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CONTROL OF PHOTO-OXIDATION AND REDUCTION OF ASCORBATE BY PHYTOCHROME OR SIMILAR COMPOUND

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Abstract—The oxidation of ascorbate and reduction of its oxidized forms that occur when leaves are illuminated with green light (500–520 nm) are prevented if the leaf is previously exposed to either far-red radiation (λ_{max} 730 nm) or blue light (λ_{max} 421 nm). After such treatment the leaf is said to be in an inactive state, but can be transformed into an active state by subsequent exposure to red light (λ_{max} 655–660 nm). The wavelength of blue or far-red light capable of rendering the leaf inactive corresponds closely with the reported absorption of the P_{fr} form of phytochrome in etiolated seedlings, that of red light which reverses this effect closely with the reported λ_{max} of the P_r form of phytochrome. It has also been shown that green light (λ_{max} 514 nm) can, under certain specified conditions, prevent the development of the inhibition induced by blue or far-red light. The process of photoreduction, which alone is observed in cyanide poisoned leaves, is also controlled by the red–far-red light reaction. The latter reaction is not affected by the photosynthetic poison DCMU which does, however, inhibit the primary reaction of photo-oxidation and reduction. The reactions of oxidation and reduction of the ascorbate system that occur in the darkened leaf are not controlled by the red–far-red light reaction. The identity of this controlling system with phytochrome is discussed.

INTRODUCTION

IT HAS been shown[1,2] that the oxidation of ascorbate, and the reverse process the reduction of the oxidized forms of the vitamin that occurs in leaves when illuminated, are separate photochemical reactions. More recently we have shown[3] that these photochemical reactions (referred to in this paper as the primary photochemical reactions) are themselves conditioned by previous exposure of the leaf to either red or far-red light. Prior illumination of the leaf with red light (600–700 nm) ensured a positive and, illuminating with far-red (> 700 nm) a negative response, to green light (500–540 nm). In the former case the leaf may be said to be in an "active" and in the latter in an "inactive" state.

In these earlier experiments[1,2] the leaves used were initially in an "active" state since we have subsequently shown that daylight produces the same effect as red light. Nevertheless although we were measuring changes in oxidation–reduction of the ascorbate system that occurred after short periods of illumination (0–60 sec), it was possible that some of the effects described[1,2] may have been due to changes induced by the red or far-red light stimulated reactions (here called the secondary photochemical reactions), rather than to the primary photochemical reactions themselves. The present paper deals with a reappraisal of the results of our earlier experiments, and an extension of our observations on the effect of the red–far-red light reaction on the ascorbate system using leaves of cultivated strawberry (*Fragaria vesca* var. Gladstone).

[1] L. W. MAPSON, *Biochem. J.* **85**, 360 (1962).
[2] L. W. MAPSON, *Phytochem.* **3**, 429 (1964).
[3] L. W. MAPSON and T. SWAIN, *Nature* **204**, 886 (1964).

RESULTS

The reaction of photoreduction may be distinguished *inter alia* from that of photo-oxidation by the fact that only the former is inhibited by low concentrations of cyanide ($\sim 10^{-5}$ M), and by its more heat-labile nature.[1] Both of these primary photochemical reactions are inhibited by the photosynthetic poisons (DCMU). *o*-phenanthroline and hydroxylamine, but may be observed in leaves in an atmosphere of nitrogen.[1]

In the first instance we examined whether these various treatments exerted their effect on the primary photochemical reactions or whether they acted by influencing the secondary (red–far-red light) reactions. We were able to separate the two sets of reactions using green light of wavelength λ_{max} 514 nm (HW, bandwidth at 50 per cent transmission, 10 nm) to initiate the primary photochemical reactions. We were able to show that such light used for the short periods (1–120 sec) necessary to initiate and establish the primary photochemical reactions, did not itself reverse the inhibitory effect of far-red light, or alter the effect of red light.

Cyanide Poisoned Leaves

Cyanide (5×10^{-5} M) poisoned leaves show photoreduction but no photo-oxidation. This behaviour is not affected by prior exposure to red light but is completely prevented by prior exposure to far-red light. Moreover red light, as with normal leaves, reverses the effect of far-red. At this concentration cyanide does not therefore interfere with the secondary photochemical reactions, but suppresses the primary reaction of photo-oxidation.

Sensitivity to Heat

Photo-oxidation uncompensated by photoreduction is observed in leaves heated at 58° for 5 min, the consequence of which on prolonged exposure to light leads to an extensive photo-oxidation of ascorbate.[2] This abnormal behaviour is not due however to any effect on the secondary reactions, for if the heated leaf is exposed to far-red, photo-oxidation is inhibited when the leaf is subsequently illuminated with green light, and red reverses the effect of far-red light. This heat treatment therefore affects only the primary photochemical reactions.

Leaves Poisoned with Dichlorophenylmethyl Urea (DCMU)

Similarly leaves poisoned with DCMU (10^{-6} M) are as sensitive to the influence of red and far-red light as are normal leaves. In poisoned leaves photoreduction, although reduced in rate compared with normal leaves, occurs initially on illumination.[2] Prior exposure of the leaf to far-red light, inhibits this photoreduction, and the effect may be reversed by red light. Again we may conclude that DCMU exerts its effect on the primary photochemical reactions and not on the secondary reactions.

Oxidation and Reduction in the Darkened Leaf

In the darkened leaf the enzymic reactions of oxidation and reduction of ascorbate occur on changing the oxygen tension. In air in strawberry leaves a small but steady-state level of dehydro-ascorbate (15–20 μg/g) is normally maintained. These dark reactions[1] which are independent of those of photo-oxidation and -reduction are also completely independent of any control by the red–far-red light reaction. Thus the rate of reduction of dehydro-ascorbate in the dark when the leaf is transferred from air to nitrogen is as rapid in a leaf previously

exposed to far-red light as in one exposed to red. Moreover the rate at which the oxidation occurs in the dark when air is re-admitted is the same under both conditions. The final steady-state level of dehydro-ascorbate attained is also unaffected by the prior exposure of the leaf to red or far-red light.

Action Spectra

By exposing the leaf first, to either red or far-red light for 5-min periods and then to light of various different wavelengths for the same period, we were able to determine the effectiveness of such light in reversing the red or far-red light reaction. After a dark period of 10-min

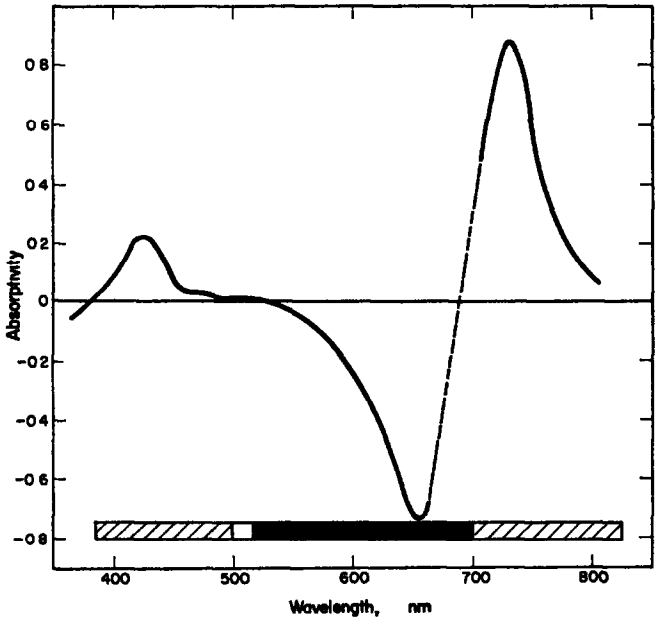

FIG. 1. CORRESPONDENCE BETWEEN THE DIFFERENCE SPECTRUM FOR PHYTOCHROME ($P_{fr}-P_r$), HENDRICKS AND BORTHWICK (1963), AND WAVELENGTH OF LIGHT EFFECTIVE IN INHIBITING OR REACTIVATING THE PRIMARY REACTIONS OF SUITABLY TREATED LEAVES.

■ Wavelength of light effective in inhibiting primary reactions previously activated by red.
■ Wavelength of light effective in reactivation of the far-red inhibited leaf.
□ Neutral region.

duration, the activity of the primary reactions was determined by their response to illumination by green light (λ_{max} 514 nm) for periods of 5 or 60 sec. With such light (intensity equal to 100 ergs/cm^2/sec) on a leaf in a fully active state, 5 sec illumination is sufficient to reduce the dehydro-ascorbate from the normal 15 μg/g to zero (photoreduction), and after a further 55 sec the concentration of dehydro-ascorbate then increases to a new steady-state level equivalent to 90–100 μg/g of leaf (photo-oxidation). With a totally inactive leaf, no significant change occurs in the concentration of dehydro-ascorbate even after much longer periods of illumination (15 min).

Results showing the wavelength of light capable of reversing red light activation are shown in Fig. 1 and indicate that light either between 390–490 nm or above 700 nm is capable of rendering the leaf inactive. Conversely, light between 530–700 nm reactivates after

exposure to far-red light. Light of wavelengths ~500–520 nm represents a change-over point, which neither reverses the effect of far-red nor of red light, and is therefore neutral in character. A further but sharper change-over point occurs at and around 700 nm.

These results are consistent with the hypothesis, though they by no means prove, that phytochrome is the active component concerned in the red–far-red light reaction studied here as can be seen from the comparison with the difference spectrum of the two forms of phytochrome (P_f–P_r) as reported by Hendricks and Borthwick[4] for etiolated maize seedlings

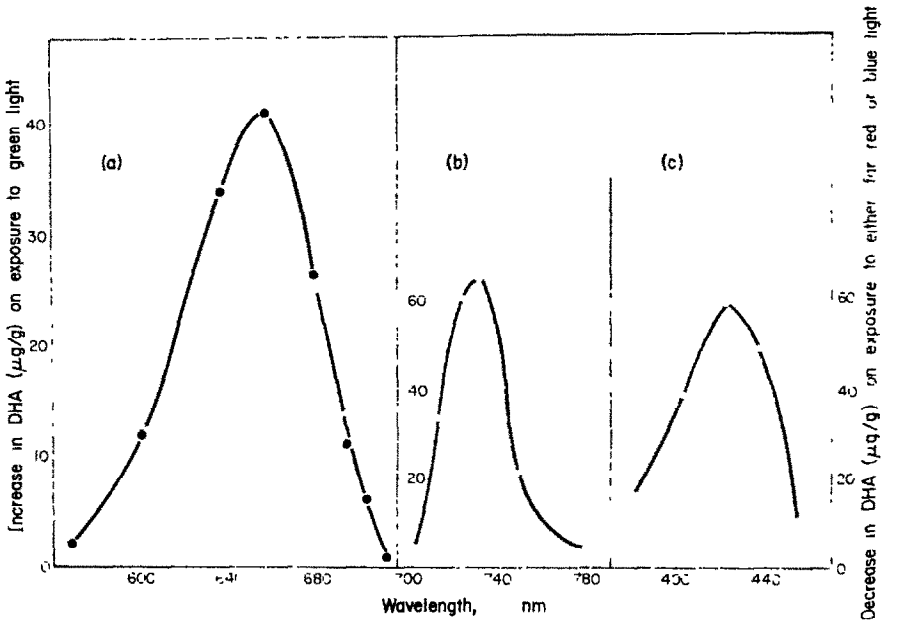

FIG. 2. ACTION SPECTRA OF BLUE, FAR-RED AND RED LIGHT.

(a) Reversal of the inhibiting effect of far-red or blue light by red light. ●—Leaf exposed 5 min to far-red light (723 nm) to completely inactivate the leaf, then exposed to light (5×10^{-12} einsteins cm^2/sec) of wavelength indicated for 1 min. After a dark period of 10-min duration the activity tested by determining response to green light (514 nm). ○—Leaf treated with blue light (421 nm) to completely inactivate, this followed dark period of 10 min then treated red light for 1 min. After a further dark period (10 min) activity tested as above. Red light of different wavelengths between 580–700 nm = 5×10^{-12} einsteins/cm^2 sec.

(b) and (c) Wavelength of far-red or blue light effective in inhibiting photo-oxidation of ascorbate. Leaf exposed to green light (510–530 nm) for 2 min then exposed 1 min to light of wavelength indicated. Blue light of different wavelengths between 380–450 nm = 5×10^{-12} einsteins cm^2 sec. Far-red light of different wavelengths between 690–780 nm = 1×10^{-11} einsteins cm^2 sec.

(Fig. 1). The interesting finding is that light in the blue region of the spectrum acts like far-red light in inhibiting the primary reactions.

Light of wavelength between 570 and 700 nm which is capable of reversing the inhibition induced by blue or far-red light has therefore been examined in more detail. The leaf was first rendered completely inactive by exposure to far-red (723 nm) for 5 min and then exposed for 1 min to light of different wavelengths between 570 and 700 nm of the same energy level (4×10^{-12} einsteins/cm^2 sec). After a dark period the response of the primary reactions to

[4] S. B. HENDRICKS and H. A. BORTHWICK, (1963) from D. VINCE, *Biol. Rev.* **39**, 506 (1964).

green light were determined. The same procedure was adopted for measuring the activation by red light after exposure to blue light (421 nm), with the exception that an additional dark period was interposed between the removal of the blue and application of the red light. The results are illustrated in Fig. 2(a) and show that the optimum response (determined by measuring the rate of increase in the concentration of DHA) in reversing the inhibition by either far-red or blue light was obtained with light of approximately 655–660 nm the corresponding action spectra were also very similar. There is in fact no evidence to suggest that two separate photochemical reactions involving two different phytochrome-like compounds were necessary, one to reverse the inhibition due to far-red light, the other to reverse that due to blue light.

In a similar manner the wavelength of either blue or far-red light most active in inhibiting the response of the leaf to green light was determined. In these experiments the leaves were illuminated by light (510–530 nm) and after the steady-state level of dehydro-ascorbate had been obtained, the leaf was illuminated *in addition* with blue or far-red light of different wavelengths of comparable energy levels. The rate of decrease in the concentration of DHA was followed. These results (Fig. 2(b) and (c)) show that for blue light λ_{max} is in the region of 421 nm and for far-red light in the region of 730 nm.

The Relative Efficiency of Far-red or Blue Light

The results of exposing the leaf in the active state to varying levels of radiation by either far-red or blue light, show that the conversion of the leaf to the inactive state (as determined by response to subsequent illumination by green light) depends on the total radiation determined by the product of intensity and duration (Table 1). Equally the results show that the

TABLE 1. RELATION BETWEEN INTENSITY AND DURATION OF BLUE, FAR-RED OR RED RADIATION

	Intensity (ergs/cm²/sec)	Duration (sec)	Total radiation einsteins × 10^{-12}/cm²	Response to green light after dark period*: Increase in DHA μg/g⁻¹	Response to green light after dark period*: State of activity
	Exposure to red light				
Leaf pretreated with far-red light to convert to inactive state	9	45	215	35	++
	45	9	215	32	++
	135	3	215	34	++
	9	60	287	70	+++
	45	12	287	69	+++
	135	4	287	76	+++
	Exposure to far-red light				
Leaf in active state	9	30	164	25	++
	45	6	164	20	++
	9	45	245	1	−
	45	9	245	7	−
	135	3	245	9	−
	Exposure to blue light				
Leaf in active state	9	45	147	4	−
	45	9	147	11	−
	100	4	147	6	−

* Leaf exposed to green light (λ_{max} 514 nm 25 ergs/cm²/sec) for 2 min after elapse of 10-min dark period. +++, Completely active; ++, partially active; −, inactive or only slightly active.

same applies for the reverse process, namely the conversion of the leaf from an inactive to an active state by red light radiation.

The efficiency with which blue or far-red light inactivates the leaf with regard to the primary reactions was then determined. The leaves in the active state were transferred to dark and then illuminated with low intensities of either far-red or blue light, for varying times. After a succeeding period in the dark (10 min) the leaves were tested for their response to illumination by green light (λ_{max} 514). The duration of illumination by either blue or far-red light (9 ergs/cm^2/sec) was increased to a point at which complete conversion of the leaf

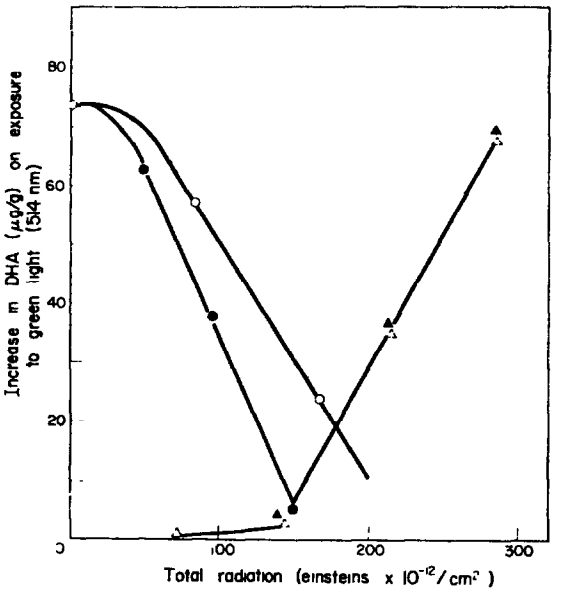

FIG. 3. EFFICIENCY OF BLUE, FAR-RED AND RED LIGHT.

I. Leaves from daylight ("active state"): ●—Illuminated with blue light (λ_{max} 421 nm, 3.5×10^{-12} einsteins/cm^2/sec) to give total radiation as indicated, this followed by dark period (10 min) before response to green light determined. ○—Illuminated with far-red light (λ_{max} 723 nm, $5{\cdot}5 \times 10^{-12}$ einsteins cm^2/sec) to give total radiation as indicated, this followed by dark period (10 min) before response to green light determined.

II. Leaves illuminated with far-red to convert to "inactive state": ▲ - Far-red (λ_{max} 723 nm, 15×10^{-12} einsteins/cm^2/sec) for 2 min; △—far-red (λ_{max} 723 nm, 5×10^{-12} einsteins cm^2/sec) for 1 min. Illuminated with red light (λ_{max} 655 nm, 5×10^{-12} einsteins cm^2 sec) to give total radiation as indicated, this followed by dark period (10 min) before response of leaf to green light determined.

Response to green light (λ_{max} 514 nm) measured by determining changes in the concentration of DHA after exposure to this light (25 ergs/cm^2 sec) for 2 min.

to the inactive state was achieved. The results showing the relation between einsteins of incident blue or far-red light and the degree of inactivation are given in Fig. 3. These indicate that blue light was more effective than far-red light, and furthermore that an absorption of ~ 40 einsteins $\times 10^{-12}$/cm^2 of far-red light energy and somewhat smaller absorption of radiation by blue light (~ 20 einsteins $\times 10^{-12}$ cm^2) occurred before any significant inactivation resulted.

Efficiency of Red Light

The leaf was first inactivated with far-red light (26 ergs/cm^2/sec) for 2 min and then exposed for varying times to red light (635 nm, 9 ergs/cm^2/sec). After a succeeding dark period (10 min) the degree of reactivation achieved was measured by determining the increase in concentration of DHA when the leaf was exposed to green light for 2 min. The results (Fig. 3) show that in this case an even greater radiation of red light energy appears to be necessary ~ 140 einsteins $\times 10^{-12}$ before any significant change in the degree of activation of the leaf is achieved. Thereafter, however, the relation between the radiation and response was found to be similar to that observed with far-red light. Further experiments indicated that if the far-red radiation was increased three times above the minimum necessary for complete inactivation, this did not alter the radiation energy of red light necessary to restore the leaf to the active state.

Reversion in the Dark

One of the characteristics of the phytochrome system in plant tissue is the instability of the P_{fr} form of phytochrome when the tissue is placed in the dark, either because of reversion

TABLE 2. DEVELOPMENT OF THE INACTIVE STATE IN THE DARK FOLLOWING EXPOSURE TO RED LIGHT

	Pretreatment	Dark period at 15° (hr)	Response to green light* photo-oxidation (increase in DHA μg/g) 30 sec	60 sec
1	Red light,† 2 min	0·2	32	65
2	Red light,† 2 min	24	28	65
3	Red light,† 2 min	46	14	54
4	Red light,† 2 min	70	8	30
5	As (4) but re-exposed red light, 2 min	0·2	35	72
6	Red light, 2 min	72	6	—
7	Daylight, 5 min	76	40	72
8	Daylight, 5 min	84	—	76
9	As (8) but re-exposed 2 min, red light	0·2	37	78
10	Daylight, 5 min	96	42	79
11	Daylight, 5 min	120	41	—

* Green light, λ_{max} 514 mμ, 25 ergs/cm^2/sec.
† Red light, λ_{max} 634 mμ, 250 ergs/cm^2/sec.

to the inactive P_r form or to destruction of P_{fr}. Dark reversion of P_{fr} to P_r has been demonstrated in cauliflower florets,[5] artichoke hearts and parsnip roots,[6] and destruction of P_{fr} in etiolated seedlings.[7] No data are available, however, concerning the stability of the P_{fr} form in the green leaf. In the present study the active condition of the leaf, induced by exposure to red radiation, changed on keeping the leaf in the dark. The results (Table 2) show that the leaf lost 80 per cent of its activity after being held for 75 hr at 15° in the dark. The rate of inactivation was negligible over the first 24 hr of darkness, but increased with time. Brief re-exposure of the leaf to red light restored the activity to its initial value indicating that reversion to the inactive state had occurred during the dark period. These results are in

[5] W. L. BUTLER, H. C. LANE and H. W. SIEGELMAN, *Plant Physiol.* **38**, 514 (1963).
[6] W. S. HILLMAN, *Am. J. Botany* **51**, 1102 (1964).
[7] P. J. DE LINT and C. J. P. SPRUIT, *Mededel. Landbouwhogeschool Wageningen* **63**, 1 (1963).

agreement with the characteristics of the phytochrome system described above, although the periods of darkness here found to be necessary for inducing inactivation are longer than those reported for other tissues, suggesting that if phytochrome is involved its P_{fr} form is more stable in leafy tissue. The small loss of activity in the first 24 hr of darkness compared with the greater rate of loss in succeeding periods may indicate that the concentration of the P_{fr} form in the leaf is higher than the minimum necessary to maintain the primary reactions at their maximum rate, and these are not seriously affected until the concentration of the P_{fr} form falls below this threshold level. This is possibly the reason for the observation that there is no inactivation by far-red or blue light immediately on exposure but only after exposure to a certain amount of radiation (cf. Fig. 3).

Of interest was the different behaviour of the leaf when this was exposed to daylight rather than to red light before the ensuing period of darkness. Under these conditions, although the leaf was left for periods up to 120 hr in the dark, there was no decrease in the photo-oxidative activity of the leaf in its response to illumination by green light. The reason for this different behaviour must await further experimentation.

In contrast to the instability of the leaf in the active condition after red radiation, the inactive state (induced by previous exposure of the leaf to far-red radiation) was completely stable. After period of up to 144 hr in darkness at 15°, there was only a slight reversion to the active state (< 10 per cent of the initial activity) but complete reactivation could be achieved

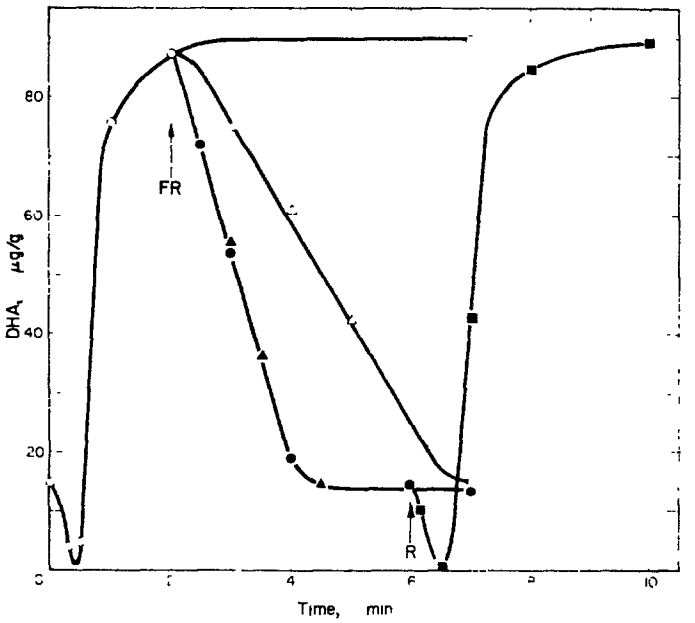

FIG. 4. INHIBITION OF PRIMARY REACTIONS IN PROGRESS BY FAR-RED LIGHT AND REVERSAL BY RED LIGHT.

At zero time primary reactions initiated by green light (510–535 nm, 25 ergs cm^{-2} sec).

● After 2 min far-red light (700–800 nm) applied (600 ergs cm^{-2} sec).

▲ After 2 min far-red light applied, green light removed.
After 2 min leaf returned to the dark.

■ Treatment as ● but after 6 min far-red light removed replaced by red light (600–700 nm, 200 ergs cm^{-2} sec).

at any time by brief exposure to red light. This result is also consistent with the known properties of phytochrome.

Effect of Red, Far-red or Blue Light on the Primary Reactions in Progress

In most of our previous experiments a dark period was interposed between the exposure of the leaf to red or far-red light before being illuminated with green light. We wished to study in more detail the inhibiting effect of far-red or blue light under conditions in which the primary reactions were *already in progress*. Leaves were exposed to green light (λ_{max} 514 nm) for sufficient time (2 min) to allow the primary reactions to become fully established, (DHA 88 μg/g) and at this stage the leaf was illuminated with a beam of far-red light in addition. As the results illustrated in Fig. 4 show, there was an abrupt return to the steady-state level of dehydro-ascorbate (15 μg/g) normally observed in the dark. The rate of fall of dehydro-ascorbate was found not to be increased if the green light was removed at the same time as

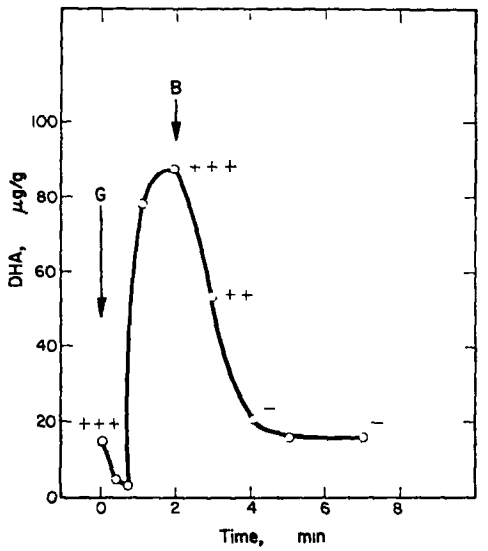

FIG. 5. CORRESPONDENCE BETWEEN INHIBITION OF PRIMARY REACTIONS BY BLUE LIGHT AND DEVELOPMENT OF INACTIVITY TO GREEN LIGHT AFTER DARK PERIOD.

+++ Completely active.
++ Partially active.
= Inactive.

Green light, 25 ergs/cm²/sec; blue light, 72 ergs/cm²/sec.

the far-red light was applied. It is noteworthy that the rate of reduction in the level of dehydro-ascorbate was about double that observed when the leaf returns to the dark level normally. Moreover no induction phase was detectable when the reaction was terminated by far-red light, but a short induction phase was always present when the reaction was terminated by removal of light. This treatment with far-red converted the leaf to the inactive state, but as with our earlier experiments the primary oxidation reactions could be re-established by illumination with red light (Fig. 4).

In view of our earlier results we expected that blue light (395–425 nm) might act very similarly to far-red light, and in many respects this has been found to be so. Thus if the primary reactions are initiated by green light and the leaf then illuminated in addition with blue light, prompt cessation of the primary reactions are observed (Fig. 5). Moreover,

coincident with the arrest of the primary reactions, the leaf changes from the active to the inactive state.

There is one major difference, however, between blue light and the far-red light. As shown in our earlier publication[2] light of wavelength of 730 nm or above cannot initiate the primary photochemical reactions, even in a leaf in an active condition, whereas blue light (in the region of 436 nm) is very active. This difference is revealed when leaves in the active condition are illuminated with either blue or far-red light alone. Only in the former case was a similar sequence of photoreduction and photo-oxidation observed. Again, as with

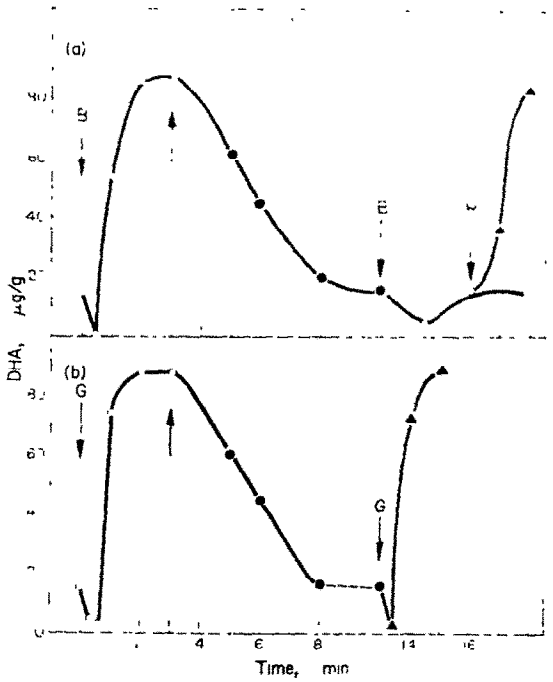

FIG. 6. EFFECT ON THE PRIMARY REACTIONS OF ILLUMINATING WITH BLUE OR GREEN LIGHT.

(a) Primary reactions initiated by blue light (λ_{max} 421 nm, 72 ergs cm^{-2} sec) at zero time. Blue light removed after 3 min, followed by dark period 10 min, when blue light reapplied. ▲—Inhibition induced by blue reversed by application of red light.

(b) Primary reactions initiated by green light (λ_{max} 520 nm, 25 ergs cm^{-2} sec) After 3 min, green light removed—followed by dark period 10 min. ▲—Primary reactions restored on reapplication of green light.

green light primary reactions continued until the blue light was removed. However if the leaf was then left 10 min in the dark, re-illumination with blue or green light gave no reaction except in some cases where a feeble photoreduction reaction has been observed (Fig. 6). Increasing the intensity of radiation of the blue light by a factor of two did not alter the situation. In fact, as previously shown, with blue light the minimum radiation necessary to render the leaf completely insensitive to further radiation by green light under the above circumstances is of the order of 160 einsteins $\times 10^{-12}$ cm (Fig. 4), even so radiation at about half this level is still capable of initiating the primary reactions. It is not therefore possible to use blue light to stimulate the secondary, without at the same time initiating the primary

photochemical reactions. Control experiments with green light, which has itself no measurable effect on the secondary reactions, showed that in this case the primary reactions were as marked during a second period of illumination following a dark period, as they were during the first period (Fig. 6).

The above results might be interpreted on the basis that with blue light the primary photochemical reactions may be observed because, owing to their more rapid response, they occurred before the initiation of the secondary reactions could be established. However if this is the whole explanation, it is difficult to see why on continued illumination (>3 min)

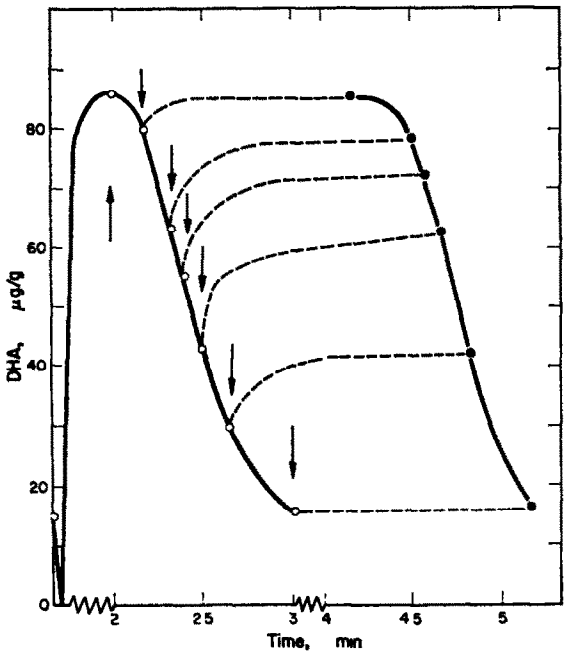

FIG. 7. RELATION BETWEEN RATE OF DECLINE OF THE PRIMARY REACTIONS AFTER REMOVAL OF LIGHT, AND RATE OF DEVELOPMENT OF INSENSITIVITY TO FURTHER ILLUMINATION.

At zero time primary reactions initiated, and steady-state level of DHA established by illumination with blue light (λ_{max} 421 nm, 60 ergs/cm²/sec).

○—○ Rate of decrease of DHA in the dark following removal of blue light.
●—● Decrease in the steady-state level of DHA obtained after a second period of illumination (2 min) following increasing intervals in the dark.

↑ Blue light off. ↓ Blue light on again.

with blue light the primary reactions are not subsequently inhibited. Blue light in this respect is thus different from far-red light and appears to be capable of converting the leaf to the inactive state *only* after a dark period has intervened, which in turn may mean that it cannot block the primary reactions if these are already in progress, a conclusion which is consistent with the results of the following experiments. After illumination with blue light the rate at which the leaf becomes insensitive to further illumination parallels very closely the rate at which the primary reactions decline on removal of light. This relationship is illustrated by the results recorded in Fig. 7 in which it is shown that the fall in the level of DHA with time after the blue light is removed, is arrested but not reversed to any appreciable extent by

subsequent illumination. Illumination thus prevents the fall that would otherwise occur if the leaf was left in the dark, but it does not restore the steady-state level to that observed in the completely active leaf.

INTER-RELATIONSHIP BETWEEN BLUE, GREEN AND FAR-RED LIGHT

Illumination by green followed by blue light. As shown above the inhibitory effect of blue light can be demonstrated, without the intervention of a dark period, by exposing the leaf to this light (72 ergs/cm^2/sec) in addition to and following green light (25 ergs cm^{-2} sec) (Fig. 5). Under these conditions the primary reactions elicited by green light are arrested, and the leaf after a dark period is then in the inactive state. The ability to arrest the primary reactions depends not only on the intensity and duration of the radiation by blue light but also on the previous extent of radiation by green light. The results (Table 3) indicate that in

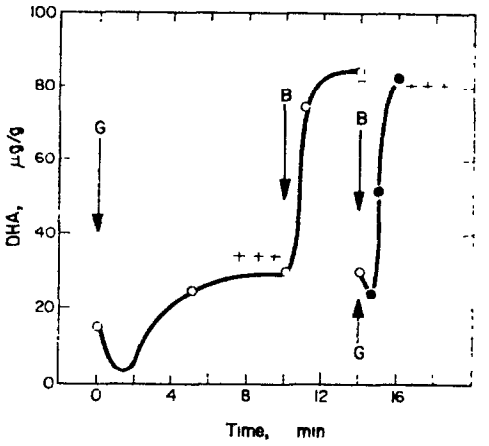

FIG. 8. EFFECT OF BLUE LIGHT ON PRIMARY REACTIONS INITIATED WITH LOW INTENSITY GREEN LIGHT.

○ Primary reactions initiated by green light (2 ergs/cm^2 sec) followed after 10 min with blue light (30 ergs/cm^2/sec) in addition.

● Same pretreatment but green light removed ¦ as blue light applied.

Condition of leaf: + + +, sensitive to green light after dark period (10 min). −, Insensitive to green light after dark period.

the majority of cases if the ratio of the product (intensity and duration) of radiation by blue light compared to that of green light exceeds a value of ~ 1·0 the primary reactions are totally inhibited. The chief exception to this occurs when the green light is of very low intensity (2 ergs cm^2/sec), and the leaf is exposed to blue light. Under these circumstances the primary reactions are enchanced, the low steady-state level of DHA achieved with the green light rises on the addition of blue to a new high steady-state level (Fig. 8). In all cases however after a dark period (10 min), when the ratio of the two radiation exceeds a value of ~ 1·0, the leaf behaves like a leaf treated with blue light alone, and does not respond or responds only weakly to illumination by green light.

Although these experiments indicate an "antagonism" exists between blue and green light, it can also be shown that the effect of blue light in inhibiting the primary reactions in progress and in rendering the leaf insensitive after a dark period to further illumination by green light, is completely *dependent* on the presence of some green light. Thus, as the results

TABLE 3. INHIBITORY EFFECT OF BLUE LIGHT ON REACTION INITIATED BY GREEN LIGHT

Type of experiment	Prior exposure to green light		Exposure to blue light		Ratio radiation (einsteins) blue/green	Response to green light* after dark period (10 min)
	Intensity (ergs/cm²/sec)	Duration (min)	Intensity (ergs/cm²/sec)	Duration (min)		
Blue light in addition to and following green light	25	2 to 10	None		0	+++
	25	2	5	3	0·1	+++
	25	2	72	3	0·15	+++
	25	2	72	1	0·8	++
	25	2	72	2	1·2	−
	25	2	72	5	1·7	−
	25	10	72	2	0·4	−
	25	10	72	5	0·8	−
	25	4	72	2	0·8	++
	10	10	72	2	1·0	−
	10	10	24	2	0·34	+++
	10	5	24	2	0·60	+++
	10	2	72	10	5·0	−
	2	10	5	4	0·6	−
	2	10	72	3	7·0	−
Green light removed as blue light applied	25	2	72	2	2·4	+++
	25	2	72	5	6·0	+++
	10	3	72	5	10·0	+++
	2	10	72	2	6·0	+++
	2	10	72	10	30·0	+++

* 25 ergs/cm²/sec for 2 min.
Blue light, λ_{max} 421 nm, HW 11 mμ; green light, λ_{max} 514 nm, HW 10 mμ.
−, Inactive; +, slightly active; ++, partially active; +++, completely active.

in Table 3 show, if when the primary reactions have become established, the green light is switched off simultaneously with the application of blue light, the leaf after a dark period remains in the active condition. These results are obtained even under conditions when the value of the ratio of the radiation of blue to green is as high as 30. As the results in Table 3 indicate only a very low level of green radiation is necessary for blue light to convert the leaf to the inactive state. This is further exemplified by the results illustrated in Fig. 8 where although the effect of blue light, on the primary reactions initiated by green light of low intensity, appear to be identical in the two experiments, the leaf which is illuminated by blue in addition to green light is converted to the inactive state whereas that exposed to blue light, with simultaneous removal of green, remains active.

We may conclude that when the primary reactions elicited by green light are in progress, blue light can only arrest these if of sufficient intensity in relation to that of green, and further-

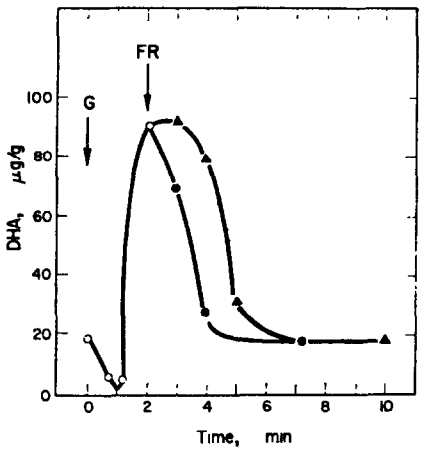

FIG. 9. INHIBITION BY FAR-RED LIGHT OF REACTIONS INITIATED BY GREEN LIGHT.
● Far-red light applied at ↓ in addition to green light.
▲ Green light removed at ↓ as far-red light applied.
Green light—25 erg/cm² sec; far red—26 erg/cm² sec.

more only in the presence of green light are the primary reactions arrested directly and the leaf converted to the inactive state. Green light thus has a "synergistic" influence with blue light in arresting the primary reactions and converting the leaf to the inactive state.

Illumination by green followed by far-red light. Although far-red light, under the same circumstances, was still capable of inhibiting the primary reactions in progress and rendering the leaf inactive (Table 4), there is some evidence that in the absence of green light it is also somewhat less effective. Thus the arrest of the primary reactions by far-red light are slightly delayed in the absence of green light (Fig. 9) and the relative intensities of radiation of far-red to green must be higher to convert the leaf to the inactive state. A ratio of radiation of far-red to green light equal to ~ 1·0 or above is sufficient for this purpose providing the leaf is also exposed to green light, but in the absence of the latter the far-red radiation must be increased about three times if the same result is to be achieved (Table 4). It should be noted that these observations were made with far-red light of low intensities, if far-red light of high intensity relative to green light is used, then as indicated in Fig. 4 there is no detectable difference in the rate at which the primary reactions are inhibited in the presence or absence of

TABLE 4. INHIBITORY EFFECT OF FAR-RED LIGHT ON REACTIONS INITIATED BY GREEN LIGHT

Type of experiment	Prior exposure to green light		Exposure to far-red light		Ratio radiation (einsteins) far-red / green	Response to green light* after dark period (10 min)
	Intensity (ergs/cm²/sec)	Duration (min)	Intensity (ergs/cm²/sec)	Duration (min)		
Far-red light in addition to and following green light	25	2	None		0	+++
	25	2	26	1	0·5	+++
	25	2	26	2	0·7	+
	25	2	26	3	0·9	−
	25	2	26	10	1·2	−
	25	10	26	2	0·24	−
	25	10	9	1	0·04	+++
	2	2	15	1	3·5	−
	2	2	15	5	7·5	−
	None		9	1	9·0	−
Green light removed as far-red light applied	25	2	26	1	0·7	+++
	25	2	26	2	1·4	+++
	25	2	26	3	2·2	+
	25	2	26	5	3·6	−
	25	2	26	10	7·0	−
	2	10	26	10	18·0	−

* 25 ergs/cm²/sec for 2 min.
Green light, λ_{max} 514 nm, HW 10 mμ; far-red light, λ_{max} 723 nm, HW 12 mμ.
−, Inactive; +, slightly active; +++, completely active.

green light. Despite these resemblances between the relation of blue to green light on the one hand and far-red and green on the other, nevertheless blue light even when used in relatively high intensity compared with green appears unable to inhibit in the absence of green, whereas far-red light can, under similar conditions, achieve this.

Prevention by Green Light of the Inhibition Induced by Blue or Far-red Light

If the primary reactions are initiated with blue light (72 ergs/cm^2/sec) and, when these have reached a steady state, the leaf is then illuminated with green light (25 ergs/cm^2/sec) in addition, in contrast to the reverse procedure, only a transitory fall in the concentration of DHA is observed, with a re-establishment of the steady-state level as illumination with green light continues. The results of a typical experiment in which the leaves were illuminated with blue light (72 ergs/cm^2/sec) for 2 min and then additionally illuminated with green light for varying times are given in Fig. 10(a). Coinciding with the transitory fall in the concentration

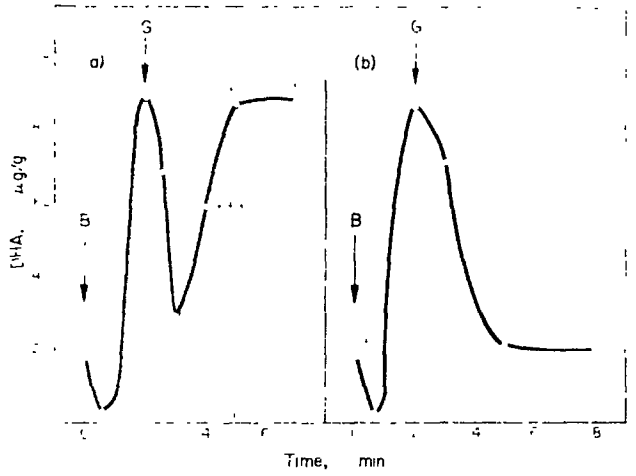

FIG. 10. EFFECT OF GREEN LIGHT ON PRIMARY REACTIONS INITIATED BY BLUE LIGHT.

Blue light (72 ergs·cm^2/sec) was used to initiate the primary reactions followed by: (a), green light 25 ergs/cm^2/sec; (b), green light 2 ergs/cm^2/sec. + + + Active, and − inactive after dark period.

of DHA following illumination with green light, the condition of the leaf changes, as the duration of exposure to green light is increased, from the inactive to the completely active state.

Other results in Table 5 illustrate, in addition, the ability of green light to prevent or reverse the inhibition induced by blue light. The effectiveness of the green light depends, as previously (Table 3), on the relative intensities of the two lights and the duration of exposure. Green light is thus more effective if blue light is removed as the green light applied. There is also some evidence to show that the *longer* the leaf is first exposed to blue light the greater the ease with which green light prevents the development of inactivation. With prior exposure to blue light (72 ergs/cm^2/sec) for 2 min, reversal by green light occurred when the ratio of green to blue radiation exceeded a value of 0·14. With longer pretreatment with blue light for 5 or 10 min this reversal by green light occurred when the value of the ratio of the two radiations exceeded 0·05 and 0·006 respectively. The results (Table 5) also show that if a dark period (10 min) is interposed between the removal of blue light and the application of

TABLE 5. PREVENTION BY GREEN LIGHT OF THE INHIBITION INDUCED BY BLUE LIGHT

Type of experiment	Prior exposure to blue light		Exposure to green light			Response to green light* after dark period (10 min)
	Intensity (ergs/cm²/sec)	Duration (min)	Intensity (ergs/cm²/sec)	Duration (min)	Ratio radiation (einsteins) green/blue	
Green light in addition to and following blue light	72	2 to 15	None		0	−
	72	2	10	3	0·1	−
	72	2	25	1	0·14	−
	72	2	25	2	0·21	+++
	72	2	25	3	0·25	+++
	72	2	25	5	0·30	+++
	72	5	2	3	0·014	+
	72	5	10	3	0·06	++
	72	5	25	3	0·16	+++
	72	10	2	3	0·008	−
	72	10	10	3	0·03	+++
	72	10	25	3	0·1	+++
	72	15	2	3	0·006	++
Blue light removed as green applied	72	2	2	3	0·05	−
	72	2	10	1	0·085	+++
	72	2	25	1	0·21	+++
	72	2	25	3	0·6	+++
	72	3 followed by	25	2	0·28	−
	72	2 10-min dark	25	3	0·6	−
	72	2 period	25	10	2·1	−

* 25 ergs/cm²/sec for 2 min.

+++, Completely active; ++, partially active; +, slightly active; −, inactive.

green, then the inactive state induced by blue light is not changed by subsequent exposure to green light.

When an intensity of green light (2 ergs/cm^2/sec), which the results in Table 5 indicate is insufficient to reverse the effect of blue, is applied to a leaf already exposed to blue light, this results in the complete arrest of the primary reactions in progress. Thus in the experiment illustrated in Fig. 10(b) the primary reactions were initiated by blue light (421 nm, 72 ergs cm^2/sec) and illumination continued for 2 min to establish a steady-state level of DHA. At this point when the leaf was illuminated with green light in addition the steady-state level of DHA fell to the value observed in darkened leaves, and in contrast to the experiment illustrated in Fig. 10(a) the leaf remained in an inactive state. These two experiments illustrate that green light, depending on its intensity, can either block completely the primary reactions initiated by blue, or reverse the effects of blue light by restoring the leaf to the active state. In the latter case only a temporary blocking of the primary reactions is observed.

There was evidence that green light was also capable of preventing the inactivation

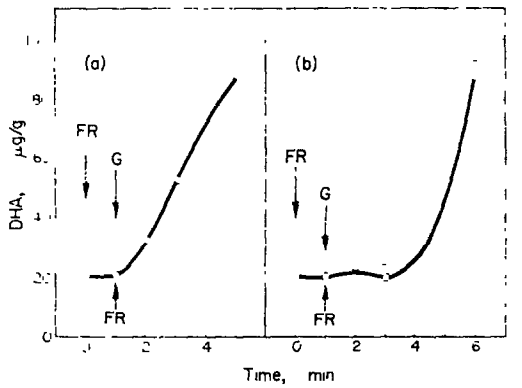

FIG. 11. REVERSAL BY GREEN LIGHT OF THE INHIBITION OF THE PRIMARY REACTIONS INDUCED BY FAR RED LIGHT.

At zero time leaf exposed to far-red light. In (a) 9 ergs/cm^2/sec, and in (b) 26 ergs/cm^2/sec. ↑FR far-red removed, ↓ green light (25 ergs/cm^2/sec) applied.
Notation as in previous figures.

induced by far-red provided that (1) the period the leaf was exposed to far-red light of low intensity was of short duration (1–2 min) and (2) that the green light was applied immediately the far-red light was removed. The results (Table 6) show that under these conditions a ratio of radiation of green/far-red in excess of 2·0 is sufficient to prevent or reverse the inhibition which would otherwise result from exposure to far-red radiation. In the same experiments it was shown that the change-over from a leaf in the inactive to the active condition corresponded with the initiation of the primary reactions by green light (Fig. 11). The green light used in these experiments had a wavelength of λ_{max} at 514 nm decreasing to 1 per cent or less transmission at 500 and 540 nm. The possibility that sufficient "red" light in the region of 540 nm was being transmitted and reversing the effect of blue or far-red could not be entirely excluded. That this was not the case however was shown by the fact that if under conditions in which green light had been shown to be effective for reversing the inhibition of far-red a dark period was interposed between the removal of far-red and application of green light, no activation by green light was observed (Table 6).

TABLE 6. CONDITIONS FOR PREVENTION BY GREEN LIGHT OF INHIBITION INDUCED BY FAR-RED LIGHT

Type of experiment	Prior exposure to far red		Exposure to green light			Response to green light* after dark period (10 min)
	Intensity (ergs/cm²/sec)	Duration (min)	Intensity (ergs/cm²/sec)	Duration (min)	Ratio radiation (einsteins) green / far-red	
Green light in addition to and following far-red	9	1 or 2	None		0	−
	9	1	25	10	1·8	−
	9	2	25	5	1·4	−
	26	2	25	5	0·5	−
	9	1	100	5	6·5	−
Far-red removed as green light added	9	1	25	1	2·0	+++
	9	1	25	10	20·0	+++
	9	5	25	5	2·0	−
	26	1	25	1	0·7	−
	26	1	25	2	1·4	−
	26	1	25	5	3·4	+++
	45	1	25	5	2·0	+++
	9	1 } followed by	25	5	10·0	−
	26	1 } a dark period	25	5	3·4	−
	45	1 } of 10 min	25	5	2·0	−
	9	5 } of 4 min	25	5	2·0	−
	9	1 }	25	5	10·0	−
	9	1 of 2 min	25	10	20·0	−

* 25 ergs/cm²/sec for 2 min.
Far-red light, λ_{max} 730 nm, HW 10 mμ.
+++, Active; −, inactive.

These results emphasize the difference between the ability of green light on the one hand and of red light on the other to prevent or reverse the effect of far-red radiation. In the first case green light acts only if it is applied at the same time or immediately after a short period of radiation by far-red; in this sense it prevents the inhibition that would otherwise develop. Red light on the other hand can reverse the inhibition induced by far-red radiation even of high intensity and after the leaf has remained for long periods in the dark.

Cyanide Poisoned Leaves

The results of a series of experiments on cyanide poisoned leaves, in which only the primary reaction of photoreduction is observed on illumination (resulting in a fall of the dark steady-state level of DHA to zero) are illustrated in Figs. 12 and 13. These show that both far-red or blue light are each capable of arresting the process of photoreduction even when the reaction is in progress. In these experiments the primary reaction of photoreduction was initiated by green light, and after the DHA had fallen to zero the leaf was additionally illuminated with far-red or blue light. The rapid return of DHA to the steady-state level of the darkened leaf which followed, indicates that the process of photoreduction has been completely inhibited. This inhibition, can as with normal leaves, be immediately reversed by substituting red for far-red or blue light. An experiment illustrating this reversal by red light is given in Fig. 12.

As in the experiments previously described the dependence of blue light on the presence of green light for blocking these reactions may also be observed. If the process of photoreduction is initiated by green light (25 ergs cm^2 sec) and when fully established this light is removed simultaneously with illumination by blue light (72 ergs cm^2 sec) only a transitory (temporary rise in DHA concentration) but no permanent arrest of the photoreduction process is observed (the concentration of DHA remains at zero) (Fig. 13(a)). Similarly if the photoreduction process is initiated by blue light and then illuminated by green light of sufficient intensity (25 ergs cm^2 sec) only a temporary arrest of the primary reactions is observed (Fig. 13(b)). If, however, green light of lower intensity (2 ergs cm^2 sec) is used, complete arrest of the reactions of photoreduction are observed (concentration of DHA increases to the value observed in a darkened leaf) (Fig. 13(b)).

The conditions under which the primary reactions are blocked by blue or green light are thus identical in both normal and cyanide poisoned leaves. In the latter case photo-oxidation has been suppressed by cyanide so that these experiments indicate that both the photoreductive and photo-oxidative reactions are controlled by essentially the same mechanism.

DISCUSSION

The characteristics of the system responsible for what we have called the secondary photochemical reactions are in several respects similar to those reported for phytochrome. Equally, many of our results are susceptible of interpretation in terms of the activity of phytochrome or of a phytochrome-like compound, P. This compound, it is assumed, is capable of existence in three allosteric forms P_1, P_2 and P_3. Whilst the primary reactions may not necessarily be initiated under the influence of light energy by the P compound their functioning is dependent on the existence of the P compound in an active form. Thus the P compound, when present either as the P_1 or P_2 form, will allow the primary reactions to proceed, whereas when present as P_3 no electron transfer can occur.

Since the primary reactions of photoreduction and oxidation of ascorbate are inhibited when the P compound is in the inactive form, this positions the P compound in an inter-

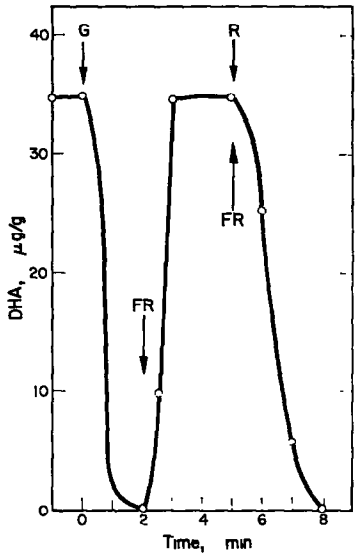

FIG. 12. INHIBITION OF PHOTOREDUCTION IN CYANIDE POISONED LEAVES BY FAR-RED AND ITS REVERSAL BY RED LIGHT.

Photoreduction initiated by green light (25 ergs/cm²/sec). After 2 min ↓ far-red light in addition (100 ergs/cm²/sec). After 6 min ↑ far-red light removed and ↓ red light applied (100 ergs/cm²/sec). Illumination by green light continuous throughout experiment.

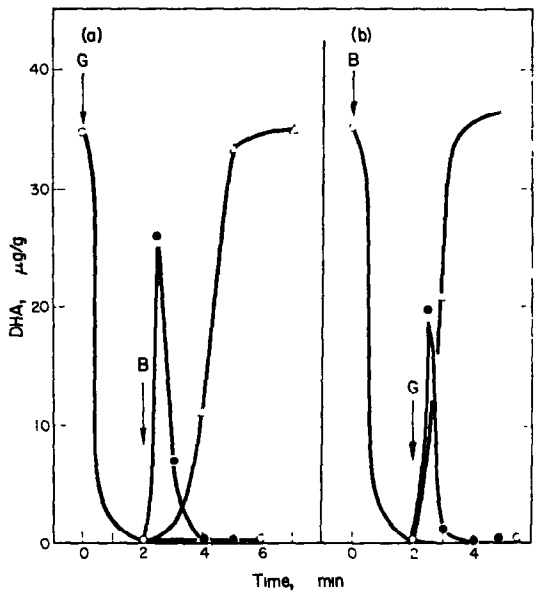

FIG. 13. EFFECT OF BLUE AND GREEN LIGHT ON CYANIDE POISONED LEAVES.

(a) Photoreduction initiated by green light (25 ergs/cm²/sec) ○—○. After 2 min △—△ blue light (72 ergs/cm²/sec) in addition. After 2 min ●—● green light removed as blue light applied.

(b) Photoreduction initiated by blue light (72 ergs/cm²/sec) ○—○. After 2 min ●—● green light (25 ergs/cm²/sec) in addition. After 2 min △—△ green light (2 ergs/cm²/sec) in addition.

mediate position between the source of electrons generated by visible light energy and the ascorbate couple. As both photoreduction and oxidation of the ascorbate couple appear to be dependent on the existence of P in an active form, this suggests that it does not control the electron flow by itself participating in it. If only photoreduction or photo-oxidation was affected when P was converted to its inactive form then it might be positioned before or after the ascorbate couple. The fact that both photo reactions are inhibited suggests that it blocks electron flow both into and out of the system by some other means. As shown in earlier publications[1, 2] the visible light energy active in this respect is that at the blue and red end of the spectrum though green light (510–560 nm) can also stimulate. Thus the absorption by the leaf of light energy of different wavelengths will have two separate effects in the reactions studied here: (1) it will generate electrons by being absorbed by other components of the leaf, e.g. chlorophyll and (2) it will modify the form of the phytochrome-like compound P so that electron flow along pathways controlled by this compound will be either inhibited or promoted.

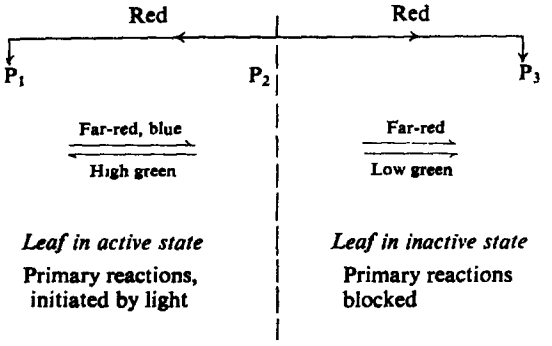

FIG. 14. EFFECT OF DIFFERENT WAVELENGTHS OF LIGHT ON THE PHYTOCHROME-LIKE SYSTEM INVOLVED IN THE SECONDARY REACTIONS.

The scheme here proposed to explain our results, especially the inter-conversion of the different forms of P catalysed by light of different wavelengths (photo-allosteric conversion), is outlined in Fig. 14.

The main features of this scheme are as follows:

1. Far-red light (730 nm), absorbed by P_1, catalyses the reaction $P_1 \rightarrow P_2 \rightarrow P_3$, the conversion of $P_1 \rightarrow P_2$ we assume to be rapid, and this is followed by a slower conversion to P_3 under the stimulus of far-red radiation.
2. Blue light (421 nm), also absorbed by P_1, converts $P_1 \rightarrow P_2$, but does not catalyse the conversion of $P_2 \rightarrow P_3$.
3. Green light (514 nm) absorbed by the P_2 form can catalyse either the reaction $P_2 \rightarrow P_3$ or the reaction $P_2 \rightarrow P_1$.
4. Red light (660 nm) catalyses the $P_3 \rightarrow P_2 \rightarrow P_1$ reaction thereby reversing the effect of far-red or blue light.
5. The ease with which the P_2 form is connected to P_3 is greater when the electron flow is low or absent, e.g. in the darkened leaf, and conversely least when the electron flow is high.

Such a scheme, put forward chiefly as a working hypothesis for future work, has the merit of rationalizing most of the results obtained. Thus in a leaf in the active state, when the

primary reactions are initiated by green light, the electron transfer path is open, (P_1 form present) and after a dark period may be reinitiated by further illumination. On the other hand, when the primary reactions are initiated by blue light, electron flow is established by the conversion of $P_1 \rightarrow P_2$ reaction. However, during a succeeding dark period, P_2 is converted to P_3 as electron flow subsides. After such a dark period the leaf becomes insensitive to further illumination because the P compound has been converted into its P_3 form. This can then only be reversed by exposure of the leaf to red light which converts $P_3 \rightarrow P_1$.

We have shown that green light when applied immediately after a short period of far-red radiation (itself sufficient to induce complete insensitivity to further illumination) can prevent the inhibition that would otherwise develop. The question as to whether the green light under such conditions has merely prevented or delayed the inhibition induced by far-red (or blue) light developing or whether it has actually reversed the inhibition is difficult to answer conclusively, since it is conceivable that the conversion of the leaf to the inactive state, under the influence of either far-red (or blue) light, may only become established during a succeeding dark period. In this respect it should be emphasized that these apparent reversal effects of green light are only observed after short periods ($\sim$1 min) of radiation by far-red light of low intensity. If this is prolonged for 5 min or if a dark period of even short duration (2–4 min) succeeds a short period of far-red radiation no reversal by green light occurs (Table 6). Such results suggest that a time factor may be involved in the development of the inactive state. This might be occasioned by the formation of an intermediate between the active form of the compound responsible and the inactive form, the conversion to the fully inactive form being a slower reaction and requiring time to develop fully. The intensities of far-red radiation used in these experiments were low, with higher intensities it seems possible that such an intermediate state would not be detected owing to its rapid transformation to the fully inactive compound. On the present hypothesis prevention of the inhibition by green light is due to the regeneration of the P_1 form from the P_2 intermediate form, produced by far-red radiation. The failure of green light to effect this change after longer periods of radiation by far-red or after a short period of far-red radiation followed by a dark interval is due to the inability of green light to affect the P_3 form once this has been formed. The same explanation accounts for the ability of green light to prevent the development of the inhibition induced by blue light. In this case it is possible to prevent this inhibition developing if the green light is applied after blue light is removed irrespective of the length of the time the leaf has been exposed to this light. This different behaviour is explicable for with illumination by blue light alone no conversion of the P_2 form to the P_3 occurs until a dark period intervenes, in which case the degree to which the green light can prevent inhibition developing decreases as the time interval between removal of blue light and application of green is increased. In fact the rate of development of insensitivity closely parallels the rate at which the primary reactions subside after removal of blue light, and this in turn suggests that the intermediate P_2 form of the phytochrome-like compound is stabilized when the electron flow is high, but as this decreases so the conversion of the P_2 to the P_3 form is promoted.

We cannot pretend that this theory completely explains all the effects observed when the leaf is exposed to mixed radiation (green or blue light). It does explain why blue light alone will not inactivate the primary reactions already in progress unless green light of low intensity is applied (stimulation by green light of $P_2 \rightarrow P_3$ reaction). It is also easy to visualize why higher intensities of green light, following blue light under the same conditions, inhibit the primary reactions only temporarily and then restore these to their original activity, moreover

the leaf after a succeeding dark period remains in the active state. This on the scheme above suggests that the P_2 form has been converted back to the P_1 form. Thus with low-intensity green light the predominant reaction appears to be $P_2 \rightarrow P_3$ with higher intensities, the predominant effect is $P_2 \rightarrow P_1$ and this result may be due to the direct antagonism between blue and green light on reaction $P_1 \xrightarrow[\text{green}]{\text{blue}} P_2$, so that with a high ratio of blue, green radiation the concentration of P_2 is high so that the $P_2 \rightarrow P_3$ reaction is favoured, whereas with green light of higher intensity in relation to blue the concentration of P_2 is reduced to a sufficiently low value by the stimulation of the $P_2 \rightarrow P_1$ reaction thus reducing or eliminating the $P_2 \rightarrow P_3$ reaction.

More difficult to interpret is why the primary reactions are arrested in the reverse procedure to the above experiment when blue light is applied to a leaf previously exposed to green light of high intensity. The answer to this would appear to reside in the fact that the intensity of green radiation necessary to prevent the inhibition due to blue light developing decreases with the length of time the leaf is exposed to blue light (Table 6). Such results suggest that the intensity of green radiation necessary to reverse the $P_1 \rightarrow P_2$ reaction decreases as the blue radiation is prolonged. In the first case under consideration the leaf was exposed to blue light for 2–3 min before the application of green, in the second case inhibition of the primary reactions occurred immediately on the application of blue. Thus the intensity of green radiation in the first experiment was sufficient in the continued presence of blue light to promote the reaction $P_2 \rightarrow P_1$, in the second experiment this was not so and the chief effect of green was to promote the $P_2 \rightarrow P_3$ reaction and thus cause an arrest of the primary reactions. We cannot at the moment offer any reasonable explanation for the apparent increase in activity of green radiation following periods of blue radiation.

It is also possible to see why, when the primary reactions are initiated with green light and blue light is applied at the same time as green light is removed, there is only a transient arrest of the primary reactions which are re-established with continued illumination by blue light. This may be explained on the scheme above as being due to the change from $P_1 \rightarrow P_2$ form under influence of blue light, but if this is so it is difficult to explain why after a succeeding dark period the leaf still remains in the active state. One would have anticipated that the P_2 form would have been converted to the inactive P_3 form in the dark, i.e. the leaf should have been rendered inactive to light as in the case when a dark period succeeds a period of illumination by blue light alone.

The fact that the leaf, activated by exposure to red light, reverts very slowly in the dark to the inactive state suggests that P_1 is converted to P_3 under such conditions. The prevention of this process when the leaf is exposed to daylight rather than red light, suggests the formation of some stabilizing factor presumably by a photochemical reaction.

We have no information as to the mechanism whereby the P compound controls the primary photochemical reactions and speculation at this stage seems pointless. One possibility is that the phytochrome-like compound exists in photochemically interconvertible allosteric forms and that one or more of the enzymes catalysing reactions involved in the electron flow also exist in allosteric forms whose configuration is determined by that of the phytochrome. Further insight into the reactions involved must await further experimentation.

EXPERIMENTAL

Estimation of ascorbic and dehydroascorbic acids. These estimations were carried out by the indophenol method previously described.[1]

Light sources. The source of light was a high intensity tungsten lamp, the far-red component of which was, where necessary, selectively absorbed by the use of a filter consisting of 1 cm of 5% w/v $CuCl_2$ solution.

Monochromatic light was obtained by (1) the use of a Monochromater (high intensity grating, Bausch & Lomb), (2) the use of selective interference filters (Bausch & Lomb) and gelatine filters (Wratten and Ilford) or (3) by combination of both.

Light energy. The intensity of monochromatic light of different wavelength used in the experiment was controlled by the use of a photo-electric selenium cell calibrated at the different wavelengths used in terms of ergs by reference to a standard thermophile.

Leaves. The leaves of the strawberry plants (var. Gladstone) grown in pots in a cool greenhouse were picked on the day of the experiment and left for 30 min in darkness before being subjected to the experimental procedures. All experiments were conducted at 15°. Cyanide treatment of the leaves was carried out as described by Mapson[1] and the concentration of cyanide used in the present experiments was 5×10^{-5} M.