Distribution of Phytochrome in Etiolated Seedlings 1, 2

W. R. Briggs and H. W. Siegelman

Department of Biological Sciences, Stanford University, Stanford, California, and United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland

The plant pigment phytochrome regulates a large number of physiological processes in higher plants (14). The pigment is most readily detected in dark-grown seedlings both because it is normally present in higher concentrations than in light-grown seedlings (8, 11), and because dark-grown seedlings contain only small amounts of other pigments absorbing light in the same spectral regions as phytochrome. The pigment is present in dark-grown seedlings in the red-absorbing form (P_r) (8, 11), which is normally stable. Following transformation to the far red-absorbing form (P_r) by red light, there is a gradual loss of detectable pigment reversibility, suggesting that P_r is labile (8, 11). The disappearance of the labile P_r is apparently enzymatic (7) and is prevented by temperatures near 0°.

A study of phytochrome distribution in seedlings is useful for several reasons. First, it allows a correlative study with respect to the distribution of growth and of various growth substances, e.g. auxin. Second, it allows comparison of pigment distribution with the distribution of light sensitivity for a given physiological process, providing a potential source of information both on the light-sensitive process and on the action of phytochrome itself. Third, it provides information on good potential sources of material both for in vivo studies of phytochrome and for pigment purification. The present paper is concerned with the distribution of phytochrome in the etiolated seedlings of 6 species fairly widely used in plant physiological investigations.

Materials and Methods

Oats (Avena sativa L., cv. Victory, Glenn, Garry, and Bamlu), barley (Hordeum vulgare L., cv. Traill), and corn (Zea mays L., cv. USDA 13) were all germinated and grown in 10 × 16 × 25 cm plastic boxes on water-saturated pads of cellulose packing material (Kimpak X-133, type 2011). Except for 1 experiment with oats, the seeds were not soaked prior to placing them in the boxes, nor were the oat or barley seeds husked. Approximately 50 ml of dry oat seed, 100 ml of barley, or about 50 individual corn seeds were grown in each box. Heavier planting in each case led to reduced germination. The boxes were covered, wrapped in a double layer of black cloth, and placed in a constant temperature dark room (25 ± 0.5°) until used. Immediately before sample preparation, the boxes were placed in a cold room (2°). Only after an hour of chilling were the seedlings exposed to room light for sample preparation.

Sunflower (Helianthus annuus L., cv. Mammoth Russian), beans (Phaseolus vulgaris L., cv. Red Kidney), and peas (Pisum sativum, cv. Alaska) were all grown in flats of washed sand. The peas only were presoaked for 4 hours before planting. The peas and beans were watered daily with dilute Hoagland's solution, the sunflowers with tap water. The flats were kept in total darkness except for brief daily exposures to dim green light during watering. The growing temperature was again 25 ± 0.5°. With these 3 species, samples were prepared under dim green light in the growth room, placed in petri dishes, and transferred to the cold room wrapped in black cloth.

The various regions of the plant being assayed on a given day were excised, like parts pooled, and standard weights of tissue obtained. These pooled samples were then chopped into pieces 1 to 2 mm in size and packed evenly in a cell with a 1 cm² cross-sectional area and a clear plastic bottom. All samples were stored in small beakers on ice until immediately before the chopping operation.

The samples were assayed for phytochrome with an instrument designed to measure the difference in OD between 660 mμ and 730 mμ in an optically dense and light-scattering system (Ratiome Oda Model R-2, Agricultural Specialty Company, Hyattsville, Maryland). The cell was maintained at 0° by an ice jacket, and the sample irradiated with between 3 and 5 minutes of actinic red light, sufficient to transform the phytochrome as far as possible into the far red-absorbing form, and to transform most of the photochlorophyll present to chlorophyll (the latter reaction would otherwise interfere with phytochrome reversibility measurements). The difference in the OD of the sample between 660 and 730 mμ (Δ OD) was recorded when actinic red light induced no further change. Actinic far red light, sufficient to drive the phytochrome as far as possible into the red-absorbing form was used next, and the Δ OD value again recorded. As the latter treatment transforms P_r into P_r, there is a decrease in the OD at 730 mμ and a corresponding increase at 660 mμ, and the Δ OD is changed. The amount of change [Δ(Δ OD)] is

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a measure of the relative amount of photoreversible phytochrome in the tissue sample. In practice, the samples were alternately exposed first to red and then to far red light at least 3 times. The 3 values of \( \Delta (\Delta \text{OD}) \) obtained following far red irradiation were then averaged, and it this average which is shown in the tables and graphs below. Though in most cases these values were just the same as those obtained in the reverse direction with actinic red light, occasionally the initial red treatment failed to transform all of the protochlorophyll present, and the red-induced changes in \( \Delta \text{OD} \) were therefore less reliable measures of phytochrome reversibility.

After a sample had been assayed for phytochrome, it was immediately ground in a mortar with 0.1 m potassium phosphate buffer, pH 7.5, for a final volume of 2.5 ml. To this slurry was added 2.5 ml of 10% trichloroacetic acid. After thorough mixing, the preparation was centrifuged at 12,500 \( \times g \), the supernatant decanted, and the pellet resuspended and centrifuged 3 times in 95% ethyl alcohol to remove various pigments, primarily carotenoids. After the final alcohol rinse, the pellet was colorless. It was then made up to 1 ml with distilled water, and 4 ml of biuret reagent (13) were added. After 30 minutes, immediately following centrifugation to clarify the solution, the OD at 560 nm was measured.

Under these conditions, a value of \( E_{1 \text{cm}} = 0.0465 \) measured with crystalline bovine serum albumin, was used to determine protein concentration.

**Results**

Influence of Sample Size on Apparent Phytochrome Reversibility. In an optically dense and light-scattering system, the magnitude of an absorption band for a fixed amount of pigment is a function of the average path length of light through the sample. Factors influencing this average path length include the size, number, and reflectance of the scattering particles, together with the degree of association and orientation of the pigment molecules with respect to the particles. Butler (6) has presented a detailed treatment of the absorption of light by pigments in turbid materials.

In measuring phytochrome absorbency changes, one takes advantage of the scattering properties of the samples to amplify the absorption bands of very small amounts of the pigment by increasing optical path length. Since the size, number, or reflectance properties of the light-scattering elements in the tissue is unknown (and since these elements are hardly homogeneous), absolute determinations of phytochrome content based solely on red- or far-red induced absorption changes in intact tissue cannot be made. Furthermore, sample thickness plays an important role in absorption-band amplification. If the thickness of a light-scattering sample is doubled, the average optical path length is more than doubled, since light is not only scattered additionally within the added sample material, but also back and forth between the original and the added material. Doubling sample thickness may triple or quadruple the optical path length, and thus triple or quadruple the apparent absorption of a pigment contained within the sample.

In the present study, therefore, it was important to measure the influence of sample size on apparent phytochrome photoreversibility in the tissue, both to determine how accurately sample size could be controlled for reproducibility, and to determine if possible a conversion factor for equating measurements made on samples of different sizes. Seven-day-old dark-grown barley shoots 6 to 7 cm in length were excised. The apical 5 cm and the basal 1 to 2 cm were assayed separately. No attempt was made to separate coleoptiles from primary leaves. Phytochrome measurements were made on samples weighing from 0.13 to 0.60 g, and the apparent phytochrome reversibility per g fresh weight of tissue calculated for each sample. The results with the apical 5 cm are shown in figure 1. If doubling sample thickness (by doubling sample weight) merely doubled the average optical path length, the curve shown should be a horizontal line. Clearly, however, doubling sample thickness must almost quadruple the average optical path length (cf. 0.2 and 0.4 g samples) since the apparent \( \Delta (\Delta \text{OD})/g \) is doubled. Almost identical results were obtained with 0.2 and 0.4 g samples of the basal regions, although the actual activity values were almost twice as high as those shown in figure 1.

In all of the following experiments, samples of identical weight (\( \pm 10 \) mg) were used where possible, for direct comparison of phytochrome measurements. When samples of identical weight could not be obtained, a tissue available in quantity was assayed at each of the various weights used, the ap-
parent phytochrome reversibility per g of tissue for each weight was calculated, and the amplification factor between light and heavy samples was determined. In this way, all results could be expressed on the basis of a single sample size, though actual measurements may have been made on samples of several different weights.

The above discussion assumes that the scattering properties of each different tissue type are identical (although unknown). This assumption is probably not entirely valid, since particle size, number, and reflectance may vary considerably between different tissues, coleoptiles and primary leaves, for instance. Nevertheless, comparisons between different regions of coleoptiles, or between different regions of primary leaves, are probably valid, and large differences between different tissue types are undoubtedly reliable.

Changes in Measurable Phytochrome Photo-reversibility with Age in Barley Seedlings. In 4 separate experiments, shoots of dark-grown barley seedlings 5, 6, 7, or 8 days old were assayed for phytochrome. In each experiment, shoots were sorted according to length. Thus, with 5-day-old seedlings, 4 size classes were obtained: 0.5 to 1.5, 1.5 to 2.5, 2.5 to 3.5, and 3.5 to 4.5 cm from seed to coleoptile apex. For the very oldest seedlings, maximum shoot length was 9 cm. All sample weights were 0.55 g. The number of shoots used in each sample was recorded for calculation of phytochrome reversibility per seedling as well as per g fresh weight. The best correlation was found between phytochrome reversibility and shoot length, rather than chronological age, as shown in figures 2 and 3. The different types of symbols represent shoots of different ages. Four cm shoots, for instance, yield the same phytochrome reversibility whether they are 5, 6 or 7 days old.

Total measurable phytochrome, expressed on a per g fresh weight basis, declines almost linearly until the shoots are approximately 5 cm long, and then begins to level off (fig 2). However, activity per seedling (fig 3) is simultaneously increasing, almost tripling as the shoots grow from 1 to 5 cm in length. As will be shown below, the activity in older shoots is quite localized.

Distribution of Phytochrome in Barley Shoots. The shoots of 6-day-old dark-grown barley seedlings were harvested, the coleoptiles split longitudinally along 1 side, and the primary leaves removed. Plants were selected in which both primary leaves and coleoptiles were 7 ± 0.5 cm long. The various sections shown in table I were excised and pooled for phytochrome and protein assay. Leaf samples weighed 0.19 g, and coleoptile samples 0.3 g. One coleoptile sample was measured for phytochrome at both weights, and the conversion factor for expressing all results on the basis of the heavier samples obtained.

The results from 1 of 2 similar experiments appear in figure 4. Phytochrome activity per g is highest in both coleoptile and primary leaf apices, with some further localization at the basal ends of both organs. In a comparable experiment using 7-

![Fig. 2. Phytochrome content of dark-grown barley shoots of various lengths and ages, expressed per g fresh weight.](image)

![Fig. 3. Phytochrome content of dark-grown barley shoots of various lengths and ages, expressed per seedling.](image)

Table I. Ratios of Measured Phytochrome Absorptivity Reversibility

<table>
<thead>
<tr>
<th>Region assayed</th>
<th>Coleoptile</th>
<th>Primary leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical cm</td>
<td>15.9</td>
<td>6.3</td>
</tr>
<tr>
<td>2nd cm</td>
<td>9.3</td>
<td>5.5</td>
</tr>
<tr>
<td>3rd cm</td>
<td>8.5</td>
<td>3.4</td>
</tr>
<tr>
<td>4th cm</td>
<td>7.1</td>
<td>3.1</td>
</tr>
<tr>
<td>5th cm</td>
<td>6.0</td>
<td>2.2</td>
</tr>
<tr>
<td>6th cm</td>
<td>6.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Basal 0.5-1.5 cm</td>
<td>6.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

All values have been multiplied by 10³.
Fig. 4. Distribution of phytochrome in dark-grown barley shoots per g fresh weight.

Instead of 6-day-old seedlings, this localization at apical and basal ends was more pronounced, the phytochrome level detected in the leaf bases being higher than in any other portion of the shoot.

Phytochrome measurements on a protein basis for the various regions of leaf and coleoptile are shown in Table I. The ratio is highest in general in those regions of leaf or coleoptile which possess the highest phytochrome activity. Basally, phytochrome levels decline more rapidly than total protein. However, the ratio is between 2 and 3 times as high for a given section of coleoptile as it is for a comparable section of primary leaf, probably partially a function of the far larger number of proplastids in the leaves than in the coleoptiles. Proplastids would be expected to contribute substantially to the total protein in the leaves.

Distribution of Phytochrome in Corn Shoots. The shoots of 6-day-old dark-grown corn seedlings were excised within 1 mm of the seed. Primary leaves were separated from the coleoptiles as described above for barley, and leaves, coleoptiles, nodes, and mesocotyls were separated and pooled. The various organs were subdivided for phytochrome and protein assay as shown in Table II. For phytochrome assay, primary leaf samples weighed 0.20 g. All other samples weighed 0.40 g, with the exception of one mesocotyl sample assayed at both weights to obtain the necessary conversion factor. The results (Fig 5) are based on samples of 0.20 g. One of 2 similar experiments is shown.

From the node upwards, the phytochrome distribution pattern is much like that found in barley, with highest phytochrome levels appearing at the apical and basal ends of the coleoptiles and primary leaves. There is high reversibility found in the nodal regions, and only very low levels of phytochrome in the mesocotyls. The general level of activity is somewhat lower than that found in barley; and unlike barley, the coleoptiles and primary leaves of corn show approximately the same activity. The basal portion of the mesocotyls showed pronounced development of root primordia in about half of the plants; but comparison of samples made exclusively with rooting sections with samples made from sections with no visible roots revealed no measurable difference between the 2. The general pattern of phytochrome distribution found resembles closely that obtained by Birth (2) for corn seedlings.

The ratios of phytochrome to protein for various regions of various organs of corn are shown in Table II. As in barley, the ratios in corn coleoptiles and leaves is highest in regions with the greatest amounts of phytochrome, and are generally higher in coleoptiles than in leaves. Nodal regions and mesocotyls have ratios intermediate between those for leaves and those for coleoptiles.

Distribution of Phytochrome in Oat Shoots. The shoots of 5-day-old oat seedlings were excised from the seeds. Coleoptiles, primary leaves, nodes, and

Table II. Ratios of Measured Phytochrome Absorbancy Reversibility

<table>
<thead>
<tr>
<th>Region assayed</th>
<th>Coleoptile</th>
<th>Region assayed</th>
<th>Primary leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1.5 cm</td>
<td>6.3</td>
<td>Apical 1.5 cm</td>
<td>1.9</td>
</tr>
<tr>
<td>Upper central</td>
<td>4.4</td>
<td>Upper central</td>
<td>1.2</td>
</tr>
<tr>
<td>0.5-1.5 cm</td>
<td>4.4</td>
<td>0.2-1.0 cm</td>
<td>0.8</td>
</tr>
<tr>
<td>Lower central</td>
<td>4.6</td>
<td>Lower central</td>
<td>0.7</td>
</tr>
<tr>
<td>Basal 1.5 cm</td>
<td>4.6</td>
<td>Basal 1.5 cm</td>
<td>0.7</td>
</tr>
<tr>
<td>Mesocotyl</td>
<td>2.6</td>
<td>Region of node</td>
<td>2.2</td>
</tr>
<tr>
<td>Apical 3-3.5 cm</td>
<td>2.1</td>
<td>0.5 cm</td>
<td></td>
</tr>
<tr>
<td>2nd 3-3.5 cm</td>
<td>2.1</td>
<td>0.5 cm</td>
<td></td>
</tr>
<tr>
<td>3rd 3-3.5 cm</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cm</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Distribution of phytochrome in dark-grown corn shoots per g fresh weight.

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mesocotyls were separated for phytochrome and protein assay as before. Sizes of the various organs and the various regions of each organ assayed are shown in table III. Mesocotyl samples weighed 0.40 g, all other samples 0.20 g. The results of 1 of 2 similar experiments are shown in figure 6. They are all expressed on the basis of 0.20 g samples.

Apical from the nodal region, *Avena* differs somewhat from corn and from barley. The coleoptile apex shows by far the greatest amount of phytochrome, and the coleoptile base contains less phytochrome than the central regions. Furthermore, the general level of phytochrome in the coleoptile is far higher than that in the primary leaf. Like corn, however, the oat mesocotyl contains only a very low level of detectable phytochrome.

The ratios of phytochrome to protein for *Avena* are shown in table III. The familiar pattern of decline in the ratio with decline in phytochrome is again evident. The considerably higher ratios in coleoptiles than in any other organs or regions are also apparent.

It has been shown (8,11) that treatment of truly etiolated seedlings of corn with red light causes a drastic decrease in the total amount of reversible phytochrome measurable during the first few hours after the light treatment. Since in the normal use of oats for the *Avena* coleoptile curvature test (20) light is used shortly after the seeds have been soaked, to suppress mesocotyl growth, it is of considerable interest to know what this early light treatment does to the level of phytochrome found in the seedlings at the age at which they are normally used for the auxin bioassay (usually about 72 hr after soaking).

Two lots of oat seeds were hulled. One lot was soaked for 2 hours in total darkness and then set out under dim green light on moist Kimpak for an additional 70 hours of growth in the dark. The second lot was soaked in the light, set on Kimpak, and then given 4 hours of fluorescent light for the twelfth through sixteenth hour after the beginning of soaking, with the remaining growth in the dark. The dark-grown plants had mesocotyls approximately 3 cm in length and coleoptiles 2.4 cm in length at age 72 hours. The light-treated plants had mesocotyls 0.3 cm or less in length, and coleoptiles about 3 cm in length. Thus, the light treatment did suppress the mesocotyls, and at the same time slightly enhanced coleoptile growth.

The phytochrome levels plus phytochrome-protein ratios for the various regions assayed from each lot of plants are shown in table IV. In both lots of plants, the general distribution of phytochrome is quite similar to that shown for 5-day-old seedlings (table III, fig 6). However, the light treatment for mesocotyl suppression caused an approximately 25% reduction in phytochrome in all regions assayed with the exception of the mesocotyls. With the latter, the phytochrome level was considerably higher in suppressed than in nonsuppressed plants. This result is not surprising, since the suppressed mesocotyls consist of cells that have failed to elongate, and hence lack the very large vacuoles of the nonsuppressed tissue. The protein has simply not been diluted by extensive vacuolation which normally accompanies cell elongation. Even so, the ratio of phytochrome to protein is lower in light-treated than in nonlight-treated mesocotyls.

**Distribution of Phytochrome in Pea Seedlings.** Dark-grown 5-day-old pea seedlings approximately 10 cm tall from cotyledon to shoot apex were subdivided as shown in table V and figure 7 for phytochrome and protein measurements. All samples weighed 0.30 g. While the apical node and bud showed the highest level of phytochrome per g fresh weight of tissue of any region of any plant investigated, the decline in measurable phytochrome basally was also the most dramatic found (fig 7). In general, the distribution pattern agrees well with that found by Furuya and Hillman (12) for 7-day-old
Table IV. Distribution of Phytochrome and Ratios of Measured Phytochrome Absorbency Reversibility

(Δ (Δ OD)/g fr wt) to protein (mg/g fr wt) for various regions of 72-hr-old dark-grown oat shoots with or without light-induced mesocotyl suppression. Ratio values have been multiplied by 10^3.

<table>
<thead>
<tr>
<th>Region assayed</th>
<th>Phytochrome reversibility</th>
<th>Ratio, Δ (Δ OD)/g</th>
<th>phytochrome/protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf, total, 2.4 cm</td>
<td>0.023</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Coleoptile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical 0.8 cm</td>
<td>0.206</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Central 0.8 cm</td>
<td>0.110</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>Basal 0.8 cm</td>
<td>0.069</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Node, 0.3 cm</td>
<td>0.057</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Mesocotyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical 1.5 cm</td>
<td>0.026</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Basal 1.5 cm</td>
<td>0.024</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Mesocotyls suppressed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf, total, 3.0 cm</td>
<td>0.018</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Coleoptile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical 1.0 cm</td>
<td>0.154</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Central 1.0 cm</td>
<td>0.079</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Basal 1.0 cm</td>
<td>0.054</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Node, 0.3 cm</td>
<td>0.039</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Mesocotyl, total, 0.3 cm</td>
<td>0.032</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

Table V. Ratios of Measured Phytochrome Absorbency Reversibility

(Δ (Δ OD)/g fr wt) to protein (mg/g fr wt) for various regions of 5-day-old dark-grown Alaska pea seedlings. All values have been multiplied by 10^3.

<table>
<thead>
<tr>
<th>Region assayed</th>
<th>Region assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bud plus 0.1-0.2 cm</td>
<td>Cotyledonary node 0.3 cm</td>
</tr>
<tr>
<td>stem</td>
<td>3.5</td>
</tr>
<tr>
<td>First node plus</td>
<td>6.2</td>
</tr>
<tr>
<td>scale leaf, 0.5 cm</td>
<td>Apical 0.5 cm 5.5</td>
</tr>
<tr>
<td>First Internode</td>
<td>Remaining 5-6 cm plus laterals 4.2</td>
</tr>
<tr>
<td>Apical 3 cm</td>
<td>4.2</td>
</tr>
<tr>
<td>Central 3 cm</td>
<td>3.9</td>
</tr>
<tr>
<td>Basal 3 cm</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table VI. Ratios of Measured Phytochrome Absorbency Reversibility

(Δ (Δ OD)/g fr wt) to protein (mg/g fr wt) for various regions of 5-day-old dark-grown bean seedlings. All values have been multiplied by 10^3.

<table>
<thead>
<tr>
<th>Region assayed</th>
<th>Region assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary leaf</td>
<td>1.1</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>3.8</td>
</tr>
<tr>
<td>Bud, epicotyl, cotyledonary node</td>
<td>1.5</td>
</tr>
<tr>
<td>Hook, apical half</td>
<td>1.9</td>
</tr>
<tr>
<td>Hook, basal half</td>
<td>1.8</td>
</tr>
<tr>
<td>Lower central 3 cm</td>
<td>1.7</td>
</tr>
<tr>
<td>Basal 2 cm</td>
<td>2.3</td>
</tr>
<tr>
<td>Upper central 3 cm</td>
<td>2.9</td>
</tr>
</tbody>
</table>

pea seedlings. The usual decline in the phytochrome-protein ratio, following the decline in phytochrome, apex to base of the shoot, is shown in Table V. The ratio does not increase again, however, from the cotyledonary node to the root apex, although the general phytochrome level does.

Distribution of Phytochrome in Bean Seedlings. Dark-grown 6-day-old bean seedlings approximately 10 cm tall from the base of the hypocotyl to the shoot apex were subdivided as shown in Table VI and Figure 8 for phytochrome and protein measurements. All samples weighed 0.30 g. In general, the dis-
tribution of phytochrome in bean seedlings was similar to that found in peas (fig. 8). The general level of phytochrome, however, was considerably lower, almost the lowest found. The phytochrome-protein ratios (table VI) were also among the lowest found. However, reasonably high phytochrome content was found throughout the hook region of the hypocotyl, and the decline in phytochrome content basal from the apex was less dramatic than in pea seedlings.

Distribution of Phytochrome in Helianthus Seedlings. Dark-grown 4-day-old Helianthus seedlings approximately 4 cm in length from apex to hypocotyl base were subdivided for phytochrome and protein assay as shown in table VII. Once again, all samples weighed 0.30 g. Extensive browning of tissue fragments even at 0° made measurements of protein content unreliable, a problem that had not arisen with any other plant tested. Therefore, table VII shows only the phytochrome measurements, obtained from a pair of experiments. The cut surface browning did not seem to affect the phytochrome measurements. Samples which had been cut and allowed to brown for as much as 3 hours gave the same phytochrome measurements as those that were assayed immediately after cutting. A decline in the level of phytochrome is once again obvious from the apical bud to the base of the hypocotyl.

Discussion

There are several clear features which emerge from the phytochrome survey presented above. First, the greatest amount of phytochrome in a given seedling is invariably found either in meristematic regions or in regions which were recently meristematic. Thus, coleoptile tips, leaf tips, and coleoptilar nodes (the latter including the primary leaf base and the shoot apex) contain the highest levels of phytochrome found in the 3 grass seedlings investigated. Mesocotyls, however, despite their known sensitivity to red light, contain unusually low levels of detectable pigment reversibility. Similarly, the apical regions of the 3 dicots have higher phytochrome levels than the elongating and maturing regions.

Second, in regions of high phytochrome concentration, the phytochrome-protein ratio is almost always considerably greater than in regions of low pigment concentration. Preliminary measurements had failed to show this change in the phytochrome-protein ratio (5), but the more recent replicated experiments shown above always revealed it. It seems possible that the regions with a high ratio are those which are most actively synthesizing phytochrome, though further experiments are needed to test this hypothesis.

Phytochrome-protein ratios for the most active regions of 5 of the 6 plants investigated are shown in figure 9. The ratios are highest for barley and oats, intermediate for peas, and lowest of all for corn and beans. In terms of suitability as a source for phytochrome, barley and oats would appear to be the best. Though the highest phytochrome levels detected were actually in peas, these levels were confined to the most apical region of the shoot, in tissue containing a great deal of other protein and pigment. Oats are more suitable than barley for the practical reason that they may be harvested by cutting them off in the mesocotyl region, and all tissue with high phytochrome concentration is thus obtained. With barley, the lack of an elongated mesocotyl means that harvesting shoots just above the seed leaves behind both the leaf and coleoptile bases, each of which is fairly rich in phytochrome.

The very high level of phytochrome found in coleoptile apices is of considerable physiological interest. The coleoptile apex is the primary source of auxin in both corn (3) and oat (19) seedlings. It is also the region of maximum phototropic sensitivity (16, 18). Furthermore, it is very sensitive to red light. The phototropic sensitivity of coleoptiles to

![Fig. 9. Phytochrome-protein ratios (see table 1 for units) for the regions of various seedlings with the highest phytochrome content.](https://example.com/phytochrome-protein-ratios.png)
blue light is known to be strongly altered by pre-
treatment with red light (10,21), itself phototropi-
tically inactive. The phenomenon shows the char-
acteristic features of a phytochrome-mediated re-
response, and is far red reversible (4,9). Chon (9)
has found that with corn coleoptiles, the extreme apex
is the most sensitive region for red light induction
of phototropic sensitivity change. She has also found
that red light sensitivity in this system roughly par-
relates the distribution of phytochrome in the shoot.

In a paper some years ago, Araki and Hamada
(1) noted that the nodal region was the most sensi-
tive part of the oat seedling for light-induced sup-
pression of mesocotyl elongation (they evidently used
an incandescent spot lamp without filter). It is thus
not surprising to find a high level of phytochrome in
this region. The fairly high level of phytochrome and
the high phytochrome-protein ratios found in the
bean hook correlate well with the well-known sen-
sitivity of bean hooks to red light. However, the
bean hook response is in many ways anomalous (15).
and is not clearly phytochrome-mediated, at least in
the context of our present understanding of phyto-
chrome behavior in vivo. In general, there is ex-
cellent correlation between the distribution of phyto-
chrome and the distribution of red light sensitivity,
at least in the few cases in which such localization has
been attempted. A causal relationship between high
phytochrome content and high light sensitivity is yet
to be established, however.

Finally, in at least the 3 cases studied, the dis-
tribution of phytochrome roughly parallels the dis-
tribution of auxin. In oat coleoptiles (19), corn
coleoptiles (3), and etiolated pea epicotyls (17), the
greatest amounts of auxin are found in the apical
regions, declining steeply basipetally.

Summary

The distribution of phytochrome was determined
by in vivo spectroscopy in dark-grown barley, corn,
oat, pea, bean, and sunflower seedlings. In general,
the highest concentrations of phytochrome were found
in tissues which were either meristematic or which
had recently been meristematic. Regions with the
highest levels of detectable phytochrome also had the
highest phytochrome-protein ratios, suggesting that
these regions might be active sites of phytochrome
synthesis. The distribution of phytochrome was com-
pared with that of other physiological systems, and
found to parallel auxin distribution, phototropic sen-
sitivity, and red light sensitivity, at least roughly.
The suitability of the various species as sources of ex-
tractable phytochrome is discussed.

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