Physiology of Movements in the Stems of Seedling *Pisum sativum* L. cv Alaska[1]

III. PHOTOTROPISM IN RELATION TO GRAVITROPISM, NUTATION, AND GROWTH

STEVEN J. BRITZ AND ARTHUR W. GALSTON
Light and Plant Growth Laboratory, Beltsville Agricultural Research Center, United States Department of Agriculture, Beltsville, Maryland 20705 (S. J. B.); and Biology Department, Kline Biology Tower, Yale University, New Haven, Connecticut 06511 (A. W. G.)

ABSTRACT

Phototropic response in etiolated pea (*Pisum sativum* L. cv Alaska) seedlings is poor. However, the curvature induced by unilateral blue light can be hastened and increased in magnitude by a previously administered red light pulse followed by several hours of darkness. Phytochrome is involved in the red light effect. Phototropic response was almost completely inhibited by removal of the apical bud and hook, but it was restored if exogenous indole-3-acetic acid was applied apically to the cut stump. Therefore, the stem contains both the phototropic photoreceptor and response mechanism. Perception of gravity and gravitropic response were also localized in the stem, but gravitropism was scarcely inhibited by decapitation. It was also observed that the kinetics and curvature pattern of gravitropism differed greatly from those of phototropism. Like phototropism, stem nutation required auxin and was promoted by red light. Unlike phototropism, photoenhanced nutational curvature required the apical hook and was propagated as a wave down the stem. Naphthylphthalamic acid inhibited, in order of decreasing effect, nutation, phototropism/gravitropism, and growth. Phototropism, gravitropism, and nutation appear to represent distinct forms of stem movement with fundamental differences in the mechanisms of curvature development.

The modification of tropistic response by light (*e.g.* Refs. 8 and 31) is a useful approach to the study of primary processes of stimulus perception and transduction in plants (*e.g.* Ref. 2). Of course, light effects may also involve changes in secondary processes related to growth regulation (9, 30). In dark-grown Alaska pea seedlings, red light was reported to enhance gravitropism and phototropism (19, 20, 23). While an action of light on growth regulatory steps was indicated for both processes, the mechanisms differed. Light-promoted gravitropism was thought to involve a photoinhibition of ethylene release (19), whereas light-promoted phototropism was suggested to involve an increase in tissue sensitivity to auxin (20).

We have recently found, based on presentation time studies, that a prior red light treatment increased graviperception in pea seedlings (2). Contrary to the earlier report (19), we were unable to find any promotive effect of light pretreatment on the rate or magnitude of gravitropic response under continuous gravistimulation (2, 3). However, we used intact plants, whereas the previous authors used excised stem segments in which light-promoted gravitropism probably represented the alleviation of wound inhibition by photo-induced suppression of ethylene release.

The dramatic enhancement of phototropism by red light does take place in intact pea plants under conditions in which the kinetics and magnitude of gravitropism are not greatly altered. It would therefore seem there must be basic differences in the way light effects the two tropistic responses. Phototropism and gravitropism are often assumed to share the same secondary (*i.e.* growth regulatory) processes (11, 25), so an initial hypothesis would be that red light modifies early steps in the phototropic reaction sequence. We have tested this hypothesis through a more detailed comparison of phototropism and gravitropism.

Pea stems also have oscillatory bending movements (nutations) which are strongly stimulated by red light (3, 4, 14). Darwin and Darwin (7) contended that tropisms were asymmetrically modified nutations. Although we have found that gravitropism and nutation in pea stems are relatively independent of one another (3), the possible relation between phototropism and nutation remains to be explored.

MATERIALS AND METHODS

Pea (*Pisum sativum* L. cv Alaska) seedlings (Asgrow Co.[2]) were raised and treated individually in glass vials in the dark at 27°C and 80% RH as described in detail elsewhere (3). Red light treatments were given 6 d after the start of imbibition, during which time the third internode was elongating. Either of two red light sources were used: (a) four 15-w fluorescent lamps (Sylvania F15T8/R) wrapped in two layers of red cellophane (DuPont); or (b) five 20-w fluorescent lamps (Sylvania F20T12/GRO) wrapped in two layers of red filter (Roscolux 1019; Rosco Co., Port Chester, NY). Far red light was obtained from six 20-w fluorescent lamps (Sylvania F20T12/232) wrapped in two layers of Roscolux 1019 and one layer of Roscolux 1076. Depending on light treatment, plants are referred to as DP[3] or RP$_x$ (or RP when x is between 16 and 24 h). All plants were exposed to dim green light during watering (4 d after imbibition), experimental light treatment, and set-up. Green light was obtained from fluorescent lamps (Sylvania F15T8/G) wrapped in four layers of green celluloid (two layers of Roscolene 874 and two layers of Roscolene 877). The light source

[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[3] Abbreviations: DP, dark-grown plants; RP$_x$, plants irradiated with red light x hours before the experiment; NPA, naphthylphthalamic acid.

for phototropism consisted of either a 20-w or 40-w blue light-emitting fluorescent lamp (Sylvania F20T12/B or F40T12/B) filtered through one layer of blue light-transmitting acrylic (Rohm and Haas 2424). This source emits less than 0.1% of its power as far red and IR light. Fluence rates were altered by changing slits or distances and were measured with a radiometer (YSI model 65).

Phototropism, gravitropism, and hook angle were measured from shadowgraphs (see Refs. 4 and 14). Growth and nutation were measured from time-lapse photographs as described elsewhere (3, 14). The pea seedling has bilateral symmetry with the plane of symmetry (the ∥ -plane) bisecting the apical hook (3). Nutational movements are most pronounced in this plane and consequently interfere with measurements of tropistic response. These were therefore induced orthogonally to the ∥ -plane by stimulation of the seedlings with gravity or with unilateral light directed at right angles to the plane of the hook. Gravitropic induction was continuous in all cases and started with the seedlings tilted horizontally. Gravitropism, nutation, and growth were measured under growth conditions. Phototropism was conducted in a separate dark room not regulated for temperature or humidity.

Techniques for the removal of the apical bud and plumule or the hook and for the treatment of the cut stems with lanolin (Fisher) ± IAA (Sigma) are described elsewhere (4). In some experiments, intact plants were daubed unilaterally with approximately 5 mg of lanolin applied with a syringe. NPA was obtained as the sodium salt (ALNAP, Uniroyal) and prepared as an aqueous solution with 0.1% Tween-20 (Sigma) as a surfactant (see Ref. 3).

RESULTS

The kinetics of phototropic response in peas have not been described previously, so the initial approach was to record the time course of curvature in the third internode of seedlings exposed continuously to unilateral blue light (75 mw m^{-2}). DP reacted very poorly and required a lag time of about 2 h between the onset of illumination and the initiation of response (Fig. 1). Even after 4 to 5 h of light, curvature in DP was only about 20° (data not shown). Longer irradiations (about 10 h) resulted in increased
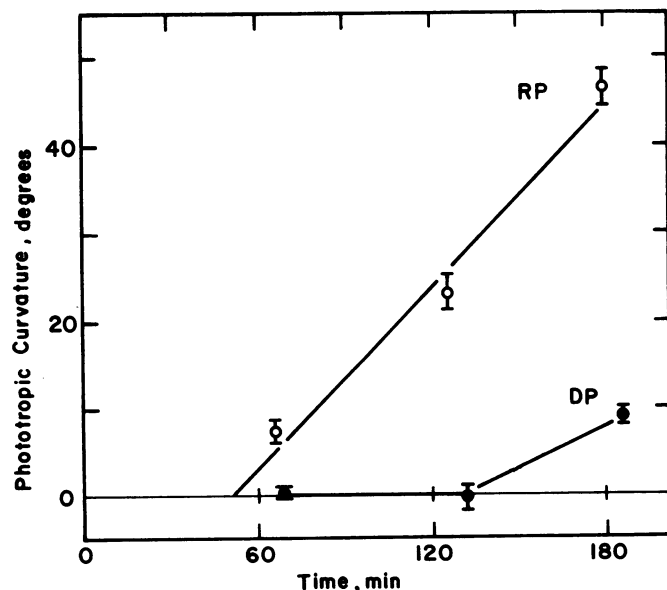

FIG. 1. Kinetics of phototropic response of DP (●) and RP$_{15}$ (O). The red light treatment was for 4 min (760 J m^{-2}). The abscissa indicates the time span of blue light exposure (75 mw m^{-2}). The vertical bars indicate 1 SE.

response, but this long-term effect could be supplanted by a brief red light pulse followed by a dark incubation of 15 h. The resultant RP responded phototropically more rapidly and to a greater extent than DP, but they still required a lag period (Fig. 1). A red light fluence of 760 J m^{-2} saturated the pretreatment effect.

The detailed blue light irradiance dependence of phototropism in peas is not known, but it is unlikely that phototropism in either RP or DP is light limited, since the responses were not improved at a higher fluence rate (500 mw m^{-2}). Continuous irradiation did appear to be required, since shorter duration exposures (1 s to 1 h) over a range of fluence rates were unable to induce phototropic curvature in subsequent darkness.

Previous studies of red light-promoted phototropism in peas did not report on the effects of far-red light (20, 23). We found that far-red light, while having a significant effect by itself, was able to reverse the action of red light, indicative of photochrome involvement (Table I). Interactions between blue light photoreceptors and phytochrome have been reviewed elsewhere (22).

The time course for the development of phytochrome enhancement of phototropism is shown in Figure 2. Plants were given saturating red light pulses at zero time and incubated in darkness. Separate groups of plants were removed from darkness at intervals and assayed for hook angle, phototropism, or gravitropism. DP exhibited a consistent, low level of phototropic response throughout the time course, whereas red-irradiated plants demonstrated an enhanced response starting roughly 4 h after the red light pulse and continuing for more than 16 h. Phytochrome-regulated hook opening paralleled the enhancement of phototropism (Fig. 2), but phytochrome-promoted nutational amplitude appeared under comparable conditions to be complete largely within about 8 h of a red light pulse (4). In contrast to phototropism and nutation, the magnitude of gravitropism was only slightly affected by prior red light (Fig. 2; see also Refs. 2 and 3).

Stem movements in pea seedlings differed in a number of respects aside from the effect of red light. In RP, for example, phototropic curvature developed evenly over an extended zone of the epicotyl (Fig. 3A) whereas the initial apical portion of gravitropic curvature straightened and only a basal curvature remained (Fig. 3B; see also Ref. 3). Note that the basal gravitropic curvature resulted even though the induction period was briefer and the response was smaller than for phototropism. In contrast to both tropisms, red light-stimulated nutational curvature was restricted to the ∥ -plane and, furthermore, was propagated as a wave down the stem (3). Patterns of growth in the epicotyl were also affected by light. In RP, the zone of elongation was spread over a greater length of stem than in DP (3).

Phototropism, gravitropism, and nutation also diverged in response to removal of the apical bud and/or the hook. Excision of both these organs followed by a 150-min incubation period almost completely inhibited phototropism (Fig. 4), but the loss of response was largely prevented by the addition of IAA (100 μg g^{-1}) to the lanolin paste used to coat the cut end of the stem. The effect of excision on gravitropism was mainly to increase the response time

Table I. *Effect of Far Red Light on the Red Light-Enhanced Phototropism of Pea Seedlings*

Induction for 3 h with 300 mw m^{-2} unilateral blue light began 18 to 19 h after the time of red (135 J m^{-2}) and/or far-red (475 J m^{-2}) light treatment.

Treatment	Phototropism[a]
	degrees
Dark controls	11 ± 2
2 min far red	21 ± 2
1 min red	50 ± 4
1 min red—2 min far red	24 ± 1

[a] Values are means ± SE.
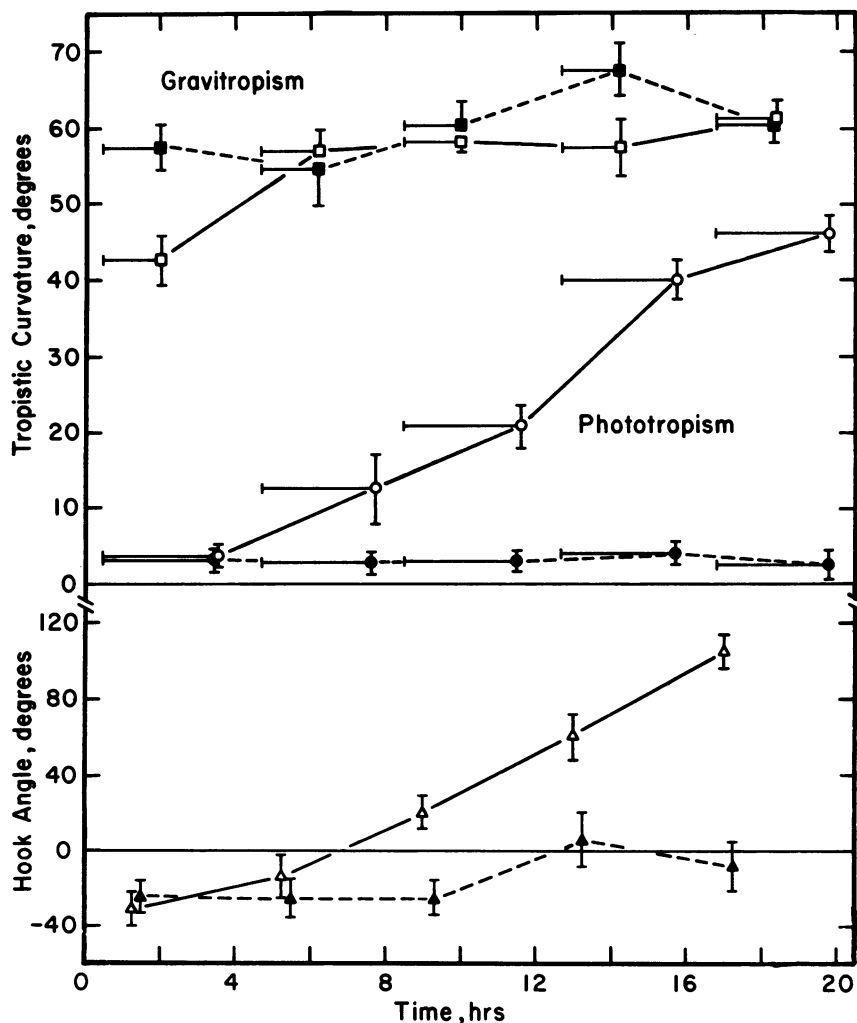
FIG. 2. Time course for red light effects on gravitropism (□,■), phototropism (○,●), and hook angle (△,▲) in DP (■,●,▲) and RP$_x$ □,○,△). Four min of red light (760 J m^{-2}) were given at zero time when the plants were 6 d old. The horizontal bars indicate the duration of tropistic induction, 1.5 h for gravitropism and 3 h for phototropism. The vertical bars indicate 1 SE. Blue light fluence rate was 75 mw m^{-2}.

and to decrease the rate of curvature (data not shown, but see Ref. 18 for comparable results). Exogenous IAA had little influence on these effects which were generally unapparent if one considered only final gravitropic curvature after long-term stimulation (e.g. Fig. 4). The complete reaction chains for phototropism and gravitropism in RP are, therefore, contained in the epicotyl below the hook, but the two responses must differ dramatically in their quantitative requirement for auxin. This result is quite different from that in coleoptiles where decapitation inhibited both gravitropism and phototropism and where the inhibition of both was reversible with added IAA (29). Auxin and decapitation effects in RP are summarized in Table II. Note that nutation in decapitated seedlings has a strong dependence on exogenous IAA, as does phototropism, but that the hook is required for an IAA effect on nutation (4).

NPA, an inhibitor of auxin transport (21, 28), had comparable effects on gravitropism and phototropism (Fig. 5). These results also confirmed earlier reports that nutation was more sensitive to NPA than was gravitropism (3) and that straight stem growth was relatively insensitive to NPA (5). It is not clear why phototropism and gravitropism have similar NPA sensitivity but dissimilar requirements for exogenous IAA.

It was previously reported that restoration of growth after removal of the apex was more sensitive to exogenous IAA in red light-treated plants than in dark controls (20). However, the IAA

in lanolin was applied directly to the cut surface of the stem. Red light can lead to decreased activity of IAA oxidase (a special problem at cut surfaces) and also to decreased wound inhibition of growth (such as would occur after cutting). We therefore tested light effects on the auxin sensitivity of intact plants by applying IAA in lanolin paste unilaterally to the third internode of the epicotyl just below the hook. The resultant curvatures were measured after a 2-h incubation in the dark (Table III). DP reacted significantly better than did RP over the range of 30 to 100 μg g^{-1} IAA. By 300 μg g^{-1} of IAA, curvature was saturated in DP and the effect in RP was equal to that in DP.

DISCUSSION

Tropistic responses in dicotyledonous plants have received increasing attention in recent years and certain patterns have emerged. For example, the photoreceptor for phototropism is present in the responding stem tissue in peas as well as in other species (1, 13, 16, 26). As in peas, phototropism in dark-grown seedlings of sunflower (13) and cress (16) is poor compared to that in deetiolated seedlings. However, cress seedlings require prolonged exposure to light before phototropism is potentiated. Phototropism in peas and in cress is also poor compared with that in coleoptiles, but in some species (e.g. lentils and radish) the phototropic response is quite similar to that of coleoptiles in terms of
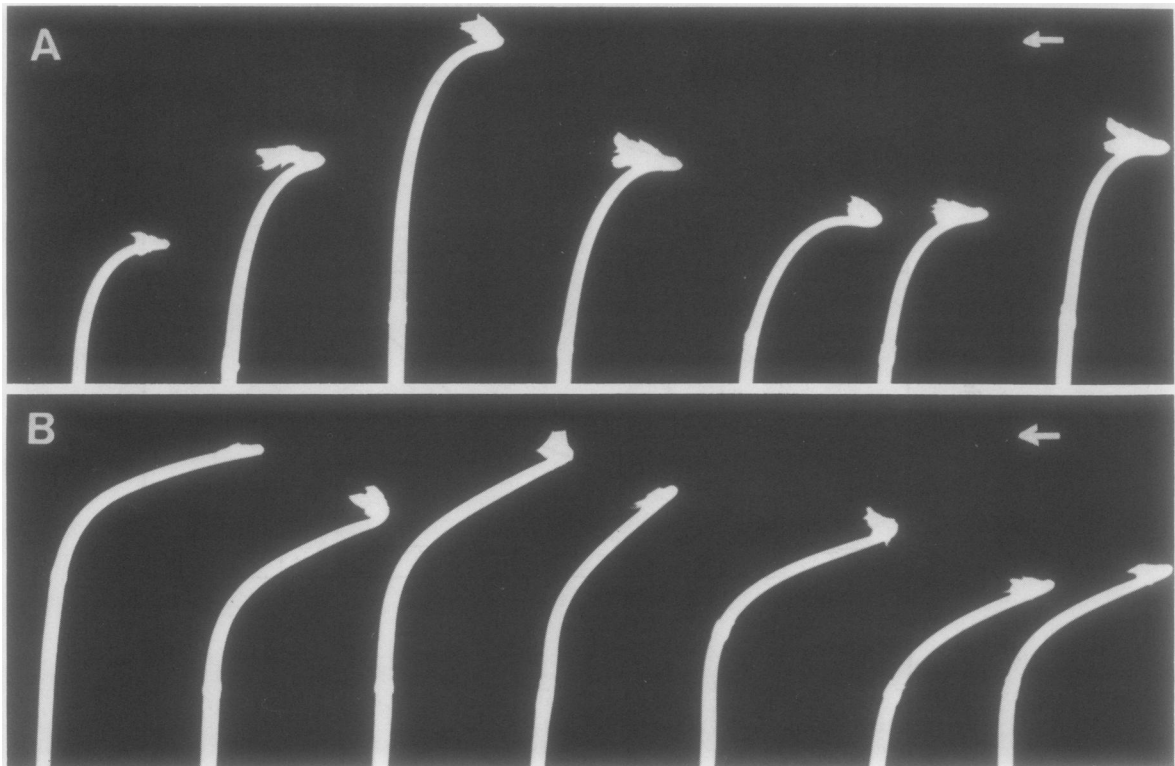
FIG. 3. Shadowgraphs of phototropically stimulated (A) and gravitropically stimulated (B) RP$_{18}$. Conditions of tropistic induction were the same as in Figure 2. The arrows indicate the direction of light or gravitational attraction. Average phototropic and gravitropic curvatures (± SE) were 78 ± 3° and 62 ± 3°, respectively.
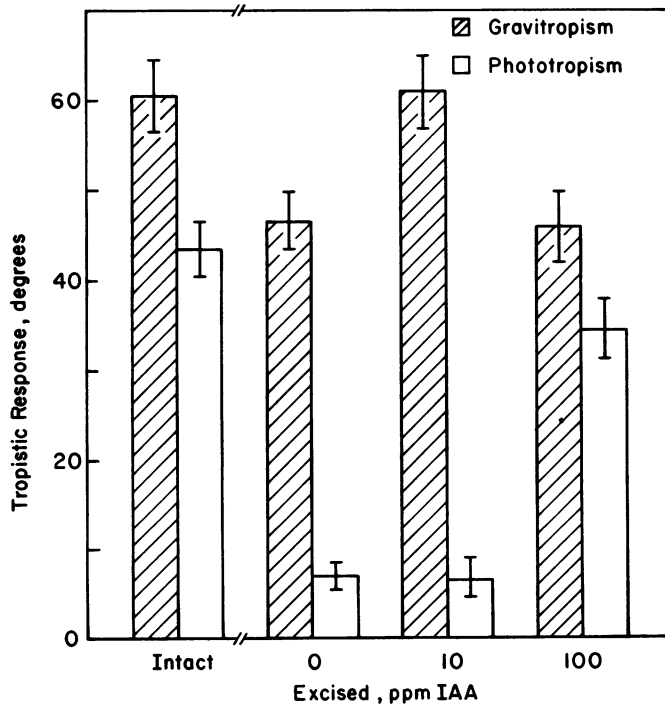

FIG. 4. Effect of exogenous IAA on gravitropic (hatched bars) and phototropic (open bars) responses in intact and decapitated RP$_{17}$. Among the latter plants, both hook and bud (plumule) were removed and replaced with a lanolin paste ± IAA at the indicated concentration (ppm = μg g^{-1}). Plants were incubated 150 min before initiation of tropistic reactions (same induction conditions as in Fig. 2). The vertical bars indicate 1 SE.

Table II. *Effect of Decapitation and Exogenous IAA on Nutation, Phototropism, and Gravitropism in Red Light-Treated Pea Seedlings*

Data summarized from this paper and References 2, 3, 4, and 19. The IAA treatments, 100 μg g^{-1} in lanolin paste, should not be construed as optimal. The gradations of response are approximate and determined as follows: ++++, no inhibition with respect to intact plants; +++, slight inhibition; ++, strong inhibition; +, almost complete inhibition.

Response	Bud and Hook Excised		Bud Only Excised	
	−IAA	+IAA	−IAA	+IAA
Nutation	+	+	+	+++
Phototropism	+	+++	++/+++	+++
Gravitropism	+++	+++	++++	++++

kinetics, blue light sensitivity, existence of first and second positive curvatures, and red light modulation (10, 27). Of course, we cannot compare the light sensitivity of peas with other species because we used saturating fluence rates. We do note that epicotyls of Alaska peas (6) and hypocotyls of cress (15) have a sensitive and rapid blue light-growth response with the photoreceptor localized in the responding stem tissue.

Seedlings responding phototropically are subjected to gravitropic induction in the opposite direction. This effect may be significant as is indicated by the potentiation of phototropic response during clinostat treatment (24). Although pea seedlings curve more rapidly in response to gravity than they do to unilateral light, we can rule out diminished gravitropic response as a cause of red light-enhanced phototropism (Fig. 2; see also Ref. 17). In fact, the light-treated seedlings actually appear to be more gravi-sensitive (2). Whereas our detailed studies of gravitropism were conducted in the dark or with dim-green light, preliminary experiments failed to show an effect of blue light on gravitropic re-
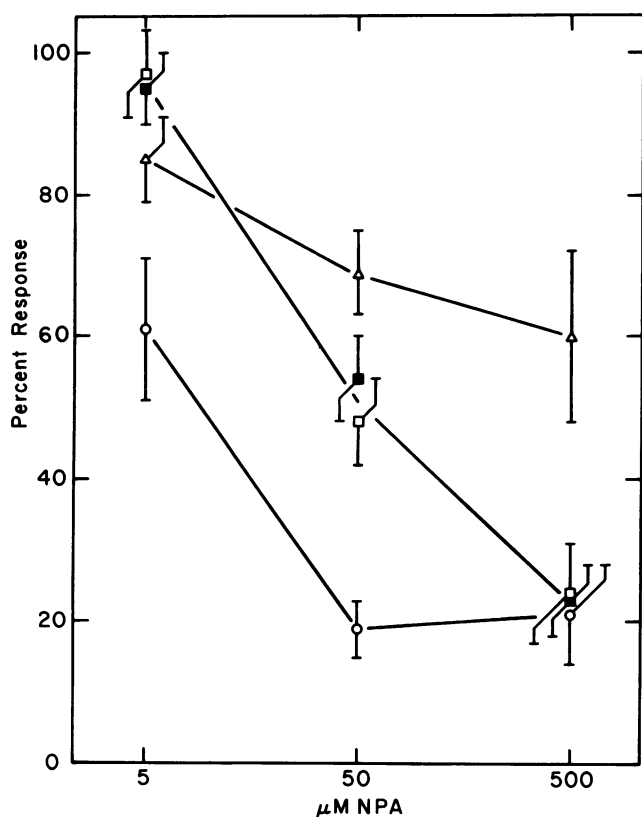
FIG. 5. Inhibition of nutation (O), gravitropism (■), phototropism (□), and growth (△) in RP₁₆ by NPA. Plants were dipped into the appropriate solution (see Ref. 3) and incubated 5.5 h (4 h for phototropism) before reactions were started. Nutations (observed in the ‖ -plane), growth, and gravitropism were measured over a 1.5-h span. Phototropism was induced with a 3-h blue light irradiation (75 mw m⁻²) and was initiated 1.5 h in advance of the other responses in order to account for the longer lag period. Control experiments showed that NPA effects were constant at least between 4 and 7 h after treatment. Tween-20 controls (100% response ± 1 SE) gave the following values: growth, 3.1 ± 0.2 mm; gravitropism, 69 ± 3°, phototropism, 56 ± 4°; nutation, 45 ± 2°. The vertical bars indicate 1 SE.

Table III. *Induction of Curvature by Unilateral Application of IAA in Lanolin to the Third Internode of Pea Seedling Epicotyls*

Approximately 18 h after time of red light (4-min pulse, 540 J m⁻²), plants were daubed with about 5 mg of lanolin ± IAA and incubated 2 h in the dark under the same conditions as for phototropism.

IAA	Curvature[a]	
	Dark controls	Red-irradiated
$\mu g\ g^{-1}$	*degrees*	
0	6 ± 3	6 ± 3
3	9 ± 2	4 ± 3
10	11 ± 2	9 ± 2
30	16 ± 2	3 ± 1
100	26 ± 2	14 ± 3
300	23 ± 2	25 ± 2

[a] Values are means ± SE.

sponse.

Auxin and auxin transport seem to be important for phototropism (Figs. 4 and 5). However, we can probably rule out increased sensitivity to auxin in stems of irradiated plants as a cause of red light-stimulated phototropism (Table III). In fact, red light appears to make seedlings less sensitive, although possible differences in

the penetration of IAA through the cuticle cannot be discounted.

In this regard, the question of lateral transport of auxin during phototropism should be reconsidered (*e.g.* Ref. 12). Recent studies with sunflower seedlings failed to demonstrate lateral transport of auxin during phototropism (13). Although unilateral blue light was reported to cause increased accumulation of radiolabeled IAA on the shaded side of pea epicotyls (20), there are some problems in interpretation. Radioactive IAA was added to cut surfaces, but the recovered radioactivity was not checked to verify that the label remained in IAA. Moreover, the differential distribution was determined several hours after the start of curvature and was not influenced by red light which altered the magnitude of the response.

Inasmuch as red light does increase the sensitivity of pea seedlings to gravity (2), it is still reasonable to speculate that red light may also increase the sensitivity of the blue light photoreceptor. Unfortunately, we can use neither gravitropic response nor nutation as controls to check for changes in secondary processes. Variations in the pattern of bending and in the effects of light, decapitation, and inhibitors indicate that phototropism, gravitropism, and nutation incorporate differences in the control of stem curvature. These differences emphasize the complexity of endogenous regulation of growth in plants.

LITERATURE CITED

1. BRENNAN T, JE GUNCKEL, C FRENKEL 1976 Stem sensitivity and ethylene involvement in phototropism of mung bean. Plant Physiol 57: 286–289
2. BRITZ SJ, AW GALSTON 1982 Light-enhanced perception of gravity in stems of intact pea seedlings. Planta 145: 189–192
3. BRITZ SJ, AW GALSTON 1982 Physiology of movements in stems of seedling *Pisum sativum* L. cv Alaska. I. Experimental separation of nutation from gravitropism. Plant Physiol 70: 264–271
4. BRITZ SJ, AW GALSTON 1982 Physiology of movements in stems of seedling *Pisum sativum* L. cv Alaska. II. The role of the apical hook and of auxin in nutation. Plant Physiol 70: 1401–1404
5. CHING TM, RH HAMILTON, RS BANDURSKI 1956 Selective inhibition of the geotropic response by N-1-naphthylphthalamic acid. Physiol Plant 9: 546–558
6. COSGROVE DJ 1981 Rapid suppression of growth by blue light. Occurrence, time course and general characteristics. Plant Physiol 67: 584–590
7. DARWIN C, F DARWIN 1881 The Power of Movement in Plants. D Appleton & Co, New York
8. DENNISON DS 1979 Phototropism. *In* W Haupt, ME Feinleib, eds, Encyclopedia of Plant Physiology, New Series, Vol 7. Springer-Verlag, Heidelberg, pp 506–566
9. ELLIOTT W, J SHEN-MILLER 1976 Similarity in dose responses, action spectra, and red light responses between phototropism and photoinhibition of growth. Photochem Photobiol 23: 195–199
10. EVERETT M 1974 Dose-response curves for radish seedling phototropism. Plant Physiol 54: 222–225
11. FILNER B, R HERTEL, C STEELE, V FAN 1970 Some aspects of geotropism in coleoptiles. Planta 94: 333–354
12. FIRN RD, J DIGBY 1980 The establishment of tropic curvatures in plants. Annu Rev Plant Physiol 31: 131–1481
13. FRANSSEN JM, J BRUINSMA 1981 Relationships between xanthoxin, phototropism and elongation growth in the sunflower seedling *Helianthus annuus* L. Planta 151: 365–370
14. GALSTON AW, AA TUTTLE, PJ PENNY 1964 A kinetic study of growth movements and photomorphogenesis in etiolated pea seedlings. Am J Bot 51: 853–858
15. HART JW, IR MACDONALD 1980 Photoregulation of hypocotyl growth: geotropic evidence for the operation of two photosystems. Plant Cell Environ 3: 189–193
16. HART JW, IR MACDONALD 1981 Phototropic responses of hypocotyls of etiolated and green seedlings. Plant Sci Lett 21: 151–158
17. HART JW, IR MACDONALD 1981 Phototropism and geotropism in hypocotyls of cress (*Lepidium sativum* L.). Plant Cell Environ 4: 197–201
18. HEATHCOTE DG 1981 The geotropic reaction and statolith movements following geostimulation of mung bean hypocotyls. Plant Cell Environ 4: 131–140
19. KANG BG, SP BURG 1972 Relation of phytochrome enhanced geotropic sensitivity to ethylene production. Plant Physiol 50: 132–135
20. KANG BG, SP BURG 1974 Red light enhancement of phototropic response in etiolated pea stems. Plant Physiol 53: 445–448
21. KATEKAR GF, AE GEISSLER 1980 Auxin transport inhibitors. IV. Evidence of a common mode of action for a proposed class of auxin transport inhibitors: the phytotropins. Plant Physiol 66: 1190–1195
22. MOHR H 1980 Interaction between blue light and phytochrome in photomorpho-

genesis. *In* H Senger, ed, the Blue Light Syndrome. Springer-Verlag, Berlin-Heidelberg, pp 97–109

23. NAUNOVIĆ G, M NEŠKOVIĆ, D GRUBIŠIĆ 1980 Relationship of light and growth regulators in the phototropic reaction of dwarf pea stems. *In* J DeGreef, ed, Photoreceptors and Plant Development. Antwerpen University Press, pp 393–397

24. PICKARD BG, K DUTSON, V HARRISON, E DONEGAN 1969 Second positive phototropic response patterns of the oat coleoptile. Planta 88: 1–33

25. SCHMIDT W, J HART, P FILNER, KL POFF 1977 Specific inhibition of phototropism in corn seedlings. Plant Physiol 60: 736–738

26. SHUTTLEWORTH JE, M BLACK 1977 Role of cotyledons in phototropism of deetiolated seedlings. Planta 135: 51–55

27. STEYER B 1967 Die Dosis-Wirkungs Relationen bei geotroper und phototroper Reizung: Vergleich von Mono- mit Dikotyledonen. Planta 77: 277–286

28. SUSSMAN M, MHM GOLDSMITH 1981 The action of specific inhibitors of auxin transport on uptake of auxin and binding of N-1-naphthylphthalamic acid to a membrane site in maize coleoptiles. Planta 152: 13–18

29. WENT FW 1942 Growth, auxin, and tropisms in decapitated *Avena* coleoptiles. Plant Physiol 17: 236–249

30. WILKINS H, RL WAIN 1975 The role of the root cap in the response of the primary root of *Zea mays* L. seedlings to white light and to gravity. Planta 123: 217–222

31. WILKINS MB 1979 Growth-control mechanisms in gravitropism. *In* W Haupt, ME Feinleib, eds, Encyclopedia of Plant Physiology, New Series, Vol 7. Springer-Verlag, Heidelberg, pp 601–626