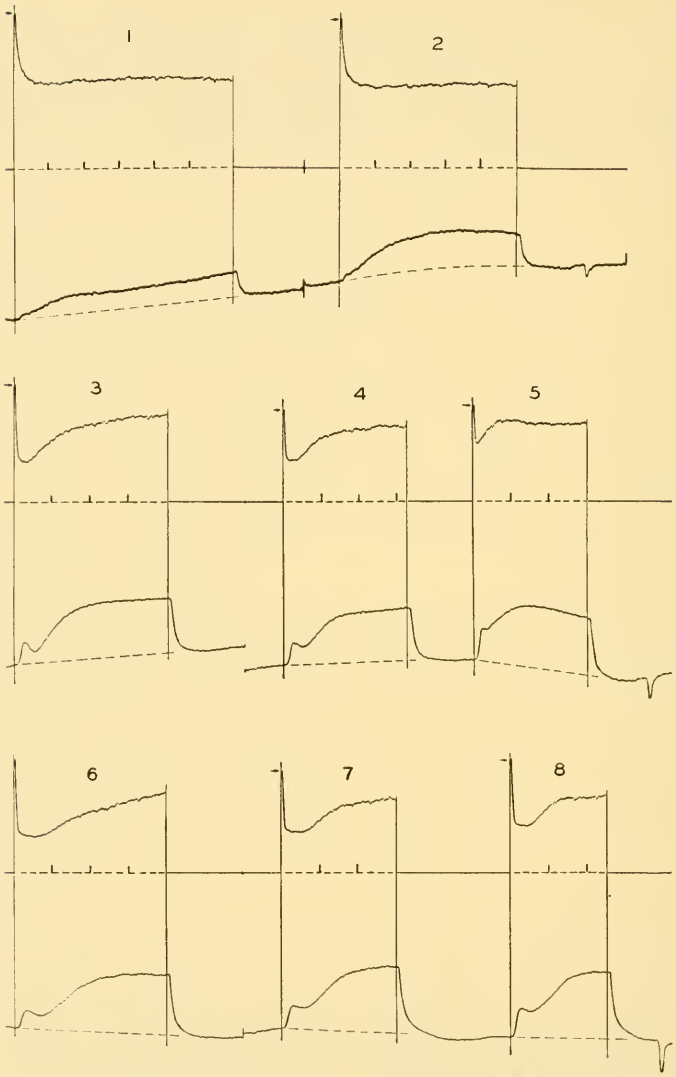

FIG. 8.—The induction behavior in high light of *Chlorella* cultured under two different conditions. The effect of the preceding dark rest.

1 and 2. *Chlorella* grown in 4.0 percent carbon dioxide, studied in 0.24 percent carbon dioxide.
1. after 10 minutes light, 20 minutes dark rest;
2. after 6 minutes light, 10 minutes dark rest.

3, 4, and 5. *Chlorella* grown in and studied in 0.03 percent carbon dioxide.
3. after 3 minutes light, 40 minutes dark rest;
4. after 4 minutes light, 10 minutes dark rest;
5. after 3 minutes light, 2 minutes dark rest.

6, 7, and 8. The same suspension of *Chlorella* as 3, 4, and 5 (above), left overnight in 0.33 percent carbon dioxide and low light (10×10^4 ergs/cm.^{2}/sec.). Studied in 0.03 percent carbon dioxide, high light.
6. after 30 minutes light, 60 minutes dark rest;
7. after 4 minutes light, 10 minutes dark rest;
8. after 3 minutes light, 3 minutes dark rest.

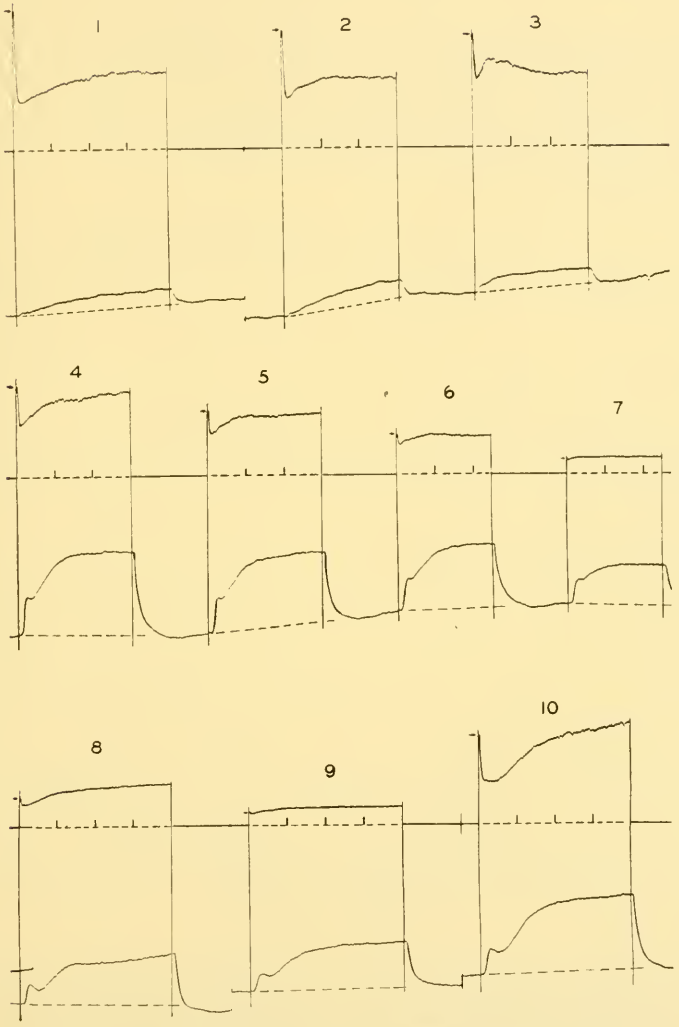

FIG. 9.—The induction behavior of *Chlorella* grown in air.

1, 2, and 3. In 0.33 percent carbon dioxide and high light after dark rests of 25, 10, and 2 minutes respectively.

4, 5, 6, and 7. In 0.03 percent carbon dioxide and after 2-minute dark rests. Sequence of light intensities: (in relative units) 100, 74, 49, 21 respectively.

8, 9 and 10. In 0.03 percent carbon dioxide and after 10-minute dark rests. Sequence of light intensities of: (in relative units) 49, 21, 100 respectively.

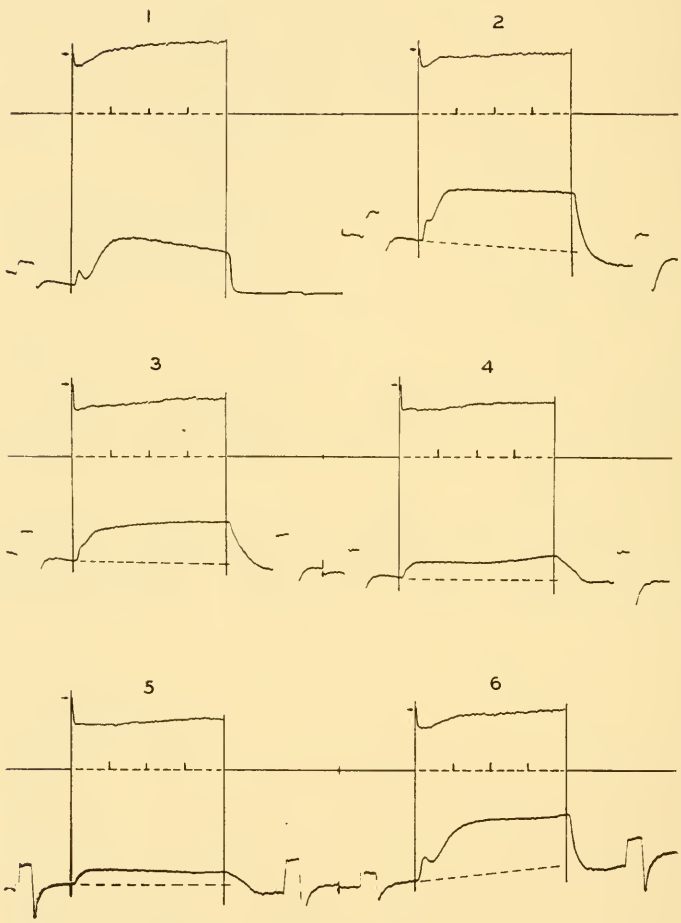

FIG. 10.—The induction behavior in high light of *Chlorella* grown in 0.03 percent carbon dioxide. The effect of carbon dioxide pressure. Each record taken after 5 minutes light in 0.03 percent carbon dioxide, 10 minutes dark with the carbon dioxide pressure specified.

1. In 0.03 percent carbon dioxide.
2. In 0.008 percent carbon dioxide.
3. In 0.003 percent carbon dioxide.
4. In 0.0003 percent carbon dioxide.
5. In zero carbon dioxide.
6. In 0.03 percent carbon dioxide (repeat of curve 1).

the period in high light is greater than the rate measured before illumination—as has been previously pointed out in the literature (cf. Emerson and Lewis, 1939).

In order to determine accurately the course of fluorescence intensity in the first seconds of illumination a few experiments have been made with a very fast galvanometer.[6] Three of these are shown in figure 11. The recording drum was speeded up to give these

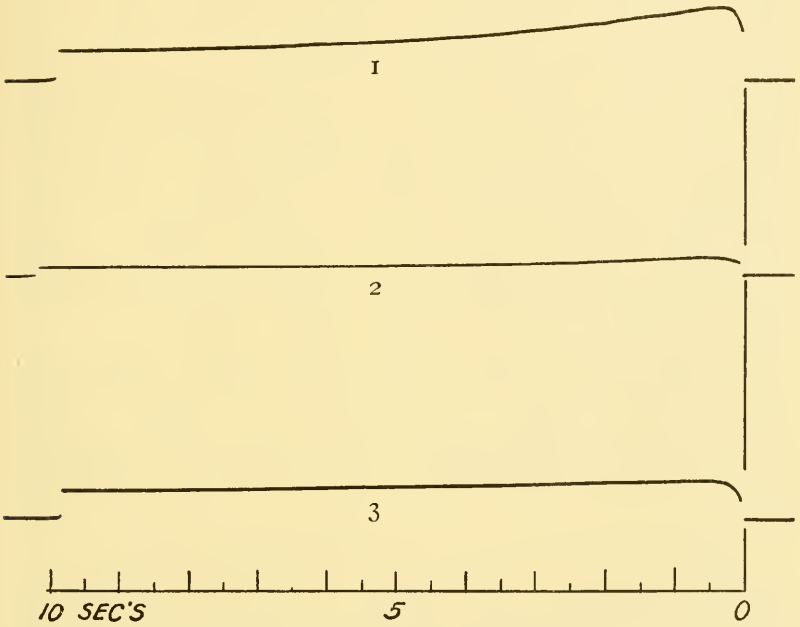

FIG. 11.—The fluorescence behavior of wheat and *Chlorella* in air during the first 10 seconds of illumination.

1. *Chlorella* in high light.
2. *Chlorella* in low light.
3. Wheat in high light.

records, which are reproduced nearly full size. For curve 1 the photocell was placed immediately in back of a suspension of *Chlorella* illuminated with full intensity (60×10^4 ergs/cm.^{2}/sec.). These conditions were duplicated in curve 2 except that the light intensity was cut in half. (The galvanometer sensitivity decreased between these two records owing to a drop in the battery current actuating the field electromagnet, so that the curves cannot be compared

[6] Kipp and Zonen "torsion string" galvanometer with electromagnet, 1/100-second period. Loaned by the Department of Terrestrial Magnetism, Carnegie Institution of Washington, courtesy of Dr. M. A. Tuve.

directly as to ordinate height.) Curve 3 was obtained with a layer of attached wheat leaves, one leaf thick, placed in front of the photocell. Further experiments along this line are now in progress.

DISCUSSION

The induction curves for carbon dioxide assimilation of *Stichococcus bacillaris* obtained by Aufdemgarten (1939a, 1939b)[7] have been essentially duplicated by our curves for *Chlorella* (figs. 8, 9, 10). His observations on the effects of different nutrient media on the initial maximum in the induction curves are being further investigated in this laboratory. The curves for carbon dioxide assimilation by wheat are consistent with those reported in previous papers (McAlister, 1937, 1939). Changes in technique (constant-flow method, increased sensitivity, and continuous recording) have made it possible to observe two effects not apparent before: 1, a rapid initial uptake of carbon dioxide (fig. 3, curves 1 and 2), and 2, a secondary depression in the rate of carbon dioxide uptake under high concentrations of carbon dioxide (figs. 4 and 5).

Our fluorescence curves for wheat are in almost complete agreement with those obtained by Franck and Wood (1936) on various excised leaves (cf. fig. 1, curves 1 and 2, etc.). Their observation of a rapid initial rise followed by a slower increase to the maximum is duplicated by our experiments using a very fast galvanometer (fig. 11).

Comparison of our fluorescence curves with those of other workers is more difficult owing to the different conditions employed. Kautsky and Marx (1937) obtained for various excised leaves a smoothly rising fluorescence curve approaching a maximum in 3.4 seconds. Their data could be fitted to a straight line by plotting the logarithm of the "fluorescence quenching" against time. In later work from the same laboratory Kautsky and Hormuth (1937) reported fluorescence curves followed during the first 50 seconds of illumination. Although these show a quick rise followed by a slower decay, the magnitudes of the changes involved are much smaller than those obtained by Franck and Wood and by us. These differences can be accounted for, at least in part, by the fact that Kautsky used as the exciting source ultraviolet radiation of probably rather low intensity.

[7] It is interesting to note here that the minimum in photosynthesis in intermittent light of about 1-minute intervals reported by McAlister (1937) for wheat has been confirmed by Aufdemgarten (1939a) for both *Hormidium* and *Stichococcus*.

In our studies over a wide range of intensities it is apparent that at low light the proportionate changes in fluorescence intensity are small.

The fluorescence curves obtained by Wassink and Katz (1939) for *Chlorella vulgaris* are also not directly comparable with ours because of the very low light intensities employed. Their highest light (1.9×10^4 ergs/cm.^{2}/sec.) was only about 1/25 of the intensity used in most of our experiments (as in figs. 8, 9, and 10). Their fluorescence curves show a rapid initial rise, followed by a slower rise to a maximum reached in 2 or 3 seconds; a decrease to a minimum at about 5 seconds; a rise to a second maximum at about 20 seconds; and a gradual decay continuing for several minutes. As in the case of Kautsky and Hormuth, the variations observed were proportionately small. Our equipment does not permit us to work effectively at light intensities as low as theirs. However, several of our curves obtained at lower intensities (ca. 10^5 ergs/cm.^{2}/sec.) show some similarity. Curve 5 of figure 1 is very similar to their curves except for the absence of their first maximum; curve 9 of figure 9 is also similar to their curves except for their final decay. Most of the work of Wassink and Katz was devoted to a study of the various phases of the fluorescence curve as functions of temperature, light intensity, and oxygen pressure on cells in which photosynthesis was totally inhibited by cyanide. Such a treatment completely prevents the final decay in fluorescence as clearly shown by their figure 4 (p. 153). Similar experiments have not yet been tried in this laboratory.

Our exploratory data make it clear that a wide range of induction phenomena is exhibited by *Chlorella* and by wheat under various conditions of the plants and their environment. More intensive and quantitative studies now being undertaken are necessary to make clear the complex interplay between the various external and internal factors and their effects on the induction behavior. This discussion will therefore be chiefly phenomenological, considering the relationships of the various observed effects rather than the mechanism by which they are brought about.

INTERPRETATION OF INDUCTION CURVES

The induction curves must first be examined in order to separate instrumental effects from the true plant behavior. The fluorescence curves obtained with the fast galvanometer (period 1/100 second) may be considered very close to the true course of the fluorescence intensity, since the time lag in recording is here very small (fig. 11).

The galvanometer used in the rest of the experiments (figs. 1 to 10) had a period of 1.2 seconds, and this slight lag must therefore be considered in interpreting the fluorescence curves. The recording of the rate of carbon dioxide assimilation is not nearly so rapid. Time is required for the gas stream to pass from the plant chamber to the absorption cell, for the thermocouple response, and for the response of the primary and secondary galvanometers. Fortunately these time lags are not entirely additive. However, there is in addition an integration or "smearing out" of the response. For instance the volume of air in the absorption cell at any instant represents a finite time period of the plant's activity. The galvanometer system may be thought of as responding in overlapping units of time of about 4 seconds each. During the quick initial "gulp" of carbon dioxide (curves 1 and 2 of fig. 3) the peak change in carbon dioxide passes through the absorption cell in much less than 4 seconds. The initial movement of the galvanometer is therefore at least partially a ballistic response with a correspondingly reduced sensitivity and shortened time of indication during this important part of the curve.

A number of the curves shown above have been redrawn in order to eliminate as far as possible the instrumental lag in the recording of the rate of carbon dioxide assimilation. This has been done by making tracings of the original curves and moving the carbon dioxide curve to the left a distance equivalent to the time of transit between the plant chamber and the absorption cell. The curves are also corrected for the decreased sensitivity at higher carbon dioxide concentration which is characteristic of the spectrographic method. In addition a broken line (·–·–·–) has been added to show the probable course of carbon dioxide assimilation in the plant which would give the recorded curve. This is appreciably different from the recorded curve only during the rapid changes of the first 10 seconds of illumination. The fluorescence curves have not been altered since they are reasonably accurate except for the first 2 or 3 seconds.

Perhaps the simplest type of induction behavior is that exhibited by wheat at low light intensity (fig. 1, curve 5) or following a very short dark rest (fig. 1, curves 6 and 7). These curves approximate linear behavior, i.e., induction effects are here approaching a minimum. It was under light intensities even lower than those in curve 5 of figure 1 that the fluorescence curves of Wassink and Katz and of Kautsky and Hormuth were obtained. It is possible that with greater resolution in our recording apparatus similar effects might be observed here. In any case these effects must be small compared to those obtained at higher intensities.

Figure 12 illustrates the behavior of wheat under high light intensities. The simpler case is shown in curve A obtained under low oxygen pressure. Here there is a strictly inverse relationship between the rate of carbon dioxide assimilation and the intensity of fluorescence. (However, the almost perfect mirror-image relationship of the two curves is purely fortuitous as to ordinate height since both intensity of fluorescence and rate of carbon dioxide assimilation are recorded in arbitrary and independently chosen units. This fact must be borne in mind in the examination of all the curves.) This inverse relationship is one which would be expected if it were assumed that the intensity of fluorescence is always a constant fraction of that part of the energy absorbed by chlorophyll which is not taken up by photochemical mechanisms. Although we have no factual basis for such an assumption, we shall adopt this point of view since it will simplify the discussion without greatly limiting its generality.

On the other hand the induction in air (20 percent oxygen) is more complex and has led us to superimpose the curves in low oxygen as broken lines (-----) for comparison in curve B. Let us assume that the broken line (behavior in low oxygen) represents an approach to an idealized case in which a strictly inverse relationship exists between rate of carbon dioxide assimilation and intensity of fluorescence. The hatched area between the lines would therefore represent the extent of a reaction which decreases both the intensity of fluorescence and rate of carbon dioxide assimilation. Since this reaction is associated with increased oxygen pressure it seems most logical to consider it a photooxidation, sensitized by chlorophyll, which (like photosynthesis) cuts down the intensity of fluorescence, but which also cuts down the rate of carbon dioxide assimilation by producing carbon dioxide or some intermediate which can be used in place of carbon dioxide in photosynthesis.

The essential point involved in the consideration of figure 12, *B*, is that for wheat in normal air there are two types of reaction affecting the induction curve of carbon dioxide assimilation. In one of these the rate of carbon dioxide uptake and intensity of fluorescence are related inversely; in the other, directly. This point makes no implications as to the nature of the two processes.

Several other cases of induction behavior are examined in figures 13 and 14. The same conventions have been used in presenting these curves as in figure 12 except that here we have no experimentally determined "ideal" curves for comparison. The drawn-in broken lines (-----) have been arbitrarily located so as to give an inverse

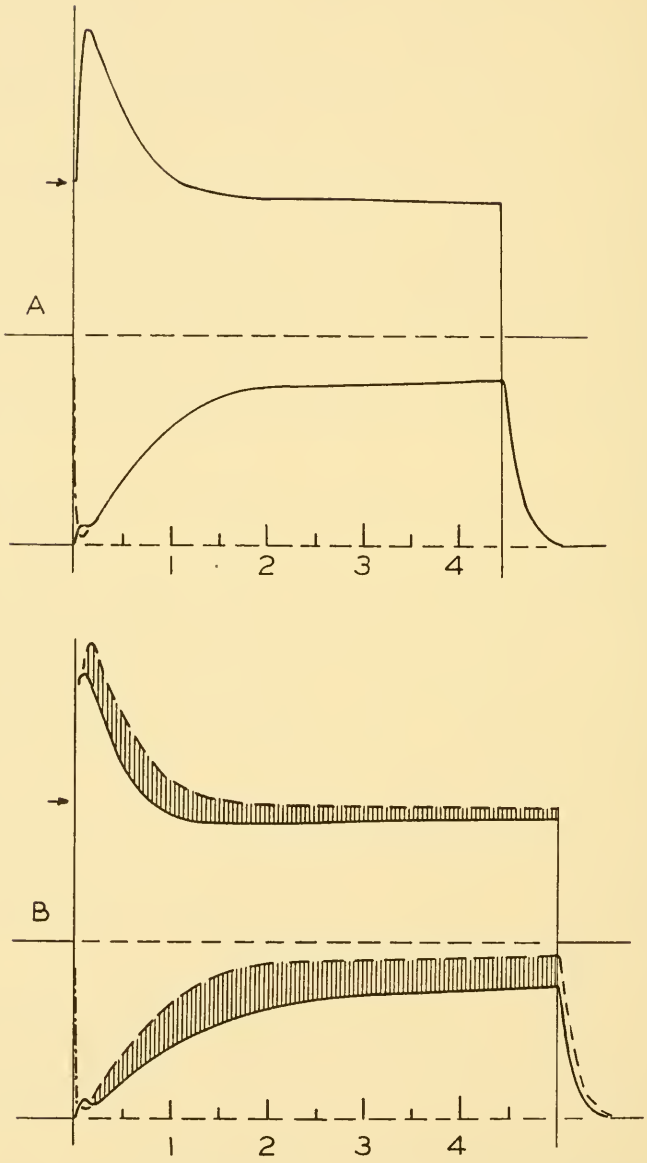

FIG. 12.—Induction behavior of wheat in low (A) and in normal (B) oxygen pressure. In 0.03 percent carbon dioxide, high light, and after 30-minute dark rests. The curves are derived from 2 and 1 of fig. 3.

relationship between the fluorescence and carbon dioxide curves. For instance the broken line superimposed on the fluorescence curve was first drawn so that no intensity less than the final value is reached. A broken line was then superimposed on the carbon dioxide curve so that the indicated rate of uptake always bears an inverse relation to the fluorescence intensity. The enclosed hatched areas therefore represent the minimum extent of reactions bearing a direct relationship to fluorescence.

In the case of wheat in high carbon dioxide (fig. 13, *A*) it is clear that a reaction takes place during the time of the minimum in fluorescence which involves a direct carbon dioxide-fluorescence relationship. For the rise in fluorescence following the minimum is accompanied by an increase in the rate of carbon dioxide assimilation. On the other hand, the inverse relationship between the second maximum in fluorescence and the second minimum in carbon dioxide assimilation is perfectly clear. This relationship is further borne out in the series of curves of figure 5. Here the second maximum in fluorescence and the second minimum in carbon dioxide assimilation are seen to be similarly affected by progressive changes in carbon dioxide concentration. A certain similarity to this behavior of wheat may be shown by *Chlorella* (fig. 13, *B*) although the first minimum in fluorescence is less marked and the second maximum is much smaller and more drawn out.

The peculiar behavior of *Chlorella* grown in air is marked by the predominance of a direct relation between carbon dioxide assimilation and fluorescence. In figure 14, *A*, the broken lines have been drawn arbitrarily straight across and parallel to the base lines. This is another case in which fluorescence data are of direct aid in interpreting the carbon dioxide assimilation curves. By itself, such a carbon dioxide assimilation curve may be interpreted in either of two ways: 1, photosynthesis starts out with a sudden "gulp" of carbon dioxide, then slows, and finally builds up to a steady rate; or 2, photosynthesis starts off at the maximum rate, but a carbon-dioxide-producing reaction occurs for a short period, causing a minimum in the assimilation curve. The fluorescence data reject the first interpretation.

A number of cases have been observed in which the induction in wheat approaches that of *Chlorella* grown in air. One of these is illustrated in figure 14, *B*. Here a distinct minimum in fluorescence is accompanied by a small but obviously correlated inflection in the assimilation curve. The importance of the "intermediate" cases such as figures 13, *B*, and 14, *B*, is that they deny the existence of any real

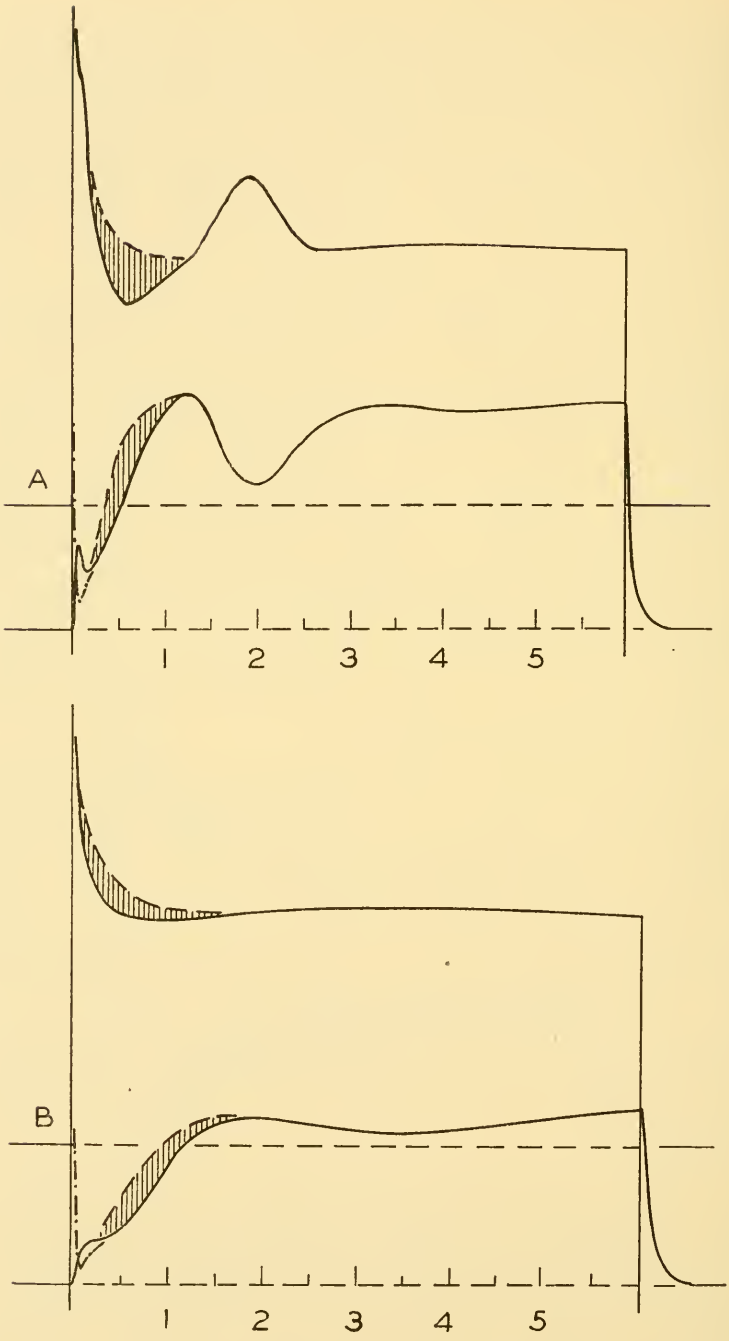

FIG. 13.—Induction behavior in 0.24 percent carbon dioxide and high light of wheat after 10 minutes dark rest (A) and of *Chlorella* grown in 4 percent carbon dioxide after 20 minutes dark rest (B). The curves are derived from 1 of fig. 5 and 1 of fig. 8 respectively. For convenience the ordinate scale for rate of carbon dioxide uptake in A has been reduced to $\frac{1}{2}$.

differences between the photochemical mechanism in *Chlorella* and in wheat. At first glance the induction phenomena shown by *Chlorella* grown in air (fig. 14, *A*) and wheat (fig. 12, *B*) appear very different. However, the occurrence of intermediate cases makes it clear that

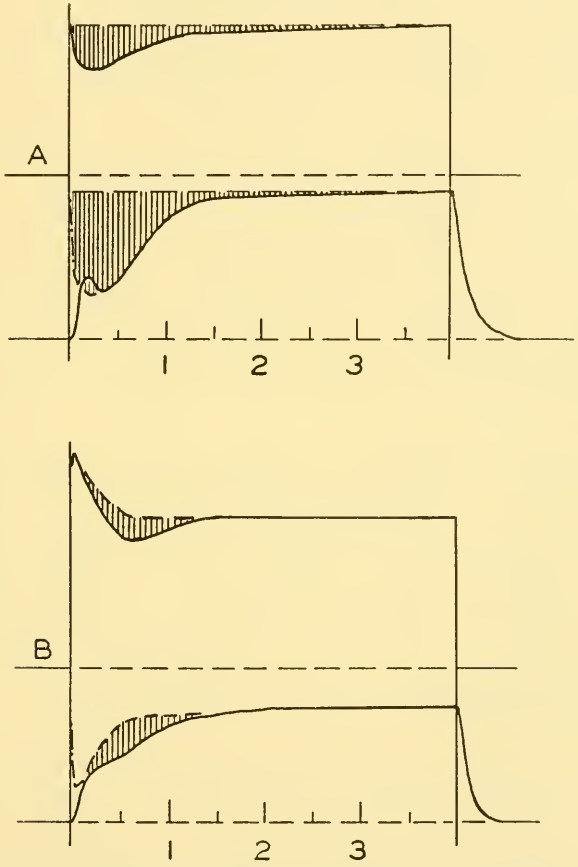

FIG. 14.—Induction behavior in 0.03 percent carbon dioxide and high light after 10 minutes dark rest for *Chlorella* grown in air (A) and for wheat (B). The curves are derived from 6 of fig. 10 and 5 of fig. 3 respectively.

this difference is due merely to the predominance of one or the other of at least two different processes in these extreme cases.

STEADY-STATE RELATIONS

Measurements of the steady-state conditions of fluorescence and rate of carbon dioxide assimilation were made with the hope of ob-

taining additional data which might be of help in the interpretation of the relationship between fluorescence and photosynthesis shown by induction studies. Similar studies have been made by Wassink et al. (1938), who have measured the intensity of fluorescence and rate of oxygen production under steady-state conditions. They report no change in the state of fluorescence (i.e., intensity of fluorescence proportional to incident intensity) in passing from a light-limiting condition of photosynthesis to light-saturation. Nor was fluorescence intensity influenced by any of a number of conditions which markedly affected the rate of photosynthesis (temperature, partial inhibition by cyanide, oxygen pressure). However, when photosynthesis was partially inhibited by urethane, the intensity of fluorescence was clearly raised. In a later paper Wassink and Katz (1939) showed an increase in intensity of fluorescence due to complete inhibition of photosynthesis by cyanide. Their highest incident intensity was less than 2×10^4 ergs/cm.^{2}/sec. Complete saturation was apparently reached at this intensity in the number 9 carbonate-bicarbonate buffer of Warburg (1920).

In the present experiments on wheat [8] we find a marked change in fluorescence in passing from light-limiting to carbon-dioxide-limiting conditions. This is seen in both figures 15 and 16. Figure 15 shows the rate of carbon dioxide assimilation and intensity of fluorescence for wheat at 23° C. and 0.03 percent carbon dioxide in both air and tank nitrogen as a function of incident intensity. For the two cases in air (duplicate experiments) the intensity of fluorescence is seen to rise above the initial straight line concurrently with a marked departure from light-limiting conditions.

Figure 16 exhibits a similar behavior in a comparison of intensity of fluorescence under 4 percent and 0.03 percent carbon dioxide. Here, presumably, the wheat was entirely under light-limiting conditions at 4 percent carbon dioxide, but at 0.03 percent carbon dioxide a carbon dioxide limitation begins at relatively low incident intensity (cf. fig. 15) and the fluorescence intensity rises above the initial line.

Warburg (1920) has shown that *Chlorella* in high light and high carbon dioxide produces oxygen at a considerably greater rate in 2 percent than in 20 percent oxygen. A similar behavior as to carbon dioxide assimilation in wheat is shown by figure 15. Here under carbon-dioxide-limiting conditions the rate is 30 percent higher in

[8] Similar quantitative experiments on algae have not been feasible with our present equipment. Such work is anticipated.

0.5 percent than in 20 percent oxygen. As Warburg pointed out, this suggests that the rate of a reaction involving oxygen and opposing photosynthesis is diminished in passing from high to low oxygen, and consequently the rate of carbon dioxide assimilation is increased. The intensity of fluorescence is lower in this experiment in 0.5 percent oxygen than in normal air. However, the

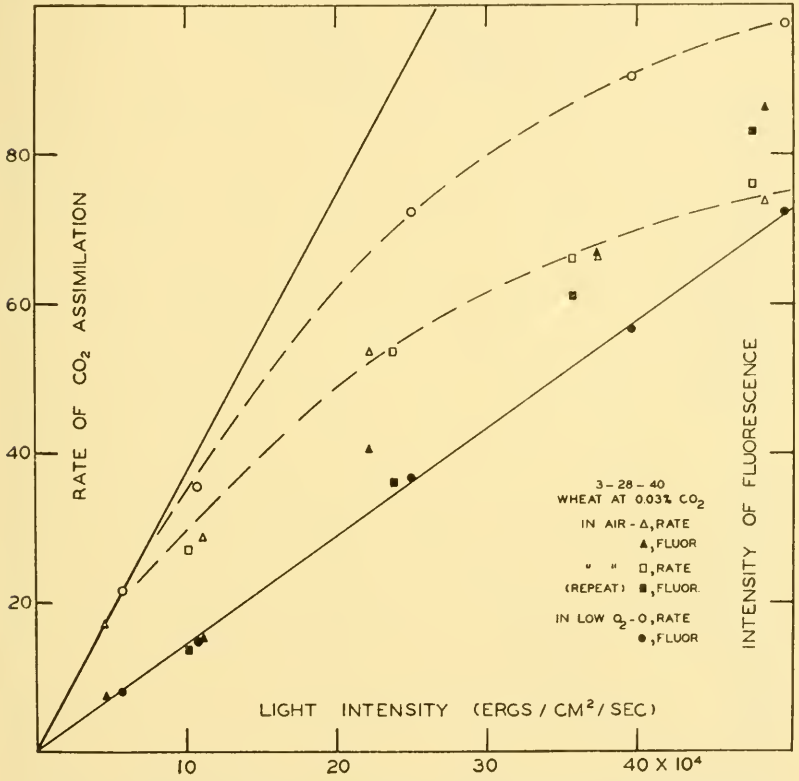

FIG. 15.—Carbon dioxide assimilation and intensity of fluorescence versus incident light intensity. A comparison of low and normal oxygen pressures for wheat at 0.03 per cent carbon dioxide.

intensities of fluorescence in these two cases of 0.5 percent and 20 percent oxygen are reversed in the steady state as compared to their relationship in and immediately following the induction period when the fluorescence is higher for the low oxygen condition (see fig. 12, *B*). Thus the fluorescence data suggest a different possibility, namely, a transition from the situation in the induction period already discussed to a case in the steady state where oxygen is able

to inhibit photosynthesis. Reducing the oxygen pressure then allows a greater rate of photosynthesis and the fluorescence is consequently reduced. Further experiments, wherein the transition from the induction phase to the steady state is followed more closely are

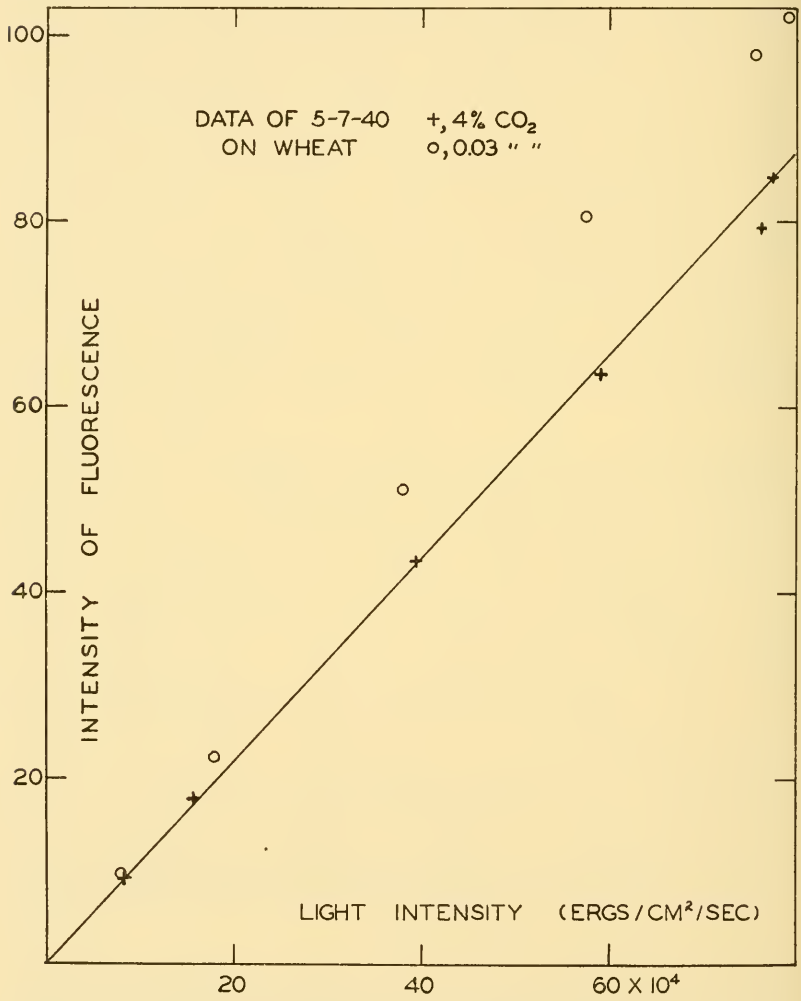

FIG. 16.—Intensity of fluorescence versus incident light intensity for wheat at 4 percent and at 0.03 percent carbon dioxide.

anticipated and should distinguish between these two opposing explanations.

It is interesting to note that this oxygen effect of relatively large proportions occurs under natural growing conditions and hence may

be of importance in studies involving vegetative growth. As yet it has been observed only in wheat, but other plants should be examined to determine the generality of the phenomenon. Because of its magnitude and intimate relationship with photosynthesis it should not be considered as an effect of light on the normal (dark) respiration.[9] Curve 3 of figure 7 indicates, as was previously reported by McAlister (1939), that respiration of wheat in light (in the absence of photosynthesis) is observed to be of the same magnitude as in darkness.

In comparing our data on wheat with those of Wassink et al. (1938) on *Chlorella,* it must be borne in mind that their incident intensities were nearly two orders of magnitude smaller than ours. Although our equipment does not permit accurate work at such low intensities, in this region we likewise find no marked departure from a linear relationship between intensity of fluorescence and incident light intensity. The only marked departure that we do find appears to be due to a carbon dioxide limitation, whereas with the number 9 buffer and the low intensities used, Wassink et al. were not far from light-limiting conditions in most of their experiments. In none of their experiments did they attain a carbon dioxide limitation. Consequently our data are not at great variance with theirs.

The young wheat used in our experiments requires very high intensity to reach light-saturation. In experiments, as yet incomplete, we have used incident intensities as high as 140×10^4 ergs/cm.^{2}/sec., which are twice as high as those of figure 16 and three times the intensity in visible solar radiation. At this highest intensity we have found that carbon dioxide assimilation in 0.4 percent carbon dioxide is still increasing though not at the light-limited rate. In the experiments of Hoover et al. (1933) incident intensities up to about 70×10^4 ergs/cm.^{2}/sec. were used. It is important to point out here that their Mazda radiation was filtered only by a 2.5-mm. layer of saturated copper sulphate solution. Consequently the incident intensities effective in producing photosynthesis in their experiments were from 20 to 30 percent of the values reported. However, their experiments clearly indicate that a very high value for light-saturation is to be expected for young wheat.

THE "DARK PICK-UP" OF CARBON DIOXIDE

A dark pick-up of carbon dioxide is apparent in all the assimilation curves obtained with the continuous-flow method. That it is greater than the instrumental lag is seen when it is compared to this lag,

[9] See also Gaffron (1940).

as for example in figure 1, curve 4, figure 5, curves 4 and 6, and figure 8, curves 2, 5, and 8. The sharp dip at the end of these curves (in darkness) represents the sweeping out of the system of a 15-seconds' accumulation of respired carbon dioxide upon resumption of the air flow. This gives a measure of the total instrumental lag in the case of wheat and of the instrumental plus liquid-to-gas diffusion time in the case of *Chlorella* (fig. 8).

A greatly increased time for the dark pick-up is evident when the plants have been in low carbon dioxide concentration during the light period. This is seen in curves 1, 3, 4, and 5 of figure 2, curves 3, 4, and 5 of figure 10, and particularly in curve 2 of figure 7. In this latter case, where the concentration of oxygen was also low, the dark pick-up has a half time of more than 1 minute.

Much more work remains to be done on this dark pick-up, particularly when the present type of experiment is put on a more quantitative basis. The present work with the continuous-flow method has not shown a dark pick-up lasting for 15 or 20 seconds following a high rate of photosynthesis as was previously reported (McAlister, 1939). This discrepancy is being investigated and will be reported on in the near future.

GENERAL DISCUSSION

It has been the purpose of this paper to examine a wide range of induction phenomena rather than to study any one of them in detail. The apparent relationships between these various effects have been pointed out. A correlation between the fluorescence intensity and rate of carbon dioxide uptake has been clearly shown in a number of cases, although this has involved sometimes a direct, and at other times an inverse, relationship.

Induction phenomena have been observed whenever a sudden increase in the rate of photosynthesis was brought about. Particularly significant are the fluorescence curves accompanying sudden changes in carbon dioxide concentration. These show clearly that fluorescence is somehow affected by the rate of photosynthesis. On the other hand it must be pointed out that changes in intensity of fluorescence have occasionally been observed without accompanying deviations in the rate of carbon dioxide uptake (e.g., curve 4 of fig. 2, and curve 3 of fig. 9, and others not shown).

Our data make it clear that the induction of photosynthesis is not a single or simple process. It is conceivable that during this period three types of reactions are proceeding toward the steady-state condition: 1, photosynthesis, involving the reduction of carbon dioxide;

2, photooxidation, an oxidation of certain more stable intermediates or products of photosynthesis, sensitized by chlorophyll; and 3, oxidation, a direct burning of unstable intermediates of photosynthesis. Of these the first two are dependent, the third independent, of energy transfer from activated chlorophyll.

The simple viewpoint which we have taken of the transfer of energy from activated chlorophyll is that such energy may be: 1, contributed to a photochemical process; 2, re-emitted as fluorescence; or 3, lost as heat. It is thus possible that the intensity of fluorescence may be influenced by either photosynthesis or photooxidation or by both processes simultaneously. Our data show that some such relationship does exist, but the details of the relation are obscured by our lack of knowledge of the mechanism of the quenching of fluorescence.

Another line of evidence comes from the dependence of the induction effects on the length of the previous dark rest. This well-established phenomenon may be accounted for by either of two alternate hypotheses: 1, Some material, formed by respiration, gradually accumulates (up to a maximum level) in the dark. In its subsequent photooxidation in light, transient intermediates are formed which somehow aid in the emission of fluorescence. 2, Some intermediate or product of photosynthesis, formed in light, inhibits fluorescence and increases the rate of photosynthesis. In the dark the concentration of this substance progressively decreases.

Either of these two hypotheses can be made to account for the induction in both carbon dioxide uptake and fluorescence of wheat in 0.03 percent carbon dioxide (fig. 12). However, the implications of the two hypotheses are very different. The first[10] postulates that photosynthesis starts out immediately at about the level of the steady rate. The induction in carbon dioxide uptake is due to the carbon dioxide (or carbon-dioxide-sparing substance) produced by the initial photooxidation. This also means that there is a direct relationship between rate of carbon dioxide *production* and intensity of fluorescence, i.e., an *inverse* relationship between rate of carbon dioxide *uptake* and intensity of fluorescence.

The second hypothesis, on the other hand (as suggested above, p. 23), supposes that the rates of both photosynthesis and photooxidation bear an inverse relationship to the intensity of fluorescence. There is an initial "gulp" of carbon dioxide and momentary quenching of fluorescence which corresponds to the amount of "intermediate"

[10] Suggested to us by Dr. James Franck.

adsorbed in the neighborhood of the chlorophyll and not destroyed in the dark. Thereafter the concentration of this intermediate first decreases until its rate of formation equals its rate of utilization, then it slowly increases to the steady-state value. Correspondingly, the fluorescence rises abruptly to a maximum and then slowly decays, while the rate of carbon dioxide assimilation falls sharply to a minimum and then rises gradually to the equilibrium rate.

Neither of these hypotheses will account for all the data obtained. The first is certainly inconsistent with the burst of fluorescence caused by a suddenly increased carbon dioxide concentration (curves 3 and 4 of fig. 6) and makes difficult any interpretation of the several observed cases in which carbon dioxide and fluorescence are directly related (figs. 13 and 14). The second does not explain the inverse relation between the second carbon dioxide minimum and the second fluorescence maximum found in wheat at high carbon dioxide concentration (fig. 13, *A*). However, because it seemed more consistent with the other data, we have taken this viewpoint as a basis for discussion.

From this second viewpoint the induction shown by wheat in 0.03 percent carbon dioxide in nitrogen is chiefly the building up of the rate of photosynthesis accompanied by a decay in fluorescence.[11] In air a photooxidation occurs as a secondary process, prolonging the induction in carbon dioxide uptake.

In *Chlorella* either one or the other of these two processes may be made to predominate. This alga quickly adapts itself to environmental conditions. Cells grown in 4 percent carbon dioxide show, when first studied, an induction generally similar to that of wheat (as fig. 13, *B*). After a few hours of light in 0.03 percent carbon dioxide the fluorescence curve develops a noticeable minimum during the induction period. After 24 hours in 0.03 percent carbon dioxide the induction behavior has changed completely to that shown by cells which have never been in high carbon dioxide (as fig. 14, *A*). During this time the secondary photooxidation progressively attains a more important role in the induction behavior until all other effects seem to be obscured.[12]

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[11] This decay may also be brought about in zero carbon dioxide if oxygen is present (curves 4 and 6 of fig. 3). This may be interpreted as due to the opposing effects of photosynthesis and photooxidation, for which the carbon dioxide uptake curve is the net result.

[12] This experience may be related to Aufdemgarten's observation (1939b) that the minimum in the induction curve of carbon dioxide uptake is dependent on the composition of the nutrient media used.

SUMMARY

Simultaneous measurements of intensity of fluorescence and rate of carbon dioxide assimilation during and following the induction period in wheat and in *Chlorella* are reported. While these observations are to be regarded as exploratory and preliminary, they permit the following conclusions:

1. Any sudden change in conditions of illumination or of carbon dioxide concentration that produces a large increase in the rate of photosynthesis also gives rise to a "burst" in the intensity of fluorescence. When this change is from darkness to high light, the burst of fluorescence produced can be resolved into three parts, as was previously shown by Franck and Wood. The intensity of fluorescence rises instantly (less than 0.01 second) to a height about equal to the final equilibrium value, then more slowly (in about 1 second) rises two or three times higher to a maximum which is followed by a decay (lasting about 1 minute) to the equilibrium value. The simultaneously observed rate of carbon dioxide assimilation follows a curve that is inversely related to the changes in fluorescence. When the recorded curve is corrected for instrumental effects it is apparent that the rate of carbon dioxide uptake starts at a value at least as high as the equilibrium rate, then quickly drops to a low minimum (but not negative) value, followed by a rise asymptotically approaching the final rate. At low oxygen pressures (less than $\frac{1}{2}$ percent) the curves of fluorescence and rate of carbon dioxide uptake are almost exact mirror images of each other (as to time).

2. The changes in rate of carbon dioxide assimilation observed in the induction period of wheat under normal air conditions (20 percent oxygen and 0.03 percent carbon dioxide) are caused by two processes, of which one exhibits an inverse relation to intensity of fluorescence and the other is directly related. The dependence of this second type on oxygen pressure and the observation of a greater rate of carbon dioxide uptake under low oxygen pressures suggests that this second type of reaction is a photooxidation.

3. In *Chlorella* the induction behavior is greatly influenced by the previous conditions of culture. Cells grown in high carbon dioxide show a behavior quite comparable to that of wheat. In the induction shown by cells acclimated to low carbon dioxide, the photooxidation type of reaction predominates to such an extent that pronounced minima are produced in both the fluorescence and carbon dioxide uptake curves.

4. Under carbon dioxide concentrations greater than that of normal air the induction phenomenon in wheat is complicated by a second maximum in fluorescence occurring after about 1-minute illumination. At the same time a minimum in rate of carbon dioxide uptake is observed which clearly bears an inverse relationship to this second maximum of fluorescence.

5. Curves relating intensity of fluorescence and rate of carbon dioxide uptake to incident intensity have been obtained from measurements made under steady-state conditions following the induction period. These show a marked change in fluorescence in passing from light-limiting to carbon-dioxide-limiting conditions. The intensity of fluorescence rises above the initial straight line concurrently with a marked departure from light-limiting conditions.

The rate of carbon dioxide assimilation in wheat in high light and 0.03 percent carbon dioxide is 30 to 50 percent higher in 0.5 percent than in 20 percent oxygen. This suggests that for young wheat a reaction of large proportions opposing photosynthesis is always depressing the rate of carbon dioxide assimilation under natural growing conditions.

LITERATURE CITED

Aufdemgarten, H.

1939a. Zur Kenntnis der sogenannten Induktionsvorgänge bei der Kohlensäureassimilation. Planta, vol. 29, pp. 643-678.

1939b. Weitere Untersuchungen mit dem Gaswechselscheiber über die Kohlensäureassimilation. Planta, vol. 30, pp. 343-352.

Emerson, R., and Lewis, C. M.

1939. Factors influencing the efficiency of photosynthesis. Amer. Journ. Bot., vol. 26, pp. 808-822.

Franck, J., and Herzfeld, K. F.

1937. An attempted theory of photosynthesis. Journ. Chem. Phys., vol. 5, pp. 237-251.

Franck, J., and Wood, R. W.

1936. Fluorescence of chlorophyll in its relation to photochemical processes in plants and organic solutions. Journ. Chem. Phys., vol. 4, pp. 551-560.

Gaffron, H.

1940. Studies on the induction period of photosynthesis and light respiration in green algae. Amer. Journ. Bot., vol. 27, pp. 204-216.

Hoagland, D. R., and Arnon, D. I.

1938. The water-culture method for growing plants without soil. Univ. California Agr. Exp. Stat. Circ. 347, pp. 1-39. Also *in* Ann. Rep. Smithsonian Inst. for 1938, pp. 461-488.

Hoover, W. H., Johnston, E. S., and Brackett, F. S.

1933. Carbon dioxide assimilation in a higher plant. Smithsonian Misc. Coll., vol. 87, No. 16, pp. 16-17.

KAUTSKY, H., and HORMUTH, R.
1937. Die Abhandigkeit des Verlaufs der Fluoreszenzkurven grüner Blätter vom Sauerstoffdruck. Biochem. Zeitschr., vol. 291, pp. 285-311.

KAUTSKY, H., and MARX, A.
1937. Photographische Registrierung und Auswertung der Fluoreszenzintensität grüner Blätter. Biochem. Zeitschr., vol. 290, pp. 248-260.

McALISTER, E. D.
1937. Time course of photosynthesis for a higher plant. Smithsonian Misc. Coll., vol. 95, No. 24, pp. 1-17.
1939. The chlorophyll-carbon dioxide ratio during photosynthesis. Journ. Gen. Physiol., vol. 22, pp. 613-636.

ORNSTEIN, L. S., WASSINK, E. C., REMAN, G. H., and VERMEULEN, D.
1938. Theoretical considerations concerning the relation between chlorophyll fluorescence and photosynthesis in green plant cells. Enzymol., vol. 5, pp. 110-118.

VERMEULEN, D., WASSINK, E. C., and REMAN, G. H.
1937. On the fluorescence of photosynthesizing cells. Enzymol., vol. 4, pp. 254-268.

WARBURG, O.
1920. Über die Geschwindigkeit der photochemischen Kohlensaurezersetzung in lebenden Zellen. II. Biochem. Zeitschr., vol. 103, p. 188.

WASSINK, E. C., and KATZ, E.
1939. The initial changes of chlorophyll-fluorescence in *Chlorella*. Enzymol., vol. 5, pp. 145-172.

WASSINK, E. C., VERMEULEN, D., REMAN, G. H., and KATZ, E.
1938. On the relation between fluorescence and assimilation in photosynthesizing cells. Enzymol., vol. 5, pp. 100-109.