PHOTOXIDATION PROCESSES IN PLANTS

By J. FRANCK and C. S. FRENCH

(From the Department of Chemistry (Fels Foundation), University of Chicago, Chicago)

(Received for publication, July 9, 1941)

INTRODUCTION

Many observations indicate that the uptake of oxygen and the evolution of CO_2 by plants and plant tissue can be enhanced by irradiation with visible light. Some observers believe that a direct action of the light on the respiratory system is responsible for this effect, which they therefore call "light respiration;" others assume that the increased O_2 uptake is caused by photoxidation processes not different in principle from analogous effects in non-living matter. Taking in account the results published in the literature and the ones described in this paper we conclude that the second assumption is correct. The ultimate cause of the extra oxygen absorption after or during irradiation consists of photoxidation processes sensitized mainly by chlorophyll, but these processes are by no means simple ones. For instance, the products formed by photoxidation may interfere as substrate or as inhibitor with the normal plant metabolism. We mention as a simple example the fact that by these photoxidation processes the substrate of normal respiration can be chemically changed in such a way that during the subsequent dark period the rate of respiration is temporarily greater than its normal value. According to our opinion the introduction of the term light respiration for such an effect is unnecessary, just as it is for the well known influence which photosynthesis has on respiration in starved plants; by producing carbohydrates photosynthesis restores the absent substrate of respiration and so the rate of respiration measured during the subsequent dark period is increased. We cannot completely preclude the possibility that a genuine light respiration *i.e.*, an influence of light on the system of enzymes involved in respiration, does exist, but observations made with plants by others and by ourselves so far give no clear indication of such an effect. Attempts to find it should be made in gaseous atmospheres containing only about 1 to 2 per cent of oxygen as that oxygen concentration is great enough to saturate respiration yet not large enough to cause significant disturbances by photoxidation processes.

EXPERIMENTAL PROCEDURE AND RESULTS

The main methods of observing photoxidation processes in normal plant tissue belong to two different groups. The first is an indirect one based on early observa-

tions made by Warburg (1). Photosynthesis at different light intensities is measured as a function of the oxygen content in the gaseous atmosphere and the decrease of the rate of photosynthesis with the rising oxygen concentration is studied. That the depression of the rate under the influence of oxygen actually can be used to obtain information on photoxidation processes will be shown in this paper. The second method introduced by van der Paauw (2) is a direct one and is the method used by us. Plants are irradiated in gaseous atmospheres containing oxygen but practically no

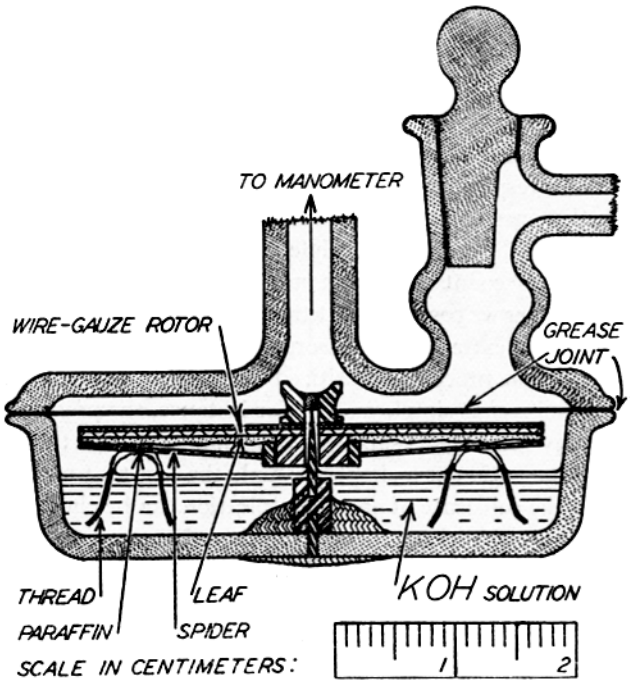


FIG. 1. A modified Warburg vessel in which a leaf is mounted on a rotor, with the lower surface containing the stomata just above a pool of KOH solution.

CO_2, the CO_2 produced by respiration and photoxidation having been removed as efficiently as possible by KOH solution. The influence of photosynthesis on the pressure readings thus becomes small under these conditions and the oxygen uptake under the influence of light can be measured manometrically. Fig. 1 shows the main part of the modified Warburg manometer used by us; some improvements and adaptations for the purposes of our experiments distinguish it from van der Paauw's original device.

A round piece of hydrangea leaf 3.7 cm. in diameter with a 0.6 cm. hole in its center was cut out with a punch and mounted under a wire gauze rotor with the underside of the leaf down. This rotor was supported on a jewel bearing by a needle so that the leaf was just a few millimeters above the surface of a layer of 10 per cent KOH

solution in the glass vessel of Fig. 1. Two paraffined threads looped over the rotor trailed in the KOH thus stirring it adequately. On top of the rotor was a magnetized iron strip which caused the leaf to rotate at a rate of 145 R.P.M. when a rotating magnetic field was applied from the outside.

The light source was a 2000 watt bulb with a spherical mirror behind it; the light was collected by a pair of 10 inch condenser lenses mounted together. An image of the condenser lens was focussed on to the bottom of the vessel of Fig. 1 contained in a glass-walled thermostat tank, with the aid of another large condenser lens and a mirror placed in the water. 2 inches of flowing water served as an infrared remover. Large Kolle culture flasks filled with $CuSO_4$ or with $K_2Cr_2O_7$ solution were used in special experiments as color filters. Calibrated wire screens were used to vary the light intensity.

(Later, the lens system was replaced by an elliptical mirror of 1 foot in diameter. This focussed the light of a 1000 watt projector bulb directly on the vessel without any lenses and gave a maximum intensity of about 80,000 lux.)

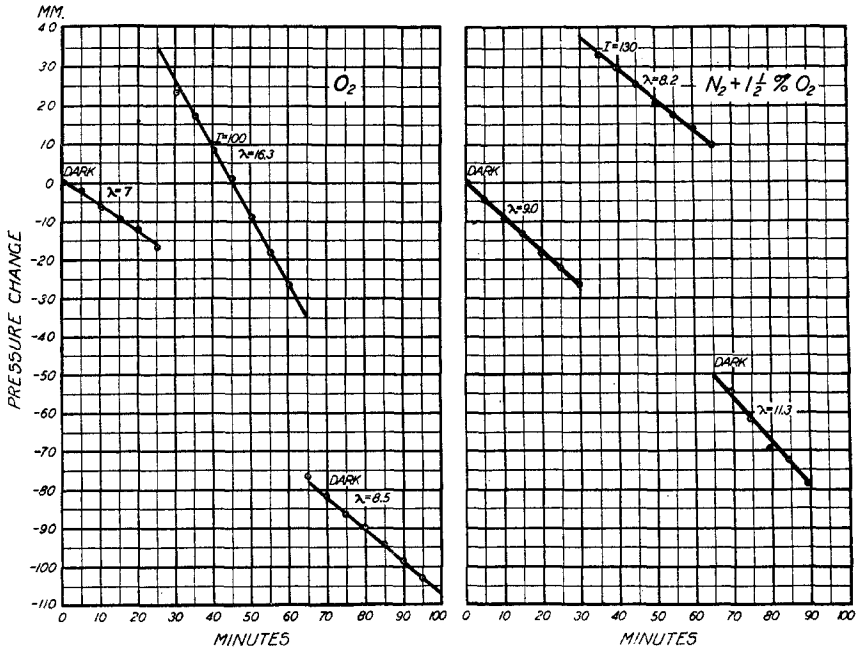
Practically all experiments were done with cuttings of hydrangea leaves. Other leaves when tested gave the same type of results. Attempts to use algae spread over the surface of moist filter paper were abandoned since the algae dried out too quickly under the influence of the strong light needed for our experiments.

Experiments with color filters showed that strong photoxidation is obtained with red or blue wavelengths, thus indicating that it is the chlorophyll which sensitizes this reaction as these are the wavelengths wherein chlorophyll absorbs most strongly.

Figure 2 *a* gives typical curves for manometer readings with pure oxygen in the dark and the light. The pressure decrease in the curves with the slopes $\lambda = 7$ and $\lambda = 8.5$ is produced by respiration, while the much steeper slope $\lambda = 16.3$ is due to the combined effects of respiration and photoxidation. The slope is furthermore influenced by the fact that when illuminated a part of the respiration CO_2 can be photosynthesized before it escapes out of the leaf and into the KOH solution. Further, the leaf becomes warm by illumination in spite of the circulation of the gas atmosphere in the thermostated vessel. This heating produces a rise in pressure which will be reversed the moment the light is turned off.[1] Generally, the pressure rise due to heating of the leaf at the beginning of an illumination is several millimeters of water greater than the pressure fall due to cooling at the end of an illumination period. An effort was made to determine whether this pressure difference could be used as a measure of the concentration of intermediate products of photosynthesis left over in the leaf from previous illumination periods in the presence of CO_2. These inter-

[1] It was not possible to calculate from the pressure changes above, the change in temperature of the leaf which occurs when the light is turned on. This temperature change was therefore measured with a thermocouple, but because of experimental difficulties these measurements were made only with non-rotating leaves. The temperature rise was 1.3°C. at the intensity most frequently used in our experiments and required about 5 minutes. The temperature dropped at an equal rate when the light was again extinguished. Of course, in a rotating leaf, the temperature change would not be as great nor would so long a time be required for the new steady state to be attained.

mediates would then be further reduced in producing oxygen under the influence of the illumination until their supply is exhausted. Since the observed pressure differences were only of the order of 1–4 mm., scarcely greater than the experimental error, we cannot be certain that the pressure changes observed were due to this effect. Certainly, it has no influence on the calculation of the rate of photoxidation, since for that purpose we have used only the slope of the curves after they had become linear. Usually, photoxidation is followed by a period of enhanced respiration. This period



2a 2b

FIG. 2. Measurements of oxygen consumption in the light and dark in an atmosphere of oxygen as compared with measurements in nitrogen containing 1.5 per cent oxygen.

is not to be confused with the time necessary to reach thermal equilibrium. It takes but 2–3 minutes to balance the thermal effects whereas the enhanced respiration may last 10–30 minutes and even longer under certain conditions. The explanation of this was briefly mentioned in the introduction and will be discussed further below. Finally, one must take into account the fact that the pressure measurements are influenced by photosynthesis. Indeed, this effect is larger than we expected it to be under the conditions of our experiment. The photochemical reduction of CO_2 formed by respiration was measured in an atmosphere of nitrogen containing just enough oxygen so that the rate of respiration is not limited by the O_2 tension. In the case of hydrangea leaves about 2 per cent oxygen is enough as may be seen from Fig. 3, wherein the rate of respiration is plotted as the function of oxygen pressure.

Fig. 2*b* gives a set of curves as measured in $N_2 + 1\frac{1}{2}$ per cent of O_2 in the dark and in the light. It shows that the photoxidation is negligible under these conditions while photosynthesis compensates for a part of the respiration.

From the foregoing, it appears that since the rate of photoxidation in oxygen is calculated from three independent rate measurements, the final accuracy of the result is small. The precision is further reduced by the fact that the rate of photoxidation is not constant but decreases with time. The velocity of the decay depends upon the previous life history of the leaf, the light intensity, and the oxygen concentration. A leaf which has been well fed by photosynthesis or whose sugar supply has been enriched by immersing its stem into glucose solution shows a somewhat stronger photoxidation which also remains constant for a longer period. (In measuring the degree to which photoxidation of a given leaf depends upon external factors such as light intensity or oxygen

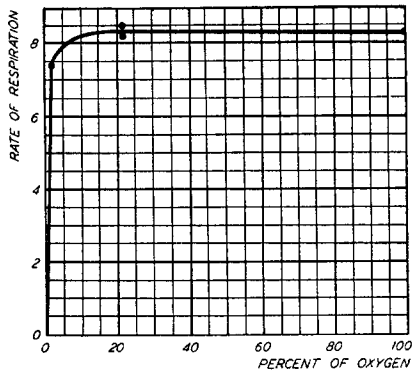


FIG. 3. Dependence of the respiration rate on the partial pressure of oxygen

concentration, one must be careful not to use too long irradiation periods. One can insure the absence of this error in the curves obtained by measuring the various points on the curve in a random order.)

The curve showing the rate of photoxidation as a function of oxygen concentration is of the same type in all leaves and is independent of their previous treatment. Fig. 4 presents an example.

The ordinate represents the rate of oxygen consumption by photoxidation corrected for respiration and photosynthesis; the abscissa is the percentage of oxygen contained in the atmosphere which was at normal pressure. For the reasons mentioned above, the accuracy is not great, but it is sufficient to determine the shape of the curve. The rate rises rapidly at first with the oxygen concentration. Later, the slope becomes smaller and finally one reaches O_2 saturation; the rate becomes independent of further increase in the oxygen concentration. The saturation point is reached when about 60 per cent of the gaseous atmosphere is O_2.

Curves in which the rate of O_2 uptake is plotted against the light intensity

reveal the fact that the rate rises less than linearly with the light intensity at least at high intensities. It was impossible for us to measure the shape of the curve accurately enough to determine whether the rate is proportional to the square root of the intensity. (At high intensities the rate of photoxidation declines too rapidly with time for precise measurement.) To establish beyond doubt that the deviation from linearity is not merely a deception the following procedure was chosen. The rate was measured in bright, intermittent light

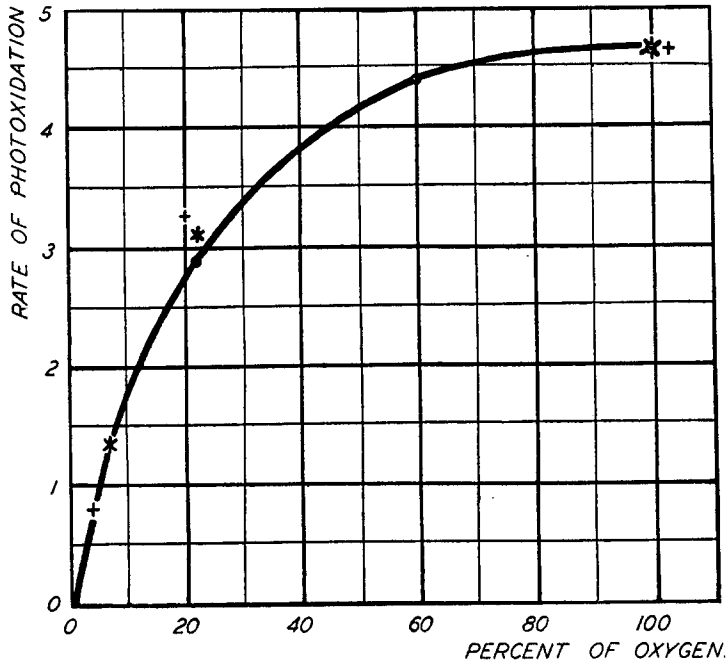


FIG. 4. Relative rate of different photoxidation processes plotted *versus* O_2 pressure. Circles, our data for photoxidation in hydrangea leaves; crosses, Warburg's data for the decrease in rate of photosynthesis with O_2 pressure; stars, Gaffron's data for the photoxidation of serum sensitized by adsorbed porphyrin.

and then immediately afterwards in continuous light of the same average intensity. Since the average illumination is the same in both cases there is no change in the temperature of the leaves and therefore no transient effect on the manometer readings such as would accompany a large increase or decrease in steady illumination. Any break in the pressure *versus* time plot must be attributed to a change in the rate of photoxidation. It is obvious that a change will occur only if the rate varies in a non-linear manner with the light intensity.[2]

[2] We furnished our brightest light with a large shutter pivoted on a bearing so that it could be lifted out of the light beam periodically and then dropped back. The shaker mechanism of the Warburg tank drove the shutter by a chain fixed with a stop so that the dark time was about 4 times the light period. The maximum intensity

Table I shows some observations which indicate that the yield becomes lower at high intensities.

No particular effort was made to get exact data for the quantum yield of the photoxidation in leaves, since the effect varied so much with the conditions. But a comparison of the rates of photoxidation with the rates of photosynthesis which is obtained in these leaves makes it possible to estimate the order of magnitude of the quantum yield. Under favorable conditions it turns out to be $\leqq 1/100$ in an atmosphere of pure oxygen.

Experiments in which leaves were allowed to take up substantial amounts of sugar, by soaking the stem or the whole leaf in sugar solution, did not show much increase in photoxidation. However, if the corrections for respiration

TABLE I

Photoxidation in Continuous and Intermittent Light of the Same Average Intensity

Average rate per 30 min. period in mm./5 min.			Photoxidation corrected for respiration	
Dark	Continuous	Intermittent	Continuous	Intermittent
−3.8				
	−8.8		−4.0	
		−5.9		−1.1
	−6.8		−2.0	
		−5.6		−0.8
	−5.0		−0.2	
		−4.6		+0.2
	−5.5		−0.7	
−4.8				
	−6.5	−5.3	−1.7	−0.5

and photosynthesis were applied, the decay of photoxidation with time does appear to be slowed down.

A sugar-fed leaf irradiated in oxygen free from carbon dioxide shows a remarkable increase in its respiration, as may be seen by Table II.

In the case shown here, the respiration rose more than 100 per cent. The enhancement of the respiration lasts for hours provided the photoxidation period was long enough and the light during that period strong enough. Obviously the conclusion seems to be that under the influence of photoxidation, hexose is chemically changed into products which are more easily oxidized by the normal respiratory catalysts or are themselves autoxidizable. It is known

---

was 12.8 times the average intensity. The flashes came at the rate of 66 per minute. Now the average intensity of the alternate light and dark periods was measured by the increase in pressure (84.7 mm.) in the manometer vessel when a black paper was substituted for the leaf. It was found that screens transmitting 7.8 per cent of the continuous light made the intensity of the continuous light very nearly the same (85.6 mm.) as that of the integrated flashing light.

that in chemical oxidation processes hexose shows such behavior, but it is uncertain whether it is justifiable to compare such auto-oxidations *in vitro* with the respiration processes in the leaf.

Prolonged photoxidation damages the photosynthetic apparatus and finally kills the leaf, whereas with a short exposure to light and oxygen, in the absence of CO_2, the damage is reversible. This fact (which is in agreement with results of Myers and Burr (3) to be discussed later) was revealed in the following

TABLE II

Oxygen uptake in 10 minutes mm. Brodie solution	Respiration	Photoxidation (corrected)
	Before exposure in pure O_2...12.1	
		1st photoxidation period lasting 25 min..... 8.4
	After 1st photoxidation period.....14.5	
	Returning after 2 hrs. in the dark to.....12.7	
		2nd photoxidation period lasting 25 min..... 8.6
	After 2nd photoxidation period.....22.5	
	Constant over a period of 90 min.....22.5	
		3rd photoxidation period lasting 25 min.....10.0
	After 3rd period.....24.0 (Measured 30 min.)	

way: leaves were exposed to light in the presence of 1 per cent CO_2 and the photosynthetic rate was measured. Then they were subjected to photoxidation for a short time by exposure in CO_2-free oxygen (generally about 20 minutes) and afterwards photosynthesis was again measured. The photosynthetic activity recovered slowly. The time necessary for the recovery depends on the illumination; intensities just great enough to produce saturation of photosynthesis give much quicker recoveries than the smaller ones.

Fig. 5 gives an example of such measurements. Before the treatment the induction loss is unobservably small (upper curves); after treatment (lower curves) the induction period lasts about 15 minutes but the rate comes back to its old value. Recovery of photosynthesis in the region where the damage

is reversible can be accomplished not only by photosynthetic activity but also by subjecting the leaf to long dark periods. In some cases where photoxidation was followed by a long dark period we observed that on reillumination, photosynthesis was resumed with an induction loss no greater than the usual one in normal untreated leaves.

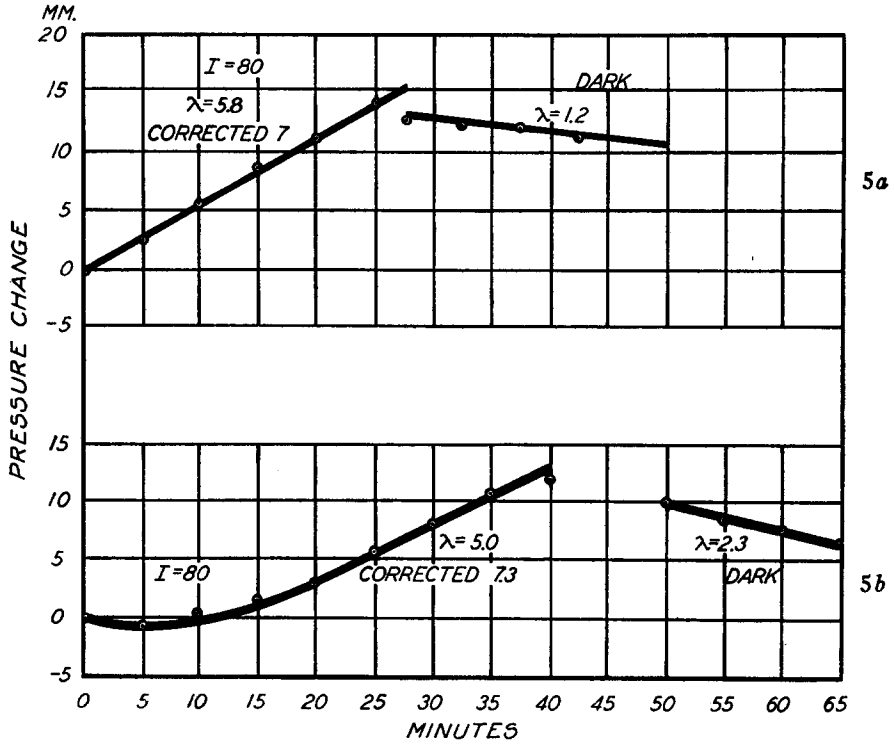
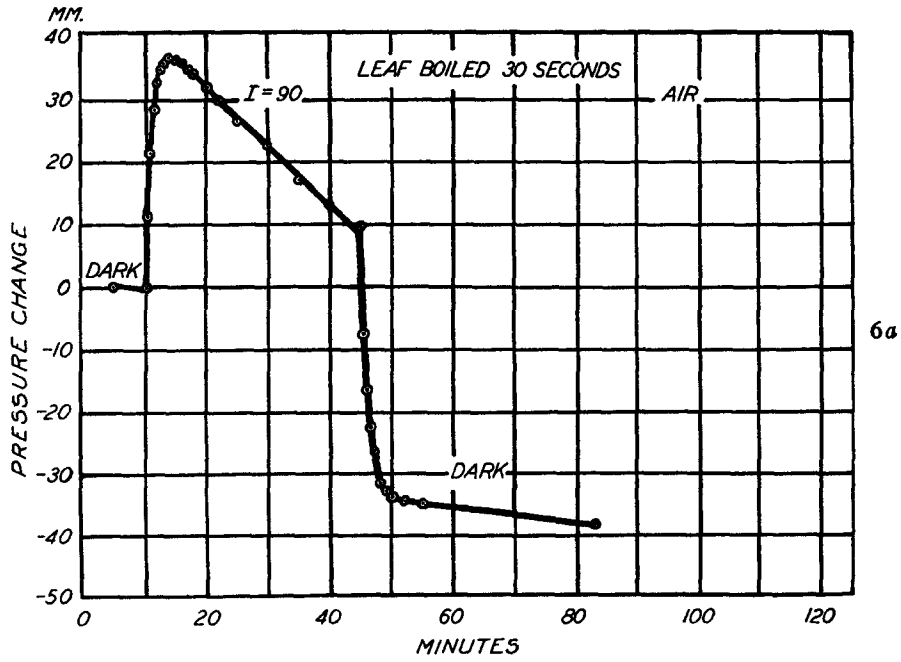


FIG. 5. Photosynthesis measured in the presence of sufficient CO_2 both before (5 *a*) and after (5 *b*) a photoxidation period.

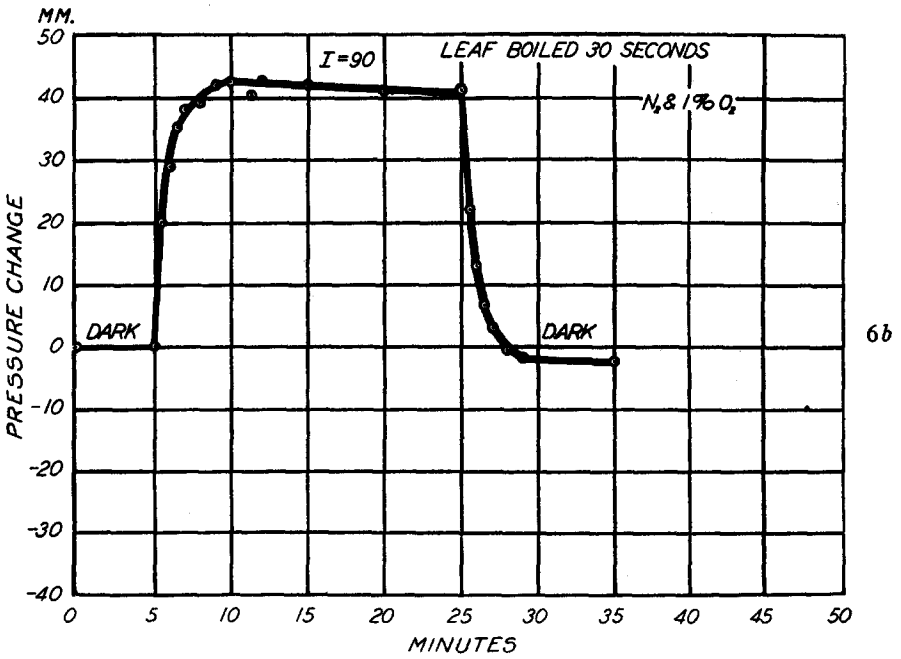
A series of experiments was made to find out whether photoxidation in leaves depends on the action of heat-labile enzymes. For that purpose leaves were killed by submerging them for 20 seconds to 5 minutes in boiling water. The results show that photoxidation takes place in dead leaves just as well as in live ones. (Compare Fig. 6*a* and *b*.) Also, the dependence of the rate of photoxidation upon light intensity is unaltered within the accuracy of the experiments. The rate of photoxidation in the boiled leaves seems a little smaller than in the fresh ones.

Finally, some experiments done in this laboratory[3] may be mentioned which

[3] The experiments were carried out by T. Puck and C. S. French.



6a



6b

FIG. 6. Measurements of oxygen consumption in a boiled leaf in the light and dark in an atmosphere of oxygen (6 *a*) as compared with similar measurements in nitrogen containing 1.5 per cent oxygen (6 *b*).

show that plant juices containing whole chloroplasts or only their macerated contents (mostly consisting of chlorophyll adsorbed on its native protein) show photoxidation. The rate of oxygen consumption is smaller than in leaves, but can be made even stronger than in leaves by addition of easily oxidizable substances like ascorbic acid. Ascorbic acid is also oxidized in the dark but at a much lower rate, so the rate of photoxidation can be calculated from the enhancement of the oxygen uptake during the illumination period. The experiments are in accordance with the observations made by French (4) in water extracts of purple bacteria.

Comparison of the Results with Older Observations

It has been known for a long time as mentioned in the introduction, that photoxidation processes occur in plants. Noack (5) showed already in 1923 that substances whose oxidation products can be recognized by their color can be photo-oxidized in plants into which they are artificially introduced. The same author stated that O_2 absorption is increased in leaves illuminated for 84 hours in absence of CO_2. Van der Paauw whose method of observation is comparable to ours undoubtedly observed photoxidation as well as its enhancing influence on respiration in the dark directly following an illumination period. Van der Paauw himself comes to the conclusion that in his observation the normal tissue respiration is enhanced by "plasmogeneous influences." We think that his point of view is not tenable for the following reasons: as was discussed above, the O_2 uptake depends on the partial pressure of oxygen in a manner entirely different from that of respiration. Furthermore, killed leaves which do not respire and plant juices show a comparable O_2 uptake in the light. The same remarks apply to the conclusion which Shri Ranjan (6) draws from similar observations. Likewise, the papers of Fökler (7) and Fökler and Montfort (8) are not convincing in their conclusions based on analogous observations. In respect to these papers we agree with Gaffron's (9) criticism.

The work of Mevius (10) is related to photoxidation in plants exposed to light in a CO_2-free atmosphere. This author studied the behavior of leaves of higher plants still connected with the intact plant. A part of the plant was exposed to light in a CO_2-free atmosphere while other parts of the plants were irradiated in normal air and were therefore able to photosynthesize. Mevius found that leaves could stand long exposures to light in the CO_2-free atmosphere without observable damage provided that the photosynthesizing leaves supply enough food for the exposed leaves. If that flow of nourishment stopped or became too small, starch and sugar were consumed and the leaves turned yellow with the destruction of proteins.

All other conclusions on the occurrence of photoxidation are based on observations of the rate of photosynthesis. The most important contributions of this type are those of Warburg (1) and of Myers and Burr (3). Warburg's

first observations of the influence of oxygen concentration on the rate of photosynthesis yielded the result, which has often been confirmed since, that even in the presence of sufficient CO_2 the rate of photosynthesis at light saturation depends strongly on the oxygen concentration. Under some conditions the rate may be reduced to 60 per cent if an atmosphere of N_2 (containing enough oxygen to saturate respiration) is replaced by an atmosphere of pure oxygen. Sometimes, on the other hand, the retarding influence of oxygen is quite small. It is known that the difference in behavior depends on internal factors such as food concentration and respiration. At low intensities neither Warburg nor others (compare McAlister and Myers (11); Myers and Burr) found any influence of the oxygen concentration on the rate of photosynthesis.

An exception is found in the results of Katz and Wassink and coworkers (12), who observed that at low intensities as well as at high ones, a diminishing of photosynthesis occurs with increase in the oxygen concentration.

Most authors do not relate these strong influences of oxygen concentration on the rate of photosynthesis to photoxidation, but a comparison of Warburg's results with ours unmistakably reveals an intimate connection between these effects. The most direct proof is that the variation of the photosynthesis rate with oxygen pressure follows the same sort of curve as does our photoxidation data. Moreover, this curve is similar to the one Gaffron (13) observed in 1926 for the photoxidation processes occurring in an aqueous solution in which porphyrins or chlorophyll were adsorbed at the surface of the proteins. In Fig. 4 are recorded Gaffron's observation (marked with stars) together with those of Warburg (marked with crosses), and our own. The fact that the significant variations occur in the same range of oxygen pressures for all three sets of observations is a strong indication that the phenomena are related. We see a further connection in the fact that after a long irradiation the difference between the rates of photosynthesis in N_2 and O_2 respectively is greatly reduced, just as photoxidation in leaves goes down with the irradiation time.

A great quantitative difference exists, however, between the results of the direct and the indirect methods of photoxidation measurement. The diminishing of the rate of photosynthesis by oxygen is usually 10 to 20 times greater than the rate of photoxidation itself; one must therefore assume that the direct uptake of oxygen by photoxidation is small compared to the indirect influence on photosynthesis. That is just the same conclusion to which Myers and Burr (3) were guided by their observations of the influence which extremely high light intensities have on the photosynthetic rate in *Chlorella*. These authors did not vary the oxygen concentration but irradiated the algae in the presence of air containing CO_2 and in some cases, cyanide as well. They observed normal saturation curves forming a flat plateau, but when the light intensity surpassed a critical value the rate started to decrease and continued to do so with further increase of light intensity. At the very highest values of the

light intensity the oxygen production ceases entirely and is replaced by an oxygen consumption which is greater than the normal respiration. The critical intensity at which the influence of the photoxidation becomes discernible depends upon conditions inside of the plants (concentration of CO_2, food, presence or absence of cyanide, etc.). Franck and Gaffron (14) have already pointed out that the critical intensity apparently coincides with the intensity region at which the photosynthetic apparatus begins to become depleted of CO_2 and hence of intermediates of photosynthesis. (See the theory of Franck and Herzfeld (15) and fluorescence experiments of Franck, French, and Puck (16).) A catalyst (called catalyst A) responsible for the formation of the compound CO_2-acceptor-molecule becomes unable to replace this substrate rapidly enough at that intensity. Further, the greater the amount by which the light intensity surpasses the saturation intensity, the more rapidly is this substrate destroyed by dissociation processes. Each shift of the region in which depletion of this substrate occurs (for instance, by addition of cyanide) produces a corresponding shift of the critical region in Myers and Burr's experiments. It is obvious that by a depletion of the photosynthetic apparatus of intermediates the bulk of the chlorophyll becomes available as a sensitizer of photoxidative processes and so produces a sharp rise in the yield of photoxidation. Myers and Burr (3) reject the idea that the consumption of O_2 by photoxidation at the highest intensities would overcompensate photosynthesis. They rather assume that photoxidation produces an inhibitor of photosynthesis. The main reason for this hypothesis is the fact that the photosynthetic activity is found to be reduced or entirely stopped after a photoxidation period when tested with light intensities so low that photoxidation no longer plays a rôle. The rate of photosynthesis recovers if the photoxidation period was not too strong and too long. These results of the indirect method agree entirely with ours measured directly. By too intensive irradiation the plant will be killed and bleached.

It does not seem necessary to enter into a discussion of further similar papers since they are mostly in accordance with the facts already discussed. (For references, compare, for instance, Myers and Burr (3), and Franck and Gaffron (14).)

THEORETICAL DISCUSSION

The experimental data discussed above prove beyond doubt that photoxidation processes occur in plants even under normal conditions. It is a photochemical process taking place together with photosynthesis, but with a much smaller yield. Normally, it does not attack vital parts of the living plants but food or intermediate products of the plant metabolism. As long as the photosynthetic apparatus is connected with intermediates of photosynthesis, the photoxidation is small, but it nevertheless reduces the rate of photosynthesis

strongly though indirectly. If by carbon dioxide limitation or some denudation process the concentration of the photosynthetic intermediates becomes small, photoxidation will become dangerous since vital parts of the plant will now be attacked. The plant can be destroyed by photoxidation, a phenomenon described by botanists as solarization of plants.

The photoxidation processes in plants take place without enzymatic dark reactions (see the experiments with boiled leaves) but probably consist rather of a photochemical process sensitized by the plant pigment, chlorophyll. The characteristics of this process are just the same as the ones observed in photoxidation processes *in vitro* sensitized by chlorophyll or porphyrins adsorbed by proteins in aqueous solution.

Two questions deserve a fuller discussion, the first concerns the process by which photoxidation acts as an inhibitor of photosynthesis and the second deals with the general chemical kinetics of photoxidation processes induced by chlorophyll adsorbed on protein surfaces.

According to Franck, French, and Puck) 16), one must assume that during the induction period of photosynthesis an excess of photo-peroxides can oxidize certain metabolites and that the resulting products in turn are capable of inhibiting photosynthesis by poisoning the catalyst responsible for photosynthesis saturation (called catalyst B). To explain the inhibition by photoxidation one need only make the hypothesis that this process results in the formation of the same or a similar inhibitor. It offers a natural explanation for the fact that the limiting influence of a high oxygen concentration vanishes at low light intensities. At these low intensities a great part of catalyst B can be inactivated by the inhibitor without influencing the rate of photosynthesis.

The dependence of the magnitude of the oxygen influence on internal factors can be understood by the fact that the concentration of the metabolic product (which is attacked by photoxidation) varies considerably in leaves. The reversal of the inhibition can occur in the dark since the inhibiting oxidation product is consumed by respiration.

We now inquire as to why the recovery time of photosynthesis is much longer (several hours) than the lifetime of the inhibitor (about 1 second, see Franck, French, and Puck). The abnormally long induction periods of photosynthesis and the still longer dark recovery periods required after photoxidation have to be explained in a different manner. We believe that they both are caused by the denudation of the photosynthetic apparatus of intermediates. A normal rate of photosynthesis is only possible if the supply of intermediates is not too small and if the different intermediates all have the same concentration. If intermediates are lacking at the beginning of an irradiation period in the presence of carbon dioxide, it takes some time to build up equilibrium concentrations by photosynthetic activity. Until that is accomplished, the photosynthetic rate is subnormal. The time observed for the duration of the induction periods (at different light intensities) after an exposure in a carbon

dioxide free atmosphere is of the expected order of magnitude. According to Franck and Herzfeld (15) the intermediates can also be very slowly restored in the dark by a reversal of photosynthesis (not identical with normal tissue respiration). We believe that this process is responsible for the recovery in the dark.

We will now discuss the chemical kinetics of photoxidation sensitized by chlorophyll adsorbed on protein in an aqueous solution. The fact that the reaction becomes oxygen-saturated at an oxygen concentration which is many thousand times greater than the one which saturates photoxidation processes sensitized by chlorophyll in organic solutions is of special interest. This means that in the aqueous solutions oxygen has to react with a very unstable molecule. One can calculate from the oxygen concentration at the saturation pressure that the lifetime is equal to or somewhat greater than 10^{-8} to 10^{-9} seconds. The obvious explanation that the molecule in the unstable state is the chlorophyll molecule excited by light absorption must be rejected for two reasons. In the first place the lifetime of the excited chlorophyll molecule (compare Franck and Herzfeld (17)) in the leaves is still 10 to 100 times smaller than the above value and secondly, the fluorescence of the chlorophyll in leaves is not, or at least is practically not, quenched by an atmosphere of oxygen. To explain the behavior of the chlorophyll fluorescence in relation to photoxidation processes in organic solutions, Franck and Livingston (18) had to introduce the hypothesis that chlorophyll can be transformed by light absorption processes into an unstable tautomeric configuration with a lifetime much longer than 10^{-8} seconds. But according to Livingston's (19) observations this lifetime will be shortened by impacts with molecules of many organic substances which catalyze back reactions leading to the stable modification of chlorophyll. In the present case the chlorophyll is in permanent contact with the protein, a fact which easily can account for the reduction of this lifetime to the desired value. We, therefore, propose that impacts between the chlorophyll tautomer and oxygen are also responsible for the photoxidation. By the reaction of the unstable tautomer with oxygen, monodehydrochlorophyll and HO_2 are formed (as in organic solutions—compare Weiss (20) and Franck and Livingston (18)). The radical HO_2 is able to oxidize organic matter but its oxidation reactions have to compete with back reactions in which the stable modification of chlorophyll and molecular oxygen are restored. In very dilute, true solutions the chance for back reactions is small but since in our case the chlorophyll is adsorbed in a high concentration on complicated interfaces of a protein, the back reactions become very significant. The HO_2, especially at higher light intensities, has ample opportunity to collide with monodehydrochlorophyll before it can attack the substrate to be oxidized. Calculations yield the result that under the conditions mentioned the quantum yield must be small for small concentrations of the oxidizable substrate and that the oxygen consumption will rise with the square root of the light intensity. The first result is in

accordance with our observations and the last one is at least not in contradiction to the results here presented, within the limits of error of the experiments.

SUMMARY

1. Photoxidation in leaves is measured by exposing them to light in an atmosphere free from carbon dioxide but containing varied percentages of oxygen.

2. Photoxidation is observed in living leaves as well as in dead ones and in plant juices. Its rate is only slightly enhanced by feeding the leaves with sugar, but the respiration (autoxidation) becomes considerably enlarged during the exposure and the following dark period.

3. The rate of photoxidation rises slower than linearly with light intensity; its dependence upon oxygen pressure has the character of a saturation curve. Oxygen saturation occurs at about 6/10 of an atmosphere of oxygen. A similar dependence on oxygen pressure has been observed by Gaffron for photoxidation *in vitro* sensitized by chlorophyll adsorbed on proteins and by Warburg for the depression of the saturation rate of photosynthesis.

4. The influence of photoxidation on photosynthesis and the chemical kinetics of photoxidation are discussed.

BIBLIOGRAPHY

1. Warburg, O., Katalytische Wirkungen der lebenden Substanz, Springer, 1928, p. 346, Fig. 2.
2. van der Paauw, F., *Rec. trav. bot. néerl.*, 1932, **29,** 497.
3. Myers, J., and Burr, G. O., *J. Gen. Physiol.*, 1940, **24,** 45.
4. French, C. S., *J. Gen. Physiol.*, 1940, **23,** 469.
5. Noack, K., *Z. Bot.*, 1923, **12,** 273; *Naturwissenschaften*, 1926, **14,** 385.
6. Shri Ranjan, *J. Indian Bot. Soc.*, 1940, **19,** 20.
7. Föckler, H., *J. wissensch. Bot.*, 1938, **87,** 45.
8. Montfort, C., and Fökler, H., *Planta,* 1938, **28,** 515.
9. Gaffron, H., *Biol. Centr.*, 1939, **59,** 288.
10. Mevius, W., *Jahrb. Bot.*, 1935, **81,** 327.
11. McAlister, E. D., and Myers, J., *Smithson. Misc. Coll. 99*, No. 6, 1940.
12. Wassink, E. C., Vermeulen, D., Reman, G. H., and Katz, E., *Enzymologia*, 1938, **5,** 100.
13. Gaffron, H., *Biochem. Z.*, Berlin, 1933, **264,** 251.
14. Franck, J., and Gaffron, H., Advances in enzymology, 1941 Interscience Publishers, 1941, **1,** 199.
15. Franck, J., and Herzfeld, K. F., *J. Physic. Chem.*, 1941, **45,** 978.
16. Franck, J., French, C. S., and Puck, T., *J. Physic. Chem.*, 1941, in press.
17. Franck, J., Herzfeld, K. F., *J. Physic. Chem.*, 1937, **41,** 97.
18. Franck, J., and Livingston, R., *J. Chem. Physics*, 1941, **9,** 184.
19. Livingston, R., personal communication.
20. Weiss, J., *Naturwissenschaften*, 1935, **35,** 610; *Tr. Faraday Soc.*, 1939, **35,** 48. Carter, A. H., and Weiss, J., *Proc. Roy. Soc., London, Series A*, 1940, **174,** 359.