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Phytochrome-mediated Electric Potential Changes in Oat Seedlings[1]

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ABSTRACT

Brief exposures to red light induce far red-reversible changes of 5 to 10 millivolts magnitude in the upper 1 centimeter of etiolated *Avena* coleoptiles. The changes begin within 15 seconds of the start of illumination and they continue for at least 12 minutes. The changes were measured using a flowing solution of 10 mM KCl to contact the surface of the coleoptile. A dark-grown coleoptile shows no change in response to far red light unless it first receives red light treatment. The second of two red light exposures is ineffective without an intervening far red treatment. Some characterization of these electric responses to light is presented.

In investigating phenomena associated with the transformation of phytochrome in plants, one problem of interest is to determine the earliest effects of the transformation of phytochrome by red and far red light. It is possible that such transformation will produce observable electric changes, particularly if an early action of the transformed phytochrome is on a membrane (6, 12). Indeed, Tanada clearly showed that red light would cause isolated root tips of barley (13) or mung bean (14) to adhere to a negatively charged glass surface; subsequent far red light then caused their release. The results suggested that red light was causing the apical end of the root tip to become electropositive with respect to its base, an electrical change which could be negated by subsequent far red light. Yunghans and Jaffe (17) characterized the phenomenon in some detail for mung bean roots. Jaffe (7) further demonstrated by direct electrical measurement that the predicted electrical changes did indeed occur. Red light caused the apical end of the root tip to become increasingly positive, while subsequent far red light had the reverse effect. A second red light exposure brought about a second positive change, though it was far smaller than the first. The damping of the response was attributed to exhaustion of available oxygen by the confined root tip.

The present work was designed to repeat and extend Jaffe's electrical measurements. Conclusive proof for the involvement of phytochrome in these electrical changes requires a demonstration that far red light alone is without effect, and that a second or subsequent red light response can be obtained (assuming saturating red light treatment) only if the immediately previous light treatment was far red. It was decided to make electrical measurements on the *Avena* coleoptile instead of isolated root tips for several reasons. First, this organ was known to be unusually rich in phytochrome (2). Second, coleoptiles exhibit some of the most sensitive phytochrome-mediated responses known (1, 5, 16). Third, the electric techniques used here had already been found successful for measuring auxin-induced electrical changes in *Avena* coleoptiles (8). Finally, the root tip response measured by Jaffe (7) was very small, just 2 mv at the very best, and variable, and we hoped to find a larger and more reproducible response for detailed characterization studies.

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MATERIALS AND METHODS

Plant Material. Seeds of oats (*Avena sativa* L., cv. Victory) were husked, soaked in tap water for 2 hr, and placed on wet filter paper in closed Petri dishes. For the first 4 hr after soaking the seeds were exposed to 0.25 mw cm^{-2} of red light to suppress mesocotyl growth and promote coleoptile elongation (15); thereafter they remained in darkness. Forty-eight hours after start of soaking the coleoptiles were about 5 mm high and the roots about 10 mm long. The seeds were then inserted into glass tubes supported in a rack in a closed dish so that the coleoptile could grow upwards and the roots down into 10 mM KCl.

Seventy-two hours after the start of soaking, the straightest seedlings were removed and clamped at the base of the coleoptile. The coleoptiles were oriented vertically, with the roots and seed immersed in 10 mM KCl. At this stage the coleoptile was 20 to 30 mm high, the primary leaf was up to the top, and the mesocotyl was about 10 mm high. These seedlings were allowed to equilibrate for at least 2 hr before being used in experiments. During experiments over the subsequent 8-hr coleoptiles grew at a rate of about 0.8 mm/hr. Temperature throughout the growing process was 25 C. The necessary manipulation of the coleoptiles during growth was carried out with as little dim green light as possible, at most 3 min for each coleoptile.

Lights. The dim green safelight used throughout was a 20-w gold fluorescent tube (G.E. F20T 12 GO) behind 3/16 inch of deep blue No. 2424 and 3/16 inch of green No. 2092 Plexiglas (Rohm and Haas). It was about 50 cm above the seedlings and was used only when illumination was actually required.

Red or far red light, to effect phytochrome transformation, was obtained from a 500-w projector with a photographic shutter and colored filters in place of the lens. The

light was passed also through a 4-liter beaker of water to absorb infrared and to focus the light onto the coleoptile. A movable shield close to the coleoptile enabled illumination of restricted regions. The base of the coleoptile, the mesocotyl, the seed, and the roots were invariably shielded from the light. Except for 1 cm at the tip of the glass tubes carrying the contact solution to the coleoptile, the electric measuring system was also shielded from the light. The light shone horizontally onto the vertical coleoptile, in the plane defined by the two vascular bundles. Red light, of intensity 2.5 mw cm^{-2} at the coleoptile, was produced with Rohm and Haas red Plexiglass No. 2423 and a glass cutoff filter, Corning No. 3-66. These filters allowed the transmission of 0.3 mw cm^{-2} within the far-red range: this is about 15% of the red intensity. Without the cutoff filter the plastic would pass nearly 0.01 mw cm^{-2} of blue light. Twenty seconds of this light (plastic filter only) was sufficient to cause phototropic bending. Far-red light, of intensity 0.4 mw cm^{-2} at the coleoptile, was produced with Corning cutoff filters Nos. 7-69 and 3-66. Intensities were measured with an Eppley thermopile No. 3979 and a Keithley 150B microvolt ammeter. Red light treatment of the coleoptiles was given for 10 sec, and far red treatment for 65 sec. The total dose in each case was, therefore, about 25 mjoules cm^{-2}. Blue light of intensity 0.3 mw cm^{-2} was produced by Corning filter No. 5-60.

Measuring Conditions and Techniques. Measurements were made at 21 to 25 C, at high humidity in a small Lucite box fitted with sleeves to allow external manipulators to position the two contacts on the coleoptile. The contacts were placed on the ab- and adaxial sides of the coleoptile and, except where specified, at 3 to 5 mm below the apex. When at 0.5 mm, the contact was on the side of the coleoptile away from the pore.

The method of making contact with the coleoptile using flowing solution has previously been described (Fig. 1 of Ref. 8). Electric contact with the plant is made from the flowing solution in a double glass tube by a small liquid bridge. There is no solid contact with the coleoptile. Potassium chloride of concentration 10 mM, or where specified 100 mM, was used as the contact medium.

The mercury-calomel electrode in the solution at the base of the coleoptile was connected to ground and to the reference terminal of the meter (Keithley 150B microvolt ammeter). With suitable switching the mercury-calomel electrode from either of the contacts on the coleoptile could be connected to the high resistance input of the meter. Thereby the potential of either point of contact with the coleoptile could be observed and recorded on the chart recorder (Bausch and Lomb VOM 5) attached to the meter. The 10^9-ohm input resistance of the meter is sufficiently high for the present measurements, since the resistance of the coleoptile and contact system was always less than 10^7 ohms. The resistance was checked at the start of measuring each coleoptile by observing the potential change when a current of 10^{-9} ampere was passed through the system.

By switching the meter input every few seconds from one contact to the other on the coleoptile the chart recorder displayed the time variations of the potential of each contact. (Transient variations in potential lasting less than a few seconds were of no interest.) Each coleoptile, therefore, provided two separate records of changes in potential, at one or two positions below the apex. Sometimes the liquid bridge forming one of the contacts would break during a recording, caused usually by slight bending of the coleoptile at its base. The contact could easily be reestablished without affecting the record from the other contact and with loss of only 1 or 2 min of record from the broken contact. Because of the absence of data during that time the record for that particular contact for the current treatment of the coleoptile could not be used in analyzing the results of that treatment. Later records from that contact on the plant could be used, however.

RESULTS

Preliminary Observations. Preliminary observations were made on oat and rye coleoptiles and on pea epicotyls. Most of these plants had experienced excessive green light during growth, and the red light was used without the cutoff filter so it leaked a little blue (0.2% of the total energy).

A small red, far red reversible effect on the potentials on oats was observed. A rise in potential occurred after red light and a fall in potential after far red light. It appeared also that unrestricted green light in the constant temperature growth room markedly suppressed the responses to red and particularly to far red light. These observations led to the experiments reported below using more stringently dark-growing conditions and red light without any blue component.

No consistent red or far red effects were observed on either rye or peas and so no further experiments on those were undertaken, though subsequent work with peas under the more stringent conditions has revealed reproducible changes (M. S. Everett, personal communication). Blue light, even the small amount from the red Plexiglas, induced a rapid, 20 to 40 mv fall in potential of the top of an etiolated pea epicotyl. This phenomenon does not seem to be related to phytochrome transformation and it has not yet been pursued.

Blue light was not observed to have any immediate effect on the oat coleoptile potentials. The light did of course induce bending of the coleoptile and a 20-min period electric wave previously reported (8) moved down from the top. The presence of this wave made it difficult to observe subsequently the potential changes induced by red or far red light, but these phytochrome-mediated changes were not abolished by the electric wave.

The preliminary experiments on oats indicated that 25 mjoules cm^{-2} was a saturating dose for the response to either the red or the far red light. Doses one-tenth of this amount produced very much smaller responses. More detailed measurement of effects of different intensities and doses have not been made.

Although the light-induced potential changes were observable anywhere in the top 1 cm of the oat coleoptile, the best responses appeared at about 5 mm below the apex. This position was therefore chosen as the standard one.

A Phytochrome-mediated Effect. To demonstrate that the observed electric potential changes on oats were indeed mediated by phytochrome, a sequence of five red light and five far red light treatments, shown on the bottom of Figure 1, was devised. The 6-min interval between the successive treatments is insufficient to allow the full development of the response to each treatment. It does, however, allow observation of the initial rapid potential change following each treatment.

The sequence of treatments was given to each of six coleoptiles. One of them appeared damaged when observed in daylight after its measurement. All data from it was therefore rejected even though clear responses had been recorded. One of the remaining five coleoptiles had one contact at 0.5 mm instead of 3 to 5 mm so only one set of results was available from it. On some of the other coleoptiles one contact occasionally broke. Sample sizes thereby varied from seven to nine contacts for each treatment, on the five coleoptiles.

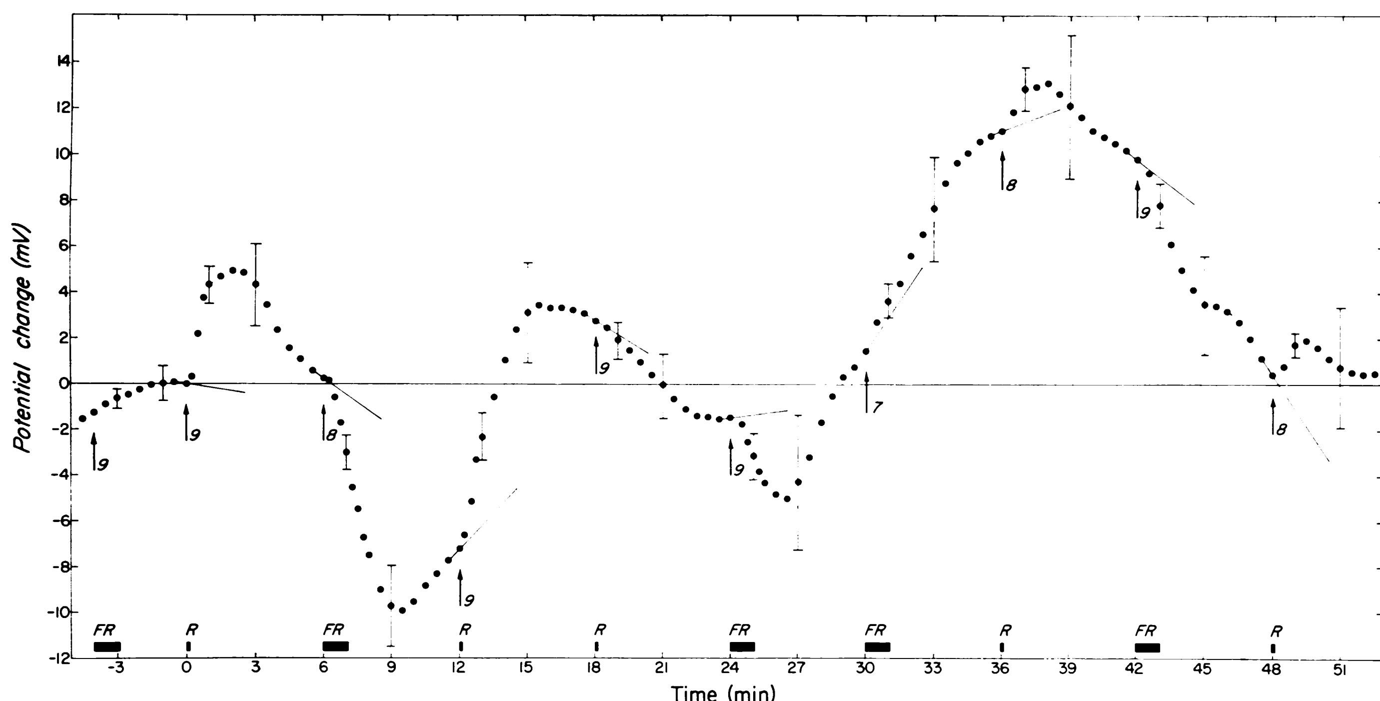

FIG. 1. Average electric responses of five oat coleoptiles to a sequence of five far red and five red light treatments. The start of each treatment is indicated by an arrow, and its duration is indicated by a bar on the bottom of the figure. Each treatment, except the first red, started 6 min after the start of the previous one. Each coleoptile had one or two effective contacts on it: sample sizes (7, 8, or 9) are shown beside the arrow for each treatment. For calculating the average response to each treatment the potential of each contact at the start of that treatment was taken as the reference zero. At the start of each treatment a straight line has been drawn through the initial point and the point half a minute earlier, and has been extrapolated forward for 2.5 min, to indicate the way in which the potential appeared to be changing as the treatment started. Plotted points are at 0.25-min or 0.5-min intervals. Ninety-five per cent confidence limits are shown at 1 min and 3 min for each treatment. The top 10 mm of the coleoptile was illuminated. Measuring contacts were at 3 to 5 mm below the apex.

Figure 1 shows the results of the sequence of red and far-red treatments. Red light induces a quick and statistically significant rise in potential; far red light induces a quick and statistically significant fall in potential. A response to red does not occur if there has been a preceding red treatment without an intervening far red. A response to far red light only occurs if the far red follows a red treatment. Figure 1 shows that the red and far red doses are indeed saturating since a repeat dose of the same color does not produce a response.

A few additional experiments showed that blue light was not able to substitute for far red in permitting a subsequent red treatment to produce a response.

Thus the phenomenon of the electric responses is clearly a phytochrome effect. Further experiments have shown no physiological evidence for reversion of Pfr back to Pr within an hour at least. If the time between the two consecutive red treatments in Figure 1 is increased from 6 to 60 min there is still no electric response to the second red light treatment unless there has been an intervening far red treatment. This result is perhaps not surprising since spectral reversion of phytochrome has never been observed in coleoptiles despite several studies (3, 9, 11).

Since the far red treatment preceding the initial red appears to cause no significant potential change, the origin of the graph axes in Figure 1 is taken at the start of the initial red treatment.

Time Course of Response to Red Light. Fifteen coleoptiles were given the initial 10-sec red light treatment, and the potential changes were followed for 12 min. On five of the coleoptiles the potential changes were followed for an additional 6 min. The results are shown in Figure 2.

The variability of the observations, measured by the standard deviation, is small early after treatment, so that the initial change is quite well defined. Standard deviations are much larger later on, remaining at about 5 mv after about 12 min. Much of this variability seems to be due to the slow

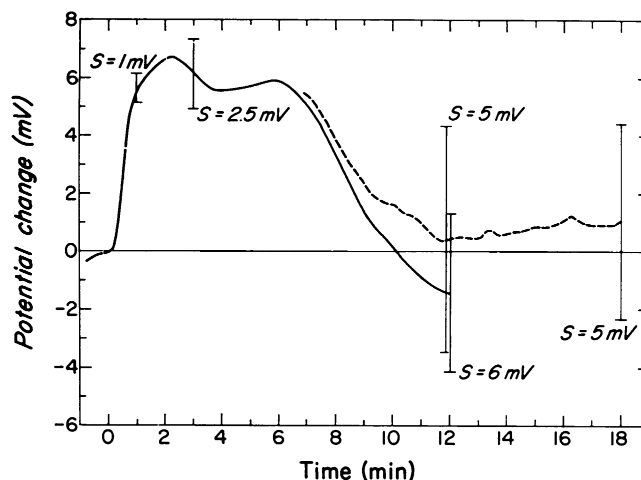

FIG. 2. Mean potential changes at 5 mm following intial red illumination for 10 sec at time zero. Solid line: data obtained for 12 min; sample size: 18 contacts from 15 coleoptiles (including the five measured for the longer time). Dashed line: data obtained for 18 min; sample size: eight contacts from five coleoptiles. This curve is plotted only after 7 min. Before that time it differed from the larger sample, of which it is part, by less than 0.3 mv. Both curves are drawn through points computed at 0.25-min intervals. Ninety-five percent confidence limits, staggered for clarity, are shown at 1, 3, 12, and 18 min. The standard deviations, S, of the data at those times are given alongside the confidence limits.

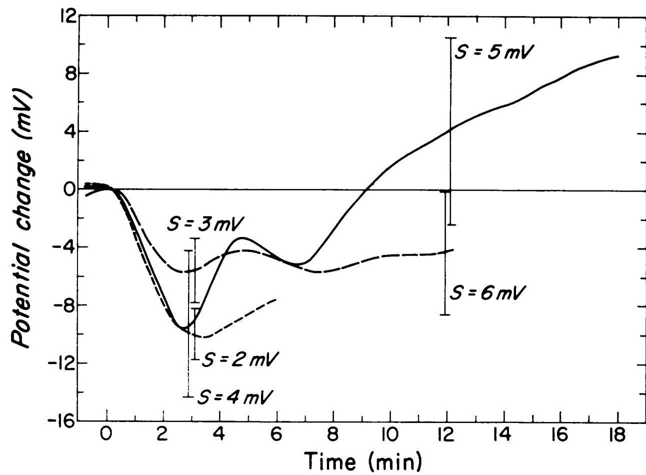

FIG. 3. Average potential changes at 5 mm following 65 sec of far red light starting at time zero. Short dashes: treatment was 6 min after initial red light (data from Fig. 1); sample size: eight contacts from five coleoptiles. Long dashes: treatment was 12 min after initial red light; sample size: 10 contacts from 10 coleoptiles. (These coleoptiles had been used for some 12-min measurements for Fig. 2.) Solid line: treatment was 18 min after initial red light; sample size: six contacts, from four coleoptiles. (These coleoptiles had been used for some 18-min measurements for Fig. 2.) The curves are drawn through points computed at 0.25-min intervals. Ninety-five per cent confidence limits (staggered for clarity) are shown at 3 min and 12 min. The standards deviations, S, of the data at those times are given alongside the confidence limits.

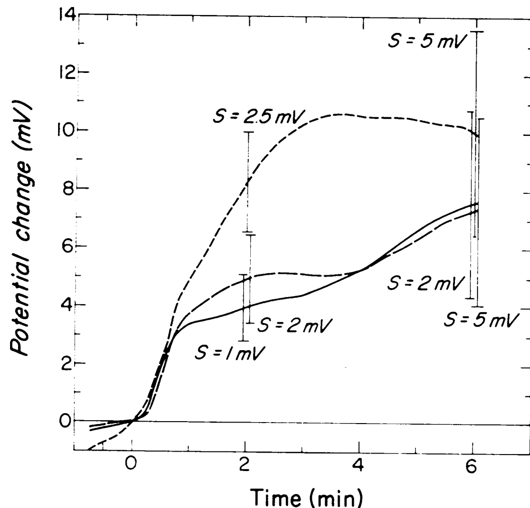

FIG. 4. Average potential changes at 5 mm following 10 sec of red light at time zero. Short dashes: treatment was given 6 min after far red which was 6 min after initial red. Data are from Figure 1. Sample size: nine contacts from five coleoptiles. Long dashes: treatment was given 12 min after far red which was 12 min after initial red; sample size: 10 contacts from nine coleoptiles. Solid line: treatment was given 18 min after far red which was 18 min after initial red; sample size: six contacts from five coleoptiles. Curves are drawn through points computed at 0.25-min intervals. Ninety-five per cent confidence limits (staggered for clarity) are shown at 2 and 6 min. The standard deviations, S, of the data at those two times are given alongside the confidence limits.

drift in potential that each coleoptile shows (*e.g.*, dashed line, Fig. 7C). Nevertheless the average behavior is clear and the changes induced by red light are roughly complete by about 12 min.

Measurements on a few coleoptiles at high meter sensitivity and at high chart speed showed that the electric potential changes induced by the red light could be detected within 10 sec after the start of illumination. For these experiments the red light was not turned off after the 10 sec, but was left on for a minute. The responses from these coleoptiles were not different from those of the sample used in Figure 2.

Time Course of Response to Far Red Light. The form of the response to far red light depends on the length of time since the preceding red treatment. The 65-sec far red treatment was given to samples of coleoptiles 6, 12, or 18 min after a single initial 10-sec red light exposure. The results are shown in Figure 3. The 12-min curve should be regarded as the definitive curve for the response to far red light; the 18-min curve, because of small sample size is not significantly different from it.

At 3 min after treatment the potential of the 6-min sample is just significantly lower than the potential of the 12-min sample (5% level). This decrease is possibly because the potential fall induced within 3 min by far red light may be having added to it the potential fall occurring (Fig. 2) between 6 and 9 min after the red light (see below).

Within the 1st min all curves in Figure 3 are closely similar. Inspection of the records from each treatment shows that the response to far red light begins within 15 sec of the start of illumination. This delay is longer than that for the response to red light, perhaps because the far red light source had lower intensity.

By inspection of the original records from each coleoptile, the peak occurring at about 5 min in the 12- and 18-min curves in Figure 3 appears to be a real phenomenon. After about 8 min much of the variability indicated by the confidence limits in Figure 3 is due to an apparent slow drift of potential superimposed on what would show otherwise as significant changes.

Response to Red after Far Red Light. After the initial red and far red treatments, illustrated in Figures 2 and 3, with observations lasting 6, 12, or 18 min, the coleoptiles were given a second red treatment. Results of this treatment are shown in Figure 4. The responses, at 1 min, are two-thirds the size of the responses to the initial red (Fig. 2). Otherwise the time course of the response is roughly the same within the first 6 min.

The 6-min curve in Figure 4 is just significantly higher (5% level) than the others at 2 to 3 min. This may be because for it the red treatment was given only 6 min after the far red: it can be seen that the potential was rising at a rate of more than 1 mv min^{-1} at the time zero for the 6-min curve in Figure 4.

Response to Red with Immediate Far Red. Figure 5 shows the average potential changes following three successive treatments, separated by 12 min, of red light (10 sec) immediately followed by far red (65 sec). Twelve minutes after the third, a fourth treatment gave a similar but smaller response. After the 1st min of each response the variability of the data becomes quite large: the standard deviation is about 5 mv after 12 min in each case.

The potential in Figure 5 falls more rapidly than it does in Figure 2 for which the red was not immediately followed by far red light. This difference raises the question of how the coleoptile produces the Figure 5 response from its reactions to red and to far red light. Figure 6 is a re-presentation of data from previous figures. The solid line in Figure 6 is the same as the solid line in Figure 5 (first treatment); the dotted line in Figure 6 is the algebraic sum of the first red and the first far red 12-min curves from Figures 2 and 3, respectively, with 15-sec displacement of the far red curve to

the left. (A 10-sec displacement would have been correct, since the far red treatment in Fig. 5 started 10 sec after time zero, but the available data points were at 15-sec intervals. The consequent 5-sec error is trivial.) The agreement between the two curves in Figure 6 is such that the responses to red, followed by immediate far red, shown in Figure 5, may be simply the sum of the separate responses to red and to far red light. This observation has important implications for the mechanisms of production of the two responses.

Responses to Red and Far Red by Decapitated Coleoptiles. The possibility of a relationship between the observed electric changes and auxin was investigated using doubly decapitated coleoptiles.

The first decapitation, of about 2 mm, was performed 3.5

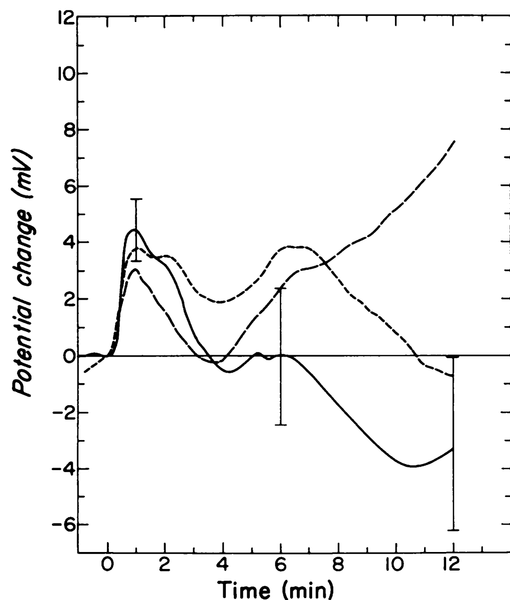

FIG. 5. Average potential changes at 5 mm due to 10 sec of red light, starting at time zero, and immediately followed by 65 sec of far red light. Solid line: first treatment; sample size: 10 contacts from five coleoptiles. Short dashes: second treatment, given 12 min after the first; sample size: nine contacts from five coleoptiles. Long dashes: third treatment, given 12 min after the second; sample size: eight contacts from four coleoptiles. (This curve is indistinguishable from the solid line at times less than 0.5 min.) The curves are drawn through points computed at 0.25-min intervals. Ninety-five per cent confidence limits are shown at 1, 6, and 12 min for the first treatment; confidence limits for the other treatments are slightly larger.

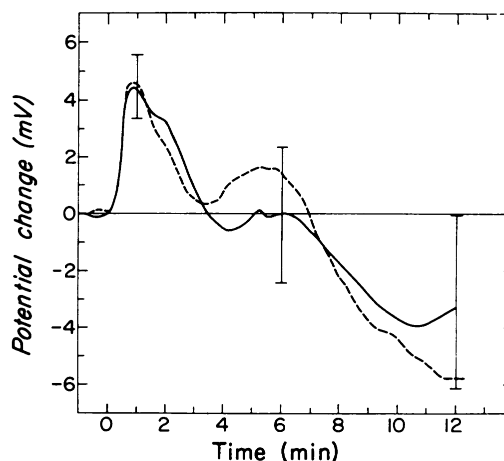

FIG. 6. Average potential changes from previous figures. Solid line: first treatment from Figure 5. Dashed line: algebraic sum of the 12-min line from Figure 2 and the 12-min line (but shifted 15 sec later in time) from Figure 3. (The two curves are indistinguishable in the first 0.5 min.)

hr before the initial red treatment. The second decapitation, also of 2 mm, was performed about 0.5 hr before the initial red treatment.

Potential changes were observed on decapitated coleoptiles both after the initial red and the following far red light 12 min later. Contact with the coleoptile was made at 1 or 3 mm below the cut top, *i.e.*, 5 or 7 mm below the initial apex.

The top two rows of Table I show the mean potential, with 95% confidence limits, at both 1 and 3 min after each treatment. The third and fourth rows of Table I give the corresponding information for decapitated coleoptiles which had been supplied with auxin. The IAA was applied as a droplet, at about 20 mg/liter of concentration, to the cut top, about 20 min before the initial red treatment. At the conclusion of its measurement, each IAA-treated coleoptile had clearly grown, in response to the auxin.

Table I (top four rows) shows the small responses of decapitated coleoptiles to red and far red light. Though the significance is marginal at best, an appropriate slope change was always observed on the recording 10 to 15 sec after the start of illumination. The IAA treatment is apparently without effect on the responses from the decapitated coleoptiles. Hence all available data for decapitated coleoptiles have been combined in rows 5 and 6 of Table I. They may be compared with the data, taken from Figures 2 and 3 for intact coleop-

Table I. *Electric Potential Changes on Decapitated Coleoptiles*

Potential changes are shown at 1 and 3 min after red or far red treatment of coleoptiles, with and without decapitation, and with and without applied IAA. Sample sizes and 95% confidence limits for the means are given.

Treatment			1 Min after Treatment		3 Min after Treatment	
Light	Coleoptile	Hormone	Sample size	Potential	Sample size	Potential
			contacts	*mv*	*contacts*	*mv*
Red	Decapitated		8	0.88 ± 0.62	6	0.50 ± 1.44
Far red	Decapitated		6	−0.38 ± 0.45	6	0.25 ± 2.21
Red	Decapitated	IAA	8	0.70 ± 0.69	8	0.38 ± 1.30
Far red	Decapitated	IAA	8	−0.48 ± 0.58	8	−1.30 ± 0.51
Red	Decapitated	± IAA combined	16	0.79 ± 0.41	14	0.43 ± 0.82
Far red	Decapitated	± IAA combined	16	−0.41 ± 0.30	16	−0.62 ± 0.79
Red	Intact		18	5.68 ± 0.50	18	6.16 ± 1.22
Far red	Intact		10	−2.02 ± 1.01	10	−5.58 ± 2.20

tiles, in rows 7 and 8 of Table I. The near-elimination of the responses by double decapitation might suggest that auxin is required for the phytochrome-initiated potential changes to take place. This explanation seems unlikely, however, because application of sufficient IAA to cause good growth does not restore the responses. Little can be said at this stage about how the decapitation affects the responses.

Localization of the Responses. On seven coleoptiles the response at 0.5 mm from the top was compared with the response at the standard 5-mm position. One minute after the red treatment, the response at 0.5 mm was 72 ± 16% of the response at 5 mm. Similarly, on five coleoptiles, the response, at 1 min after red treatment, at 10 mm from the top was 59 ± 21% of the response at 5 mm. In making these observations, care was taken only to illuminate at least the region including the two contacts on the coleoptile. Variations in the size of this illuminated region may account for some of the variability in the results. Thus the electric response to red light may be observed quite strongly from contacts anywhere on the top 10 mm of the coleoptile, though the response is largest near 5 mm below the apex.

The remaining experiments have to do with the localization of the response within the tissue. For a few observations the concentration of KCl in one contact was changed to 100 mM. This concentration change did not appear to have any effect on the subsequent responses to red or far red light. The change in concentration of the contact solution of course produces a change in potential measured by the system. The change was a decrease of about 40 mv and it was complete, apart from a slow drift, within 5 min. A prior treatment by red or by far red light had no significant effect on the time course of this response to concentration change or on the time course of the response to the reverse concentration change back to 10 mM.

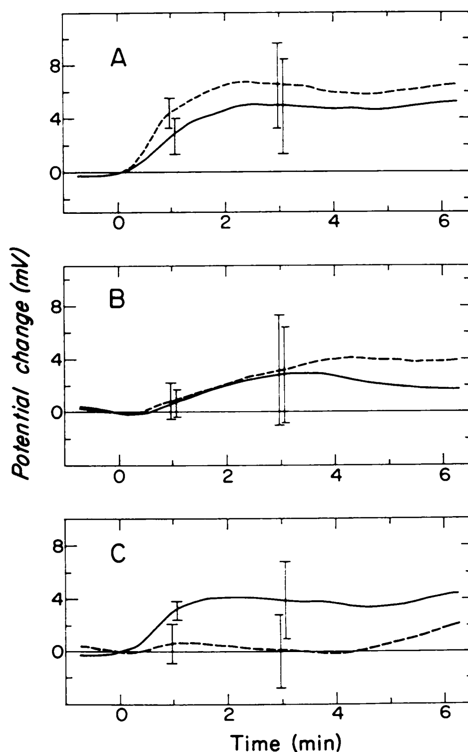

FIG. 7. Average potential changes following three successive 10-sec exposures to red light. Dashed line: contact at 5 mm; solid line: contact at 2 mm. The curves are drawn through points computed at 0.25-min intervals. Ninety-five per cent confidence limits (offset slightly for clarity) are shown at 1 and 3 min on each curve. Sample sizes: 5 or 6. A: Initial red treatment to the region around the 5 mm contact, *i.e.*, from 4 mm to 6 mm below the apex; B: repeat of the above red treatment 12 min later; C: Third red treatment after further 12 min: whole of the top 8 mm of the coleoptile illuminated.

On six coleoptiles contacts were placed at 2 and 5 mm from the apex. A 2-mm region around the 5-mm contact was given the 10-sec red light treatment. Twelve minutes later this red treatment was repeated. A further 12 min later the whole of the top 8 mm of the coleoptile was given red treatment. The three responses of the two contacts are shown for 6 min each in Figure 7. Although it is in the dark, the contact at 2 mm responds nearly as well to the initial red (Fig. 7A) as does the illuminated 5-mm contact. The illuminated region is of course within the electric path for the measurement from the top contact. Both contacts appear to give a marginally significant (5% level) response (Fig. 7B) to the second restricted red light exposure. This response, if real, is slightly slower in rising than the normal response. When the illumination is extended to include the 2-mm contact, it gives a good response (Fig. 7C). Although the response in this case from the 5-mm contact is not statistically significant, inspection of the records suggests that it is nevertheless real because each record shows a slight increase in slope at 0.5 min after treatment. No change, even of this small size, was observable in records from coleoptiles given a second red treatment, following a first, over the whole top 8 mm of the coleoptile (see Fig. 1). These results provide some evidence that the responses to red and far-red light do not take place exclusively at the plasmalemma of those epidermal cells bathed by the contact medium.

DISCUSSION

Electric potential changes in oat coleoptiles have the red, far red reversible characteristics of phytochrome-mediated processes. The transformation of phytochrome to Pfr and back to Pr has an effect that is observable within 15 sec. Following each light treatment the potentials have stabilized sufficiently after 12 min to allow another light treatment to be given and usefully assessed.

Initially the phytochrome is in the Pr form and hence an initial far red treatment (Fig. 1) has no immediate effect. From Figure 1 there may possibly be some later effect of the initial far red in causing the potential following the initial red treatment to fall to zero after only 6 min instead of after 12 min, as shown in Figure 2. The difference between the two figures is statistically significant (5% level) at 6 min.

A possible mechanism to account for the observed potential changes may be given in the following terms: transformation of phytochrome affects, directly or indirectly but nevertheless quickly, a membrane system. The effect is on membrane permeability or on ion pumps in the membrane. If there is an effect on permeability with consequent ion movement, this movement may be irreversible even though the phytochrome can be transformed back. Thus the electric responses may well show a kind of fatigue, as observed both here and by Jaffe (7) (who attributed it to oxygen deficiency), even though the phytochrome transformation may not (4). Excessive green light during growth has been found to decrease the size of the responses. The green does not have to affect phytochrome transformation; it may merely decrease the membrane's ability to respond to the transformed phytochrome. Green light, however, does transform phytochrome *in vivo* in corn coleoptiles (11), albeit very inefficiently, so a phytochrome-mediated green light effect is not excluded.

In describing Figure 6 it was noted that the observed potential changes in Figure 5 could be just the sum of the changes shown by the 12 min curves in Figures 2 and 3. If this is so, it implies that the electric changes are produced by physically distinct, independent sources or processes. These are either two electric current generators in parallel, or two voltage generators in series. The following model is the kind that might satisfy this requirement: transformation of phytochrome to Pfr first initiates a (perhaps transient) lowering of a membrane permeability to an ion (cation one way or anion the other way), and secondly turns off a pump that was maintaining that ion out of electrochemical equilibrium. Transformation of the phytochrome back to Pr by far red light will turn on the pump again but does not alter the sequence of membrane permeability changes which continue to their completion independently.

It might be suggested that the membrane in question could be the plasmalemma of the epidermal cells and that the ion could be potassium (12). Several experiments, however, argue strongly against such participation of the epidermal cells in the observed responses. The lack of interaction between changes in contact medium (KCl) concentration and the phytochrome-induced potential changes is one minor argument. Stronger argument comes from Figure 7. In that figure a strong response comes from a contact which is *not* illuminated (though some light scattering is possible). Hence the observed potential changes do not arise entirely at the points of electrical contact with the coleoptile, and nothing can be said about ion specificity. The results of Figure 7 are perhaps best explained by assuming that the response is generated, in a polar fashion, within the illuminated region of tissue. If the response is associated with the plasmalemma it will be that of the transverse, not tangential, walls; moreover, it will be on the top (or in opposite fashion, on the bottom) part of the membrane of each parenchyma cell.

The experiments reported above, giving evidence on localization and mechanisms, are really of a preliminary nature. Confirming evidence may be obtained from experiments with intracellular microelectrodes, with different contact media, with many contacts on the coleoptile at different levels, and with localized illumination. Electron microscopic evidence on the localization of phytochrome within the cell would be extremely helpful. Pratt (10) has recently used an immunological technique to demonstrate localization of phytochrome in oat coleoptile tissues. Though the tissue localization is elegantly demonstrated, inadequate fixation prevents making any firm conclusions concerning subcellular localization except that cells which contain phytochrome have it widely distributed. Intimate association of phytochrome with the plasmalemma is yet to be directly shown.

If the observed phytochrome-initiated responses are associated with the upper, or lower plasmalemma, or both, they might be expected to be affected by auxin. From the present data, however, the relationship, if any, is obscure. One can only conclude that the sites of the auxin-mediated electric wave (Fig. 3 of ref. 8) are different from the sites of the electric responses to phytochrome transformation.

In conclusion, *Avena* coleoptiles exhibit phytochrome-mediated electric changes of sufficient magnitude and reproducibility to justify further experimentation. The complexity of the responses to red and far-red light, and the relationships between them, preclude any simple model involving only red-induced permeability increase followed by cation efflux. Further experimentation with different contact media, varying both ionic species and concentration, and intracellular recording, are clearly required for any definitive model-building.

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