Planta (Berl.) 87, 193—205 (1969)

Role of Growth Regulators in the Bean Hypocotyl Hook Opening Response

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Received January 29/April 7, 1969

Summary. The opening of the hypocotyl hook in bean seedlings is due to a rapid elongation of cells on the inner side of the hook elbow. Red light promotes hook opening by inducing this cell elongation.

Opening is inhibited by low concentrations of indoleacetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), and higher concentrations of these auxins cause a closure of the hook. In darkness, opening is induced slightly by *p*-chlorophenoxyisobutyric acid (PCIB), whereas in red light this auxin antagonist promotes opening only when IAA is added simultaneously to inhibit opening.

The amount of diffusible auxin released by the hook tissue is not affected by red illumination that is sufficient to induce maximal hook opening.

Gibberellic acid (GA) promotes the hook opening. The magnitude of its effect is, however, rather small, especially in darkness. (2-Chloroethyl)-trimethylammonium chloride (CCC) and 2′-isopropyl-4′-(trimethylammonium-chloride)-5′-methylphenyl piperidine-1-carboxylate (Amo-1618) inhibit hook opening in red light, and this inhibition is completely overcome by addition of GA.

Cytokinins and abscisic acid at rather high concentrations inhibit hook opening in light but produce no significant effect in darkness.

Hook opening is promoted by Ca^{++} and K^{+}, and notably by Co^{++} and Ni^{++}.

It is concluded that 1. endogenous gibberellin assists in hook opening, but light does not act by changing the gibberellin level; 2. light does not act by decreasing the endogenous auxin level; and 3. cytokinins or abscisic acid do not seem to have a special role in the response.

Introduction

The terminal portion of the shoot axis of dark-grown seedlings in many dicotyledonous plants is shaped as a hook. In the hypocotyl of bean seedlings, opening of the hook is mediated by phytochrome (KLEIN *et al.*, 1956; WITHROW *et al.*, 1957), exposure of the hook to red light bringing about opening, this effect being reversible by far-red light.

The light-induced hook opening is inhibited by both the presence of the terminal organs (i.e., intact hooks) and the addition of 3-indoleacetic acid (IAA) to excised hook segments. These observations led to the speculation that the terminal organs supply a diffusible auxin to the hook (KLEIN *et al.*, 1956), and that this auxin prevents opening. Furthermore, the observation that an antiauxin induced hook opening in the absence of red light led to the hypothesis that red light acts by reducing the level of auxin in the hook (KLEIN, 1965).

This paper presents results of an investigation of the hypothesis that light induces hook opening by influencing the hormonal balance of the hook tissue.

Materials and Methods

Plant Materials. 6-day-old dark-grown seedlings of *Phaseolus vulgaris* L., cv. Black Valentine, were used. The seeds were planted in vermiculite in a plastic tray and germinated in complete darkness at $26 \pm 1°$ and $70 \pm 2\%$ relative humidity. Under a dim, green safelight the terminal organs were removed and the hook was cut at its apical end in such a way the initial angle of the hook was 0°, meaning that the hook was curved through an angle of 180° from the direction of the straight portion of the hypocotyl.

Two principal methods were employed to incubate hooks for the experimental period, usually 24 hr, or 20 hr in the case of continuous red illumination: a) 10 hooks with approximately 2-cm shanks (basal ends) attached were placed on a double layer of filter paper in a 10 cm Petri dish containing 20 ml of distilled water or test solution; b) 10 hooks with approximately 4-cm shanks attached were placed vertically with their basal ends inserted in 1% agar in a dish. In the latter case, test substances were applied in an agar block which was placed on the apical cut surface of the hook. The set of hooks was then kept in a glass chamber (volume about 10 liters) to prevent the agar block from drying out but not interfere with red illumination. The hooks were shadowgraphed at the end of the test period, and the angle of opening was measured on the shadowgraphs. Results are given as mean angle of opening $\pm$ standard error of the mean.

Illumination. The red-light source was a pair of 4-watt fluorescent lamps covered with 6 layers of red cellophane (DU PONT) which transmitted light above 600 nm. The light intensity was 375 erg cm^{-2} sec^{-1}. All manipulations were carried out in a dim green safelight (WITHROW and PRICE, 1957). They were usually completed within 30 min. Such exposure did not significantly affect subsequent hook opening.

Bioassay of Diffusible Auxin. Diffusible auxin from the hook was assayed by the *Avena* curvature test (WENT and THIMANN, 1937). In order to obtain appreciable, reproducible yields of diffusible auxin from the tissue, it was necessary to prevent enzymatic inactivation of auxin at the cut surface by using 0.005 M KCN (STEEVES *et al.*, 1953). An agar block ($2.7 \times 2.7 \times 1.5$ mm) was attached to the basal cut surface of each hook for a 2-hour diffusion period in a water-vapor-saturated Petri dish in complete darkness and used for the test immediately after the diffusion period.

The distribution of elongation along the inner and outer surfaces of the hook (Fig. 1) was measured by applying a series of fine projecting lanolin "hairs" spaced about 2 mm apart along the two sides of an isolated hook standing with its base in agar. IAA, where administered, was applied in an agar block to the apical end. The marked hooks were shadowgraphed at the beginning and end of the 24-hr growth period, and the fractional change in distance between each of the marks was determined by measurements upon the shadowgraphs.

Results

Effects of Light and Auxin on Elongation. The distribution of elongation activity along the length of isolated hook segments, measured as described above, is illustrated in Fig. 1. Light induces a spectacular elongation on the inner side of the hook, and IAA inhibits this elongation.

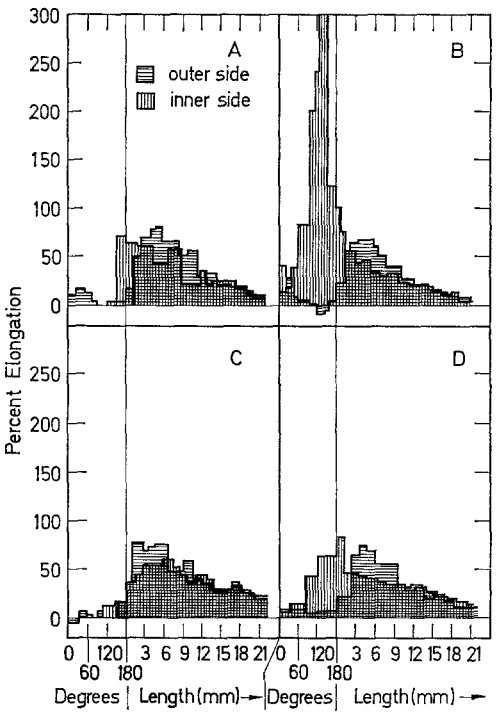

Fig. 1 A—D. Distribution of elongation along the inner and outer sides of isolated hypocotyl hooks and the subjacent shank under treatment with darkness (A), red light (B), IAA (0.5 µg/ml) in darkness (C), and IAA plus red light (D). Abscissa; distance from apical end of the hypocotyl expressed in terms of degrees in hook portion considered as a semicircle (0° meaning apical end, 90° meaning vertical peak, and 180° meaning basal end of the semicircle), and expressed in terms of length of the shank starting from the basal end of the hook

Photomicrographs were made from microtome sections cut from paraffin-embedded specimens of hook tissue that had been fixed at zero time and after 20 hr in red light. By measurements on these photographs the mean lengths of cortical cells on the inside of the hook elbow region was estimated, and was found to be approximately 2.5 times greater after the light-induced period of hook opening. The agreement between this figure and the directly measured elongation on the inside surface of the hook elbow (Fig. 1) supports the statement of KLEIN (1959) that this growth response involves cell enlargement not accompanied by any appreciable cell division.

Results of treatment with different IAA concentrations are collected in Fig. 2. They show that IAA application specifically inhibits elongation

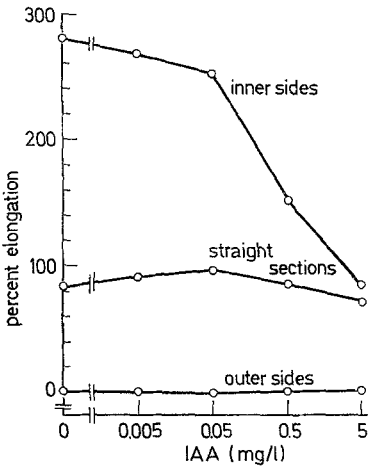

Fig. 2. Effect of IAA on elongation of the inner and outer sides of isolated segments of hook elbow and straight sections of the hypocotyl shank in red light for 24 hr

on the inner side of the hook and has little or no effect on elongation in the straight part of the hypocotyl or on the (slight) elongation of the outer side of the hook.

Fig. 3 shows dependence of opening angles on concentrations of IAA and 2,4-dichlorophenoxyacetic acid (2,4-D). Both these auxins strongly inhibit hook opening in red light and in darkness. The small discrepancy between the growth curve (Fig. 2) and the opening curve in terms of IAA concentrations is probably because of the different method employed in the latter case where hooks were placed in IAA solution in a Petri dish. Auxin concentrations above 1 mg/l induce a negative curvature, meaning that the hook becomes curved in the reverse of the normal direction of opening.

Fig. 4 shows that *p*-chlorophenoxyisobutyric acid (PCIB), an auxin antagonist, induces hook opening in darkness to a certain extent, but has no such effect in red light. KLEIN (1965) reported a similar effect of naphthylmethylsulfide acetate. When IAA was added in the medium in red light, PCIB partially reversed the IAA inhibition, the magnitude of its effect being somewhat greater than that in darkness without IAA (Fig. 4).

Effect of Light on the Yield of Diffusible Auxin. Results of bioassays of the diffusible auxin from the hook are summarized in Table 1. Red light had no significant effect on the amount of diffusible auxin released by the hooks. Neither pretreatment with red light from 1 hr up to 16 hr nor treatment with red light during the 2-hr diffusion period caused any

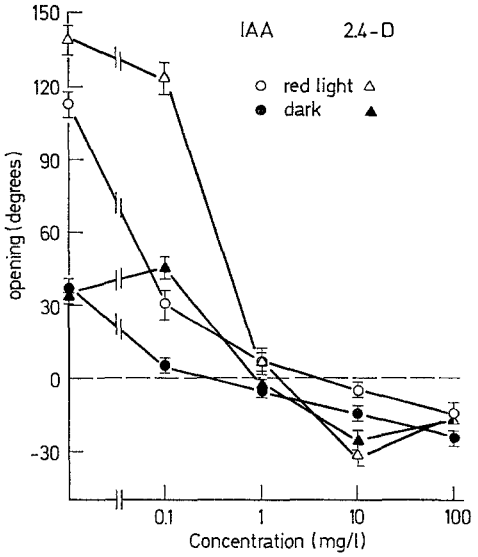

Fig. 3

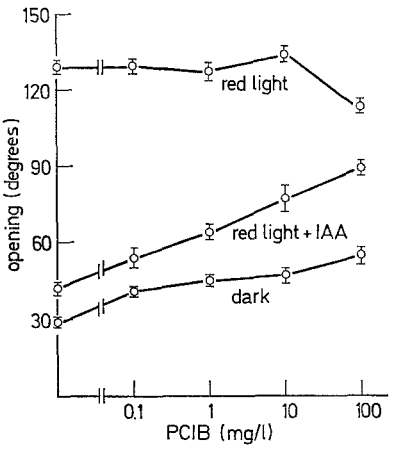

Fig. 4

Fig. 3. Effects of concentrations of IAA and 2,4-D on hook opening. Vertical bars represent the range of standard errors

Fig. 4. Effect of PCIB on hook opening in dark, red light, and red light plus IAA (0.1 mg/l)

appreciable difference in the yield of diffusible auxin compared with that from the dark control. The amount of diffusible auxin from excised hooks actually declines rapidly with time; 4 hr after the excision the amount of diffusible auxin is almost nil in both red light and dark. When the terminal organs (cotyledons and shoot apex) were not removed, diffusible auxin could still be collected after 16 hr of red light or dark treatment, indicating that the terminal organs are supplying auxin to the hypocotyl. The red-illuminated hooks opened about 45° at 16 hr, whereas the dark hooks remained closed.

Involvement of Gibberellic Acid. As shown in Fig. 5, gibberellic acid (GA) promotes hook opening in red light but has little effect in darkness. We were completely unable to confirm the substantial induction of hook opening in the dark by very high concentrations (10^{-3} M) of gibberellin that was reported by KLEIN (1965). However, we found, as Table 2 indicates, that an effect of GA in darkness can be brought on by treatment with PCIB.

KLEIN (1965) reported that the degree of hook opening is a function of shank length, and suggested that GA is transported acropetally and red light causes the GA transport. The data presented in Table 3 confirm

Table 1. *Effect of red light on the diffusible auxin yield of the hook tissue*

Exp't. No.	Experimental condition[a]	Degrees of *Avena* curvature[b] (mean ± standard error)	
		Dark control	Red light
1	Excised hook. 1 hr light treatment followed by 2 hr diffusion in dark	15.0 ± 0.82	13.4 ± 0.92
2	Excised hook. 4 hr light treatment followed by 2 hr diffusion in dark	2.1 ± 1.11	0.9 ± 0.66
3	Excised hook. 2 hr light treatment during diffusion period	14.2 ± 0.54	14.2 ± 0.73
4	Intact hook with terminal organs. 16 hr light treatment followed by 2 hr diffusion in dark[c]	17.0 ± 1.11	15.9 ± 1.01
5	Excised hook. 4 hr light treatment with IAA (10 mg/l) in agar block. Agar block removed at end of the 4 hr. Diffusion in dark for subsequent 2 hr	18.3 ± 0.28	18.3 ± 1.26

[a] Red light intensity, 9400 erg cm^{-2} sec^{-1}.

[b] 0.05 mg/l IAA in agar block yielded curvature of 16.7 ± 1.04, and 0.25 mg/l IAA yielded 26.6 ± 2.12.

[c] Red light treated hook opened about 45° at 16 hr.

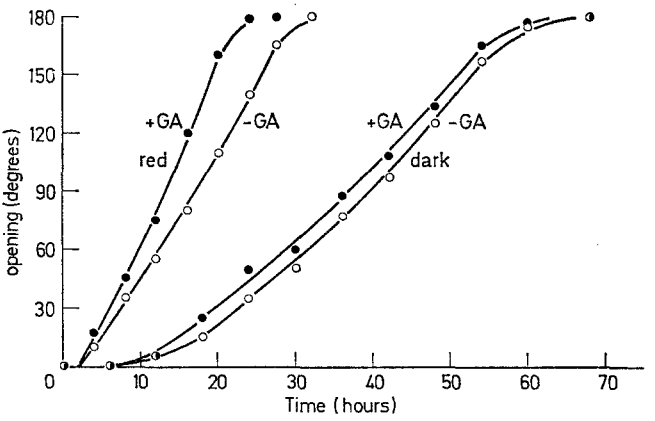

Fig. 5. Time course of opening in red light and dark, with and without GA (0.055 μg per hook)

his observations that hook sections with longer shanks opened to a greater extent than those with shorter shanks. However, the Table also shows that a saturating concentration of GA (cf. Fig. 6) causes equal

Table 2. *Enhancement of GA response by PCIB*
Hook opening, degrees.

Condition	H_2O	GA (10 mg/l)	PCIB (100 mg/l)	GA (10 mg/l) + PCIB (100 mg/l)
Dark	16 ± 2.9	24 ± 3.5	36 ± 2.5	67 ± 2.5
Red light	120 ± 2.9	143 ± 1.8	94 ± 3.8	123 ± 3.0

Table 3. *Effects of the shank length and GA on hook opening in red light*
Figures show hook opening in degrees $\pm$ standard error of the mean.

Shank length (cm)	GA (mg/l)	
	0	20
3	91 ± 1.6	121 ± 2.3
8	121 ± 3.1	166 ± 1.7

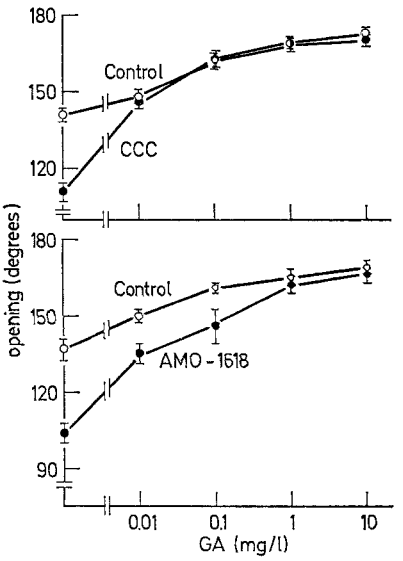

Fig. 6. Reversal of CCC and Amo-1618 inhibitions of hook opening by GA in red light. CCC (10^{-2} M) and GA were applied in solution simultaneously to the hooks. Plants were pretreated on the 3rd day of germination with Amo-1618 (200 mg/l) in a soil drench and GA was later applied to the hooks

promotions of opening when given to hooks with short and long shanks, indicating that the effect of the shank cannot be ascribed to its providing a source of GA.

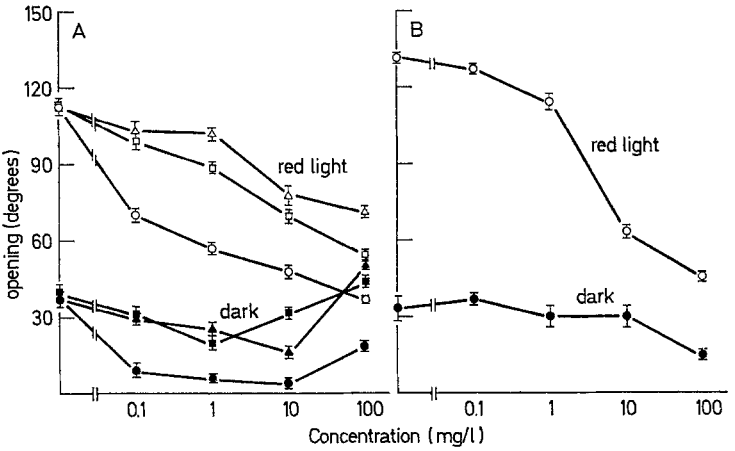

Fig. 7 A and B. Effects of cytokinins (A) and abscisic acid (B) on hook opening
o Benzyladenine; △ Pyranosylated Benzyladenine; □ 6-Furfurylaminopurine

The growth retardants (2-chloroethyl)trimethylammonium chloride (CCC) and 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate (Amo-1618), which inhibit GA synthesis (HARADA and LANG, 1965; DENNIS *et al.*, 1965), reduce hook opening in red light. This inhibition can be completely overcome by addition of GA in the medium (Fig. 6).

Effects of Other Growth Regulators. 6-furfurylaminopurine (kinetin), benzyladenine, and pyranosylated benzyladenine have inhibitory effects on hook opening in red light, but very slight effects in darkness (Fig. 7 A). Cytokinin treatment did not relieve the inhibition of hook opening by auxins.

Abscisic acid inhibited hook opening at fairly high concentrations (Fig. 7 B). Tested at 10 mg/l abscisic acid did not reduce the promotive effect of 10 mg/l GA nor the inhibitory effect of 0.1 mg/l IAA.

Effect of Cations. Co^{++}, as found previously by KLEIN (1959), and Ni^{++} promote hook opening both in red light and in darkness (Fig. 8). At 10^{-3} M these cations induce hook opening in darkness quite dramatically. Higher concentrations are inhibitory in both light and darkness.

Time-course measurements (Fig. 9) showed that the promotive effect of Co^{++} is exerted on the initial phase of the response and that even at the optimally promotive concentration Co^{++} gradually becomes inhibitory and prevents attainment of complete straightening of the hook. However, 10^{-3} M $CoCl_2$ in darkness cause initially as rapid a rate of hook opening as can be induced by treatment with red light. Even so, the Co^{++}-induced hook opening can initially be further promoted substantially by red light (see Fig. 9), and it appears from these data that the reduced red-

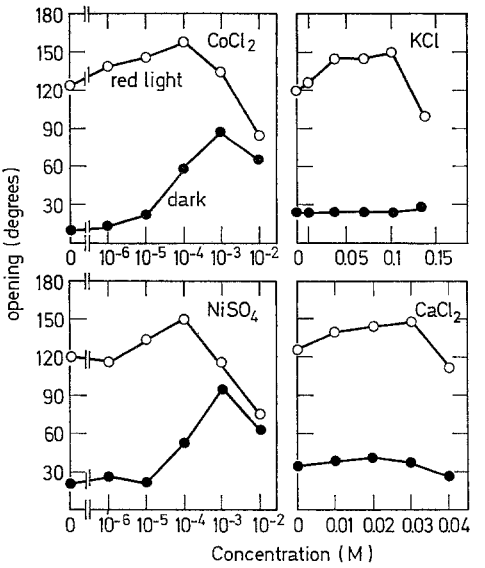

Fig. 8. Effects of some cations on hook opening

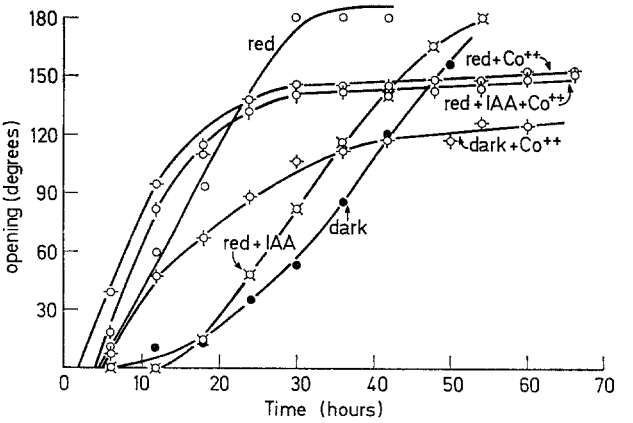

Fig. 9. Time course of hook opening under a combination of 10^{-3} M $CoCl_2$ and 2 mg/l IAA

light response recorded at the optimal Co^{++} concentration in Fig. 8 is actually due to the later, inhibitory effect of Co^{++} on hook opening. These observations supplement the finding of KLEIN (1959) that Co^{++} interacts synergistically with red light in this response.

Ca^{++} and K^{+} exert a small but consistent stimulation of hook opening in light, but unlike Co^{++} and Ni^{++} they do not induce opening in darkness

Table 4. *Effects of salts on hook opening responses to GA and IAA*
Figures given in the first two horizontal rows are the average results from three separate experiments, all of which showed comparable promotions by Ca^{++} and GA and a comparable arrest of these promotions under the combination (Ca^{++} + GA). Results in the remaining rows are from single experiments.

Treatment	Control	Hook opening, degrees	
		GA (10 mg/l)	IAA (0.1 mg/l)
Red light	126	154	45
Red light, 0.03 M $CaCl_2$	140	119	83
Red light, 0.01 $MnCl_2$	89	143	34
Red light, 0.01 M LiCl	49	87	21
Dark	30	33	−3
$CoCl_2$ 0.001 M in dark	90	92	92
$NiSO_4$ 0.001 M in dark	95	103	59

(Fig. 8). Na^+, Li^+, Mn^{++} and Mg^{++} had no appreciable effect on hook opening in light or dark at concentrations up to 10^{-3} M; Li^+ and Mn^{++} were inhibitory at 10^{-2} M (cf. Table 4) whereas Na^+ and Mg^{++} were not.

When the effects of these cations upon the response to growth regulators were tested some striking results were obtained, as illustrated in Table 4 which summarizes data from a number of experiments. Ca^{++}, at a concentration optimally promotive of hook opening, completely suppressed the promotive effect of GA on the process, and indeed in the presence of Ca^{++}, GA became somewhat inhibitory. The data can also be viewed as saying that GA prevents the promotive effect of Ca^{++}. Mn^{++} and Li^{++}, on the other hand, did not prevent the promotive effect of GA, and it even appeared that GA essentially reversed the inhibitory effect of Mn^{++}. None of these ions prevented the inhibitory effect of IAA, but Co^{++} completely blocked and Ni^{++} greatly reduced the effect of IAA. This effect of Co^{++} was seen both in darkness (Table 4) and in red light (Fig. 9).

Inhibition by Mannitol. The effect of mannitol on hook opening in red light is shown in Fig. 10. Inhibition occurs in the osmotic concentration range and the inhibition curve resembles rather closely that for growth of pea stem segments (THIMANN *et al.*, 1950). In view of Fig. 10 it is remarkable that 0.2 osmolar (0.1 M) KCl and 0.1 osmolar (0.03 M) $CaCl_2$ promoted rather than inhibited hook opening (Fig. 8). Galactose and mannose, at 0.03 M, inhibited hook opening only to about the same extent (10—25%) as an equal concentration of mannitol.

Discussion

The responses to IAA, GA and kinetin of the bean hypocotyl hook are, in general, similar to those of the etiolated pea epicotyl hook (NAKAMURA

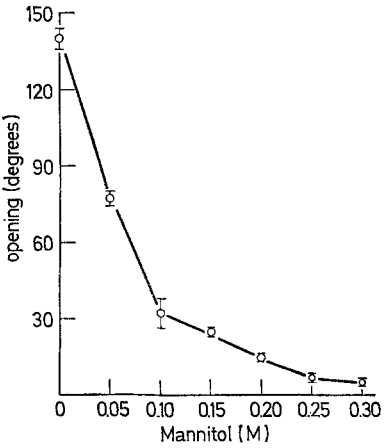

Fig. 10. Inhibition of hook opening in red light by mannitol

et al., 1966), except that auxin-treated bean hooks do not noticeably enlarge in the transverse direction. Also, NAKAMURA and TAKAHASHI (1968) reported that PCIB inhibits pea hook elongation, whereas this auxin antagonist promotes bean hook opening and reduces the inhibition thereof by IAA.

Any hypothesis that increase in gibberellin content is the factor responsible for light-induced opening of hooks can be ruled out by the small effect of GA applied in the darkness. However, the inhibitory effects of CCC and Amo-1618 and their reversal by GA suggest that gibberellin is required for the growth that is involved in light-induced hook opening. Similar results with CCC were obtained for pea epicotyl hooks by NAKAMURA and TAKAHASHI (1968).

There are some indications that seem to support the hypothesis that red light acts on opening by reducing the level of endogenous auxin. These include a) the opposing effect of auxin and red light on hook opening; b) the promotion of hook opening by removing cotyledons and plumule (KLEIN *et al.*, 1956), which as shown here are the major source of auxin for hook tissue; c) the induction of opening by PCIB in darkness; d) the enhancement of the GA effect by PCIB in darkness, and by red light, but not by PCIB in red light; e) the promotion of hook opening by longer shanks which might use up or draw more of the endogenous auxin from the hook, and f) the reduction of the auxin level by red irradiation in bean seedlings reported by FLETCHER and ZALIK (1964).

However, it seems quite clear from the results of the assays for diffusible auxin that there is no direct correlation between red light and yields of diffusible auxin from the hook tissue. Red light treatment that

is sufficient to induce substantial opening of hooks did not significantly change the amount of diffusible auxin collected from the hook elbow tissue. The dramatic promotive effect of light on opening of excised hooks extends over a period of 24 hr or more even though the yield of diffusible auxin of such hooks has fallen to nil after about 4 hr; whereas light induces opening of intact hooks bearing cotyledons and plumule even though their yield of diffusible auxin remains substantial after many hours and is still unaffected by light. It seems certain, therefore, that the opening behavior of the hook is not determined simply by its level of diffusible auxin, and that light does not induce hook opening by causing a decrease in this level. It can be asked, of course, whether red light affects a kind of auxin other than the diffusible form. The depression of auxin content that was reported by FLETCHER and ZALIK (1964) in bean seedlings after red irradiation, was measured in terms of total methanol extractable IAA. The prevalent view today, however, is that diffusible auxin is the physiologically active hormone (GILLESPIE and THIMANN, 1963; PICKARD and THIMANN, 1964).

It may be surmised, however, that the eventual slow opening of excised hooks in darkness results from depletion of their endogenous auxin whereas the rapid opening in red light is attributable to some other mechanism. Opening of excised hooks in darkness is not due merely to a loss of responsivity to auxin. This is shown by the observation that when IAA was applied to hooks in the dark 24 hr after excision, it still prevented opening.

The various lines of evidence about the relation between auxin and the red light response might be reconciled at least in part by the hypothesis that light decreases the sensitivity of hook cells towards inhibition of growth by auxin. Such a hypothesis was advanced by BRAUNER (1966) in an effort to explain the effect of gravity in geotropism. This kind of hypothesis, which is difficult to test directly, seems to be needed as a result of findings regarding the involvement of ethylene in the response (see KANG and RAY, 1969a). The participation of ethylene also affords an explanation for the remarkable effects of Co^{++} and Ni^{++} on hook opening and on its response to IAA.

The curious effects of Ca^{++} and Mn^{++} that were observed on the response to GA suggests that the effect of GA on this system may involve ion relations of the growing cells. Aspects of the response to GA are considered further by KANG and RAY (1969b).

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