LIGHT PRODUCTION BY GREEN PLANTS*

By BERNARD L. STREHLER and WILLIAM ARNOLD

(From the Biology Division, Oak Ridge National Laboratory, Oak Ridge)

(Received for publication, January 26, 1951)

The present communication offers evidence which suggests that the photosynthetic reactions are at least slightly reversible, inasmuch as plants which have been irradiated give off light for a considerable period of time after illumination. It also seems likely that this reaction is quite distinct from fluorescence or simple phosphorescence in its behavior.

The phenomenon here described was uncovered in an attempt to demonstrate the formation of energy-rich phosphorus during photosynthesis using the firefly luminescent system as an indicator of ATP formation (1). A mixture of chloroplasts and purified firefly extract gave appreciable light following illumination. However, the chloroplasts were found to be capable of doing this in the absence of the firefly extract, thereby ruling out, at the present, any conclusions as to phosphate intervention.

Materials and Methods

The light was measured with a 1P21 photomultiplier followed by two stages of amplification feeding into a Brown recorder. In order to give constant Brown recorder readings a circulating system was used. (See Fig. 1.)

Chlorella, *Scenedesmus*, and *Stichococcus* were grown in Knop's solution at 20°C., aerated with 5 per cent CO_2, and illuminated with fluorescent tubes.

Characteristics of Chlorella Luminescence

The simple experiment of starting the pump in the circulating system (Fig. 1) at various times after the exciting light had been turned off was sufficient to show that light was emitted at room temperature for as long as 30 seconds after illumination. Light production could be demonstrated for as long as 2 minutes after illumination when quanta were counted with the photomultiplier at liquid nitrogen temperatures. The decay curves shown in Fig. 2 were obtained by replacing the Brown with a Brush recorder (which has a flat frequency response from D. C. to 100 cycles per second). In these curves the zero values have been set equal to 1 although in the 28°C. experiment much more light was emitted (see Fig. 6). It will be seen that the temperature has a profound effect on the shape of the curve. A good fit is obtained for a bimolecular reaction at 6.5°C. while at 28°C. the best fit is obtained when the reaction order is 1.6.

* Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

Fig. 3 gives the intensity of the luminescence as a function of the exciting light intensity. The cells were illuminated in a Carrel flask and then pumped into the photomultiplier housing. The intensity of illumination was controlled

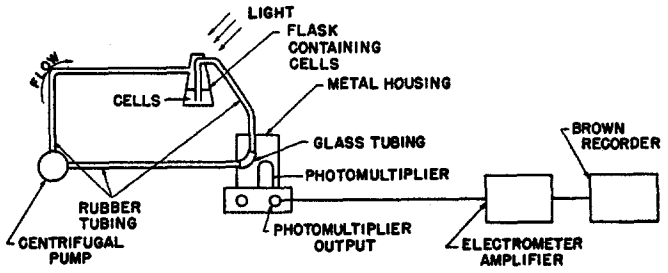

FIG. 1. Schematic diagram of apparatus used to measure light production by green algae.

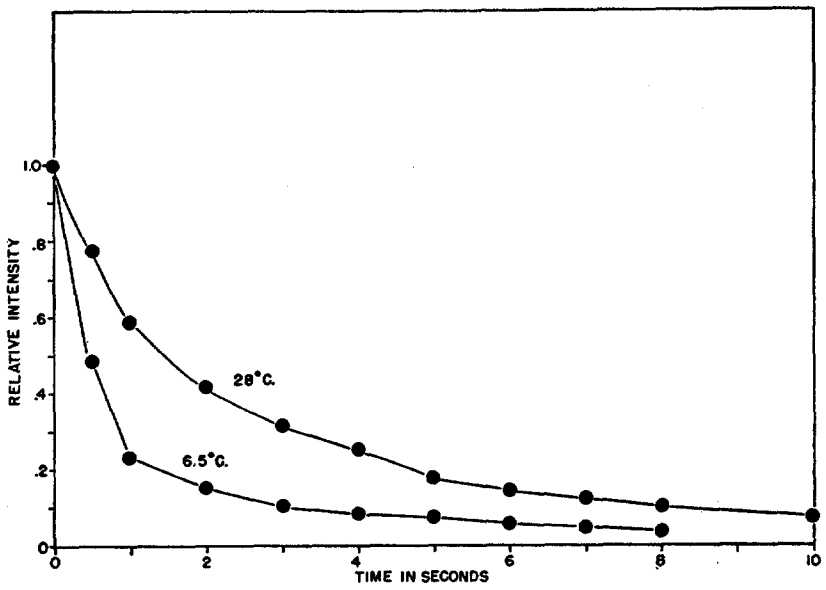

FIG. 2. Decay curves of *Chlorella* luminescence at two different temperatures (28°C. and 6.5°C.).

with an iris diaphragm while the relative light intensity was determined with a photocell.

It is obvious that this emitted light from plants must be of very low intensity or it would have been reported by earlier workers. By letting the suspension of cells in the circulating system flow in a glass tube very close to a type 1 N

spectroscopic plate it is possible to obtain considerable darkening in a few hours. From the geometry, the time of exposure, the cell density, and the sensitivity of the plate as given by Eastman Kodak Company (2) we calculate

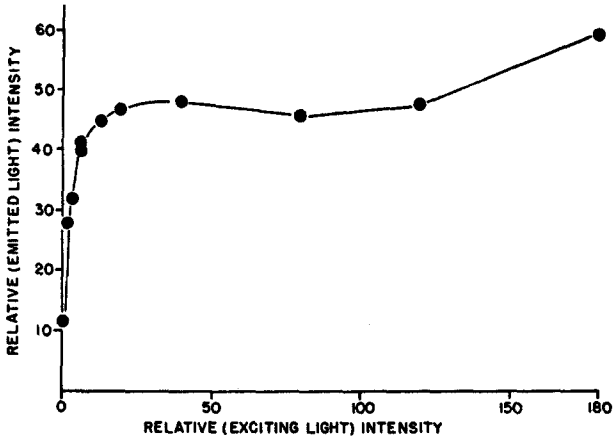

FIG. 3. Exciting light intensity *versus* luminescence intensity of *Chlorella*.

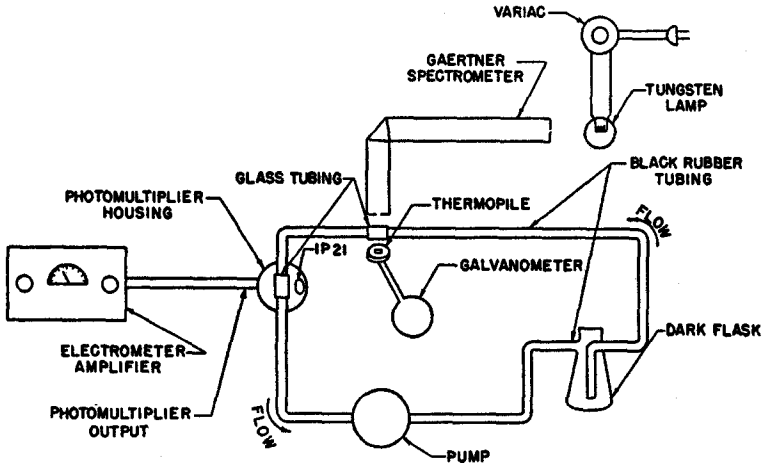

FIG. 4. Schematic diagram of apparatus used to determine the action spectrum of *Chlorella* luminescence.

the emission for each *Chlorella* cell to be 7 quanta per second near the beginning of the decay curve. While no great accuracy is claimed for this figure we believe it is correct to an order of magnitude. This is about one millionth of the absorbed energy below saturation.

The action spectrum for *Chlorella* luminescence was determined by using

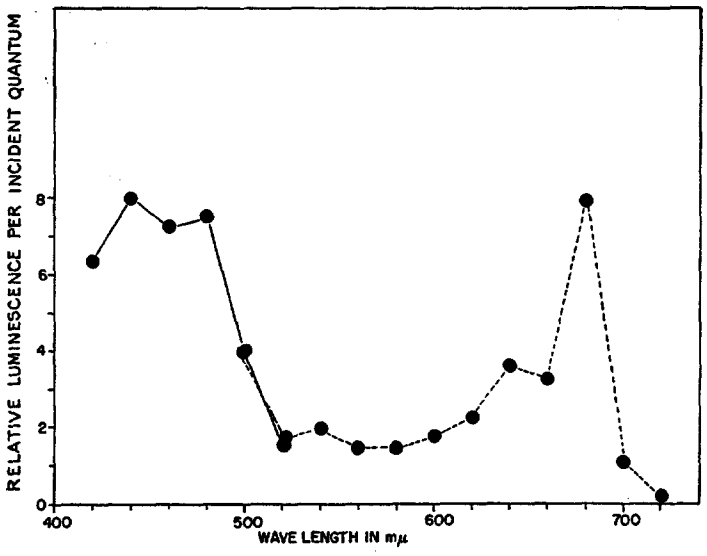

FIG. 5. *Chlorella* luminescence action spectrum. Abscissa, wave length of exciting light; ordinate, relative luminescence per incident quantum.

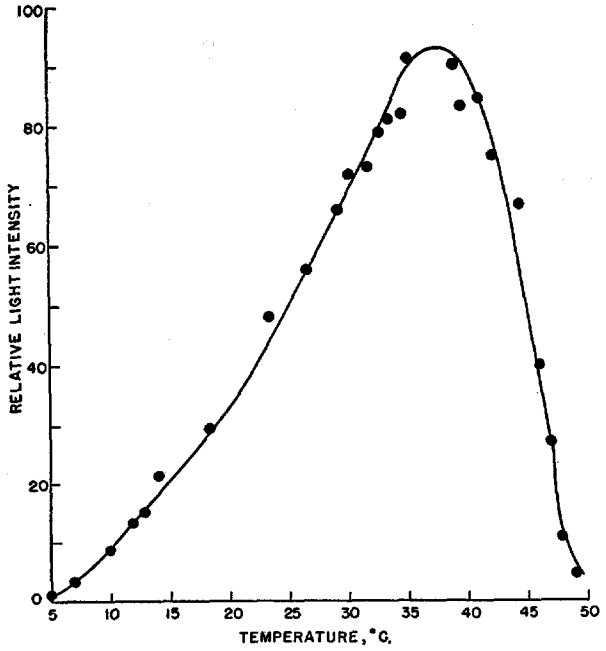

FIG. 6. Temperature *versus* luminescence of *Chlorella*.

the apparatus illustrated in Fig. 4. The intensity of the emitted light was determined as a function of the intensity of exciting light at 20 mμ intervals from 420 mμ to 720 mμ (band width—20 mμ to 40 mμ). The values thus obtained were plotted and the initial slopes of the curves at each wave length setting were divided by the wave length to give relative values in quanta rather than radiant energy. The slopes at each wave length, thus corrected to equal numbers of quanta were then plotted as a function of wave length. Fig. 5 represents the action spectrum obtained in this way for *Chlorella*. A comparison

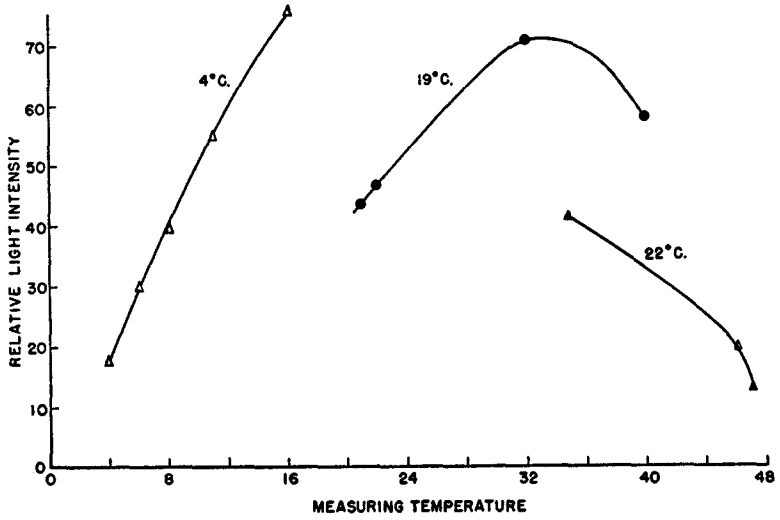

FIG. 7. Effect of illumination at a constant temperature and light emission measurement at a series of different temperatures. Temperatures indicated on curves are illumination temperatures while temperatures at which luminescence was measured are indicated on abscissa (for *Chlorella*).

of a photosynthetic action spectrum (3) with Fig. 5 leaves little doubt that the action spectra for photosynthesis and luminescence are strikingly similar.

Two separate methods of comparing the color of the fluorescence and luminescence have been used. First, a spectrograph utilizing a microscope objective (speed f .6) was used to photograph the fluorescence (excited by blue light) and luminescence and within the resolving power of this instrument the colors are identical. Second, by using an extremely sensitive quantum-counting apparatus employing a Beckman spectrophotometer as a monochromator, the colors of the two lights were found to be extremely similar if not identical. It can be said with some assurance that both lights encompass the same region of the spectrum although exact details of the shapes of the curves are not as yet established.

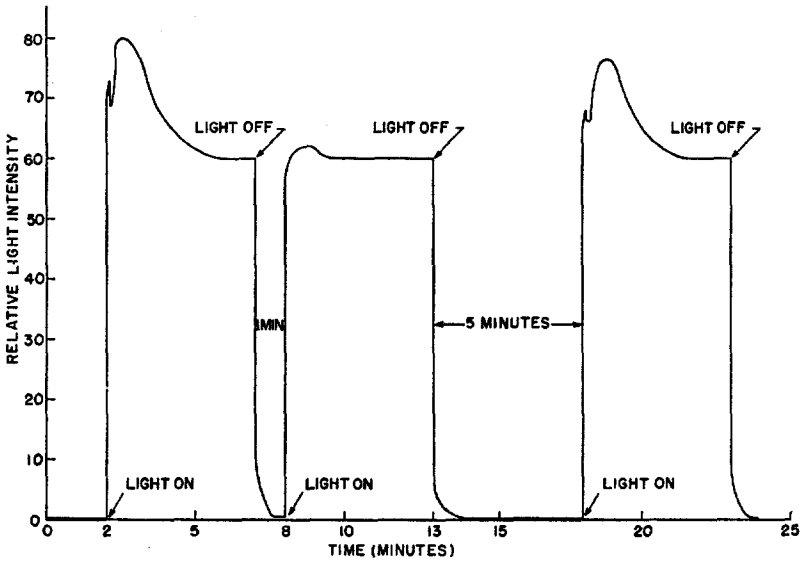

FIG. 8. Effect of dark and light periods on *Chlorella* luminescence.

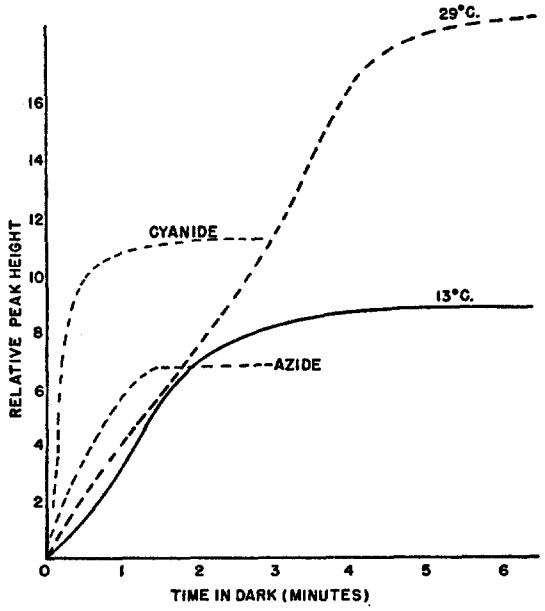

FIG. 9. Height of *Chlorella* luminescence induction burst *versus* preceding time in dark.

Effect of Environment on Luminescence

Temperature.—Two aspects of the effect of temperature on luminescence were investigated: (1) the simple temperature dependence of the over-all reaction, and (2) the effect of illumination at one temperature and light emission measurement at another. Fig. 6 shows luminescence as a function of temperature. It is apparent that the curve is similar to that obtained for an enzyme-catalyzed reaction. The activation energy as determined by a plot of the reciprocal temperature against the log light intensity is approximately 19,500 calories.

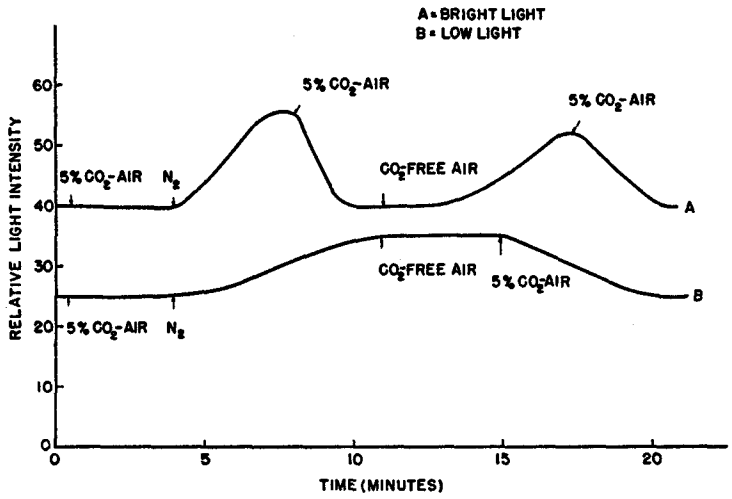

FIG. 10. Effect of CO_2-free air, 5 per cent CO_2-air, and nitrogen on intensity of *Chlorella* luminescence.

Fig. 7 shows the effect of illumination at a constant temperature and measurement of light at a series of different temperatures. The suspension was first cooled to an appropriate temperature by passing it through a coil of copper tubing in a cooling bath, then illuminated, and finally passed through another copper coil in a bath whose temperature could be varied rapidly. The results indicate that the rate-limiting step is not one associated with the primary light absorption but rather that it is one of the succeeding reactions.

Continuous Illumination.—Fig. 8 shows the effect of continuous illumination after dark periods, while Fig. 9 illustrates the effect of the preceding dark period on the height of the initial spike. The amplitude and shape of these curves are strikingly affected by added chemical agents.

Added Chemical Agents.—The effect of nitrogen, and of CO_2 addition are illustrated in Fig. 10. It should be noted that an appreciable time is required for these effects to reach completion.

Both azide and cyanide have a stimulatory effect at low concentrations (see Table I). In addition, these poisons change markedly the rate and nature

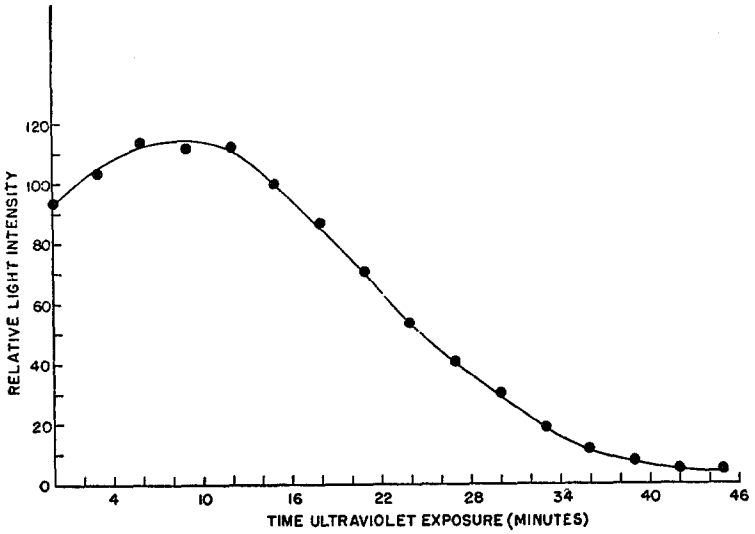

FIG. 11. Effect of ultraviolet light on *Chlorella* luminescence.

TABLE I

Effect of Inhibitors on the Luminescence of Green Plants

Inhibitor	Concentration ($M \times 10^{-4}$)	Luminescence (per cent of uninhibited)
Sodium azide	6	135
Sodium azide	15	114
Sodium azide	150	27
Potassium cyanide	6	114
Potassium cyanide	15	119
Potassium cyanide	45	151
Potassium cyanide	250	50
Sodium fluoride	305	100
Sodium fluoride	1220	60
Hydroxylamine-HCl	3.8	85
Hydroxylamine-HCl	18	5
2-Methyl-1,4-naphthoquinone	0.3	75
2-Methyl-1,4-naphthoquinone	30	14
Dinitrophenol	2	50
Dinitrophenol	5	10

of the dark recovery process. At intermediate concentrations the continuous illumination spike disappears, while at slightly higher concentrations it again appears, the time required for dark recovery being shortened to about 15 per

cent of its original value (see Fig. 9). Hydroxylamine and dinitrophenol are extremely potent inhibitors of luminescence and show little or no stimulation at low concentrations. 2-Methyl-1,4-naphthoquinone, which is a powerful inhibitor of bacterial (4) and firefly luminescence (5) as well as of other cytochrome by-pass systems (6), inhibits at all concentrations although not so strikingly as some of the other agents. Fluoride shows only a gradual inhibition with increasing concentration. The dark period required for recovery is doubled, however, when this inhibitor is used in higher concentrations.

Non-specific poisons such as ethyl alcohol and thymol inhibit at high concentrations but stimulate at low concentrations.

Ultraviolet Irradiation.—Ultraviolet light destroys the ability of the cells to luminesce at a rate comparable to its influence on photosynthesis. Fig. 11 shows graphically the effect of irradiation at 2537 A.

Occurrence of the Phenomenon

Light has been obtained from all green plants tested. These include three microorganisms, *Chlorella*, *Scenedesmus*, and *Stichococcus*; and two higher plants, *Phytolacca americana* and *Trifolium repens*.

DISCUSSION

At this time it would be premature to state with assurance either the mechanism of light production by previously illuminated green plants or the relationship of the phenomenon to the process of photosynthesis. This difficulty arises from the fact that the results thus far obtained are capable of interpretation in any one of several ways. However, even these initial experiments strongly suggest that delayed light emission by green plants is a reflection of certain early reactions in photosynthesis which, by virtue of their reversibility, are capable of releasing a portion of their stored chemical energy through a chemiluminescent mechanism.

A potent alternative to a chemiluminescent mechanism is, of course, phosphorescence or delayed light emission from excited molecules. The essential difference between such a purely physical re-emission of trapped light energy and a chemiluminescent one lies in the fact that the latter involves chemical reactions which produce excited molecules and might be expected to involve an enzyme system.

Earlier workers (7) observed the phosphorescence of solutions of chlorophyll in organic solvents and it might be argued that the delayed emission of light depends on the solution of a small fraction of the chlorophyll of the intact plant in a lipid phase within the cell. Several facts argue against the plausibility of this view: first, the addition of such non-polar solvents as ethanol and ether in moderate concentrations actually destroys the luminescence of intact cells; second, irradiation with an ultraviolet dose sufficient to destroy light-producing ability should not alter appreciably the lipid content of the cells.

The same argument holds for the heat lability of the system, since a 50°C. temperature rapidly and irreversibly destroys the ability to luminesce.

Another and more serious objection to the enzyme hypothesis is the possibility that a protein-chlorophyll complex is the light-trapping and emitting system (8) and that this system obeys the kinetics of an enzymatic reaction although there is no real catalysis involved. Even though a completely convincing refutation of this argument is impossible with the data at hand, the marked change in the order of reaction at different temperatures suggests that there may be more than one reversible reaction feeding into the light-producing system. This fact is difficult to reconcile with any model in which the energy is trapped in one molecular species or species complex. Moreover, flashing-light experiments have shown that it is not light absorption but rather temperature-dependent enzymatic reactions which are rate-limiting for photosynthesis at higher light intensities. Since this green plant luminescence saturates at about the same intensity as does photosynthesis, it would seem apparent that the same or a similar chemical reaction and not a purely physical phosphorescence determines saturation here also.

The interpretation of the previously described observations which most simply fits the experimental details is that the light-emitting process is actually a reflection of the reversibility of early reactions in photosynthesis including at least one enzymatic reaction.

This interpretation is based on the following observations. First, the luminescent reaction shows a temperature dependence very similar to the temperature dependence of photosynthesis (9). Second, ultraviolet light destroys the light-emitting ability at about the same rate as it destroys photosynthetic ability (10). Third, chemical compounds which inhibit photosynthesis inhibit light production. Fourth, photosynthesis and luminescence saturate in the same intensity range, whereas fluorescence continues to increase long after the maximal photosynthetic rate has been reached (11, p. 297). Fifth, CO_2 suppresses luminescence as though it were draining off intermediates which would otherwise feed back into the light-producing reactions. Sixth, continuous illumination produces a drop in intensity with time. And finally, the decay curves, as previously mentioned, may be interpreted as a series of reactions feeding back to the excited state.

A comparison of the properties of the luminescent reaction with fluorescence and photosynthesis will further substantiate the argument that it is more closely related to photosynthesis than to fluorescence. Preliminary measurements of the color of the emitted light indicate that it corresponds closely to the color of the fluorescent light. It also parallels fluorescence in its response to added CO_2. Its excitation spectrum follows both the fluorescent and photosynthetic action spectrum. On the other hand, it differs from fluorescence in its rate of decay, and in the effect of added agents. The fluorescence of chloro-

phyll is enhanced when it is dissolved in non-polar solvents while both photosynthesis and luminescence are inhibited by these agents.

Likewise the fact that photosynthesis and luminescence saturate at moderate light intensities is in contrast with fluorescence which shows a nearly linear response over a much greater range of exciting light intensities. Doses of ultraviolet light sufficient to destroy photosynthetic ability and luminescence are without appreciable effect on fluorescence. Finally, photosynthesis and luminescence are inhibited by low temperatures while fluorescence is actually increased (11, p. 305).

From all the foregoing it would seem likely that the phenomenon here described is closely associated with the photosynthetic process; that the energy may be trapped even at low temperatures although temperatures characteristic of enzymatic reactions are required for the re-emission of light; and that temperature, light saturation, inhibitor, and CO_2 addition experiments may argue for the reversibility of a whole series of enzymatic reactions leading to the reduction and fixation of CO_2 during photosynthesis.

SUMMARY

1. Green plants have been found to emit light of approximately the same color as their fluorescent light for several minutes following illumination. This light is about 10^{-8} the intensity of the fluorescent light, about one-tenth second after illumination below saturation or 10^{-6} of the intensity of the absorbed light.

2. The decay curve follows bimolecular kinetics at 6.5°C. and reaction order 1.6 at 28°C.

3. This light saturates as does photosynthesis at higher light intensities and in about the same intensity range as does photosynthesis.

4. An action spectrum for light emitted as a function of the wave length of exciting light has been determined. It parallels closely the photosynthetic action spectrum.

5. The intensity of light emission was studied as a function of temperature and found to be optimal at about 37°C. with an activation energy of approximately 19,500 calories. Two-temperature studies indicated that the energy may be trapped in the cold, but that temperatures characteristic for enzymatic reactions are necessary for light production.

6. Illumination after varying dark periods showed initial peaks of varying height depending on the preceding dark period.

7. 5 per cent CO_2 reversibly depresses the amount of light emitted by about 30 per cent. About 3 minutes are required for this effect to reach completion at room temperatures.

8. Various inhibitors of photosynthesis were tested for their effect on luminescence and were all inhibitory at appropriate concentrations.

9. Irradiation with ultraviolet light (2537A) inhibits light production at about the same rate as it inhibits photosynthesis.

10. This evidence suggests that early and perhaps later chemical reactions in photosynthesis may be partially reversible.

ACKNOWLEDGMENTS

We wish to thank Mr. R. W. Koza and Mr. T. A. Love for their kind interest and most helpful suggestions in many phases of this work. Thanks are also due to Mr. J. Davidson, Mr. D. Parrish, and Mr. J. R. Jones of the Instrument division for their efficient handling of the electronic design and operation of much of the equipment used in this work. The determination of the details of the later portions of the decay curves and the comparison of the color of the phosphorescent and fluorescent emissions of *Chlorella* were made possible through the kind interest and cooperation of Mr. J. A. Ghormley of the Chemistry Division, Oak Ridge National Laboratory. We are deeply indebted to him for permitting the use of his quantum-counting apparatus in these measurements and for making the determinations. Finally, we wish to thank Dr. J. R. McNally, Jr., for the loan of the Gaertner monochromator used to determine the action spectrum.

BIBLIOGRAPHY

1. Emerson, R. L., Stauffer, J. F., and Umbreit, W. W., *Am. J. Bot.*, 1944, **31,** 107.
2. Kodak Photographic Plates for Scientific and Technical Use, Eastman Kodak Co., Rochester, N. Y. 1949.
3. Emerson, R., and Lewis, C. M., *Am. J. Bot.*, 1943, **30,** 176.
4. McElroy, W. D., and Kipnis, D. M., *J. Cell. and Comp. Physiol.*, 1947, **30,** 359.
5. McElroy, W. D., and Strehler, B. L., *Arch. Biochem.*, 1949, **22,** 420.
6. Ball, E. G., Anfinson, C. B., and Cooper, O., *J. Biol. Chem.*, 1947, **168,** 257.
7. Franck, J., and Wood, R. W., *J. Chem. Physic.*, 1936, **4,** 552.
8. Szent-Gyorgyi, A., Chemistry of Muscular Contraction, New York, Academic Press, Inc., 1947.
9. Craig, F. N., and Trelease, S. F., *Am. J. Bot.*, 1937, **24,** 232.
10. Arnold, W. A., *J. Gen. Physiol.*, 1933, **17,** 135.
11. Franck, J., Photosynthesis in Plants, Ames, Iowa State College Press, 1949.