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THE ASCORBIC ACID SYSTEM IN LEAVES: FURTHER OBSERVATIONS ON PHOTOOXIDATION AND PHOTOREDUCTION

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Abstract—Photooxidation of ascorbate and photoreduction of the oxidized forms of this compound have been demonstrated in intact strawberry leaves. Photoreduction may be separated from photooxidation by (1) the different time sequence in their initiation by light, (2) the suppression of photooxidation by cyanide, (3) the greater ease with which the photoreduction system is inactivated by heat, and (4) the ability of far red light (710–720 mμ) to activate photooxidation but not photoreduction. The relevance of these observations in relation to the possibility of the ascorbate system acting as electron donor and acceptor in the photosynthetic process is discussed.

WE HAVE already described experiments on the photooxidation of L-ascorbic acid in strawberry leaves and the corresponding photoreduction of the oxidized products in cyanide poisoned leaves.[1] We have moreover shown that these light catalysed reactions appear to be superimposed on reactions of oxidation and reduction which occur in the darkened leaf. The experiments to be described below are an extension of this work and afford additional evidence suggesting that ascorbic acid may act as electron donor, and its oxidized form as electron acceptor either in the photosynthetic process, or in reactions closely associated with this process.

Photoreduction in Normal Leaves

In our earlier work we only demonstrated the process of photoreduction in leaves which had been pretreated with cyanide (5×10^{-5} M) to suppress the process of photooxidation. We did however observe in normal leaves that the process of photooxidation was preceded by an induction phase. We have now found that during this induction phase a rapid photoreduction of dehydroascorbic acid can be demonstrated. Results of experiments with strawberry leaves illuminated with polychromatic tungsten light are shown in Fig. 1 and it can be seen that the rates of both oxidation and reduction are increased with increase in the intensity of illumination. The photoreduction process is very rapid; with an illumination equivalent to 5000 lx the steady state level of dehydroascorbic acid (14–15 μg/g) is reduced to zero in 2 sec. The process of reduction is followed by one of oxidation, and as described previously[1] the concentration of DHA rises to a new steady state level which depends on the intensity of illumination. There appears to be an exponential relationship between intensity of illumination and the rate of both photoreduction and photooxidation as shown by Fig. 2.

Because of the differences in the length of the induction phase, photoreduction may be readily separated from that of photooxidation by subjecting the leaves to a short flash of light

[1] L. W. MAPSON, *Biochem. J.* **85**, 360 (1962).

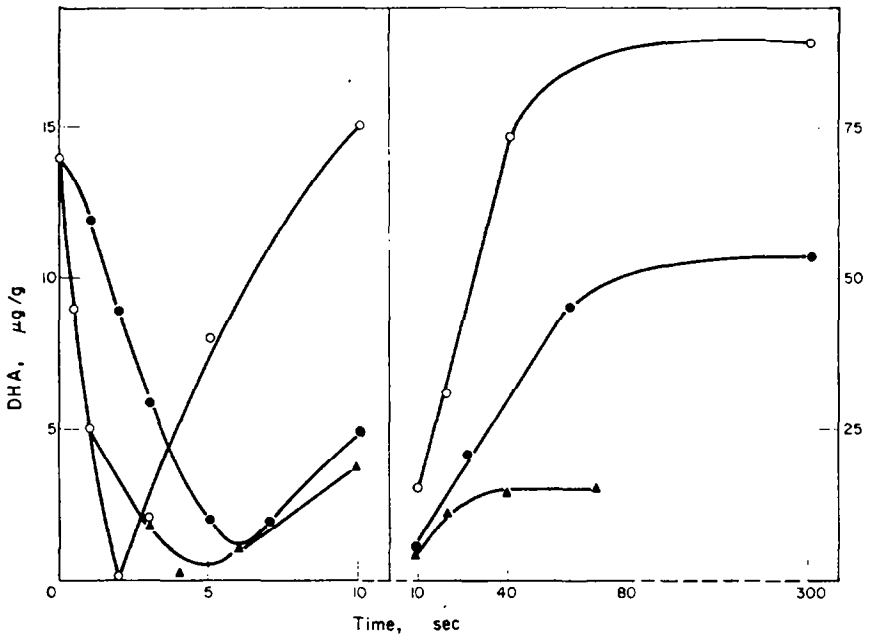


FIG. 1. PHOTOREDUCTION AND PHOTOOXIDATION IN LEAVES ILLUMINATED AT ZERO TIME WITH POLYCHROMATIC LIGHT.
Intensity of illumination, ○ = 5000 lx; ● = 500 lx; ▲ = illumination for 1 sec only, 5000 lx.

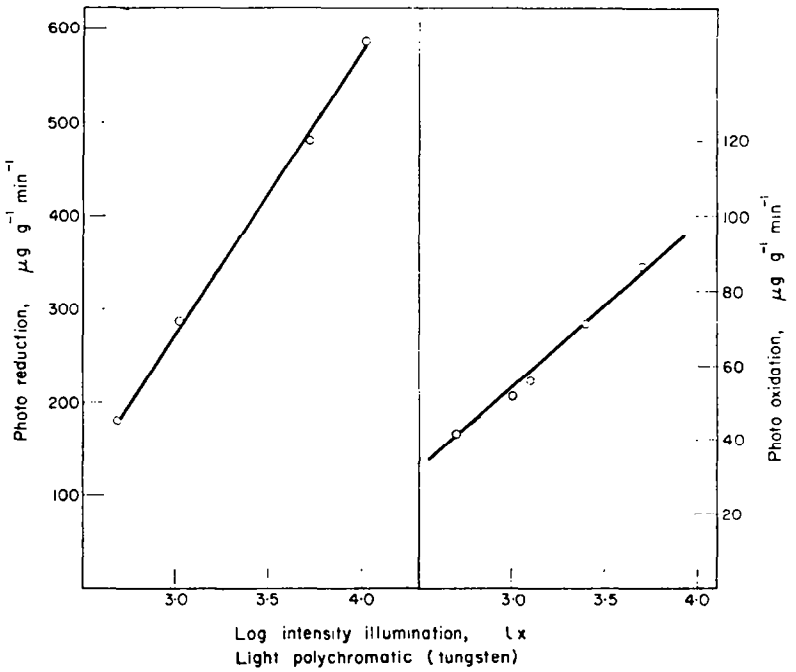


FIG. 2. RELATION BETWEEN RATES OF PHOTOREDUCTION AND PHOTOOXIDATION AND INTENSITY OF ILLUMINATION.

(5000 lx for 0·5–1 sec). Under these conditions the concentration of DHA of the darkened leaf decreases from its steady state level to a value approaching zero, to be followed by a rise again to its original level but no further photooxidation occurs and the final level appears to be dependent on an equilibrium between reactions of oxidation and reduction which exist in the darkened leaf (Fig. 1).

Relation between Induction Phase and Rate of Reaction

In the experiments described above with high light intensity (5000 lx), no induction period could be detected between the onset of illumination and the start of the photoreduction

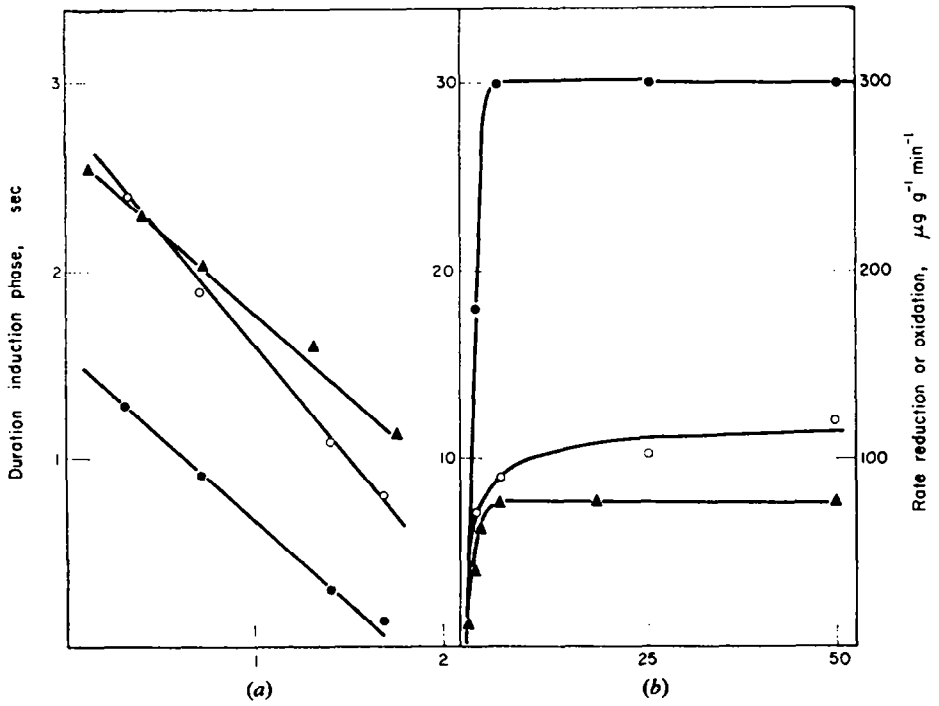


FIG. 3 (*a*) RELATION BETWEEN INTENSITY OF ILLUMINATION WITH MONOCHROMATIC LIGHT AND DURATION OF INDUCTION PHASE FOR PHOTOREDUCTION AND OXIDATION IN NORMAL AND CYANIDE POISONED LEAVES. (*b*) RELATION TO RATE OF REACTION.

● = Photoreduction in normal leaves (ordinates on left-hand side of Fig. 3*a*)
○ = Photooxidation
▲ = Photoreduction in cyanide poisoned leaves
} Ordinates on right-hand side of Fig. 3*a*.
Ordinates on right-hand side of Fig. 3*b*. Symbols as for Fig. 3*a*.

reaction, and if one exists it must be of shorter duration than 0·1 sec. With lower light intensity (500 lx) a short induction period was observed of the order of (0·1–0·2 sec). The existence of an induction phase was more readily shown in experiments in which monochromatic light (435·8 mμ) of low intensity was used, and its duration was inversely related to the logarithm of the intensity of illumination (Fig. 3). As in the former experiments the onset of the photo-

oxidation did not occur until all of the DHA had been reduced. As the results in Fig. 3*a* show, the induction phase for photooxidation was some 10–20 times longer in duration than that for photoreduction, and like the latter was related exponentially to the intensity of illumination. It will also be noted that the maximum rate of photoreduction is achieved with this monochromatic light at very low levels of illumination (5 lx). This maximum rate of photoreduction is lower than that achieved with polychromatic light of 5000 lx intensity, an observation which suggests that pigments other than that absorbing light at 435·8 mμ are effective in promoting these reactions. There appeared in these experiments to be no exact inverse correlation between the duration of the induction phase and the subsequent rate of either the photoreduction or photooxidation process. Thus with an intensity of illumination of 5 lx the maximum *rate* of photoreduction was achieved, yet the *duration* of the induction phase decreased still further as the illumination was increased. The correlation between these two factors in the photooxidation was closer but even here an increase of illumination from 5 to 50 lx halved the duration of the induction phase but only increased the rate by 30%. One further point is worthy of mention in connexion with these results, namely that the absolute rate of photoreduction is some three times the corresponding apparent rate of photooxidation. We shall return to a discussion of this observation later in this paper.

Photoreduction in Cyanide Poisoned Leaves

We have re-examined the process of photoreduction in cyanide poisoned leaves (cyanide 5×10^{-5} M), in relation both to intensity and duration of illumination. In these leaves photooxidation is suppressed in relation to that of photoreduction to such an extent that illumination of such leaves reduces the steady state level of DHA to zero which remains there as long as the illumination of the leaf continues.[1] A comparison of the rates of reduction of DHA in normal and cyanide treated leaves exposed to monochromatic radiation (435·8 mμ) shows that, here, cyanide has also some inhibitory effect on photoreduction. Thus, as the results of experiments in Fig. 3 show, the duration of the induction period is more than 10 times greater in the cyanide leaf than in the normal leaf, and the ensuing maximum rate of reduction has decreased by a factor of 4 in the poisoned leaves. Despite these quantitative differences the response to varying the intensity of illumination appears to be qualitatively similar in both cases, i.e. the maximum rate of reduction is achieved at the same level of illumination and the decrease in the length of the induction phase is related to the intensity of illumination expressed exponentially. The photoreduction in cyanide poisoned leaves like that in normal leaves may be initiated by a short flash of light, the ensuing reaction proceeding in the dark. Such experiments have the advantage that there is no interference from the photooxidative reaction. The results of experiments with monochromatic light (435·8 mμ) at an intensity of 5 lx are illustrated in Fig. 4. They show that light of this intensity, for only 1 sec duration is sufficient to initiate a maximal response, increasing the duration of the light leads only to a decrease in the length of the induction phase. With light of this intensity flashes of shorter duration than 1 sec led not only to an extension of the latent period, but also to a reduction in rate of reaction until with flashes of 0·2 sec or less, no reaction could be detected. Again, as with the experiments in which intensity of illumination was altered, there was an inverse exponential relation between duration of the induction phase and duration of the light flash (Fig. 4).

On removal of light, photoreduction is followed by re-oxidation to the steady state level of DHA existing in the darkened leaf. The whole sequence of reactions may be initiated again by a second exposure to light (Fig. 5). From the data illustrated in this figure it appears that the

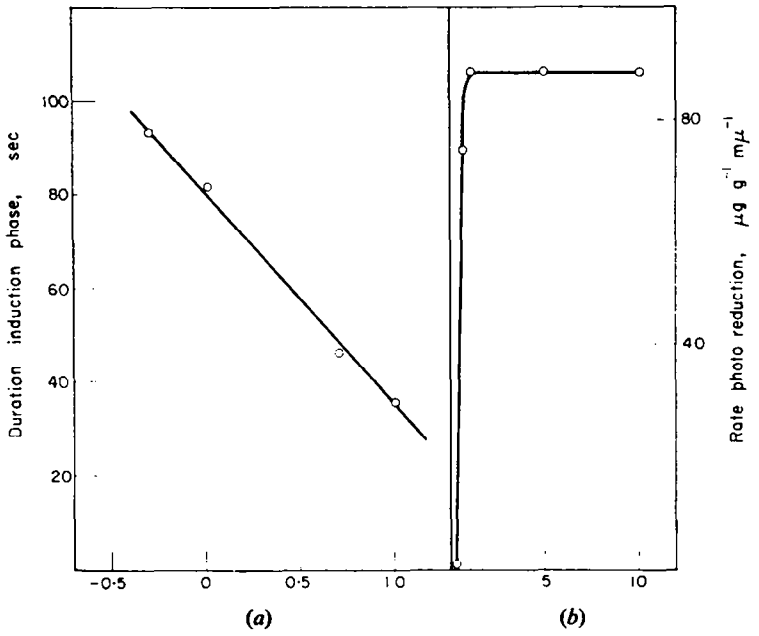


FIG. 4. RELATION BETWEEN DURATION OF ILLUMINATION WITH MONOCHROMATIC LIGHT AND (*a*) THE DURATION OF THE INDUCTION PHASE AND (*b*) ENSUING RATE OF REACTION IN CYANIDE POISONED LEAVES.

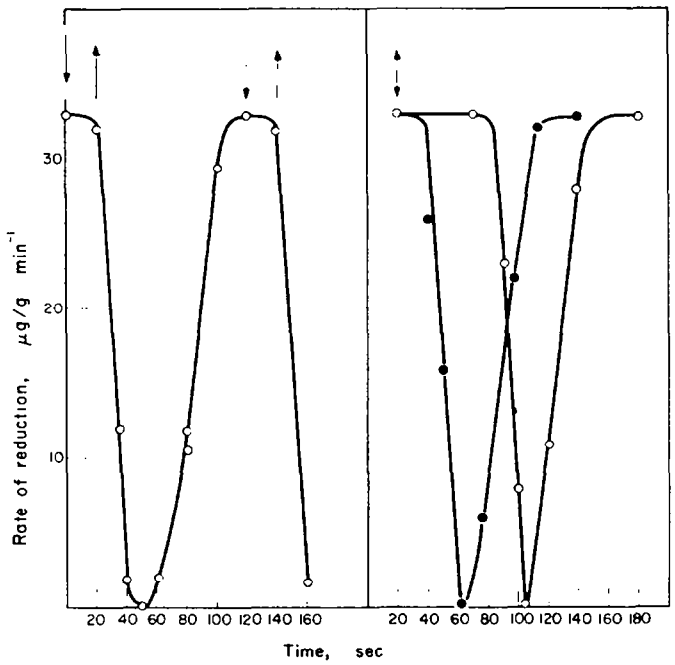


FIG. 5. EFFECT ON THE LEVEL OF DHA IN CYANIDE POISONED LEAVES ON EXPOSURE TO SHORT PERIODS OF ILLUMINATION OF MONOCHROMATIC LIGHT (435·8 mμ).

Light intensity, ○ = 5 lx; ● = 60 lx; ↓ Denotes light on; ↑ = Light off; ↕ = 1sec illumination only.

process of re-oxidation is delayed if the initial light treatment is greater than the minimum required to elicit a maximum response. For example, when the leaf was exposed to light (5 lx) for 1 sec, re-oxidation occurred almost immediately after photoreduction, but with a 10 sec exposure a delay of approximately 5 sec occurred before photooxidation was observed.

One other point is worthy of mention in the context of these experiments, namely that the maximal rate of photoreduction observed with cyanide poisoned leaves was always somewhat greater (20%) when the reaction was initiated by a short flash of light than when the leaves were continuously illuminated by light of the same intensity. This may in effect indicate that photooxidation was not completely suppressed by the cyanide treatment and was operating to some extent in the leaves illuminated with continuous light.

It has been established[2] that the steady state level of DHA in non-photosynthetic tissues is dependent on the oxygen status of the tissue. This can also be shown to be true for photosynthetically active tissues in the absence of light. The steady state level of DHA existing in strawberry leaves in the dark is rapidly reduced to zero when oxygen is removed from the external atmosphere, but readjusts itself back to its normal value on admittance of air (cf. Fig. 8). We have now shown that these dark reactions can be shown to be operating equally well in the cyanide treated leaf.

Temperature Coefficient

Primary photochemical reactions are independent of temperature. It was of interest therefore to determine if the processes of photoreduction and oxidation fell within this category. The results detailed in Table 1 show that they do, the Q_{10} for both processes being unity or

TABLE 1. TEMPERATURE COEFFICIENTS OF PHOTOOXIDATION AND PHOTOREDUCTION

Light	Incident intensity (lx)	Rate photoreduction (μg/g per min)			Rate photooxidation (μg/g per min)		
		1°	25°	Q_{10}	1°	25°	Q_{10}
Tungsten	500	180	180	1·0	96	108	1·04
,,	1000	270	300	1·04	144	160	1·04
,,	10000	600	600	1·0	225	240	1·04
,,	10000+5% CO_2	600	600	1·0	275	290	1·02
435·8 mμ	5	240	300	1·1	36	36	1·0

slightly greater. The Q_{10} for the assimilation of CO_2 on the photosynthetic process has been shown to be variable depending on whether low or high light intensities were used. With low light intensities (photochemical reactions limiting) the rate of assimilation has been found to be independent of temperature, with high light intensities especially with CO_2 at higher levels than air, higher temperature coefficients were obtained indicating that enzymic reactions were limiting the overall rate. We have therefore determined the Q_{10} of the reactions being studied here both in high and low light intensity and with CO_2 in ample supply in the external atmosphere. As the results (Table 1) show in no case did we find temperature coefficients much above unity.

The data given in Table 1 also show CO_2 at 5% level and at high light intensity stimulated the rate of photooxidation (21–22%), without affecting that of photoreduction. This stimulation was observed at both 1° and 25°.

[2] L. W. MAPSON, *Ann. N.Y. Acad. Sci.* **92**, 21 (1961).

Action Spectra

Preliminary experiments have been carried out to ascertain the effectiveness of light of different wavelengths in promoting both photoreduction and oxidation. In the absence of a suitable monochromator we were restricted to the isolation of different parts of the spectrum by the use of filters and combination of filters, and in this we were hampered by the difficulty of obtaining suitable filters capable of isolating narrow bands at the red end of the spectrum.

The light energy incident on the leaves at each of the different wavelengths was adjusted to be approximately equal to 200 ergcm^2/sec.

The efficacy of light in promoting photoreduction may be measured either (1) by determining the rate of reaction with light intensities which do not show a maximal response or (2)

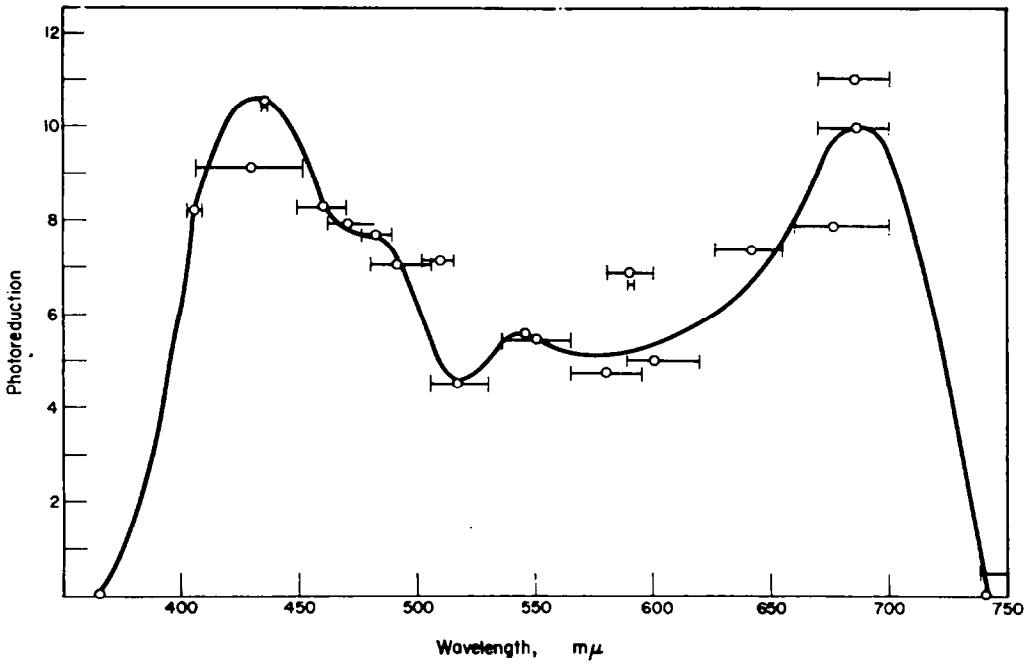


FIG. 6. ACTION SPECTRUM OF PHOTOREDUCTION IN CYANIDE POISONED LEAVES.
Ordinates: reciprocal of duration of induction phase, sec.
|—| Indicates wavelength at which at least 50% of incident light is transmitted.

by determining the length of the induction phase. In the latter case the efficiency of the light is related inversely to the duration of the induction phase. Because only a very low level of monochromatic light of effective wavelength was sufficient to give a maximum rate response, we used the second method since higher levels of illumination could be used and differences were more accurately measured. In addition we employed leaves poisoned with cyanide (5×10^{-5} M) in order to isolate the process of photoreduction from that of photooxidation.

The results of an action spectrum so determined are shown in Fig. 6 where the ordinates represent the reciprocal of the duration of the reduction phase in seconds. The spectrum shows that the effective light is confined to the visible spectrum, and that the maximum response is relegated mainly to the blue and red ends of the spectrum.

A similar determination was also carried out for photooxidation. In this case the ordinates in Fig. 7 represent the rates of the reactions. Here again the effective light is restricted to the

visible spectrum, the most effective being relegated to blue and red regions with additional activity with light around 500 mμ. Although it is hoped to obtain more precise information in the future by using a monochromator, the present experiments nevertheless suggest that the chlorophylls are implicated as initiators of these photochemical reactions, although the participation of other pigments (e.g. phytochromes) is not excluded.

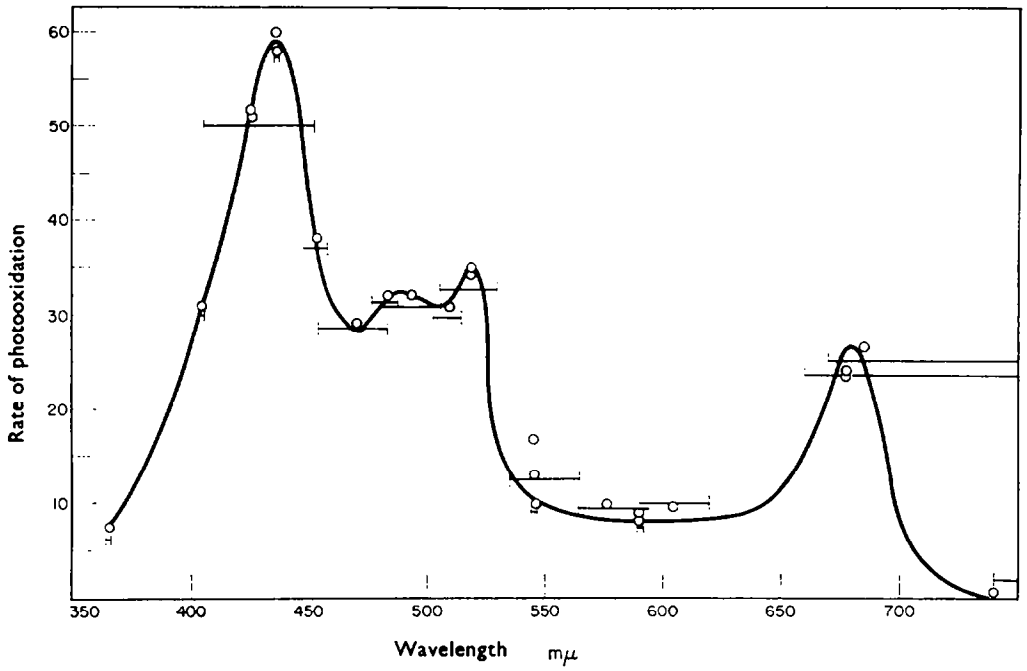


FIG. 7. ACTION SPECTRUM OF PHOTOOXIDATION.
Ordinates: rate of production of DHA on illumination (μg/g per min).
|—| Indicates wavelength at which at least 50% of incident light is transmitted.

Differentiation of Photoreduction from Photooxidation by Heat

It is possible to inactivate the ability of the leaf to photoreduce the oxidized forms of L-ascorbic acid almost completely if the leaf is subjected to heat treatment. Thus if strawberry leaves are heated for 5 min at 58° in the complete dark—the heated leaf still behaves as normal leaves in its ability to stabilize the steady state concentration of DHA at a level of approximately 14–15 μg/g. Moreover the concentration of DHA responds, as in normal leaves, to alterations in the oxygen tension of the external atmosphere (Fig. 8). There is in fact nothing to indicate that either the oxidation or reduction reactions associated with the darkened leaf are affected by the heat treatment. When however such a leaf is illuminated the ability of the tissue to photoreduce the oxidized forms of the vitamin is almost completely lost. A consequence which seems to follow from this is that once the photooxidation process sets in there is a rapid oxidation of ascorbic acid which proceeds far beyond the steady state level observed with normal leaves, to a point when most of the total ascorbic acid content of the leaf is oxidized (Fig. 9). The temperature of 58° appears to be critical as far as the strawberry leaf is concerned. At 59° and above, the leaf behaves abnormally in the dark, in that oxidation of all of the ascorbic acid occurs. This oxidation is still further accelerated by heat treatment

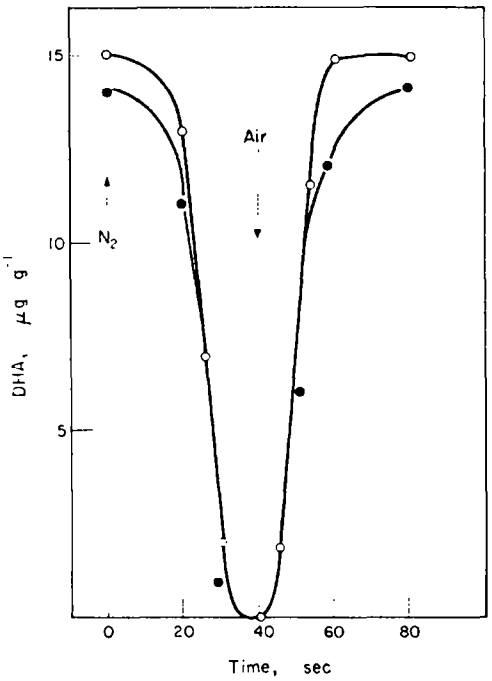


FIG. 8. RESPONSE OF NORMAL AND HEATED LEAVES TO ALTERATIONS IN OXYGEN TENSION IN COMPLETE ABSENCE OF LIGHT.

○ = Normal leaves; ● = Leaves heated 58° for 5 min.;
↑ = Into nitrogen; ↓ = Into air.

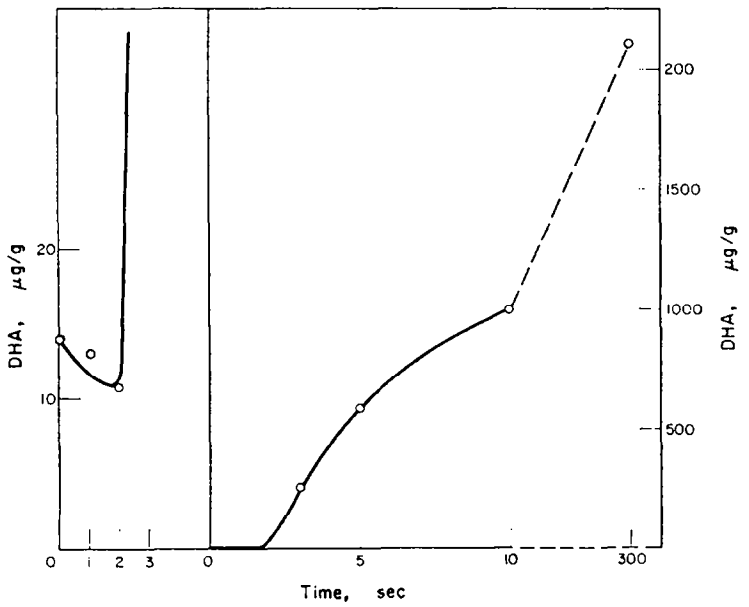


FIG. 9. EFFECT OF LIGHT ON LEVEL OF DHA IN LEAVES PREVIOUSLY HEATED AT 58°. (Polychromatic tungsten light 5000 lx) at zero time.

at 60 to 65°. At temperatures of 55° and less the leaf behaves normally both in the light and dark.

The time sequence of photoreduction and photooxidation remains the same in leaves heated at 58° as in normal leaves. Thus the induction phase prior to the onset of oxidation is of the same duration, under comparable conditions, in both sets of leaves (cf. Fig. 1 with Fig. 9). This suggests that the photooxidation process remains relatively unaffected by the heat treatment whereas the whole process of photoreduction is impaired or destroyed, rather than the alternative explanation that the photooxidation process has been hastened thus obscuring a normal photoreduction reaction.

In our earlier work[1] we visualized two factors that might operate to maintain the steady state level of DHA in illuminated leaves. The first that it represented an equilibrium between rates of reduction and oxidation and the second that the steady state level was ultimately stabilized by the fact that rate of photooxidation under conditions of high illumination was limited by the actual concentration of the vitamin at the site of the reaction. There is reason to believe, as will be shown later, that changes in this second factor may be contributing to the extensive oxidation of ascorbate observed when heated leaves are illuminated if one effect of the heat treatment is to increase the concentration of ascorbate in the 'metabolic pool'.

Differentiation of Photoreduction from Photooxidation by Light of 710–720 mμ

Emerson *et al.*[3] first showed that light absorbed in the far red end of the absorption spectra of green algae at ~ 700 mμ showed a poor yield of photosynthesis, unless supplemented with light of shorter wavelength (650 mμ). They concluded from their work that two photochemical reactions may be involved. This hypothesis has been confirmed and its interpretation extended by the work of several investigators, notably Witt *et al.*,[4–6] Duysens[7] using flash spectrophotometric methods, and by Losada *et al.*[8] from oxygen and NADPH measurements used in conjunction with certain cellular poisons. The general consensus of opinion is that electrons are transferred from excited chlorophyll molecules in two systems. The first of these systems, activated by light energy $h\nu_1$, catalyses the reduction of pyridine nucleotide and oxidation of cytochrome, the second system, activated by light energy $h\nu_2$, catalyses the reduction of plastoquinone and of cytochrome. Although the wavelength limits of these two systems differ in different organisms in general, action spectra of the isolated light reaction $h\nu_1$ shows that it is excited by light the far red limit of which is 730 mμ whilst the corresponding limit for $h\nu_2$ excitation is 705 mμ. Hence using far red light (710–720 mμ) $h\nu_1$ is almost solely excited (Witt *et al.*[6]).

If the photoreduction and oxidation reactions studied in this paper are intimately connected with these electron transfer systems the possibility existed that these separate reactions of oxidation and reduction might be differentiated by exciting one or other of these photochemical systems. We have accordingly studied the effect of light in the red end of the visible spectrum on the separate reactions of photoreduction and oxidation, using a combination of filters. We have found that light transmitted around 710–720 mμ was effective in promoting photooxidation of ascorbate, but ineffective in promoting photoreduction of the oxidized forms of the vitamin. Light transmitted from 720 mμ onwards was ineffective in both cases,

[3] R. EMERSON, R. CHALMERS, C. CEDARSTRAND and M. BRODY, *Science* **123**, 673 (1956).
[4] H. T. WITT and R. MORAW, *Z. phys. Chem. Neu Folge.* **20**, 253 (1959).
[5] H. T. WITT, A. MULLER and B. RUMBERG, *Nature*, **191**, 194 (1961).
[6] H. T. WITT, A. MULLER and B. RUMBERG, *Nature*, **197**, 987 (1963).
[7] L. N. M. DUYSENS, *Proc. Roy. Soc.* **B157**, 301 (1963).
[8] M. LOSADA, F. R. WHATLEY and D. I. ARNON, *Nature*, **190**, 606 (1961).

whereas filters transmitting light of shorter wavelength (665 mμ) promoted both photoreduction and oxidation, Fig. 10. These results indicate that the effective wavelength of light for promoting photooxidation alone is in the region of 710–720 mμ in the case of the strawberry leaf.

Confirmation that the observed results were due to the inability of light of 710–720 mμ to excite the process of photoreduction, and not merely to delay its onset so that it was obscured by that of photooxidation, was obtained in experiments on cyanide poisoned leaves.

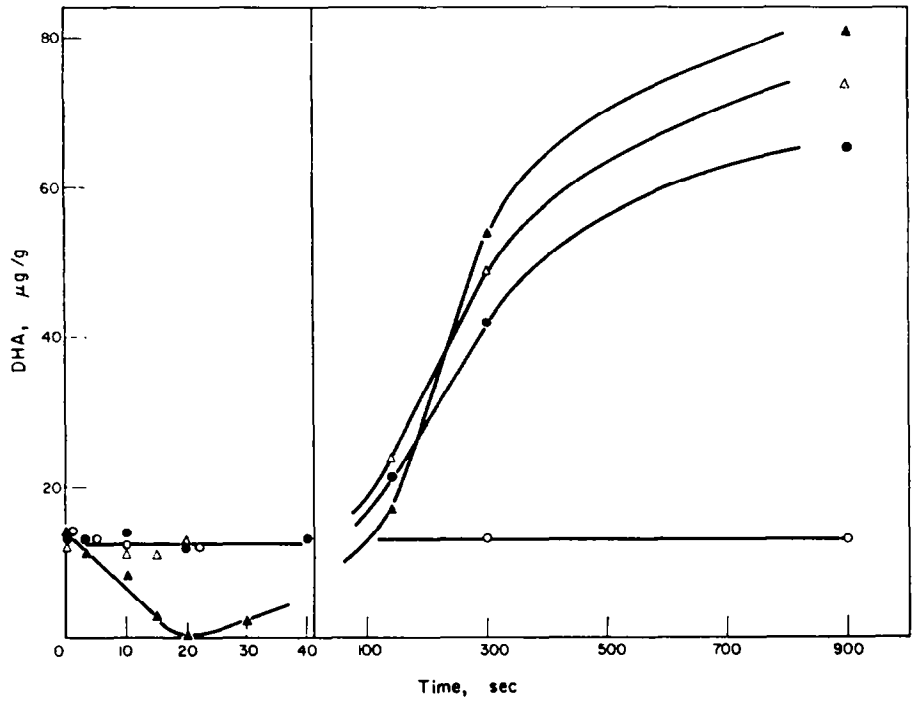


FIG. 10. EFFECT OF FAR RED LIGHT ON PHOTOREDUCTION AND OXIDATION.

○ Light transmitted by filters > 720 mμ, intensity of incident light 8000 lx.
● ,, ,, ,, ,, > 710 mμ, ,, ,, ,, ,, 4000 lx.
△ ,, ,, ,, ,, > 710 mμ, ,, ,, ,, ,, 12,000 lx.
▲ ,, ,, ,, ,, > 665 mμ, ,, ,, ,, ,, 4000 lx.

In such leaves, as already shown, photooxidation is virtually suppressed so that even small effects on photoreduction may be observed. Illumination of such leaves with light of 710–720 mμ gave no sign of any photoreduction even after illumination for several minutes. Such results indicate that light of this wavelength is incapable of initiating photoreduction.

Effect of Far Red Light on Heated Leaves

We have already shown that leaves heated at 58° respond to polychromatic illumination with a rapid photooxidation of most of the ascorbate in the leaf. In such leaves a similar sequence of events may be initiated by exposure to far red light (710–720 mμ) (Fig. 11). Unheated leaves which also respond initially in the same manner differ from the heated leaf in that the rate of photooxidation falls off with time much more rapidly than with the heated

leaf. Since in both cases photoreduction is not excited under these conditions, the difference must be due to some other property of the leaf which has been altered by the heat treatment. The property which we would suggest is altered, is that of intracellular permeability, destruction or lowering of which would allow much or all of the ascorbic acid of the leaf to gain access to

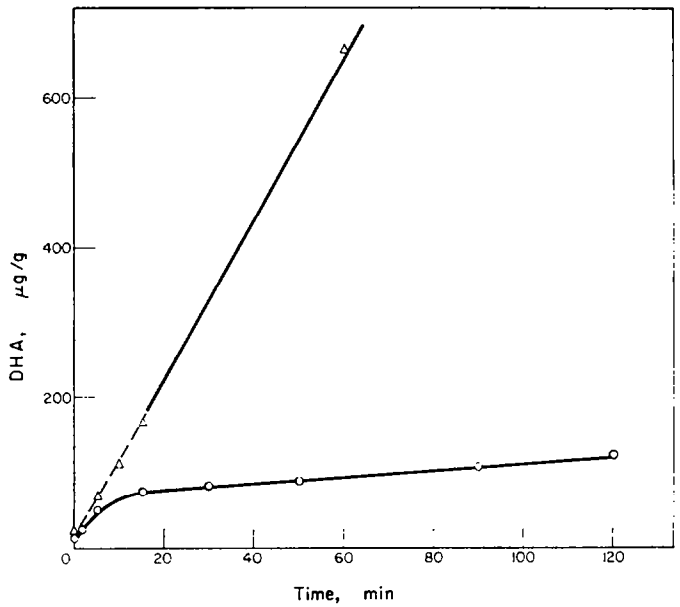


FIG. 11. EFFECT OF FAR RED LIGHT (710–720 mμ) ON PHOTOOXIDATION IN NORMAL AND HEATED LEAVES.
○ Normal leaves; △ Leaves heated 5 min at 58°.

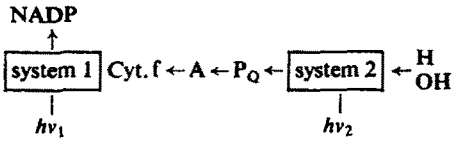
the site of these photochemical reactions. If this indeed is the case then the steady state level observed in the normal illuminated leaf is the result of an interplay of several factors including dark oxidation and reduction reactions, supplemented by those of photoreduction and oxidation, the rate of the latter being ultimately limited by the concentration of the vitamin at the photochemical site.

INFLUENCE OF CERTAIN INHIBITORS OF PHOTOSYNTHESIS

1'-(3-4-Dichlorophenyl)-1,1-dimethylurea. In our previous studies[1] we have already described experiments on the influence of 1'-(3-4-dichlorophenyl)-1,1-dimethylurea (DCMU) and substances such as *O*-phenanthroline and hydroxylamine, on the photooxidation of ascorbic acid. These studies revealed that photooxidation like photosynthesis is inhibited by these compounds. With DCMU complete inhibition with concentrations of 10^{-6} to 10^{-7} M was observed. We have now determined the effect of DCMU on the process of photoreduction. Photoreduction may be observed in a DCMU poisoned leaf when this is illuminated, the rate of the reaction has however been inhibited to about 50% of the rate obtaining in a normal leaf (Fig. 12). On continued illumination, the steady state level of DHA is maintained at zero level for a period of about 60 sec after which time there is a readjustment of the steady state level to a value obtaining in the darkened leaf and this level remains constant irrespective of any illumination. We have further shown that this phenomena can be explained as being

The further demonstration that the photoreduction reaction appears to be distinct from that of photooxidation by (1) the different time sequence in the initiation of both processes on illumination, (2) the suppression of photooxidation by cyanide, (3) the more labile nature of the system concerned with photoreduction to temperature, and (4) the difference in their excitation by far red light (710–720 mμ) emphasizes that these two reactions are motivated by different photochemical systems.

If indeed the ascorbic acid system is functioning in the electron transfer sequence of photosynthesis it must, on our results, be positioned between the two light reactions, and in view of the oxidation-reduction potential of the system: ascorbic ⇌ DHA at +0·2 V and the oxidation potential of the system ascorbic ⇌ MDHA as being between +0·2 and 0 V,[12] the most likely position is between plastoquinone and cytochrome f (position A in Scheme 1).



(Electron flow in direction of arrows)

SCHEME 1. SUGGESTED POSITION OF ASCORBATE IN THE ELECTRON TRANSFER SEQUENCE OF PHOTOSYNTHESIS.

On this scheme the oxidized forms of the vitamin would accept electrons from plastoquinol arising as a result of the activation of *system (2)* by light $h\nu_2$. Ascorbate would donate electrons to cytochrome, the flow of electrons being controlled by the activation of *system (2)* by light energy $h\nu_1$.

Three lines of evidence are consistent with this hypothesis. They are: (1) *system (2)* has been shown to be more heat labile than *system (1)*,[6] hence we should expect with *system (1)* still active that photooxidation would predominate over photoreduction and this has in fact been found to be so. (2) The far red limit for $h\nu_2$ excitation is 705 mμ and for $h\nu_1$ 730 mμ,[6] hence with light of 710–720 mμ only excitation of $h\nu_1$ should occur and on our hypothesis only photooxidation but no photoreduction; again this is in accord with our results. (3) If as postulated the ascorbic acid system is positioned at A in the sequence (Scheme 1) then we expect in the first seconds of illumination emphasis on reduction rather than oxidation since the inflow of electrons to the ascorbate system is likely to be quicker than the outflow, for this latter will not attain its maximum rate until the maximum rate of assimilation of CO_2 is achieved and that in turn after a dark period will be conditioned by the rate of build-up of intermediates such as ribulose diphosphate. It is interesting in this connexion to note that in our experiments increasing the CO_2 of the atmosphere increased the rate of photooxidation but not that of photoreduction (Table 1).

These considerations however afford no conclusive evidence that this viewpoint is correct. There is at present no experimental data showing that the ascorbic acid system is obligatory for the electron flow sequence in photosynthesis. The above results could as easily be explained if the ascorbic system was associated with, but not a necessary part of, the electron transfer sequence. It would however seem clear that the enzymic systems catalysing oxidation and reduction reactions operating in the leaf in the absence of light have access to their substrates

[12] H. STAUDINGER, K. KUSCH and S. LEONHAUSER, *Ann. N.Y. Acad. Sci.* **92**, 194 (1961).

produced in the photochemical reactions in light, for the high steady state level of DHA quickly falls, when light is removed, to the low steady state level characteristic of the darkened leaf.

The results with DCMU are also consistent with the above hypothesis. Thus the complete blockage of the electron inflow into the system would render the effect of light inoperative and thus produce the same state as exists in a leaf in the dark. DCMU poisoned leaves do in fact behave, after the first few seconds of illumination, like leaves in the dark; DCMU under these conditions does not affect the dark oxidation and reduction reaction associated with the ascorbic system. In our experiments we have also shown that photoreduction, although inhibited, can be observed temporarily in these poisoned leaves, and have explained this on the theory that a reduced component of the light activated system, is not inhibited by DCMU but reacts with DCMU when it loses an electron under the influence of light. It is of interest that an observation similar in character was observed by Trebst and Eck[13] that the phosphorylation by isolated chloroplasts, in an atmosphere of nitrogen catalysed by Vitamin K_3, becomes resistant to inhibition by DCMU when precautions are taken to add this catalyst in the reduced form, i.e. when the natural component of the tissue oxidizing Vitamin K_3 remains reduced.

EXPERIMENTAL

Light Sources

Illumination of the leaves was either by polychromatic tungsten light, or by more selective bands of the spectrum obtained by the use of various Ilford or Wratten filters used singly or in combination with this light source. In some cases monochromatic light was obtained by the use of suitable filters to isolate the various lines of the Hg spectrum, or by light from a sodium lamp. The conditions of illumination of the leaves were similar to those described by Mapson.[1]

Filters Used for Differentiation of Photoreduction from Photooxidation

The filters used for these experiments consisted of:

(1) Ilford 205+Wratten 34 for transmission from 670 mμ infrared.
(2) Ilford 206+Ilford 806 for transmission from 710 mμ infrared.
(3) Ilford 206+Wratten 45 for transmission from 720 mμ infrared.

Only a small amount of light of shorter wavelength is transmitted below the figures quoted above, these being the wavelength at which 20–25% transmission is obtained by the combination of filters. Incident light was derived from a tungsten lamp.

Heat Treatment of Leaves

The strawberry leaves (approx. 1g), obtained from plants grown in pots in the greenhouse and previously held overnight in the dark, were placed in a glass vessel, previously brought to temperature by immersion in a water bath for 15–30 min. The leaves were transferred to this vessel in the dark and kept under these conditions during the period of heating. One minute

[13] H. TREBST and H. ECK, *Z. Naturf.* **166**, 455 (1961).

was allowed for the leaves to attain the desired temperature and they were held at this temperature for a further 5 min. After heating, the leaves were removed and held for a further 10–30 min before proceeding with the experiment. All these latter operations were conducted in the dark. In the experiments in which the leaves were poisoned with inhibitors before heating these were administered 15–17 hr beforehand and the leaves kept in the dark throughout this period. The introduction of poisons into the leaf was carried out as described by Mapson.[1]

Estimation of Ascorbic Acid and Dehydroascorbic Acid

These estimations were carried out by the indophenol method described by Mapson.[1]