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Citation: The Journal of Chemical Physics **4**, 551 (1936); doi: 10.1063/1.1749905
View online: http://dx.doi.org/10.1063/1.1749905
View Table of Contents: http://scitation.aip.org/content/aip/journal/jcp/4/9?ver=pdfcov
Published by the AIP Publishing

mum and the way their dipole moments increase to a maximum in going up a particular homologous series. The change of both properties is greatest in going from the methyl to the ethyl compound and very much smaller for subsequent increases in the alkyl group. As both phenomena presumably depend on the accumulation of negative charge on the halogen atom such a correlation is not unexpected. Unfortunately, shortness of time has interfered with a thorough treatment of this subject.

In conclusion the author wishes to express his indebtedness to Dr. W. G. Penney for his very valuable criticisms and to Professor J. E. Lennard-Jones for some very helpful discussions. It should also be stated that the earlier part of the work was done at The Johns Hopkins University.

SEPTEMBER, 1936 JOURNAL OF CHEMICAL PHYSICS VOLUME 4

Fluorescence of Chlorophyll in Its Relation to Photochemical Processes in Plants and Organic Solutions

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(Received May 12, 1936)

The intensity of the fluorescence of chlorophyll in organic solutions in its relation to photochemical reactions can be explained by the assumption that the excited chlorophyll, which is free from adsorbed molecules, has a small probability of reemitting light as fluorescence, and a greater probability of predissociating into a hydrogen atom and monodehydrochlorophyll. In the presence of oxygen the product of dissociation will react with it. If acceptor molecules (*RH*) for oxygen are added to the solution, they will take over the excitation energy and protect the chlorophyll against oxidation, while they themselves will be oxidized; the first step being the dissociation of *RH* into *R* and *H*. The energy transfer may take place by impact of the second kind or as an intramolecular energy exchange within a complex molecule *H Chph RH* which will dissociate into *H Chph R* and *H*. The formation and consumption of the complex radical *H Chph R* is responsible for the change of intensity of the fluorescence with the time of irradiation which occurs in the presence of oxygen and some acceptor molecules in organic solutions. Analogous intensity time relations have been found and qualitatively described by Kautsky and his co-workers for the fluorescence of living leaves in the presence of oxygen. Quantitative measurements of these curves under various conditions form the experimental part of this paper. All observations can be interpreted by the assumption that not only photosynthesis but also photoxidation of organic substances adsorbed to chlorophyll takes place in the plant. The hypothesis is suggested that photoxidation is responsible for the so-called light saturation of photosynthesis in living plants.

INTRODUCTION

THE intensity of the fluorescence of living leaves has, as Kautsky[1] and his co-workers have shown, a complicated dependence on the time of irradiation if oxygen is present, this relation obviously having something to do with the velocity of photochemical reactions which occur in the plant. Kautsky proposed a theory which directly connected these observations with the process of photosynthesis, but Gaffron and others[2] published objections to his assumptions. One of the present authors formerly thought that the shape of the observed curves could be explained by the hypothesis that the chlorophyll must first go over into monodehydrochlorophyll by a photochemical reaction with oxygen before photosynthesis can start, but Gaffron, Gaffron and Wohl and Stoll[3] have shown that this hypothesis also is in disagreement with more recent observations, and in the course of this paper other objections to this hypothesis will be mentioned. A satisfactory explanation for the fluorescence-time relations has, therefore not been given so far, and Kautsky's careful observations while interesting, are only qualitative and

[1] Hans Kautsky, A. Hirsch and F. Davidshoffer, Ber. **65**, 1762 (1932); Hans Kautsky, A. Hirsch and W. Flesch, Ber. **68**, 152 (1935); Hans Kautsky, Biochem. Zeits. **274**, 423; **274**, 435 (1934).

[2] J. Franck and H. Levy, Naturwiss. **23**, 226 (1935); H. Gaffron, Naturwiss. **23**, 528 (1935).

[3] Gaffron, reference 2; H. Gaffron and K. Wohl, Naturwiss. **24**, 81 (1936).

therefore not sufficient for the analysis of the processes. On this account it seemed worth while to reinvestigate the whole problem of the fluorescence of chlorophyll and its relation to photochemical processes in leaves and in organic solutions.

Yield and Quenching of the Fluorescence in Organic Solutions

Studies of the fluorescence of chlorophyll in organic solutions, indicate the presence of a polyatomic molecule in which a predissociation process or quenching processes compete with the reemission of light. If irradiated with light corresponding to the three absorption regions of chlorophyll in the red, blue, and ultraviolet, it reemits red light with a small efficiency. In solutions which are supposed to be inactive in relation to the excited chlorophyll molecules, the maximum yield for fluorescent light is of the order of 10 percent.[4] In the absence of oxygen a short and weak afterglow can be observed. The fluorescence can be quenched by addition of substances such as oxygen, benzidine and iodine salts, which take up the excitation energy by impacts of the second kind.[5] The quenching by oxygen is small, an amount which corresponds to a 200 mm pressure reducing the intensity by one half, while the afterglow vanishes entirely. For benzidine and NaI the curves of concentration plotted against the intensity of fluorescence are hyperbolas as expected from their analogy to the curves obtained with fluorescent gases.

Connection between Photochemical Processes Induced by Chlorophyll and Its Fluorescence in Organic Solutions

The only photochemical processes known to be induced in organic solutions by chlorophyll are photoxidations.[6] Chlorophyll may be oxidized itself or may sensitize the oxidation of acceptor molecules for oxygen. On the other hand even in the absence of oxygen the fluorescence is weak. In solutions which are supposed to be stable in relation to excited chlorophyll and free from oxygen, 90 percent of the absorbed energy is dissipated and only 10 percent reemitted. Two explanations are possible. We may assume that the molecules of the solvent are less stable than formerly assumed and consume the energy for a dissociation process, the recombination which follows causing the afterglow as chemiluminescence. The second and more probable possibility is that the chlorophyll itself is not stable and may dissociate. Again the recombination will be responsible for the afterglow. The only dissociation process of chlorophyll which seems energetically possible if red light is absorbed is the splitting off of one of the two loosely bound hydrogen atoms whose presence the chemists have proved.[7] A reemission of 10 percent of light indicates that the photochemical process does not take over the energy immediately after the act of absorption but allows a relatively long lifetime of the excited state. The first hypothesis would require that only a very small fraction of all impacts with the solvent are effective and the latter, that the photolysis of the chlorophyll itself takes time. For a polyatomic molecule such as chlorophyll (molecular weight >1000) this behavior is to be expected; the spontaneous disintegration should occur a relatively long time after the absorption act, because time elapses before the very complicated relative movement of the atoms in the molecule produces the critical constellation of the particles and a suitable energy-distribution for dissociation.[8] The time lag will vary with the nature of the neutral solution because all molecules which surround the excited chlorophyll contribute to the complexity of the molecular system, their influence depending on the forces which couple them to the chlorophyll permanently or for the time of the impacts.

The addition of a small amount of oxygen causes a photoxidation of chlorophyll if other acceptors for oxygen are absent. The oxidation may take place as a result of impacts between excited chlorophyll and oxygen, but if one accepts the predissociation of chlorophyll, the

[4] A. Prins, Nature **135** (1935).
[5] J. Franck and H. Levi, Zeits. f. physik. Chemie **B27**, 409 (1935).
[6] See for instance H. Gaffron, Biochem. Zeits. **264**, 251 (1933) and K. Noack, Zeits. f. Bot. **17**, 481 (1925).
[7] H. Fischer, J. Chem. Soc. 245 (1934); A. Stoll, Naturwiss. **20**, 955 (1932).
[8] J. Franck, H. Sponer and E. Teller, Ges. d. Wiss. Göttingen, 1929.

main oxidation should occur by the reaction of oxygen with the products of the decomposition, and the recombination, thus hindered by oxygen, abolishes the afterglow.

If acceptors for oxygen are present, chlorophyll will be protected against oxidation, while the acceptors are photoxidized with a good quantum yield,[7] the protection occurring by seizure of the excitation energy of the chlorophyll before it reacts itself. The energy taken up by the acceptors will be used for a decomposition of these molecules and the products of dissociation will react with oxygen. The transfer of the energy to the acceptor molecules may take place by normal impacts of the second kind (as in benzidine, where the quenching is connected with the concentration by a hyperbolic curve) or while the acceptor is adsorbed on the chlorophyll forming a loosely bound complex. In this case one has a high quantum yield even if the concentration of the acceptor is small. The formation of such a complex also offers an explanation for cases in which the addition of the acceptor to be oxidized with a high quantum yield does not noticeably quench the intensity of the fluorescence and may even raise it somewhat. We may mention the photoxidation of isoamylamin as an example.[9] For the sake of simplicity we will use for an adsorption complex between chlorophyll and an acceptor the symbol *H Chph RH*. The first step for the oxidation of *RH* induced by light absorbed by chlorophyll will then be expressed by the equation

$$H\ Chph^*\ RH \rightarrow H\ Chph\ R + H.$$

The question whether the addition of *RH* will raise or lower the intensity of the fluorescence is then identical with the question whether the complex *H Chph* RH* has a longer time-lag between light absorption and chemical reaction then *H Chph** surrounded merely by the molecules of the solution. We have again to distinguish between the possibility that *H Chph** may react with the molecules of the solvent or may predissociate. On the first assumption the formation of *H Chph RH* can only then raise the fluorescence if it overcompensates the use of the excitation energy for the dissociation of *RH* by shielding off from the *Chph* the impacts of the second kind of the solvent. If, on the other hand, we assume the occurrence of spontaneous decomposition of *H Chph** we have, in order to explain the rise of the fluorescence, to ascribe to *H Chph* RH* a longer time-lag until predissociation occurs, as for *H Chph**, in spite of the two possible predissociation processes in the complex, and only one in the *Chph* free from *RH*. This is possible because the greater complexity of the system *H Chph RH* compared with *H Chph* tends to increase the time-lag between adsorption and predissociation.

[9] H. Kautsky, Ber. **68**, 152 (1935).

Changes of the Intensity of Fluorescence with the Time of Irradiation in Organic Solutions

According to results of Franck and Levi,[5] weak alcoholic or acetonic extracts of leaves irradiated with strong light in the presence of oxygen show a dependence of the intensity of fluorescence on the time of irradiation similar to that which Kautsky found for the living leaves, the essential difference being the much slower progress of the processes in the solutions. Here the intensity of the fluorescence is weak at the very beginning of irradiation, rising quickly, to a maximum after 20–30″ and then fading away in a few minutes (exponentially) almost to the low initial value. A repetition of the curve is possible only after a longer repose in the dark. Without oxygen the fluorescence shows practically a constant intensity, which is somewhat higher than the intensity at the beginning and end of the curve with oxygen, but lower than its maximum. The solution contains not only the chlorophyll but also other substances which are known to be acceptors for oxygen and easily photoxidized by chlorophyll. The photoxidation being the only photochemical process which occurs in these solutions, one must explain the shape of the curves by connecting the fluorescence-time relations with the progress of the photoxidation. According to the assumptions made above some substances when photoxidized will give, with the chlorophyll a complex of the form *H Chph RH*. We have to assume that the substances extracted from leaves belong to this class, inasmuch as the reaction proceeds quickly in spite of the low concentration in which these substances are present in the solution. The

complex *H Chph* RH* has a relatively small chance of reemitting light, most of the excited complex molecules reacting photochemically by the process

$$H\ Chph^*\ RH \rightarrow H\ Chph\ R + H.$$

The complex radical *H Chph R* produced by the light will be present only in a small concentration since it will be quickly consumed by the attack of oxygen and removal of the oxidized substance from chlorophyll. But the small amount of *H Chph R* may contribute considerably to the intensity of the observed fluorescence, as this radical must have a much greater ability to fluoresce than the original complex. After the splitting off of the hydrogen atom from *RH* the only possibility of using the excitation energy for photochemical purposes in the complex-radical *H Chph R* is the splitting off of the hydrogen atom from the chlorophyll and this process has, if it occurs at all, a small probability on account of the complexity of the molecular system. After the consumption of *RH* the *H Chph* liberated from the complex remains in the solution. This substance will again be a less effective reemitter than *H Chph R*, since if predissociation occurs this process will take place more rapidly as the molecular system is now simpler than the adsorption-complex. If there is no predissociation, *H Chph* will be inefficient for fluorescence because it has lost the protection against impacts of the second kind by the loss. The change of the intensity of the fluorescence with time is then an indicator of the change of concentration of *H Chph R* with time. The first rise of the curve is caused by the production of *H Chph R* by the light. The maximum occur in a short time since the lifetime of the radical is short, in consequence of which the equilibrium between production and consumption of *R* will be quickly reached. If an inexhaustible amount of *RH* were available, the intensity would remain constant after the establishment of equilibrium. This not being the case the slow decay is proportional to the consumption of *RH* by photoxidation.

Direct measurements of the lifetime of *R* and the dependence of the decay on the amount of *RH* present in organic solutions are planned. They should show the same results as the analogous experiments described below on the fluorescence of living leaves.

The Time-Intensity Relation of the Fluorescence in Leaves Indicates Photoxidation But Not of the Chlorophyll Itself

As was mentioned in the introduction, the starting point for this research was Kautsky's observations on the dependence of the fluorescence of chlorophyll in living leaves on the time of irradiation in the presence of oxygen. The general shape of the curve (which alone was known until now) is the same as that described for the solutions, which led one of the authors to assume that also in living leaves a photoxidation was responsible[10] for the behavior of the fluorescence. But the specific assumption that chlorophyll itself will be partially oxidized in going over to monodehydrochlorophyll in the plant and that this process is a necessary condition to start photosynthesis, is wrong. To the evidence given in the meantime by Gaffron and Gaffron and Wohl[3] (see also Stoll) we can add as a result of a few observations that a strong illumination of living leaves does not produce a considerable change of the absorption spectrum in the first few seconds of irradiation. This, however, should happen if all of the chlorophyll was transformed into monodehydrochlorophyll. In the special case of chlorophyll a splitting off of one of the loosely bound hydrogen atoms should change greatly the absorption spectrum according to the well-founded theories of Hückel and Pauling, as was pointed out to us by Dr. Corwin, who made also other helpful suggestions. The question now arises, which substances will be photoxidized in living leaves at the beginning of an illumination period, and what has this photoxidation to do with photosynthesis? Quantitative measurements of the time-intensity curves of fluorescence should offer much better information than Kautsky's qualitative ones, which however proved of great value to us in our choice of different experimental conditions, and our measurements are in accordance with his observations.

[10] J. Franck, Naturwiss. **23**, 221 (1935); Chem. Rev. **17**, 433 (1935).

General Experimental Procedures for Measuring the Time-Variation of Fluorescence of Living Leaves

Our arrangement for measuring the fluorescence of leaves was a very simple one. We excited the fluorescence with blue light emitted by a 750 watt tungsten lamp filtered through cuprammonium and focused by two large condenser lenses, on the under side of the leaf. A total-reflecting prism was used to deflect the converging cone of rays through a right angle in order to permit observation of the fluorescence in a direction perpendicular to the surface of the leaf. The blue patch of light on the surface of the leaf was observed through a red filter and the red fluorescence measured with a small photometer, constructed by Dr. Pfund, which allowed quick and exact comparisons with a beam of another source of red light whose intensity could be varied. The calibration curve of the photometer was obtained in the usual way with an optical bench. The red filter cut off all blue light reflected and scattered by the leaf so that only the intensity of the red fluorescent light was measured.

Measurement of the Decay of Fluorescence

The main shape of the curve—light intensity as a function of time—obtained in normal air and room temperature is in accordance with Kautsky. A steep rise of the fluorescence to a maximum reached in about one second is followed by a much slower decay. The decay varies a little if different kinds of leaves are used but is in the case of most of the leaves a perfect exponential function (with an asymptotic value different from zero); the decay of the intensity taking place in the course of $\frac{1}{2}$ to 1 minute could after a little practice be followed directly with the photometer and a stop-watch.

Measurement of the Rise of the Curves

We made no attempt to measure the steep rise of the curve at the beginning of an illumination period limiting ourselves to measuring the initial intensity and the intensity at the maximum. To obtain the initial value a flat metal disk of 12 cm diameter was covered with large pieces of leaf cuttings attached by glue and set in rotation by a motor. While the disk was revolving a narrow spot near the rim was illuminated with blue light. The time of exposure of each part of the leaf at the rim in the course of one revolution was only a few thousandths of a second, this short illumination not changing the conditions in the leaf, especially if the intensity of the impinging light is reduced. The fluorescence measured under these conditions can be accepted as identical with the initial fluorescence of a stationary leaf if it can be shown that the periodic repetition of the illumination is not a disturbing factor. This was controlled by longer sets of observations, which showed, that although a slow change of the fluorescence with time occurred (the general shape being the same as with constant illumination) the rate of change was so much smaller and slower that this effect could be neglected in the case of rapid observations.

Measurement of the Recovery Curves

In accordance with Kautsky's results it was found impossible to repeat the measurements with the same result, directly after a prolonged illumination, a long rest in the dark being necessary to restore the conditions as at the start. We measured the recovery in the dark as a function of time in the following way. After the final state of an extended illumination was reached, the leaf was rested in the dark for different intervals of time and the height of the maxima of the fluorescence during a very brief illumination as a function of the time of rest in the dark was measured. The measurement of each point of the curve disturbs of course the recovery, and to make this disturbance as small as possible, it was therefore necessary to employ a very brief illumination for each measurement. After making a series of measurements, considered reliable enough to show the character of the curve, control experiments were made by setting the photometer while the leaf was resting in the dark to the value to be expected for a given time of rest and comparing only the sign of the deviations. In the majority of observations the deviations were very rare and moreover very small.

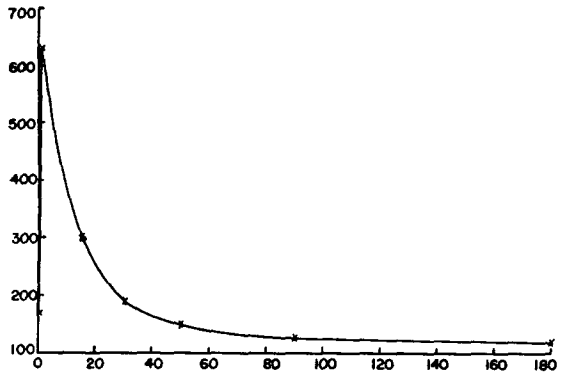

FIG. 1.

MEASUREMENT OF THE LIFETIME OF THE RADICAL *H Chph R*

In accordance with the explanation given above for the curves obtained in organic solutions, one would expect the light to produce a strongly fluorescent substance with a short lifetime, and it was possible to prove the production of this substance and to measure its life by the following procedure. Two flashes of light were applied one after another; if the first one produces a substance more able to fluoresce, the fluorescence caused by the second one will be increased by the previous illumination. The increase produced by the previous illumination will fade away if the time interval between the two flashes becomes greater. The light intensity observed at the second flash plotted against the time difference elapsed between the two flashes allows one to calculate the lifetime of the substance. The real experiment was not made with flashes but with a continuous illumination of *two* spots of the leaves near the rim of the rotating disk. For each individual revolution this is equivalent to two flashes and the time between the flashes can easily be changed by changing the distance between the two patches of light. The disk revolved with a constant speed giving a constant total duration of the illumination by the two spots of light. As sources of light we used for spot II our normal blue illumination and for spot I white light emitted by an 8-volt tungsten lamp mounted as close as possible to the surface of the rotating disk and shielded by a metal tube to avoid disturbances by scattered light. If the two patches of light are not nearer than 2–3 cm from each other this precaution is sufficient. The periodic repetition of the illumination fatigues the leaf, as was mentioned on page 11, and this influence was much greater than with the single spot of light employed before, since it was necessary to apply a much stronger illumination. The fatigue showed itself by the fact that the influence of the illumination by spot I on the fluorescence observed at spot II became smaller if the time of illumination was increased and at last vanished entirely. To avoid this disturbance the measurement for each

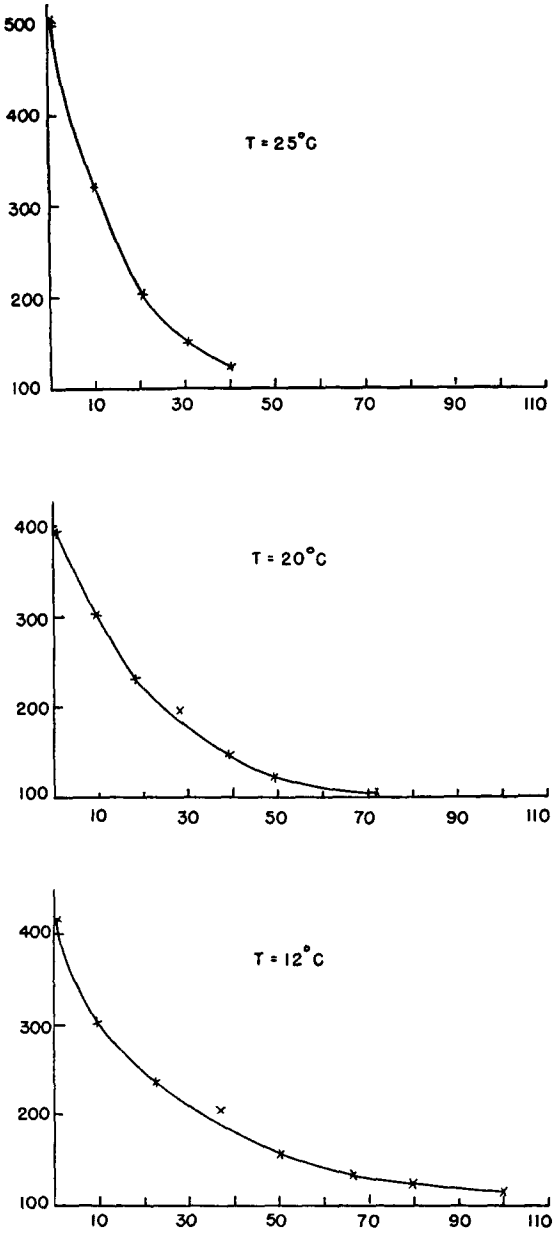

FIG. 2.

point of the curve was made with a very short exposure and the leaf allowed to recover in the dark between successive observations.

Measurement of the Influence of Different Gas Atmospheres on the Fluorescence Time Curves

Measurements on the influence of different gas atmospheres on the general shape of the curves were made with a shallow circular box of brass covered by a glass window, and rotated in the same manner as the disk, the leaf cuttings being attached to the bottom of the box. Gas was introduced through a small tube inserted in the rim of the wheel.

Measurements of the Influence of Temperature Variation on the Shape of the Fluorescence Time Curves

Our first rough measurements were made by attaching the leaf to the outer surface of a copper box filled with water at controlled temperature. This method, however, does not determine the surface temperature exactly and the final determinations were made either by immersing a test tube containing the leaf in a water bath, or by altering the temperature of the whole room.

Most of the observations were made with leaves of hydrangea or later in the winter with leaves of ficus, and several other plants (geranium, marsilia, etc.) which showed, in accordance with Kautsky, that the different plants behave in essentially the same way. The leaves were of course used only as long as they remained in a fresh condition.

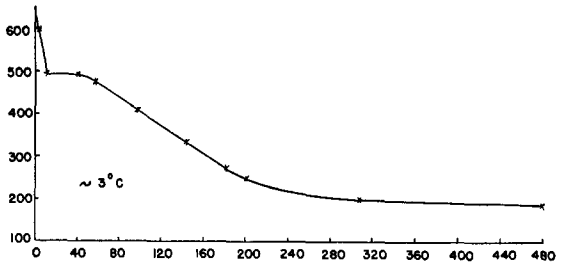

Fig. 3.

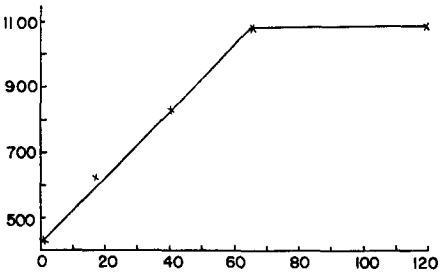

Fig. 4.

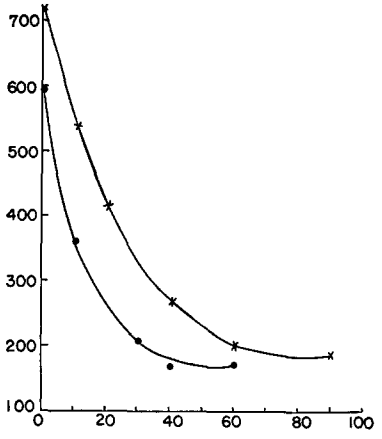

Fig. 5.

Results

In air with the normal amount of carbon dioxide the curves are as shown in Fig. 1, and, with the strong illumination employed, the intensity of the fluorescence is, always proportional to the intensity of the exciting light. The starting point has practically the same height as the asymptote reached after a long exposure, and the time constant of the exponential curves is independent of the strength of the light.

Fig. 2 shows the dependence of the decay on the temperature. The change of the time constant of the decay curves with temperatures can be interpreted by a constant temperature coefficient if one observes the curves between 25°C and 10°C. Lower temperatures gave other types of curves as in Fig. 3. The first sharp maximum cannot always be observed, and we suspect that it may be caused by temperature differences between the surface and the inner part of the leaf.

Fig. 4 shows an example of recovery curves. They are strictly linear. It seems that after the apparent equilibrium is reached (which takes the time of the order of magnitude of one minute) a very slow and small rise takes place for hours, as is indicated by the higher maximum, observed after a whole night's repose in the dark.

The dependence of the decay of the curve on the amount of CO_2 in the presence of oxygen was studied by removing with KOH the CO_2 from the air which streamed over the leaf. This treatment causing a much quicker decay of the intensity. Moreover the maximum was somewhat smaller and the fluorescence of the fatigued leaf became very weak. Fig. 5 shows two curves for a different amount of CO_2, the lower one has less CO_2, the decay is quicker and the total intensity smaller. Entirely reproducible results could only be obtained after several hours' treatment with air free from CO_2 and several illuminations and dark pauses.

Fig. 6 shows curves in a pure CO_2 atmosphere free from oxygen. Curve I shows the conditions ten minutes after the treatment with pure CO_2, Curve II half an hour later, and Curve III the final condition after several hours. For comparison purposes the normal Curve IV, obtained from the same leaf in air with the normal amount of CO_2 is given.

Observations on fluorescence as influenced by poisoning the leaves with prussic acid were made by moistening a part of the surface of the leaf with KCN solutions and the results are shown by Fig. 7. Curve 1 represents a normal leaf, curve 2

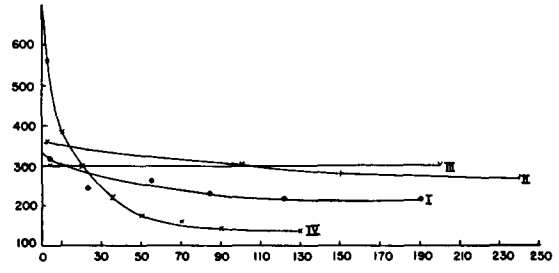

FIG. 6.

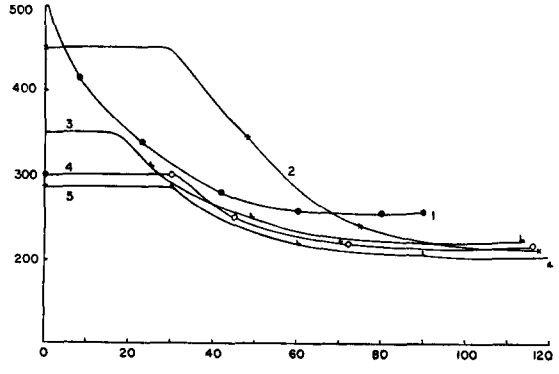

FIG. 7.

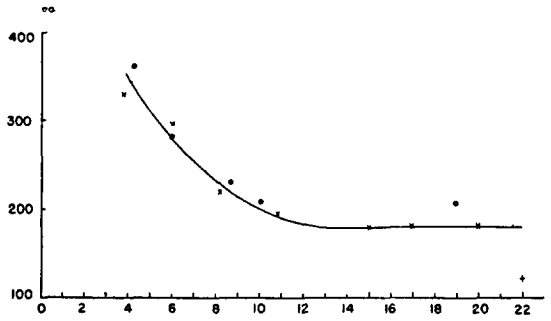

FIG. 8.

the fluorescence a short time after the poison was applied, and curves 3, 4 and 5 the influence of a stronger solution applied for longer times. The main changes are these: The fluorescence is much weaker and, while the first rise seems to be normal (not shown in the curves), the decay which follows the maximum is delayed as with very low temperatures.

Repeated applications of KCN solution followed by a ten hour repose in the dark, reduced the intensity of the fluorescence nearly to zero, and changed the color of the leaf from green to brown.

The measurements of the lifetime of the substance (*H Chph R*) by the method by the two spots of light gave results reproduced by Fig. 8. The curves obtained seem to be exponential with a half value of about 2/100 sec. The single point below the curve marked by + gives the value for the fluorescence without previous illumination. The fact that this point was always lower than the asymptotical extrapolation of the curve seems to show that the short illumination produces not only the substance with a short lifetime but causes also smaller changes which do not fade away in the time of one revolution of the disk ($\sim$ 1/6 sec.).

The time constant 2/100 sec. coincides with the time for the expiration of the Blackman reaction measured by Arnold and Emerson.[11] We expected that this coincidence was accidental because the production of the substance studied here is only detectable in unfatigued leaves after a repose in the dark, while the substance undergoing the Blackman reaction was studied by Emerson and Arnold after long periods of strong flash illumina-

[11] R. Emerson and W. Arnold, J. Gen. Physiol. **16**, 191 (1932).

tion. To prove that the lifetime measured here has nothing to do with the Blackman reaction we studied qualitatively whether prussic acid influences the lifetime. The experiments showed an independence so far as the accuracy of our experiments goes. An influence which would be as great as that caused by prussic acid on the Blackman reaction would easily be detected.

Discussion

These observations on the fluorescence of living leaves are to be interpreted in the same way as the results discussed above for the fluorescence of chlorophyll in organic solutions. In both cases a photoxidation takes place sensitized by the chlorophyll. While it is not yet possible to analyze directly the substances which undergo the oxidation, a good deal of information can be gained by the discussion of the changes of the time-intensity curves of the fluorescence observed under different outer conditions. It now seems probable that in the plant the chlorophyll is present on the surface of the chloroplasts, presumably in a monomolecular layer[12] which is in contact with the aqueous solution of the surroundings. This distribution would thus be different from the one in normal aqueous solutions of chlorophyll where the dye is distributed in colloidal particles entirely built up by chlorophyll molecules. But between the condition in normal aqueous solutions and in the leaves, there seems to be this similarity that chlorophyll in actual contact with water is unable to fluoresce. In normal aqueous solutions chlorophyll does not fluoresce at all and in plants only if the chlorophyll is protected by adsorbed organic substances, especially organic acids against impacts of the second kind with water molecules. One is forced to this conclusion by the fact that all conditions which lower the percentage of chlorophyll molecules carrying adsorbed organic substances also lower the fluorescence. In contrast to the weak fluorescence of leaves in air free from CO_2 after a short illumination, there is, for a longer period of illumination a stronger fluorescence after a longer dark period in normal air. In the first case the chlorophyll will be free from CO_2 and organic substances after a short illumination while in the second it will not only be connected with carbonic acid and the intermediate products between carbonic acid and formaldehyde but also with final products of photosynthesis and of the metabolism of the plant, which may be organic acids, as for instance tartaric acid. The slower the normal breathing process in the plant, the more of these organic acids will be present and they will be in competition with the carbonic acid and intermediate products of photosynthesis for the occupation of places of attachment to the chlorophyll. The concentration of the metabolic products will therefore be high if there is a lack of oxygen or if the breathing is reduced by low temperature or by poisoning with CN. This assumption is in accordance with Gaffron's[2] explanation of the slow start of photosynthesis if oxygen is removed from the plant. He assumes a poisoning of the photosynthetic apparatus by the products of the metabolism, and a reorganization of the photosynthetic apparatus by continued illumination. According to our point of view the plant gets rid of these products by photoxidation (which again may be a chain reaction). That chlorophyll in plants is able to sensitize oxidation processes was proved years ago by the important experiments of Noack[6] who has shown that benzidin brought in contact with chloroplasts in the living plant is readily oxidized. The shape of the curves in the presence of oxygen is then interpreted as above. The beginning of the period of illumination after a dark period finds chlorophyll not only connected with products to be photosynthesized but also with organic acids from metabolism. The fluorescence will be weak because the energy absorbed will be used in part for photosynthesis and in part for the formation of *H Chph R* and *H* out of *H Chph RH*. The radical H Chph R produced by light is, as in organic solutions, responsible for the change of fluorescence with time. If the concentration of its mother substance *RH* is reduced by photoxidation, *H Chph R* will be present in a smaller concentration and the fluorescence will again become small, since *H Chph* in contact with water will suffer impacts of the second kind, and *H Chph* connected with CO_2 and the intermediate states of photosynthesis will use the energy absorbed for the process of photosynthesis. Only if the influence of strong illumination is combined with

[12] K. Noack, Biochem. Zeits. **183**, 135 (1927); Cf. C. Zirkle, Am. J. Bot. **13**, 321 (1926).

a high supply of CO_2 one observes—as one can expect—a relatively high intensity of fluorescence after an extended period of irradiation. The concentration of sugar produced by photosynthesis now becomes so high that chlorophyll combines with the decomposition products of sugar which will directly and indirectly reduce the yield of photosynthesis by photoxidation processes.

As the experiments have shown, the fluorescence at each point on the curve is proportional to the light intensity, which is in accordance with the assumptions made, since the most active substance *H Chph R* is not only produced but also consumed in proportion to the light intensity.

The main influence of lack of oxygen, of low temperatures, or the poisoning by cyanide is, as was mentioned, to raise the concentration of the products of metabolism. This fact explains the similar aspect of the curves if one of these factors is applied to the leaves. The main difference from the normal curves is that fluorescence, after attaining its maximum remains constant for a time. This is a direct consequence of the great concentration of *RH*, since the places at the chlorophyll which will become free by oxidation of *RH* will be immediately filled again with new *RH* until a considerable reduction in the concentration of *RH* has occurred. The intensity of the fluorescence as well as the shape of these curves, fits into the picture. These curves show the highest intensity if a low temperature is used. The concentration of *RH* being great and also the amount of oxygen being higher than normal, the photoxidation will produce an abnormally high concentration of *H Chph R*. Lack of oxygen together with a great concentration of *RH* as in the curves of Fig. 6 gives a smaller intensity but the fluorescence remains constant for a much longer time, corresponding to the longer time taken for the consumption of the *RH*. Prussic acid, if applied for a considerable time, produces a great reduction of the fluorescence. This has to do with a slow decomposition of the chlorophyll itself as indicated by the brown color of the poisoned leaf.

The time used for the steep rise at the beginning of irradiation is not (or may be to a very small extent) influenced by the temperature or by prussic acid. This is to be expected since there is no reason to assume that the photochemical production of *H Chph R* or the lifetime of the radical should be influenced by low temperature or HCN. The last point is, moreover, proved by direct experiments. On the other hand, with less oxygen present the lifetime should become the longer. In this connection we cannot refer to our own experiments (because we did not change gradually the O_2 amount), but Kautsky mentions that the curve rises less rapidly and has a lower maximum when less oxygen is present.

Lack of CO_2 produces a quick decay of the curves, as the lower curve of Fig. 5 indicates. We explain this fact by a quicker consumption of *RH* because the places for attachment to the chlorophyll are free, and a competition with photosynthesis does not retard the photoxidation. On the other hand there is an exhaustion of oxygen since its new production by photosynthesis is absent in this case and also the concentration of sugar and its decomposition products will be abnormally small.

The recovery curves (Fig. 4) have an interesting shape, indicating a reaction of the zero order, which means that the restitution of the higher concentration of the products of metabolism (which corresponds to the equilibrium occurring in the dark) takes place by a catalytic surface reaction. The recovery curves show, according to our interpretation the difference between the equilibrium concentration of *RH* connected with *Chph* in the dark (equilibrium between production and consumption by breathing) and in the light, being in the latter lower since here the substances are not only attacked by normal respiration but also by photoxidation.

But not only should the products of metabolism be photoxidized but also the final products of photosynthesis. One may infer that this process becomes especially important if abnormally high concentrations of these products and of oxygen occur. We suppose in contradiction to the usual assumptions[3, 11] that the so-called light saturation has to be interpreted as an equilibrium in which photosynthesis and photoxidation balance each other to a given extent. This question and others connected with the problem of photosynthesis will be discussed elsewhere.