Modification of Apparent Phytochrome Synthesis in Pisum by Inhibitors and Growth Regulators

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Summary. The repeated exposure of Pisum (pea) plants to red light brings into operation an apparent synthesis of phytochrome which is not observed in material kept in the dark. This process shows some temperature compensation but has an optimum at 26°; it is irreversibly inhibited by 10^{-4} M cycloheximide and 10 \mu g/ml actinomycin D. It is also inhibited by the auxins indoleacetic acid, naphthalene acetic acid and 2,4-dichlorophenoxyacetic acid at 10^{-4} M but in these cases the inhibition is completely reversed when the auxin is washed out of the tissue. Antiauxins 2,4,6-tri-chlorophenoxyacetic acid and p-chlorophenoxy isobutyric acid, while strongly inhibiting growth have little effect on apparent synthesis. Other growth regulators and the precursor of tetrapyrrole synthesis, \delta-aminolevulinic acid, have no consistent effect on the process, but 3 \times 10^{-4} M cobalt (II) nitrate is inhibitory. The capacity for apparent synthesis decreases as the cells approach maturity. The results may be explained by either de novo synthesis of phytochrome, or by a transformation process resembling in some respects the dark reversion of Pfr to Pr. The physiological role of apparent synthesis is suggested.

Phytochrome, in the state which preferentially absorbs far-red light (Pfr), is labile in a variety of seedling and nonseedling tissues (5,11,13). After its production by red light the Pfr in such tissues is gradually destroyed, or, at least, rendered undetectable by the only assay currently available, which depends on photoreversible absorbance changes. In addition to this phenomenon the dark, or spontaneous, reversion of Pfr to Pr probably occurs in several tissues from dicotyledonous plants (5,7,11,15). A third process, apparent synthesis, has been described in pea stem tissue (6); it is not known whether de novo synthesis of either or both components of the holochrome is involved, or whether the process may be another type of transformation. When pea plants (Pisum) are repeatedly illuminated by red light over 6 hours and then returned to darkness, Pfr destruction is at first observed, but it is followed, 2 to 4 hours after the onset of darkness, by an increase in phytochrome. This apparent synthesis occurs only when the phytochrome concentration in the tissue has been reduced below a critical level by Pfr destruction, and only in tissue capable of growth, although growth and apparent phytochrome synthesis are separable (6). In this paper the relationship between apparent synthesis and growth, and the influence of growth promoters and inhibitors on the process, are examined in more detail.

Materials and Methods

Plant Culture. Seeds of Pisum sativum L. cultivar Alaska, obtained from the Asgrow Seed Company, New Haven, Connecticut, were grown as described earlier (7,11).

Experimental Procedure. In most experiments 7-day-old seedlings were given a standard pretreatment of three 15 minute exposures to red light separated by periods of 1 hour and 45 minutes of darkness. The red light source was described by Hillman (11). After this pretreatment a 0.5 cm segment was cut from each seedling (fig 1). The segments were bulked and subdivided into lots of 200 ± 5 mg (usually 21 segments) which were placed in petri dishes (100 × 15 mm) on Whatman Xo. 1 filter paper saturated with 5 ml of incubation medium. The standard medium was KH_{2}PO_{4}-Na_{2}HPO_{4} buffer (pH 6.7) 0.02 M with respect to PO_{4} and with 2% sucrose. All operations involved in setting up the experiments and in their subsequent harvesting were performed under dim green safelights. The excised segments were illuminated by a fourth 15 minute exposure to red light and then kept in darkness for either 18 or 24 hours before harvesting.
Phytochrome Assay. Phytochrome levels were estimated by dual wavelength spectrophotometry using a Ratiospect model R2 as described elsewhere (7,11). Apparent synthesis is most readily observed in tissue from the apical hook region of the pea stem. This region consists of young cells few, if any, of which undergo further division, but with a considerable capacity for expansion: such cells contain high concentrations of phytochrome (3,7). If, during the expansion of these cells, there is no de novo phytochrome synthesis, the phytochrome concentration will fall. A growing system such as this poses a number of problems when comparisons of tissue samples are made at different times. Some procedure must be adopted which will minimize the confusion caused by changes in weight (volume) of the sample. \( \Delta (\Delta OD) \) Readings are estimates of concentration and changes in volume and concentration should be governed, ideally, by Beer’s Law. As a simplified example, a tissue sample, grown and maintained in the dark, that reads 10 \( \Delta (\Delta OD) \times 10^3 \) initially, should read 5 after an interval of time during which its weight has doubled, if no phytochrome synthesis has occurred. This reduction in machine reading is a practical disadvantage when the phytochrome concentration being measured is low, as in our experiments, because readings are more subject to error from random noise. In practice, corrections for volume changes are complicated by changes in the total amount of measurable phytochrome during the experiment. There is a gradient of phytochrome concentration in the segments which is greatest at the terminal end where there are more cells per unit weight (7). After a 24 hour incubation the segment illustrated in figure 1 nearly doubles its weight and increases its length by 60%. If this expanded segment is divided into 2 sections of equal weight the terminal section is found to contain 70 to 90 % of the total phytochrome in the segment. If the entire segment is assayed a much lower \( \Delta (\Delta OD) \) is observed than when the terminal portion is analyzed separately. This fact has strongly influenced the adoption of the following procedure.

The initial phytochrome reading at hour 0 is based on the 200 mg weight of 20 to 22, 0.5 cm segments recently excised. In subsequent samplings the basal portions are trimmed off (fig 1) to give terminal portions, still 0.5 cm in length, with a total weight of 200 mg. The terminal 200 mg is packed into 1 cuvette and the remaining, basal sections into another. The \( 2 \Delta (\Delta OD) \) readings are added and an estimate of the phytochrome content of the entire segment is normalized to the initial weight of 200 mg.

An example of the procedure is compared with direct measurements on entire expanded segments in table 1. Since the weight (volume) of the sample has doubled, the calculated value for the entire sample is the resultant of the concentrations observed in the basal and terminal sections. In the data below the phytochrome synthesized in a given dark period, termed new phytochrome, is calculated by subtracting the \( \Delta (\Delta OD) \) measured 3 hours after the fourth light exposure, from the final \( \Delta (\Delta OD) \) at the time of harvest (for illustration see Control curve, fig 5). Hour 0 refers to the moment the fourth light exposure ends: growth refers here to increases in fresh weight.

Briggs and Siegelman (3) were unable to obtain a linear relationship between sample volume and \( \Delta (\Delta OD) \). No difficulty of this kind was experienced in the present work with pea stem segments. With segments harvested at a given time the increase in \( \Delta (\Delta OD) \) with weight was linear in the range of 120 to 340 mg. This difference may be explained by the narrower bore cuvette (6 mm) used here.

Results and Discussion

Temperature Optimum. The data in figure 2 indicate that the temperature optima of both ap-

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Table I. Examples of Phytochrome Readings Obtained from Subdivided and Entire Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt</th>
<th>( \Delta (\Delta OD) ) By subdivision</th>
<th>( \Delta (\Delta OD) ) By entire sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Terminal</td>
<td>Basal</td>
</tr>
<tr>
<td>hr 0</td>
<td>mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>200</td>
<td>0.280</td>
<td>...</td>
</tr>
<tr>
<td>24a</td>
<td>400</td>
<td>0.187</td>
<td>0.045</td>
</tr>
<tr>
<td>24b</td>
<td>400</td>
<td>0.214</td>
<td>0.061</td>
</tr>
</tbody>
</table>

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FIG. 1. Illustration of the segment, cut from 7-day-old etiolated pea plants, used in phytochrome assays.
**Figure 2**

Fresh weight and phytochrome concentration of segments incubated for 24 hours at various temperatures. Solid line: concentration of new phytochrome. Broken line: increase in weight during incubation. All values are expressed as percentages of value at 26°.

**Table II. Inhibitions of Growth and Apparent Synthesis of Phytochrome in Tissue Treated with Cycloheximide and Actinomycin D for Various Lengths of Time**

Lots 100 mg of 0.3 cm segments cut from the apex of pea stems. Inhibitor was washed from tissue by spraying with distilled water for 5 minutes and returning tissue to standard incubation medium without inhibitor.

<table>
<thead>
<tr>
<th>Treatment/time</th>
<th>New phytochrome</th>
<th>Δ Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ(Δ OD) × 10⁹/g ± S.E.</td>
<td>mg</td>
</tr>
<tr>
<td>Control</td>
<td>82.0 ± 5.9</td>
<td>116</td>
</tr>
<tr>
<td>0.1 mM Cycloheximide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ hr</td>
<td>56.2 ± 2.3</td>
<td>63</td>
</tr>
<tr>
<td>2 hr</td>
<td>39.0 ± 3.8</td>
<td>57</td>
</tr>
<tr>
<td>24 hr</td>
<td>-21.0 ± 1.4</td>
<td>28</td>
</tr>
<tr>
<td>10 μg/ml Actinomycin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hr</td>
<td>44.5 ± 2.4</td>
<td>42</td>
</tr>
<tr>
<td>24 hr</td>
<td>12.0 ± 0.7</td>
<td>33</td>
</tr>
</tbody>
</table>

Note: Both processes seem to have some temperature compensation in the range 20 to 30° where differences between weights and phytochrome concentration are not significant (P < 0.05).

**Effects of Cycloheximide and Actinomycin D.** Both apparent synthesis and segment growth are similar. Unfortunately, the correct interpretation of these results is made very elusive by the general disturbance of growth and metabolism caused by the inhibition of protein synthesis. A more precise tool is required to determine whether the appearance of new phytochrome depends on A) the synthesis of new protein to form part of the holochrome, or B) the synthesis of an enzyme in some way involved in the re-establishment of photoreversibility in a pool of nonreversible phytochrome (6). Cycloheximide has, however, been useful in demonstrating that phytochrome synthesis does not occur in dark grown segments or in those which have received only 1 exposure to red light. If synthesis were to occur in these tissues as it does in the repeatedly illuminated material, the phytochrome concentration in the segments treated with cycloheximide would be lower than in the untreated controls. The fact that this was not the case indicates that no synthesis occurred (table II). In both control and cycloheximide-treated tissue maintained in darkness there was a decline in phytochrome concentration during the 24 hour incubation period which may be accounted for partly by damage to the tissue during excision and partly by growth. In cycloheximide-treated tissue, which
2,4,6-T had inhibited values at both synthesis and 2,4,6-trichlorophenoxyacetic acid (2,4-D)-treated in these experiments.

This of the auxin-like growth inhibition was associated with some auxin action rather than auxin action. In the initial experiments with 5 mM cycloheximide, this was true. The addition of 2,4-D as an impurity, since PCIB was found not to inhibit at concentrations less than 1 mM.

Auxin/Time Interactions. The inhibition of synthesis by NAA was completely reversed if the tissue was washed for 5 minutes with distilled water and replaced in culture medium lacking auxin. This suggested the possibility of determining whether any particular phase in the entire process was especially sensitive to inhibition. The time sequence (Fig. 5) may be readily divided into two phases, the first one in which the phytochrome concentration falls due to the destruction of the Pfr formed during the red light exposure, and the second in which rapid synthesis occurs between hours 6 and 12. Tissue samples were treated for 4 hours with 0.1 mM NAA at various times before and after the light exposure, and then the auxin was washed out. Figure 5 illustrates the results of an experiment of this type. The addition of NAA during the rapid phase of synthesis resulted in an abrupt reduction in rate, and simultaneously, 2,4-D was treated.

In early experiments naphthalene acetic acid (NAA) inhibited phytochrome synthesis without inhibiting growth (6). This unexpected result has been confirmed in additional experiments which make it clear that this effect is associated with auxin action rather than with some other activity of the NAA molecule. The inclusion of 0.1 mM IAA, 2,4-D indole acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D) or NAA in the standard medium in each case inhibited apparent synthesis by 75 to 80%, although growth (increase in fr wt) was promoted by these treatments (Table IV). Further evidence of the auxin-like nature of this inhibition is provided by the fact that similar concentrations of the antiauxins p-chlorophenoxyisobutyric acid (PCIB) and 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), while inhibiting growth, had little, if any, effect on apparent synthesis (Table IV).

Figure 4 shows that both NAA and 2,4-D are strongly inhibitory at 10 μM and that there is a significant decrease in 2,4-D-treated samples at 1 μM. In contrast, 2,4,6-T had little consistent effect: the difference in values at 100 and 10 μM may have been due to

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**Table IV. Effect of Auxins and Antiauxins on Appearance of New Phytochrome**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>New phytochrome</th>
<th>Change in wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.2 ± 3.1</td>
<td>175</td>
</tr>
<tr>
<td>IAA</td>
<td>12.0 ± 0.2</td>
<td>205</td>
</tr>
<tr>
<td>NAA</td>
<td>10.8 ± 1.4</td>
<td>203</td>
</tr>
<tr>
<td>2,4-D</td>
<td>13.3 ± 0.8</td>
<td>240</td>
</tr>
<tr>
<td>2,4,6-T</td>
<td>40.6 ± 2.1</td>
<td>131</td>
</tr>
<tr>
<td>PCIB</td>
<td>58.3 ± 5.6</td>
<td>100</td>
</tr>
</tbody>
</table>

2,4-D as an impurity, since PCIB was found not to inhibit at concentrations less than 1 mM.

Auxin/Time Interactions. The inhibition of synthesis by NAA was completely reversed if the tissue was washed for 5 minutes with distilled water and replaced in culture medium lacking auxin. This suggested the possibility of determining whether any particular phase in the entire process was especially sensitive to inhibition. The time sequence (fig 5) may be readily divided into 2 phases, the first one in which the phytochrome concentration falls due to the destruction of the Pfr formed during the red light exposure, and the second in which rapid synthesis occurs between hours 6 and 12. Tissue samples were treated for 4 hours with 0.1 mM NAA at various times before and after the light exposure, and then the auxin was washed out. Figure 5 illustrates the results of an experiment of this type. The addition of NAA during the rapid phase of synthesis resulted in an abrupt reduction in rate, and simultaneously, 2,4-D was treated.

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versibly susceptible
characteristic
which
what.

If the auxin was left in contact with the tissue for
the remainder of the experiment the rate of syn-
thesis was approximately the same as in samples
which had been incubated in NAA from the start
of the experiment. If, however, the NAA was
washed out from the tissue after 4 hours the rate
characteristic of the untreated control was re-
assumed, and the rate of growth decrease some-
what. Four hour interruptions by NAA before and
during phase 1 and during phase 2 produced results
essentially similar to those in figure 5. It would
seem, therefore, that there is no point in time at
which the process is either insensitive to, or irre-
versibly susceptible to, the inhibitory action of
auxin.

Differential Effects of Auxin on Younger and
Older Cells. The stem segments used in these ex-
periments consist of cells at different stages of
development. The more immature cells in the
apical part of the segment might be expected to be
more sensitive to high auxin levels than the more
basal cells (18). Thus it seemed possible that
auxin inhibition of apparent synthesis was a second-
ary consequence of growth inhibition. An exami-
nation of relative growth rates of the terminal and
basal parts of the segments showed that 0.1 mM
2,4-D did indeed inhibit the extension of the apical
portions and that increases in the weight of whole
segments were due to increased growth in the
basal portions. Table V shows that the apical part
of the segment increased its weight and length
more than the basal in those segments incubated
in buffered sucrose (control). The addition of
2,4-D reversed this situation, inhibiting the growth
of the apical portion markedly in comparison with
the control. The increased weight per unit length
brought about by 2,4-D was most evident in the
basal part of the segment. Phytochrome assays
showed much higher levels in the terminal portion
than in the basal. This point has been discussed in
the section on methods. It is important to note,
however, that although 2,4,6-T inhibited the growth
of the apical cells, as did 2,4-D, it did not inhibit
apparent phytochrome synthesis. Thus the auxin
effect on apparent synthesis is not a simple conse-
quence of the growth inhibition of the cells in the
apical hook.

Other Growth Regulators. A number of ex-
periments with other materials have thus far yielded
essentially negative results. Neither inhibition nor
consistent promotion of apparent synthesis was
found with various concentrations of kinetin, gib-
berrellic acid (GA₃) or 2-chloroethyltrimethylam-
monium chloride (CCC). Supplying δ-aminolevu-
linic acid to the tissue had no effect on the process,
although this rate-limiting precursor in tetrapyrrole

Fig. 5. Effect of time and length of treatment with
naphthalene acetic acid (NAA) on the course of ap-
parent synthesis in excised pea stem segments. Seg-
ments incubated at 26° in phosphate buffer plus 2% su-
crose. NAA washed from tissue by spraying seg-
ments with distilled water for 5 minutes.

Fig. 6. Effect of time and length of treatment with
naphthalene acetic acid (NAA) on increase in weight of
batches of pea stem segments which weighed 200 mg
initially.
and porphyrin synthesis has been found to greatly increase protoclorophyll synthesis in barley seedlings (9). One interesting result has been the partial inhibition of apparent synthesis by 0.3 mm cobaltous nitrate (at $3 \times 10^{-4}$ M) (table VI). No explanation for this effect can be suggested but it is interesting to note that cobaltous ion promotes photosensitive growth in pea stem sections and that it may have some interrelated functions with the phytochrome system (2).

**Effect of Tissue Age.** Between hours 12 and 24 the rate of apparent synthesis slowed down considerably (fig 5). Prolongation of the incubation beyond 24 hours, after again treating the

### Table V. Growth and Phytochrome in the Terminal and Basal Parts of the Standard Assay Segment

Segments cut from intact plants were exposed to red light 4 times. Initial length was 0.5 cm. The segments were marked with a spot of lanolin/carbon black midway between the apex of the hook and the base of the segment. The incubation period was 18 hours at 26°.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Δ Length/segment</th>
<th>Δ Wt/20 segments</th>
<th>Δ Phytochrome/20 segments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Terminal</td>
<td>Total</td>
</tr>
<tr>
<td>Control</td>
<td>1.13</td>
<td>1.67</td>
<td>2.80</td>
</tr>
<tr>
<td>0.1 mm 2,4-D</td>
<td>1.29</td>
<td>0.46</td>
<td>1.75</td>
</tr>
<tr>
<td>0.1 mm 2,4,6-T</td>
<td>0.58</td>
<td>0.17</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* Close to limits of detection.

### Table VI. Effect of Cobaltous Nitrate on Apparent Synthesis of Phytochrome

The segments were incubated for 24 hours at 26°.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>New phytochrome</th>
<th>Δ Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ (ΔOD) × 10^3/g</td>
<td>% Control</td>
</tr>
<tr>
<td>Control</td>
<td>56.5</td>
<td>...</td>
</tr>
<tr>
<td>30 μM Co(NO₃)₂</td>
<td>52.5</td>
<td>93%</td>
</tr>
<tr>
<td>300 μM Co(NO₃)₂</td>
<td>27.5</td>
<td>49%</td>
</tr>
</tbody>
</table>

### Table VII. Apparent Synthesis in Tissue Incubated for Longer Than 24 Hours

<table>
<thead>
<tr>
<th>Sample time</th>
<th>Total</th>
<th>Change</th>
<th>Phytochrome</th>
<th>Total</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Δ (Δ OD) × 10^3/g</td>
<td>mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) 0.5 cm Segments incubated in buffered sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>90</td>
<td>...</td>
<td>200</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>15 min red</td>
<td>50</td>
<td>-40</td>
<td>234</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>50</td>
<td>355</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>15 min red</td>
<td>41.5</td>
<td>-58.5</td>
<td>368</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>40.0</td>
<td>-1.5</td>
<td>391</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) Decapitated intact plants: terminal 0.5 cm sampled</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>90</td>
<td>...</td>
<td>200</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>15 min red</td>
<td>55</td>
<td>-35</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>69</td>
<td>-90</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>15 min red</td>
<td>87.5</td>
<td>18.5</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>94</td>
<td>...</td>
<td>200</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>15 min red</td>
<td>60</td>
<td>-34</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>150</td>
<td>90</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>165</td>
<td>15</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>170</td>
<td>5</td>
<td>159</td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>
tissue at hour 24 with red light, failed to produce any subsequent increase in phytochrome concentration and only slight increases in weight were recorded (table VIIa). In an alternative procedure, decapitated intact plants were subjected to the standard illumination schedule and incubation period, then some of the plants were illuminated again while others were maintained in darkness for a further 24 hours. Slight, and similar, increases in phytochrome concentration were observed during the second 24 hour period in both cases (table VIIb). These results are consistent with the view that the capacity for apparent synthesis is lost as the cell matures.

Conclusions

Granted that the increase in phytochrome concentration which is observed after extensive illumination is genuinely due to a greater amount of measurable phytochrome in the system (6), it is important to decide whether the increase is caused by de novo synthesis of all or part of the holochrome, or to some other process. No evidence has so far been advanced which would allow a firm decision to be made. The failure of apparent synthesis in the presence of cycloheximide and Actinomycin D does not necessarily indicate that the protein component of Pr must be synthesized de novo, but merely that metabolism is sufficiently disrupted by the presumed absence of messenger RNA and protein synthesis to bring the process to a halt. Similar uncertainty surrounds the interpretation of the failure of δ-aminolevulinic acid to promote synthesis. It would be incorrect to assume that no new chromophore synthesis takes place. If de novo synthesis does, indeed, occur in repeatedly illuminated, but not in dark grown tissue, it is necessary to propose that at some point before our observations begin synthesis has been switched off, and that the receipt of a red light signal followed by the destruction of a large proportion of the Pfr, is active in some way in switching synthesis back on. If de novo synthesis were switched off in the dark grown tissue when a critical concentration of phytochrome or some inhibitor was reached, phytochrome synthesis, like the synthesis of porphyrins, might be subject to negative feedback or end product inhibition. The regulation of chlorophyll and heme synthesis by such controls has been described by Burnham and Lascelles (4) and Granick and Urata (10): in these cases regulation was achieved by controlling either the activity or the concentration of the enzyme δ-aminolevulinic acid synthetase. Such a mechanism may well apply to other tetrapyrrole and porphyrin syntheses. A more specific mechanism would be required, however, to accommodate the observations on apparent synthesis since the difference between the phytochrome concentration at which synthesis is turned off in the dark and that at which it is switched on again after red light treatment is nearly an order of magnitude.

An alternative hypothesis for apparent synthesis takes account of the fact that the net production of Pr raises the phytochrome from 10% of that measurable in the nonilluminated control to a little over 20%. Thus 80% of the phytochrome which was present in the tissue before the experiments began is unaccounted for. This phytochrome has undergone Pfr destruction. While the nature of destruction is unknown it is perhaps misleading to think of the Pfr as being destroyed except in the sense that it no longer undergoes photoreversible absorbancy changes. Such a loss of photoreversibility might result if the protein were to be bound to another molecule or to a membrane, or if the holochrome were to dissociate into its chromophore and protein. Apparent synthesis might then result from the re-establishment of photoreversibility in this fraction coupled with its reversion to the red absorbing form. Since there is convincing, if circumstantial, evidence that there is already a mechanism in the cells of the pea stem which can produce the conformational changes necessary to convert Pfr to Pr (16) without light input (11), it seems possible that part of this mechanism may be involved in apparent synthesis. The temperature sensitivity of the process and its inhibition by Actinomycin D and cycloheximide show that apparent synthesis is subject to metabolic control; it is unlikely, therefore, that an earlier suggestion (6) that the process might simply represent the re-establishment of a physical equilibrium between 2 fractions of phytochrome, 1 being nonphotoreversible, is correct.

The control of the rate of apparent synthesis by auxin is not necessarily of physiological significance, although it is tempting to think that it is. The young cells in which apparent synthesis is observed are relatively rich in endogenous auxin, as their growth inhibition by applied auxin indicates. The fate of auxin in the tissue grown in darkness subsequent to its exposure to light is not known, but there may be some ground for thinking that it is reduced (8,12,17). This leads to the sheer speculation that the critical level at which phytochrome synthesis, either de novo or apparent, restarts is determined by a decrease in the endogenous auxin to a level which permits synthesis to occur.

Whatever the precise mechanism of apparent synthesis it can be predicted that growing portions of the pea stem under continuous illumination will reach a point at which an equilibrium is established between Pfr destruction and the reappearance of Pr. This equilibrium has been observed and will be the subject of another paper. One of the functions of apparent synthesis may be, therefore, the maintenance of a working concentration of photosensitive receptor in those cells of the nonnetiolated plant which have yet to differentiate and mature.
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Literature Cited


