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Hormone-Solute Interactions in the Lettuce Hypocotyl Hook[1,2]

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ABSTRACT

The hypocotyl hook of the lettuce (*Lactuca sativa* cv. Grand Rapids) seedling is stimulated to a high degree of curvature through a synergistic interaction of ethylene and gibberellic acid in the light. Presentation of various inorganic salts to the seedlings caused extensive alteration of the hormone-induced curvatures, with ammonium and sulfate being the most stimulatory of curvature, and potassium and carbonate being the most inhibitory of curvature. Experiments using organic buffers indicated that the effect was not a pH response. The abilities of various cations and anions to alter the hormonally regulated curvature is suggested as further evidence of solute alteration of hormonal effectiveness. The interpretation is offered that the solutes may be influencing hormonal effectiveness through salting-in and salting-out effects on macromolecules such as proteins.

The hook which develops on the tip of the hypocotyl or epicotyl of some seedlings may serve to facilitate the emergence of the growing point from the seed coat and the penetration of the shoot through the soil. In most species, the hook type of growth is removed by red light (4). In the lettuce seedling, however, the development of the hypocotyl hook is actually enhanced by red light, and far red light brings about its removal (9). The hormonal regulation of hook formation includes a general stimulation of hook formation by ethylene (3); auxin can stimulate the hook formation in cotton (12), or in bean (15); gibberellin can stimulate hook opening in both pea and cotton (3, 12).

In a series of studies of the effects of inorganic solutes on growth and development, we have found that numerous types of processes which we have considered to be under hormonal control can be markedly altered by inorganic solutes, especially by calcium (8, 10, 11), and we subsequently established that we could alter the regulatory effects of each of the known hormones with inorganic solutes; we proposed that these effects may relate to the solutes acting as salting-in or salting-out agents on macromolecular constituents of the plant tissues (8). In the present study, we have turned to the use of the hypocotyl hook curvature of lettuce seedlings as a highly convenient growth event for the examination of hormonal controls and their alteration by inorganic solutes. Our experiments will show that hormone actions on hook curvature can be greatly amplified by certain inorganic solutes, whereas other solutes can prevent the hormone actions.

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[2] This paper is dedicated to the memory of Solon A. Gordon.

MATERIALS AND METHODS

Lettuce seeds (*Lactuca sativa* cv. Grand Rapids) were germinated on wet filter paper in the light for 1 day, at which time seedlings were selected for uniformity of the projecting radicle. For each treatment, 15 or 20 seedlings were placed in a 6-cm Petri dish containing a circle of filter paper and 2 ml of the solution of salts or hormones to be tested. In some instances, the dishes were removed to the darkroom, but in most cases they were held in the laboratory under about 100 ft-c of fluorescent light. Ethylene treatments were made by placing the dishes in glass desiccators into which ethylene was injected to give a 1 μl/l ethylene final concentration; controls were kept in separate desiccators with a mercury perchlorate-saturated paper wick inside to trap any ethylene out of the atmosphere. After 3 days of such treatment, the curvature which had developed in the hypocotyl hook was recorded by holding each seedling in front of a protractor and estimating curvature to the nearest 5°, calling an erect seedling with no hook 0°.

RESULTS

Hormonal Effects on the Hook. A survey of the effects of the five types of plant hormones on hook curvature was made as shown in Table I. A cytokinin, an auxin, ABA, and gibberellin were supplied to the seedlings, both in the absence of added ethylene and in the presence of 1 μl/l ethylene. The entire experiment was done in light, since light enhances hook curvature (9). The data show that the cytokinin depresses curvature, that IAA and ABA enhance curvature, and that GA is enormously promotive of curvature in the presence of ethylene. With ethylene alone, curvature was 65°, with GA alone it was 53°, and with both ethylene and GA the curvature was over 250°, in comparison with a control value of 29°.

In order to examine the apparent synergism between ethylene and GA further, a concentration series for GA was carried out in the presence of 1 μl/l ethylene, and the results, given in Figure 1, show that GA alone was essentially without effect, that ethylene was moderately promotive of curvature, but the combinations were very effective, with the higher concentrations of GA causing a 4- or 5-fold increase in curvature over the ethylene control.

In the case of lettuce, then, hook curving is greatly stimulated by an interaction of ethylene and gibberellic acid.

Solute Effects on the Hook. As a device for examining the effects of solutes, we utilized the interactions of ethylene and GA as the primary hormonal control over which we attempted to make alterations. First, a comparison of the relative effects of five cations upon hook curvature is given in Table II, each cation being given as the chloride salt, at two concentrations, in the presence of ethylene alone or ethylene plus GA. The data show that ammonium has a very large amplifying effect on hook curvature, especially in the presence of ethylene and GA, where a total curvature of over 400° was obtained. A lesser effect was obtained with calcium, almost no effect with

Table I. *Effects of Five Types of Hormones on Hypocotyl Hook Curvature of Lettuce Seedlings*

Experiment was done in light, all hormones were at 10^{-5} M concentration and 1 µl/l ethylene where supplied.

Solute	Hypocotyl Curvature	
	No ethylene	With ethylene
	degrees	
H_2O	29 ± 8	65 ± 5
Benzyladenine	0	53 ± 5
Indoleacetic acid	62 ± 8	96 ± 7
Abscisic acid	103 ± 11	147 ± 10
Gibberellic acid	53 ± 10	253 ± 21

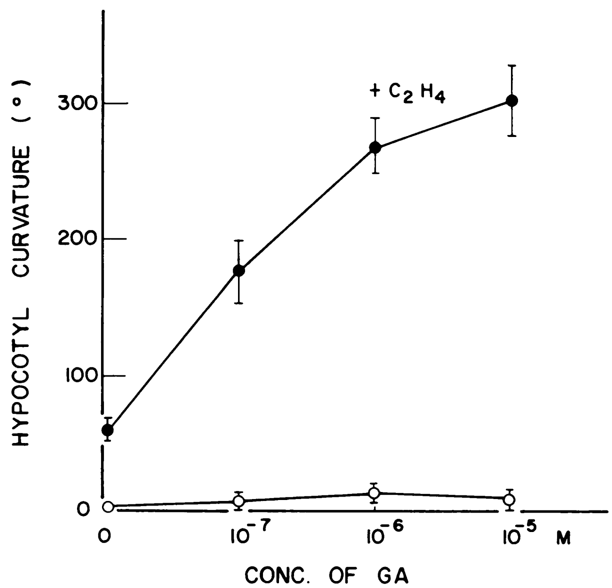

FIG. 1. Effects of gibberellic acid on hypocotyl hook curvature in the presence or absence of 1 µl/l ethylene, in the light.

magnesium, and inhibitions of curvature with sodium and potassium.

In an examination of the ammonium effect, concentration curves were determined for ammonium chloride and the interactions with ethylene, GA, or both. Results of the test are shown in Figure 2, where it is evident that ammonium chloride had almost no effect on lettuce hook curvature over the entire concentration range of 10^{-4} to 3×10^{-2} M tested. However, there was a slight enhancement of NH_4 in the presence of ethylene, a larger enhancement in the presence of GA, and a very large enhancement in the presence of both ethylene and GA, reaching a peak curvature of over 500° at 3×10^{-3} M NH_4Cl—almost two complete circles of hook. The appearance of seedlings after some of these various treatments is illustrated in Figure 3.

In contrast to the amplification of curvature by ammonium, Table II shows that potassium has a dampening effect on curvature in the presence of GA. Examination of a KCl concentration series is made in Figure 4, where it can be seen that some dampening of curvature is obtained with KCl in the presence of GA; in the presence of GA plus 10^{-3} M NH_4Cl, the dampening effect is nearly complete, reducing curvature from the 175° control value to about 40° in the presence of 3×10^{-3} M KCl, which is the same as the water control. At a higher NH_4Cl level (3×10^{-3} M), there was no significant dampening of curvature by KCl.

The experiments on inorganic solutes were undertaken in order to investigate the possibility that salting-in or salting-out solute effects might alter hormonal actions; and if salting-in or salting-out effects are involved in the observed solute effects, one would expect that anions should also have effects on the hormonal action. Selecting the ammonium ion as the strongest cation amplifier, we have compared its effectiveness in the presence of various anions as illustrated in Table III, in the presence of ethylene alone and in ethylene plus GA. The data indicate that there are strong anion effects on the hook curvature responses to the hormones, with the sulfate ion being a very effective amplifier of curvature, and lesser effects being obtained with chloride or nitrate, and some suppression of curvature being obtained with carbonate. It should be noted that the valences of the anions shown in Table III vary between 1 and 2, and hence the amount of ammonium ion will

Table II. *Comparative Effects of Five Cations on Lettuce Hypocotyl Hook Curvature*

Each cation was supplied as the chloride at 10^{-3} or 10^{-2} M, with 1 µl/l ethylene or 1 µl/l ethylene plus 10^{-5} M GA, in the light.

Solute	Hypocotyl Curvature			
	With ethylene		With ethylene + GA	
	degrees			
H_2O	46 ± 6		253 ± 27	
	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M
Ammonium	82 ± 6	114 ± 8	402 ± 22	423 ± 53
Calcium	63 ± 8	63 ± 7	301 ± 37	384 ± 23
Magnesium	66 ± 7	49 ± 6	235 ± 25	287 ± 24
Sodium	55 ± 6	57 ± 6	172 ± 33	139 ± 29
Potassium	51 ± 8	53 ± 6	99 ± 26	19 ± 3

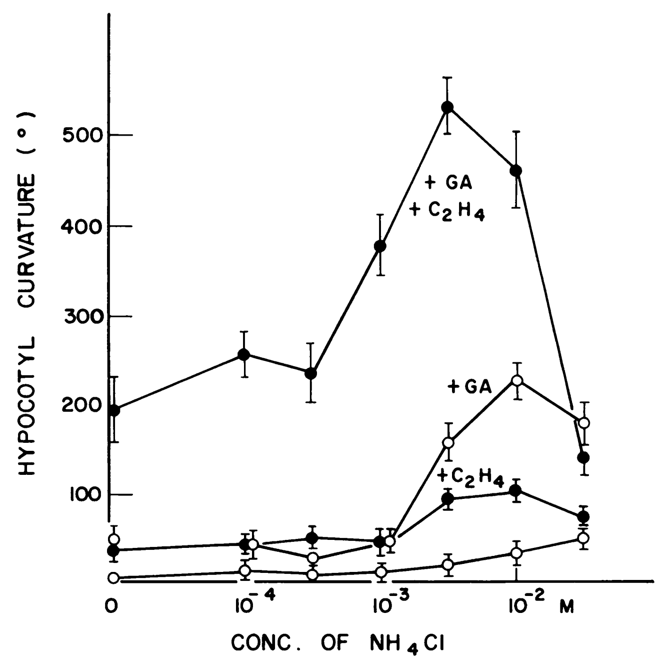

FIG. 2. Effects of ammonium chloride on hypocotyl hook curvature when supplied alone, with ethylene, with GA, or with both. Ethylene at 1 µl/l, GA at 10^{-5} M, in the light.

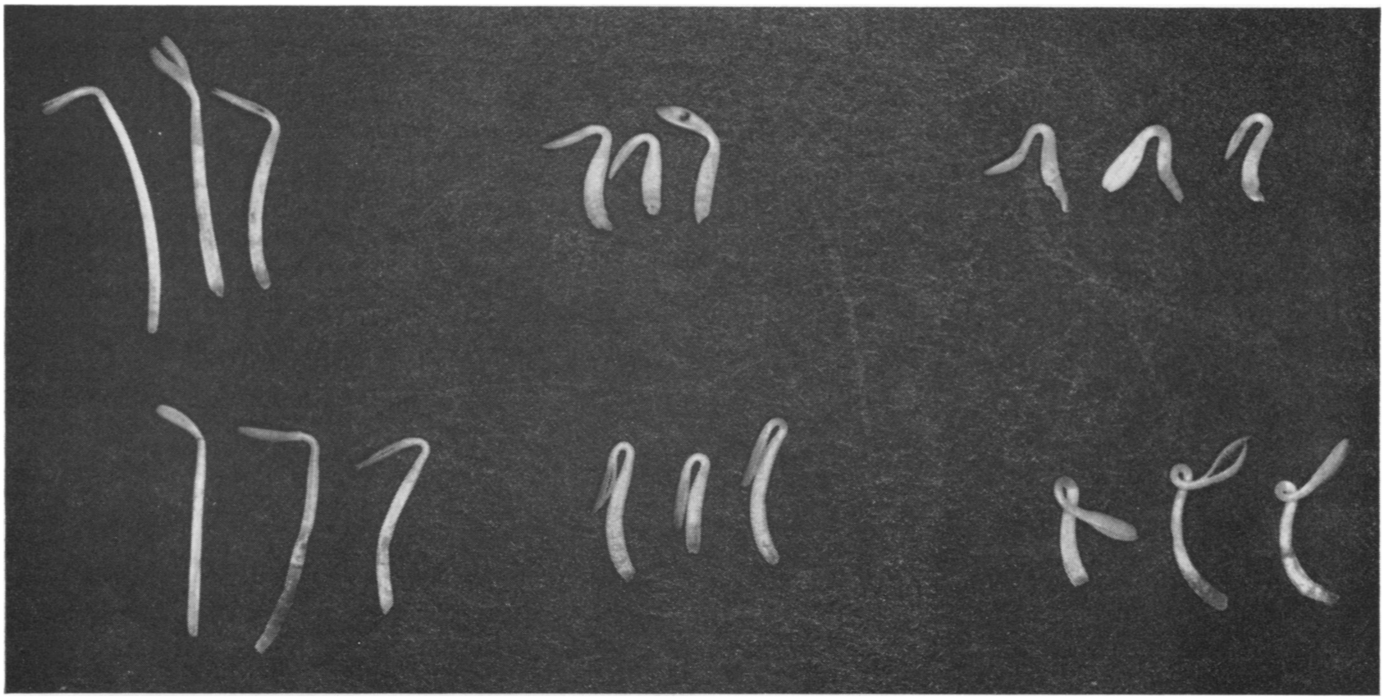

FIG. 3. Appearance of lettuce hypocotyls, showing curvature development in controls (left), ethylene treated (middle), or ethylene plus NH_4Cl (right), in the absence of added GA (above), or with GA (below). Concentrations were 1 µl/l ethylene, 10^{-2} M NH_4Cl and 10^{-6} M GA, in the dark.

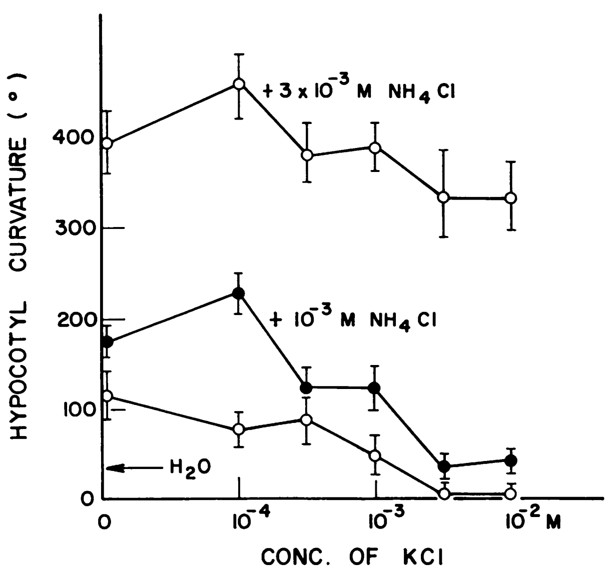

FIG. 4. Effects of potassium chloride on hypocotyl hook curvature when supplied alone, with 10^{-3} M NH_4Cl, or with 3×10^{-3} M NH_4Cl. All treatments given 1 µl/l ethylene and 10^{-6} M GA except the one labeled H_2O, in the light.

vary between the treatments reported in accordance with these valences. Nevertheless, the sulfate and the carbonate treatments are directly comparable and show that the anion can exert strong effects on hook curvature. We concluded that both cations and anions can interact with the hormonal functions in regulating the hook curvature in lettuce.

pH Effects. In all of the tests reported, the solutions being tested were unbuffered, since buffers ordinarily include other inorganic ions; consequently there is reason to ask whether the effects being observed might reflect pH alterations in the medium.

The pH of the water utilized in these experiments was pH 5.3; when any of the inorganic salts was added to a concentration of 10^{-2} M, the pH was either unaffected, or it was raised to as much as pH 5.7. Therefore, the curvature responses seem not to be related to the initial pH of the solutions. At the end of the 3 days of treatment, the pH of the test solution had ordinarily drifted up to 6.0 or down to 5.0.

A direct examination of pH effects on hook curvature would ordinarily involve the addition of inorganic salts to the test medium, which would, of course, introduce an extraneous variable. A means of circumventing this problem was achieved by utilizing two organic buffer materials, MES and tris. Without other additions, MES buffer has a pH somewhat less than 4.0; tris buffer is on the alkaline side. So titration of MES with tris was a simple means of obtaining a pH range between 4.0 and 6.0; the effects of this pH range on hypocotyl hook cur-

Table III. *Comparative Effects of Four Anions on Hypocotyl Hook Curvature*

Each anion was supplied as the ammonium salt at 10^{-3} M concentration, with 1 µl/l ethylene or 1 µl/l ethylene plus 10^{-6} M GA, in the light.

Solute	Hypocotyl Curvature (degrees)	
	With ethylene	With ethylene + GA
	degrees	
H_2O	44 ± 8	157 ± 26
Sulfate	143 ± 14	333 ± 21
Chloride	40 ± 9	230 ± 19
Nitrate	34 ± 8	201 ± 30
Carbonate	33 ± 5	66 ± 19

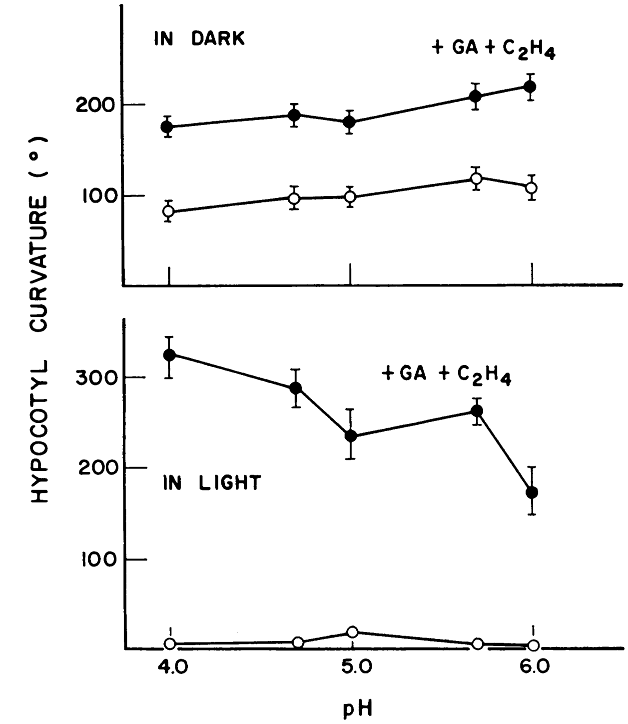

FIG. 5. Hypocotyl hook development at various pH values. pH was obtained with 10 mM MES buffer titrated to the higher pH values with tris buffer. Tests run in darkness (upper graph) or in light (lower graph), in water (open circles) or with 1 μl/l ethylene plus 10^{-5} M GA (filled circles).

vature under four types of conditions is illustrated in Figure 5, where it is evident that there was very little pH effect on either endogenous curvature in light or dark or on the effectiveness of the hormone combination of GA plus ethylene.

It seems safe to conclude, then, that pH of the medium is not responsible for the curvature responses to the inorganic solutes being examined here, as the solutes themselves had little or no effect on the solution pH and control of the pH did not alter the curvature response appreciably.

DISCUSSION

Our experiments have shown that the extent of curvature of the lettuce hypocotyl hook can be altered by the amounts of gibberellin and ethylene present, the two hormones acting synergistically to provoke curvature, especially in the light. Various inorganic ions have been shown to affect markedly the extent of curvature response to these hormones, with NH_4 being the most effective cation and SO_4 the most effective anion as stimulators of the curvature response, and K and CO_3 being the most effective suppressors of the curvature response. The solute effects are not accountable as pH effects. We suggest the interpretation that the inorganic solutes may be serving to alter the effectiveness of the hormonal regulation of curvature at least in part through their actions as salting-out and salting-in agents.

The ability of inorganic solutes to alter the conformation of proteins and nucleic acids has been described in detail by von Hippel and Schleich (19), and the effects can be attributed to the solutes altering the charged groups on the macromolecule, or altering the interactions of the macromolecule with the water lattice around it, or both. The solute effects may be either toward increased stabilization of the macromolecule (salting-out effect) or toward increased destabilization (salting-in effect). Among the common ions, ammonium and sulfate are ordinarily highly effective salting-out agents, hence the common utilization of ammonium sulfate as a protein-precipitating agent; calcium and chloride are commonly among the most effective salting-in agents, and their effects in decreasing the permeability of membranes (van Steveninck [18] for example) might be considered to be related to their salting-in effects. Through the salting-out/in effects, solutes can bring about enzyme activations, inactivations, changes in protein complexes (such as in actin and hemoglobin [*cf.* 19]), or changes in physical characteristics such as melting curves. For our purposes here, three features of these solute effects may be especially pertinent: (*a*) the solute effects on macromolecules are not specific to a given cation or anion, but there are graded effects for different cations and anions; (*b*) the solute effects ordinarily begin at 10^{-4} M or higher concentrations, and (*c*) the solute effects are ordinarily additive to one another. In the experiments reported here, we have shown that there are graded responses to series of both cations and anions (Tables II and III), the alterations of hook curvature begin between 10^{-4} and 10^{-3} M (Figs. 2 and 4), and the curvature enhancement by NH_4 can be removed by the further addition of K (Fig. 4). These evidences do not prove that salting-out or salting-in phenomena explain the solute effects described here, but they do provide a basis for offering such a suggested explanation.

Several analogous effects of inorganic ions on dynamic plant phenomena have been reported in the literature. Perhaps the most comparable report is that of Satter *et al.* (16), in which they report opposite effects for K and for NH_4 on sleep movements of leaves; their results also show graded effects for different cations as well as anions. Knypl and Rennert (6) report that the cytokinin stimulation of cucumber cotyledon growth is inhibited by NH_4 and greatly enhanced by K. Rijven and Eckhardt (14) observed a similar stimulation of fenugreek cotyledon growth with K, but there was no clear inhibition with NH_4. Rehfeld and Jensen (13) observed a great leakage of photosynthate from cotton leaf cells given NH_4, and Ca additions reversed the NH_4 effect. Another important case of reversal by two ions is the Ca inhibition of auxin stimulated growth, and its reversal with K (1). NH_4 has been found to have strong uncoupling effects on spinach chloroplasts, but we do not have evidence of its relief by other cations (5).

Interpretation of the solute effects on biological systems is complicated by the fact that there is no set pattern of their relative solute effectiveness as salting-out/in agents. Even the melting curves for purified proteins show differences in the order of solute effects with different species of proteins (17, 19). In general, however, ammonium and sulfate are among the most effective inorganic solutes favoring salting-out of proteins.

The proposed action of solutes on biological functions such as the hook curvature would of course depend upon the internal rather than the external concentrations of the applied solutes; in the case of ammonium ions, the effective range in altering hook formation lies generally between 10^{-3} and 10^{-2} M, and the natural ammonium contents of plants frequently occur at 10^{-2} M or even higher concentrations (7).

In the study of physiological mechanisms of the hook formation, Rubenstein (15) has described the phenomenon as a combined action of growth-promoting factors on one side and growth-inhibiting factors on the other side of the hypocotyl. We think that the combined effectiveness of GA and ethylene in the lettuce hypocotyl may represent such a combination of growth-promoting and growth-inhibiting factors. It is noteworthy that GA has been observed to remove hook curvature in pea (3), bean and cotton (12); in the lettuce, of course, it is a powerful stimulator of hook curvature if ethylene is present (Fig. 1).

The experiments reported here are offered as further evidence of inorganic solutes having powerful actions on plant hormonal functions, in addition to the reports we have previously made of ions altering the effectiveness of each of the five growth hormones (8), and our reports of the striking effects of Ca and other ions on developmental functions such as senescence and abscission (10, 11).

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