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INVESTIGATIONS INTO THE HORMONAL CONTROL OF
ROOT GROWTH AND GRAVITROPISM

DISSertation

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Timothy John Mulkey, B.S., M.Sc.

* * * * *

The Ohio State University
1983

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DEDICATION

Dedicated to my parents,

Calvin C. and Clytiea L. Mulkey.
ACKNOWLEDGMENTS

I wish to thank my parents and family for their love, encouragement, and belief in my abilities, without whom this dissertation would not have been possible.

I wish to acknowledge my advisor, Dr. Michael L. Evans, for his advice in preparation of this manuscript and during the research on which it is based. I wish to thank Dr. Evans for his enthusiasm, encouragement, and belief in my abilities. I have enjoyed the opportunity to work with and learn from such an exceptional scientist.

I wish to extend my gratitude to the members of my reading committee for their advice and valued opinions during the course of this research.

I wish to thank my friends; Konrad Kuzmanoff, Barbara Berrent, Lynn Mull, James McFadden, Amy Nelson, June Lee, Jody Banks; who have made the past few years interesting, if not enjoyable.
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LIST OF ABBREVIATIONS

AbA - abscisic acid (+/-, cis, trans)
ACC - 1-aminocyclopropane carboxylic acid
AVG - aminoethoxyvinylglycine
Co - cobalt nitrate, Co(NO₃)₂·6H₂O
9FCA - 9-fluorene carboxylic acid
9HFCA - 9-hydroxy-9-fluorene carboxylic acid
IAA - indole-3-acetic acid
ICA - indole-2-carboxylic acid
NAA - naphthalacetic acid
NPA - naphthylphthalamic acid
PCIB - p-chlorophenoxyisobutyric acid
SAM - s-adenosyl methionine
TIBA - 3,4,5-triiodobenzoic acid
Chapter I. Introduction

There is much debate concerning the hormonal factors involved in the control of root growth and gravitropic response. In 1936 K. V. Thimann first applied the newly identified plant hormone indole-3-acetic acid (auxin) to roots. He found that auxin stimulated root growth in a manner similar to that observed in stems and coleoptiles. However, the response was not as great as that exhibited by stem and coleoptile tissue, and stimulation of root elongation by auxin occurred at much lower concentrations and over a narrower concentration range. Roots were described as being more sensitive to IAA than shoots with optimal stimulation of root growth at approximately 0.1 nM IAA as compared with optimal stimulation of hypocotyl and coleoptile growth at approximately 1 to 10 uM. Since this early report of stimulatory indole-3-acetic acid action on root growth, many researchers have tried to repeat these results but have met with variable degrees of success (see reviews by Scott, 1972 and Aberg, 1957).

Larson (1955) found that auxin at $10^{-13}$ to $10^{-8}$ M could stimulate growth of roots but the stimulation was
very species dependent. Weston and Street (1968) found considerable stimulation of excised tomato roots in sterile culture by the synthetic auxin NAA (1-napthylene acetic acid). Other researchers have found promotion of root growth by IAA to be very mild (Audus and Shipton, 1952; Audus and Thresh, 1953; Edwards and Scott, 1974). Generally IAA has been found to be inhibitory to growth more often than stimulatory (Aberg, 1952 and 1957). Thus the classical view of auxin action on root growth which has developed is that the auxin content of roots is **supra-optimal** at most stages of root development. According to this view root tissues is more sensitive to auxin than is hypocotyl or coleoptile tissue, requires less auxin for enhancement of growth, and therefore root tissue is inhibited by much lower levels of auxin than is required to inhibit hypocotyl and coleoptile tissue.

Burstrom (1950) interpreted auxin action in terms of two phases. The first phase involved auxin increasing wall deformability and the second phase involved auxin increasing synthesis and intussusception of wall materials. There is evidence to support Burstrom's hypothesis. Wheat roots exhibit an increase in elongation rate with a decrease in final length after application of $10^{-8}$ M to $10^{-7}$ M IAA. The transient increase in rate may be attributed to an increase in wall deformability. The decrease in final length may be the result of reduced wall
synthesis which prematurely terminates elongation.

Burstrom proposed that root growth was the result of these two opposing phases; stimulation by auxin of the first phase and inhibition by auxin of the second phase. The second phase is thought to be inhibited to a greater extent than the first phase is stimulated by auxin which results in a net inhibition of root elongation over a wide auxin concentration range.

Concomitant with the inhibition of longitudinal growth upon application of auxin is a characteristic lateral swelling of the root. This swelling was first described by Cholodny (1931). The swelling is not due to a decrease in number of mitoses (Levan, 1939) or a decrease in plastic extensibility (Amlong, 1939). The swelling is strikingly similar to that observed by Neljubov (1901) in etiolated pea plants treated with ethylene. As little as 0.1 ul l⁻¹ of ethylene reduced the rate of elongation within 10 to 15 min and induced lateral expansion of the tissue.

Due to the similarity between auxin-induced and ethylene-induced swelling, several groups have investigated the possibility that auxin inhibits growth and induces lateral swelling by stimulating ethylene biosynthesis (Burg and Burg, 1968; Chadwick and Burg, 1967; Sankhla and Shukla, 1970). It has been known for over 45 years that ethylene inhibits root elongation and
induces lateral swelling (Zimmerman and Wilcoxson, 1935). However, auxin- and ethylene-induced inhibition are not identical. Auxin-induced growth inhibition is readily reversible on withdrawal of the hormone, while ethylene-induced inhibition is reversible only under certain circumstances (Andreae et al, 1968). Other problems with equating auxin- and ethylene-induced inhibition of root growth include differing kinetics and magnitude of inhibition by the two hormones, differing effects of tissue age, calcium and pH, and differing response to ethylene concentration-solution volume ratios when the solution volume and/or IAA concentration is altered. Chadwick and Burg (1970) accounted for these discrepancies between auxin and ethylene inhibition at low auxin concentrations and concluded that inhibition over the lower range of inhibitory concentrations of auxin resulted from auxin-induced ethylene biosynthesis while inhibition by higher levels of auxin (greater than or equal to $10^{-6}$ M), was not ethylene-related. Thus, models for ethylene-mediated modification of growth by auxin have been proposed (Osborne and Mullins, 1969) but the models have received very little attention.

With the elucidation of the ethylene biosynthesis pathway (Adams and Yang, 1979) a physiological basis for auxin-ethylene interaction was disclosed. Auxin was found to stimulate ethylene production through its effect on the
enzyme which converts s-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid, the direct precursor of ethylene (Jones and Kende, 1979; Cameron et al, 1979; Yu and Yang, 1979). Cytokinin and calcium (Lau et al, 1977) were also found to promote this enzymatic step, thus implicating the potential involvement of ethylene in the action of all three of these growth factors. The action of auxin on ethylene biosynthesis strengthens the original proposal of auxin-ethylene interaction in growth.

Although auxin-induced ethylene biosynthesis has been proposed to account for the inhibitory effect of auxin on growth the mode of action of lower concentrations of auxin in the promotion of growth is unresolved. Auxin has been suggested to promote growth in two phases (Vanderhoef and Stahl, 1975). The early phase of promotion has been suggested to be by the action of IAA at the membrane level, with sustained growth in the presence of auxin resulting from a second phase of auxin action which probably involves protein synthesis.

The initial response of tissues to auxin is an enhanced rate of proton efflux from the cytoplasm into the cell wall space. In the cell wall, the hydrogen ions are thought to perform one or more functions related to cell wall loosening (CWL). A lower cell wall pH may be optimal for the activity of a CWL enzyme. Alternatively, the release of protons into the cell wall may displace cations
such as calcium from the cell wall (Leopold, 1975) which would decrease cell wall rigidity. Another possible role for hydrogen ions is the direct hydrolysis of the cell wall. This concept has become known as the acid growth hypothesis. The acid growth hypothesis was formulated in its current form by Rayle and Cleland (1970) and by Hager et al (1971).

Several predictions of the acid-growth hypothesis have been proven for stem and coleoptile tissue. These include the following: 1) acidification of plant tissue by the application of an acidic solution stimulates growth (Bonner, 1934), 2) inhibition of cell wall acidification by introduction of neutral buffers inhibits auxin stimulated growth (Durand and Rayle, 1973; Cleland and Rayle, 1978), 3) auxin rapidly induces hydrogen-ion secretion from coleoptile tissue (Cleland, 1973; Vesper and Evans, 1979), 4) metabolic inhibitors which block auxin-induced growth also block auxin-induced hydrogen ion secretion (Cleland, 1973; Hayle, 1973). Even with the ever increasing amount of evidence in support of the acid growth hypothesis, there is no direct evidence proving the mechanism by which hydrogen ions function in CWL. Also the majority of evidence in favor of the acid-growth hypothesis is limited to stem and coleoptile tissue, though there is some evidence that the acid growth hypothesis may also be valid for auxin action on roots.
Edwards and Scott (1974, 1976, 1977) and McBride and Evans (1977) demonstrated that roots of maize and lentil respond to a decrease in pH by increased growth. However, if the acid growth hypothesis of auxin action were valid for roots one would predict that growth promoting concentrations of IAA would stimulate hydrogen ion secretion from root tissue while growth inhibiting concentrations of IAA would inhibit hydrogen ion secretion from roots. Until recently, this has been difficult to demonstrate. McBride and Evans (1977) were unable to detect auxin-induced proton movement in lentil roots.

Another difficulty with the acid growth hypothesis is the direct relationship between auxin action and the acid-growth hypothesis. No other hormone has been suggested to function through promotion of hydrogen ion movement. If protons are released from the protoplast into the cell wall allowing for cell wall loosening, other hormones which promote growth should also stimulate proton pumping. Appreciable acidification of the bathing media around hypocotyls and coleoptiles has not been observed during gibberellin induced growth (Stuart and Jones 1978). However, growth stimulating agents such as fusicoccin (Marre et al, 1972 and 1973) and naphthyl acetate (Vesper and Evans, 1979) have been found to induce proton secretion. The problem with the hydrogen ion secretion induced by fusicoccin and naphthyl acetate is a lack of
correlation between the hydrogen ion secretion and the stimulation of elongation observed. Both napthyl acetate and fusicoocin stimulate hydrogen ion secretion to a greater extent than the compounds stimulate elongation. The apparent discrepancies concerning the lack of hydrogen ion secretion induced by growth stimulating hormones such as the gibberellins and the over-stimulation of hydrogen ion secretion in relation to the promotion of elongation by fusicoocin and napthyl acetate exemplify the problems existing with the acid-growth hypothesis.

The initial action of auxin may be the stimulation of acid secretion. However, application of acid to tissue promotes growth for only 30 min to several hours, whereas the effect of auxin lasts for many hours. In addition to its early stimulation of hydrogen ion efflux, auxin must affect other processes within plant tissues which would sustain growth. This second phase of auxin action is probably the stimulation of protein synthesis. Theologis and Ray (1982) and Zurfluh and Guilfoyle (1980, 1982a) have found that auxin stimulates the synthesis of messenger RNA within 20 min of application. This new mRNA may play a key role in maintaining auxin-induced growth. Zurfluh and Guilfoyle (1982b) also found that inhibitory concentrations of auxin and ethylene promote messenger RNA synthesis. Several of the messenger RNAs induced by IAA or ethylene appear to be the same while others
are different. The differences may be due to the relatively high hormone levels applied or the differences in auxin- and ethylene-induced mRNA species may indicate dissimilarities in the mode of action of the two hormones. Grierson et al (1982) found that in vitro RNA synthesis in auxin treated mung bean hypocotyl nucleoli was due to auxin-induced ethylene effects. The auxin-induced ethylene enhanced RNA synthesis through the promotion of the number of RNA polymerase I molecules bound to the rRNA genes in the nucleoli. As previously noted, Chadwick and Burg postulated that auxin inhibition of growth was similar to ethylene inhibition of growth only at lower IAA concentrations.

Until the mid 1950's, IAA was considered to be the major hormone regulating root growth. In 1953, Bennet-Clark and Kefford suggested that the b-inhibitor complex may control root growth. This inhibitor complex was found in roots (Bennet-Clark and Kefford, 1953; Hemberg, 1961) and was found to be inhibitory to growth (Howell, 1954). In 1965, abscisic acid (AbA) was identified as a major component of the b-inhibitor complex (Cornforth et al, 1965), thus AbA is considered to be another hormone involved in the control of root growth.

Abscisic acid is thought to control root growth through inhibition of elongation. Several groups have found that AbA is inhibitory to growth when applied to
roots (Pilet and Chanson, 1981; review by Audus, 1983), but others have found AbA to be promotive (Yamaguchi and Street, 1977; Gaither et al, 1975; Abou-Mandour and Hartung, 1980; Smith and Ho, 1982). Other researchers could find no effect of AbA on root growth (de la Torre et al, 1972).

The problems that exist with the proposed role for AbA acting as a root growth inhibitor are similar to those with the proposed role for IAA acting as a promoter of root growth, i.e. variable and inconsistent inhibition of growth, concentration dependent and species dependent inhibition. This can partially be resolved by the fact that abscisic acid inhibition of root elongation is very species dependent. Thirty-percent inhibition of growth can be achieved in cultured roots of tomato by as little as 0.09 uM of AbA (El-Hinnawy, 1973), but 509 uM AbA is required to achieve the same degree of inhibition in intact roots of the Orla 264 cultivar of maize (Pilet and Chanson, 1981). Even within a single cultivar of maize (LG11), there is wide variation in the amount of AbA reported to be required to achieve the same level of inhibition (from 1.3 to 81 uM) (Rivier and Pilet, 1981). Thus the nature of AbA action on root growth is as controversial as the role of IAA action on root growth.

At the center of the controversy concerning AbA and IAA action in roots is the identity of the hormone
involved in regulation of asymmetric growth during gravitropic curvature. The hormone which controls root growth is the most likely candidate for controlling growth during tropistic curvature. The Cholodny-Went hypothesis (Cholodny, 1926; Went, 1926) was accepted as the first working model for hormonal action during gravitropic curvature. This hypothesis proposed that root gravitropism was controlled by the lateral movement of a plant hormone across a plant organ when oriented horizontally in a gravitational field. In shoot tissues an increased hormone concentration promotes growth on the lower surface inducing upward curvature. In roots, an increased hormone concentration along the lower surface inhibits growth, inducing downward curvature. With the discovery that indole-3-acetic acid was the diffusible plant growth hormone studied by Cholodny and by Went, IAA was proposed as the hormone involved in tropic processes. The high sensitivity of the root to auxin described by Thimann further supported the potential involvement of indole-3-acetic acid in the differential inhibition of growth during root gravitropism.

In 1957, Audus and Brownbridge (1957 A, B) proposed the "special inhibitor" theory for root geotropism (gravitropism). This theory was essentially a modification of the Cholodny-Went hypothesis. A special inhibitor was proposed to replace IAA. They felt a new
theory for root gravitropism was necessary because growth 
was found to decrease on both sides of the root during 
gravitropic curvature and low concentrations of IAA were 
found to promote curvature by promoting the growth rate of 
both sides of the root by the same proportion. The 
Cholodny-Went hypothesis implies that the lower side of a 
root would be inhibited by accumulation of IAA on in lower 
half of a horizontal root undergoing gravitropism and the 
upper surface of the root would grow at the same rate or 
at an accelerated rate due to the lower auxin 
concentration. If IAA is the hormone undergoing 
asymmetric redistribution during gravitropism, application 
of IAA to the lower surface of the root should have a more 
pronounced effect on the lower surface of the root than on 
the upper surface of the root due to the buffering effect 
of the downward flux of auxin. Thus Audus and Brownbridge 
discounted the role of auxin as the inhibitor involved in 
root gravitropism in favor of an unknown, undescribed 
inhibitory factor. With the discovery of AbA, the special 
inhibitor theory evolved into the model currently referred 
to as the "root cap inhibitor" model. This model is 
basically a restatement of the Cholodny-Went hypothesis 
with AbA replacing IAA as the inhibitory hormone. 

In the later refinement of the "root-cap" inhibitor 
model, AbA was proposed to be synthesized in the root cap 
and transported to the elongation zone asymmetrically,
with higher concentrations of AbA along the lower surface of the root. AbA has been identified in the root cap (Rivier et al, 1977) as has IAA (Rivier and Pilet, 1974; Feldman, 1980). Though both IAA and AbA have been identified in root tissue, only circumstantial evidence exists concerning the possible synthesis of either in roots. Abscisic acid and its metabolite xanthoxin have been found to decrease in the subapical 3-cm region of roots within 8 hr after decapitation (Bottger, 1978 A and B). Similar studies have suggested that IAA may be synthesized in actively dividing meristems (Davidson, 1960 and 1961).

It has been suggested by some researchers that the AbA and/or the IAA which is involved in the development of gravitropic curvature is synthesized in the root. However, the presence of IAA (Kirk and Jacobson, 1968; Scott and Wilkins, 1969; Aasheim and Iverson, 1971) and AbA (Hartung and Behl, 1974; Hartung, 1976) in roots can be accounted for by the acropetal transport of the hormones. There is little evidence for basipetal movement of either IAA (Davies et al, 1976) or AbA (Hartung, 1976). When basipetal movement of labeled IAA or AbA away from the root tip have been reported, positive identification of the radioactive hormone after it moves from the tip to the elongation zone has not been achieved.
The establishment of lateral gradients of either hormone in the elongation zone is highly debatable. Supporters of the "root cap inhibitor" model tend to find AbA accumulation along the lower surface of the root (Keeble et al, 1931; Wilkins et al, 1970; Shaw and Wilkins, 1973; Pilet, 1974, 1976). Other researchers find the opposite with higher levels of AbA along the top of horizontally oriented roots (Suzuki et al, 1979; Hartung, 1976).

There have been several studies of IAA redistribution in roots. Some studies have found that IAA accumulates along the lower surface of the root (Davies et al, 1976). Others have found little asymmetry in IAA distribution in roots undergoing gravitropic stimulation (Most, 1962) or have found IAA at higher concentrations on the upper surface of the root (Lahira, 1968).

The current state of knowledge concerning the effect of plant growth hormones on growth and their involvement in the gravitropic response has many unresolved questions. How does IAA affect root growth? Is ethylene involved in auxin-induced inhibition of growth? Do growth promoting concentrations of auxin induce proton pumping in roots thus acting initially through the mechanism proposed by the acid-growth hypothesis? Is auxin the only hormone which acts through the mechanism proposed by the acid-growth hypothesis? Is abscisic acid promotive,
inhibitory or ineffective in altering growth of roots?
Which hormone, IAA or AbA or both, is involved in inducing asymmetric growth required for gravitropic curvature of roots?

The purpose of the research reported in this dissertation was to test these questions using new high-resolution techniques to measure growth and hydrogen ion efflux of roots in response to: 1) the hormones indole-3-acetic acid, abscisic acid, and ethylene, 2) antagonists of auxin action and auxin transport, 3) inhibition of ethylene biosynthesis and 4) gravitropic modification of growth patterns.

**Plant Material.** Grains of maize, *Zea mays* L. cv. Bear Hybrid WF9x38 (Customaize, Momence, IL), cv. LG11 (Sica LG Services, Riom, France), cv. Orla (Association Selection Suisse, Lausanne, Switzerland), sunflower, *Helianthus annuus* L. cv. Russian (Boulevard Gardens, Columbus, Ohio), peas, *Pisum sativum* L. cv. Alaska (Green Thumb Garden Center, Springdale, ARK), and okra, *Abelmoschus esculentus* L. Moench cv. Clemson Spineless (Green Thumb Garden Center, Springdale, ARK) were soaked in aerated tapwater for 8 hr (the water changed each hr for sunflower and okra) and placed between wet (demineralized water) paper towels between opaque plastic trays (24 x 32 x 1.5 cm; length, width, height). The seeds were germinated at room temperature (20-24 C) under fluorescent room lighting (intensity approximately 175 uE/m²'s). Although the trays were kept under laboratory lighting, the seedlings received little light and were etiolated. The seedlings were used 3.5 days after planting when the primary roots were approximately 1.5 cm long.
In experiments using dark grown seedlings, the seed was soaked in the dark using the procedure described above, and planted on clear plastic trays between wet (demineralized water) paper towels. They were kept in the dark at 26°C for 24 hr before exposure to fluorescent "ruby red" lamps (F20T12-R; General Electric, Lexington, KY) for 8 hr (intensity about 0.06 uE/m²·s). They were returned to the dark and used for experiments 3.5 days after planting.

**Measurement of Elongation.** Root elongation was measured using an auxanometer system (Evans 1976, Muley et al 1982). The seedling was mounted vertically in a Plexiglas chamber with the root immersed in a modified Hoagland's solution (1.5 mM Ca(NO₃)₂; 1 mM each MgSO₄, KH₂PO₄, KNO₃; 20 μM H₃BO₃; 3.8 μM ZnCl₂; 0.18 μM MoO₃; 0.14 μM CaCl₂; pH 6.4) which was continuously oxygenated with pure oxygen. The auxanometer utilizes a Metripak model 33-04 rotary variable differential transformer (Gould, Cleveland, OH). The analog voltage from the transformer was directed both to a recorder and, after analog-to-digital conversion of the signal, to a PCS-42 microcomputer (IMSAI, San Leandro, CAL). Using the microcomputer system, length measurements were made at the rate of 500 per sec with a resolution of +/- 2 μm. The microcomputer-based growth rate was calculated based on the relative change in length over 20 sec time periods.
Measurement of Proton Efflux. Hydrogen ion influx and efflux was measured using 60 1-cm apical sections cut from the tips of the primary roots. The root segments were placed in a glass vial (63 mm height, 17 mm diameter, total volume 17 ml) containing 3.5 ml of 1mM KH$_2$PO$_4$/K$_2$HPO$_4$ buffer (initial pH 6.3). A semi-micro combination pH electrode (No. 2885; Markson Science, Del Mar, CA) connected to a Corning Model 7 pH meter (Corning Scientific Instruments, Medfield, MA) was used to monitor the pH. The output from the pH meter was recorded on an SRLG strip chart recorder (Sargent-Welch Scientific, Skokie, IL) at 0.1 mv full-scale sensitivity. Full scale displacement on the chart was calibrated to 1 pH unit using an attenuating back-voltage from a zero suppression source. Using the zero adjust capabilities of the recorder, a pH range of 4.5 to 6.5 could be monitored. The buffer containing the root segments was continuously oxygenated with pure oxygen at the rate of 26 l/h through a 22-gauge (0.4 mm ID) heart syringe needle. The solution was continuously stirred using a Teflon-coated micro-magnetic spin bar (8mm x 1.5 mm) placed at the bottom of the vial. The spin bar was separated from the root segments by a disk of plastic mesh mounted 5 mm from the bottom of the vial. A sleeve of plastic mesh was placed around the semi-micro pH electrode so as to extend
past the electrode tip (approx. 5-7 mm) to prevent direct contact of the electrode with individual root segments and with oxygen bubbles. Test solutions were added directly to the vial using a micropipette and concentrated stock solutions.

A further modification was incorporated in many of the later experiments. The 60 1-cm root segments were cut and placed in 100 ml of 1mM KH$_2$PO$_4$/K$_2$HPO$_4$ buffer (initial pH 6.4) containing 1.0 μM aminoethoxyvinyl glycine (AVG) and 0.1 mM cobalt nitrate (CO) to inhibit ethylene production. The segments were incubated in this solution for 1.5 hr with continuous oxygenation. The sections were then transferred to the small vial containing 3.5 ml of the same solution. The pH electrode was inserted into the vial and the pH was monitored for an additional 1.5 hrs with continuous oxygenation and stirring. After the 3 hr incubation period, the test solution was added to the vial.

**Agar Dye Visualization of Proton Efflux.** A technique for visualization of hydrogen ion influx and efflux was modified from the method of Weisenseel et al (1979). The agar-dye medium upon which the plant material was placed consisted of 0.6% non-nutrient agar (Type IV, Sigma Chemical Co., St.Louis, MO) for root experiments and 0.4% agar for hypocotyl and coleoptile experiments. The
agar-dye medium was prepared by adding the appropriate concentration of agar, 0.71 mM bromocresol purple indicator dye (Sigma Chemical Co., St. Louis, MO.) and inorganic nutrients (1.5 mM Ca(NO$_3$)$_2$; 1 mM each MgSO$_4$, KH$_2$PO$_4$, KNO$_3$; 20 uM H$_3$BO$_3$; 3.8 uM ZnCl$_2$; 0.18 uM MoO$_3$; 0.14 uM CuCl$_2$) in distilled water and adjusting the solution to pH 5.0 with 0.1 N HCl. The solution was boiled for 5 min and the pH rechecked. The agar-dye was poured to a depth of approximately 4 mm in 100 x 15 mm disposable Petri plates (15 ml per plate). The plates were cooled to room temperature (20-24 C), and the seedling was placed on the surface of the agar with the organ to be observed embedded so that half to two-thirds of the circumference was in contact with the agar-dye medium. Prior to placement on the agar-dye medium, coleoptiles and hypocotyls were abraded with mirror grinding abrasive (No. 40016, Edmund Scientific, Barrington, NJ) and rinsed with distilled water to remove the cuticle. Proton efflux in roots was observable without abrasion. The plates were oriented as appropriate for the particular experiment and photographed at intervals using 35 mm color slide film (Ektachrome, Eastman Kodak, Rochester, NY).

The bromocresol purple indicator dye exhibits color changes over the pH range of 3.5 to 10. It is yellow from pH 3.5 to 4.8, orange from pH 4.8 to approximately 5.5,
red from pH 5.5 to 6.4, and increasingly darker red to violet from pH 6.4 to 10. To obtain optimum visualization of the color patterns, the plates were viewed and photographed on a light box. The initial color pattern was apparent within 3 to 5 minutes and reached full intensity in vertical roots within 45 to 60 min.

**Cinemagraphic Techniques.** The time course and pattern of gravitropism was determined using time lapse super-8-mm films. A super-8-mm camera (Yashica SU-60 E) with a 1:1.8, f 8-48 mm zoom lens was controlled by an air-driven electronic timer. The film was Ektachrome 7244, Type A (Eastman-Kodak, Rochester, NY). Seedlings were mounted inside a closed Plexiglas chamber (37x41x52 cm; width, depth, height) with an access port for the camera lens. The humidity in the chamber was maintained at 100% by a distilled water mist aspirator and a circulating fan. Humidity levels below 100% saturation resulted in irregular growth and poor gravitropic response. The chamber temperature was maintained at 20 +/- 2 C. The chamber was illuminated by a white fluorescent light box (cool white, F20T12-CW; General Electric, Cleveland, OH) at an intensity of 6.9 μE m⁻²·s⁻¹.

**Measurement of Ethylene Production.** The ethylene level within root tissue under the various treatment regimes was
measured using the vacuum extraction method of Beyer and Morgan (1970). Twenty grams of root tissue was placed in a cheese cloth bag under an inverted funnel topped with a graduated 15 ml conical centrifuge tube. This was placed inside a Plexiglas extraction chamber (31x10 cm; height, internal diameter) which was filled with degassed, saturated ammonium sulfate solution. A vacuum of 25 inches of mercury was pulled for 3 min. The gas samples were analysed using a Hewlett Packard Model 5750 gas chromatograph (Hewlett Packard, Palo Alto, CA) with a 1.83 m x 0.318 cm ID alumina-packed copper column. The column was maintained in the isothermal mode at 100 C with the injection port and flame ionization detector temperature at 110 C. Nitrogen was used as the carrier gas at a flow rate of 3.5 cc/min.

To measure ethylene evolution in root segments pretreated with test chemicals, 80 1-cm apical root segments were cut and immediately placed into a 15 ml glass vial containing 3 ml of 1 mM KH₂PO₄/K₂HPO₄ buffer (initial pH 6.4) with or without the test chemical. The vial was purged with pure oxygen and sealed with a septum. The vial was incubated at 24 C for 3 hr. Gas samples were removed from the air space over the tissue samples in the enclosed vials and analysed by gas chromatography as previous described.
**Chemicals.** Aminoethoxyvinyl glycine (AVG) was obtained through the courtesy of Dr. G. Lee Benson, Maag Agrochemicals, HRL Sciences Inc., Vero Beach, FL, and from samples provided by Dr. Richard Gladon, Department of Horticulture, Iowa State, University, Ames, IA. Indole acetic acid (IAA), colbalt nitrate (Co), bromocresol purple, L-canaline, mimosine, silver nitrate, sodium thiosulfate, a and b naphthylacetic acid (NAA), 2-indole carboxylic acid (ICA), MES buffer (2(N-morpholino)ethane sulfonic acid) and para-chlorophenoxy isosobutyric acid (PCIB) were purchased from Sigma Chemical Co., St. Louis, MO. Abscisic acid (+/- cis,trans) (AbA) was purchased from Sigma Chemical Co and from Calbiochem-Behring Corp, LaJolla, CA. TIBA (3,4,5,-triiodobenzoic acid) was purchased from Polysciences Inc, Warrington, PN. Naphthylphthalamic acid (NPA) was purchased from Pfaltz and Bauer, Stamford, Conn. The morphactins, 9-fluorene carboxylic acid (9-FCA) and 9-hyroxy-9-fluorene carboxylic acid (9-HFCA), were purchased from Aldrich Chemical Co, Milwaukee, WI. DPX-1840 was a gift from Dr. Elmo Beyer, Jr., Dupont Experimental Station, Wilmington, DL. Sodium orthovanadate was a gift from Dr. Robert Saftner, USDA, Beltsville, Maryland. Bromoxynil phenol and bromoxynil octanoate were gifts from Rhodia Chemical Co.
Silver thiosulphate was prepared with silver nitrate and sodium thiosulphate. Sodium thiosulphate was dissolved in distilled water at eight times the final concentration of silver nitrate. After the sodium thiosulphate was completely dissolved, the silver nitrate was added very slowly with continuous, vigorous stirring. The solution turned brown but did not precipitate if the silver nitrate was added slowly. The resulting silver thiosulphate solution was used immediately.
Chapter III. Auxin Action on Proton Influx in Maize Roots and its Correlation with Growth.

Introduction

Increasing evidence exists suggesting that auxin regulation of the initial phase of growth in stems and coleoptiles is mediated by modification of hydrogen ion fluxes from the cytoplasm to the cell wall (Rayle and Cleland, 1977). The accumulating evidence concerning stem and coleoptile tissue poses the question of the role of hydrogen ion secretion in auxin-regulated growth in other tissues.

The growth of roots is known to be affected by auxin (see Chapter I). Concentrations of indole-3-acetic acid greater than 0.1 nM have been reported to be inhibitory to root growth. Since root growth is promoted by low pH and inhibited by high pH (Edwards and Scott, 1974; Evans 1976), auxin action on root growth may involve hormonal-induced alteration in wall pH.

Maloney and coworkers (1979) found that the "anti-auxin" p-chlorophenoxy isobutyric acid (PCIB)
stimulated elongation of corn roots while inducing proton efflux into the bathing solution. The "anti-auxin" PCIB is thought to act by reducing the effective level of auxin in the tissue through antagonism of auxin action. The action of PCIB in stimulating growth and hydrogen ion secretion in root tissue supports the hypothesis that auxin may influence cell wall pH. These findings support the long-standing hypothesis that auxin action is similar in root tissue and shoot tissue (Thimann, 1937).

If auxin regulation of growth is mediated by cell wall pH, growth promoting concentrations of auxin should decrease wall pH while growth inhibitory levels of auxin should cause an increase in wall pH. McBride and Evans (1977) were able to detect very weak, transient shifts in the pH of the external medium surrounding lentil roots upon application of IAA.

In this chapter I will report findings of experiments designed to determine the kinetics of indole-3-acetic acid action on root elongation and on hydrogen ion secretion from roots. These findings indicate that roots are extremely sensitive to applied auxin. Exogenous auxin at concentrations greater than $10^{-9}$ M inhibited root growth. Concentrations of auxin which inhibited root growth induced hydrogen ion uptake from the surrounding medium. This finding is consistent with the acid growth hypothesis.
Materials and Methods

For a detailed description of the materials and methods used, consult Chapter II.

Results

Auxin concentrations of $10^{-10}$ M to $10^{-6}$ M added to intact roots of Zea mays (cv WF9x38MS) resulted in either no change or in inhibition of the rate of elongation of the roots (Fig. 1). Concentrations of less than $10^{-10}$ M IAA were neither promotive nor inhibitory to root growth (similar results were obtained with $10^{-11}$ M and $10^{-12}$ M auxin). Very slight but not significant inhibition of growth was observed with $10^{-9}$ M IAA. Treatment with concentrations of IAA greater than $10^{-9}$ M resulted in increasing inhibition of the rate of elongation. At $10^{-6}$ M IAA and greater ($10^{-4}$ M and $10^{-5}$ M not shown) application of the hormone inhibited root growth by 90% within one hour.

According to the acid growth hypothesis, auxin at concentrations which promote elongation should promote $H^+$ efflux if auxin acts in a manner comparable to its action in stems and hypocotyls. Since no IAA-induced promotion of root growth was observed in roots it was not possible to test the correlation between growth promotion and
Figure 1.

**Dosage Response Curve for IAA Action on the Elongation of Intact Primary Roots of Maize.**

Intact primary roots of maize were exposed to concentrations of $10^{-6}$ to $10^{-10}$ M IAA. Growth was monitored using the auxanometer system. Elongation of treated roots was compared to the elongation rate of control roots and plotted as % of the control rate. Standard deviations are indicated for each concentration using a minimum of 5 roots per treatment.
Figure 1.
hydrogen ion efflux in these early experiments. However, the correlation between growth inhibition and hydrogen ion movement was examined using the higher range of auxin concentrations. Figure 2 (top) illustrates the effect of a growth inhibiting concentration of auxin (2 uM) growth and hydrogen ion movement in roots. Inhibition of growth upon application of 2 uM IAA began with a lag of approximately 11 min and was maximal after 40 min. Similar results were obtained using the synthetic auxin a-NAA (2 uM) except that maximal inhibition occurred after 75 min (Fig. 3, top). When 2 uM IAA was added to excised root tips, the external medium became alkaline after an average lag of 8.6 min (Table 1 and Fig. 1, bottom). The pH of the bathing medium increased by an average of 3.6 pH units within 69 min before becoming constant. This higher pH value was maintained for 80 min before decreasing over the subsequent three hour period to a value slightly above the original pH. Almost identical results were obtained with the active synthetic auxin a-NAA, except that the alkaline pH once established was maintained (Table 1 and Fig 3, bottom) at about pH 6.15 for over 14 hours (the longest period monitored).

Inactive auxin analogs should not affect root growth or H+ efflux/influx if the responses observed with the active auxins, IAA and a-NAA, are specific responses resulting from the auxin activity of the compounds.
Figure 2.

Effect of IAA on Growth and Hydrogen Ion Influx in Primary Roots of Maize.

Top: The effect on elongation of 2 uM IAA added to the medium surrounding an intact primary root of maize as measured using the auxanometer system. Bottom: The effect of 2 uM IAA on the external pH of 60 1-cm excised apical corn root segments placed in a 3-ml volume of phosphate buffer.
Figure 2.
Figure 3.

Effect of \( \alpha \)– and \( \beta \)– NAA on Growth and Hydrogen Ion Influx in Maize Roots.

Top: The effect of 2 \( \mu \)M \( \alpha \)– and \( \beta \)–NAA on growth of intact maize roots when added to the medium surrounding the root tissue. Bottom: The effect of 2 \( \mu \)M \( \alpha \)– and \( \beta \)–NAA on the external pH surrounding 60 1-cm excised apical primary root segments placed in a small volume of phosphate buffer.
Figure 3.
Table 1.

**IAA- and NAA- Induced pH Changes in Media Containing Excised Corn Root Tips.**

Duration refers to the time between addition of the hormone and when the final (higher) pH was reached. Latent period refers to the time between addition of the hormone and the initiation of the pH change. All values are averages from 12 experiments with standard deviations indicated. The hormone concentration was 2 uM.
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Latent period (min)</th>
<th>Initial pH</th>
<th>Final pH</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>8.6 ± 2.2</td>
<td>5.95 ± 0.10</td>
<td>6.3 ± 0.12</td>
<td>68.8 ± 14.3</td>
</tr>
<tr>
<td>α-NAA</td>
<td>5.8 ± 1.6</td>
<td>5.76 ± 0.14</td>
<td>6.15 ± 0.17</td>
<td>67.7 ± 20</td>
</tr>
</tbody>
</table>
Figure 3 compares the effect of the active synthetic auxin a-NAA, to that of its inactive position isomer, b-NAA. The inactive b-NAA did not affect growth or H+ uptake in corn roots. Identical results were obtained using the inactive structural relative of IAA, indole-2-carboxylic acid (data not shown).

Discussion

The results are consistent with previously reported data which demonstrated that auxin does not promote root elongation. IAA and a-NAA inhibited root elongation at relatively high concentrations (greater than $10^{-9}$ M) while having no effect on elongation at lower concentrations.

Root segments treated with inhibitory auxin concentrations caused alkalinization of the medium bathing the segments, indicating that growth-inhibitory levels of auxin may cause hydrogen ion influx from the free space into the cytoplasm. This increase in pH of the external medium was observed only in response to active auxins. The inactive auxin analogs, b-NAA and indole-2-carboxylic acid, had no effect on elongation or on the pH of the external medium.

In an earlier study from this laboratory utilizing lentil roots (McBride and Evans, 1977) hydrogen ion uptake
induced by growth-inhibitory concentrations of auxin was not observed. The failure to observe hydrogen ion influx in these experiments may have been because the root segments were not oxygenated. Evans and Vesper (1980) later found that oxygenation enhances auxin-induced acid efflux in stem tissue by at least 2 fold. Oxygenation apparently plays a major role in increasing the magnitude of IAA-induced hydrogen ion movement in plant tissues.

This data correlating auxin-induced inhibition of growth and induction of hydrogen ion influx suggests that auxin induced changes in wall pH are linked to auxin effects on root growth. The hormone-induced increase in external pH occurred with an average latent period significantly shorter than the average latent period for hormone-induced growth inhibition.

The inability to promote root elongation with indole-3-acetic acid prohibited the determination of whether auxin-induced stimulation of root growth is accompanied by a decrease in the external pH as predicted by the acid-growth hypothesis. Attempts at promoting the growth of intact roots by auxin yielded only minor and inconsistent success. Proton efflux was occasionally observed, but since elongation and secretion could not be monitored in the same tissue, it was difficult to correlate these observations.
The exact nature and role of the external pH changes occurring in response to auxin are difficult to interpret. The hydrogen ion concentration external to the wall should be similar to the wall pH, and this, along with the observation that growth rates are strongly dependent on wall pH, indicates that auxin-induced shifts in wall acidity may be crucial to the control of elongation by the hormone. However, the data presented are correlative in nature, and do not prove a cause/effect relationship between auxin-induced modification of wall pH and modification of growth rates. The possibility remains that hydrogen ion movement in roots may be a phenomenon which accompanies but does not play a causal role in hormone-induced root growth. In order to further investigate the correlation between wall pH, growth rates, and auxin action the following sets of experiments were performed: 1) Alteration in the growth pattern of roots were induced by subjecting the roots to gravistimulation, and correlations between shifts in growth pattern and shifts in hydrogen ion efflux were examined (Chapter IV), 2) Ethylene biosynthesis inhibitors were used to allow expression of auxin-promoted growth in roots, and correlated enhancement of hydrogen efflux by the hormone was examined (Chapter V).
Chapter IV. Induction of Asymmetric Hydrogen Ion Secretion From Roots of Maize By Gravistimulation or Chemical Treatment.

Introduction

Considerable evidence exists (see Chapter I and III) that auxin-induced growth responses in plant tissues are controlled by acid movement as proposed by the acid-growth hypothesis (Rayle and Cleland, 1977). This hypothesis has been questioned because much of the evidence in favor of the hypothesis was obtained using excised tissue segments. In stem and coleoptile tissue, the surface cells and cuticle were removed to facilitate hydrogen ion movement. Both excision and abrasion to remove surface cells and cuticle are capable of inducing wound alterations in the tissue.

To avoid wound alterations and damage to tissues, a pH indicator dye bromcresol purple can be used to detect surface pH changes (Weisenseel et al., 1979) thus eliminating the need to excise tissue segments to measure hydrogen ion efflux. Using the pH indicator dye method,
qualitative changes in pH pattern can be monitored in intact unaltered root tissue. This indicator-dye system can be used to determine the effect of hormonally and environmentally induced changes in the pH pattern along the surface of the intact unaltered root.

Since wall pH is strongly implicated in regulating root growth, one would expect to see acid efflux along the rapidly growing regions of the root. In non-growing regions, no acid efflux should occur. If the acid growth hypothesis is correct, the elongation zone should secrete acid and no acid efflux should occur from the regions apical to or basal to the elongation zone. When the growth of roots is inhibited by high concentrations of auxin, inhibition or reversal of hydrogen ion efflux should occur within the elongation zone (see Chapter 3).

If acid efflux patterns are correlated with hormonally regulated growth patterns in plant organs, any modification of growth should induce a subsequent modification in the hydrogen ion secretion pattern surrounding the plant organ. Alteration of growth by gravitropic stimulation should also produce a modified proton secretion pattern. If the Cholodny/Went hypothesis of gravitropism is correct and auxin moves to the lower surface of gravistimulated roots where it causes inhibition, the lower surface of the root should exhibit reduced or reversed hydrogen ion secretion. The upper
surface of the root should continue to acidify the adjacent medium. Hence, an asymmetric acid secretion pattern should result.

Studies of correlation in the modification of growth and hydrogen ion secretion by growth-regulating chemicals should also help elucidate hormonal involvement in the establishment of asymmetric growth during gravitropism. According to the Cholodny/Went hypothesis, auxin moves laterally across stems and roots toward the lower surface of the organ. This model is supported by reports that inhibitors of auxin transport such as naphthylphthylamatic acid (NPA), 2,3,5-triiodobenzoic acid (TIBA), DPX-1840, and morphactins inhibit gravitropism in roots and in hypocotyls (Gaither, 1975; Gaither and Abeles, 1975). If auxin is involved in root gravitropism, auxin transport inhibitors should block the lateral movement of auxin and the curvature of the root. Since auxin should not accumulate along the lower surface of the root in the presence of these inhibitors, no alteration in the hydrogen ion secretion pattern should occur.

Auxin antagonists such as p-chlorophenoxyisobutryic acid (PCIB) should block auxin action and prevent alteration of the acid secretion pattern around gravitropically stimulated roots. Moloney and coworkers (1979) reported that PCIB stimulated growth of intact maize roots and hydrogen ion secretion from root segments.
Thus PCI8 should increase hydrogen ion secretion from the elongation zone of intact roots if the hydrogen ion secretion is closely tied to growth rate.

If the development of asymmetric hydrogen ion secretion patterns is involved in growth and gravitropic curvature, inhibiting hydrogen ion secretion should inhibit both processes. Sodium orthovanadate (vanadate) has been shown to inhibit auxin-induced hydrogen ion secretion and growth in pea epicotyls and coleoptiles (Jacobs and Taiz, 1980). Vanadate inhibition of auxin-induced hydrogen ion secretion should allow separation of auxin- and non-auxin-related hydrogen ion secretion in root tissue.

In this chapter I will report results of experiments which show: 1) growth occurring in the elongation zone of roots is associated with hydrogen ion secretion, 2) asymmetric growth resulting from gravitropic curvature is associated with asymmetric hydrogen ion secretion, and 3) inhibitors of auxin transport and of hydrogen ion secretion inhibit gravitropic curvature and asymmetric hydrogen ion secretion.
Materials and Methods

For a detailed description of the materials and methods used, consult Chapter II.

Results

When corn seedling were placed on agar-dye plates to allow visualization of surface pH patterns using the method of Weisensel et al (1979), the color pattern along the surface of the root developed within 3 to 8 min (Plate I, A). The agar-dye medium along the surface of the root in a position corresponding to the elongation zone developed a bright yellow appearance indicating acidification below pH 4.8. In contrast, a dark red color developed in the region behind the elongation zone (the maturation zone) and around the meristematic region at the tip on the root, indicating an increase to a pH above pH 6.4 in these regions. Thus, the actively growing region (the elongation zone) appeared to secrete acid while the non-growing regions (the meristematic and mature zones) apparently took up acid.

Two exceptions to this pattern are worth noting. A small circular yellow zone was frequently observed at the tip of the root and a yellow zone often appeared in the
Plate 1.

**Surface pH Patterns Along the Primary Root of Maize.**

The surface pH pattern along the primary root of maize is shown 2 hr after placing the root on the agar-indicator dye medium in the (A) absence and (B) presence of 2 μM IAA. Regions of acidic pH from pH 3.5 to 4.8 are yellow. Orange regions represent pH from 4.8 to approximately 5.5 while red regions are areas of pH greater than pH 5.5. Under normal conditions (A) there are regions of acid secretion in the elongation zone and in the area of root hair growth. When root growth was inhibited with 2 μM IAA (B) there is no acid secretion in the elongation zone. Instead, the agar dye in the region surrounding the elongation zone became red indicating acid influx into the elongation zone. Root hairs, which are not inhibited by 2 μM IAA, continued to elongate and secrete acid producing a small yellow region in the maturation zone (B).
Plate 1.
apical region of the maturation zone. The yellow zone at
the root tip may have resulted from secretion by the root
cap of slime containing acidic polysaccharides. An
alternative explanation was that the localized zone of
hydrogen ion efflux is due to the elongation of root cap
cells. The zone of yellow in the apical portion of the
maturation zone may have resulted from acid secretion from
elongating root hairs formed in this region.

When the root was placed on the agar-dye medium
containing growth inhibiting concentrations of IAA, the
growth of the root was severely retarded. In Plate 1B, it
is apparent that 2uM IAA inhibited the formation of the
yellow region adjacent to the elongation zone. Instead of
secreting acid, the elongation zone appeared to take up
acid, as indicated by the dark red to purple zone
surrounding the normal region of elongation. This is
consistent with the observation that growth-inhibiting
concentrations of auxin caused hydrogen ion movement into
root segments bathed in an aqueous medium (described in
the previous chapter). Thus, inhibition of root
elongation by IAA is accompanied by a reversal of the flow
of hydrogen ions from the elongation zone.

In roots treated with inhibitory concentrations of
IAA there was a zone of apparent hydrogen ion efflux
associated with the region of active root hair elongation.
In the apical region of the zone of maturation, root hairs continued to form and elongate even when exposed to 2μM IAA. Associated with this elongation, was the development of a yellow color in the surrounding agar-dye medium, indicating that acid efflux accompanied the elongation of the root hairs.

Effect of Gravity on Secretion Patterns. *Zea mays* cv. WF9x38MS roots exposed to gravitropic stimulation developed surface pH patterns which appeared to be related to gravity-induced changes in growth patterns. The alteration of surface pH patterns began prior to the onset of curvature. Plate II shows a time course of the changes in the surface color pattern along the primary root of maize placed horizontally on an agar-dye medium. Within two min (Plate II A), a definite region of apparent hydrogen ion secretion developed along the elongation zone. This pattern was comparable to the pattern observed in vertically-oriented roots displaying symmetric hydrogen efflux. After 20 min, prior to the normal onset of curvature, there was extension of the zone of hydrogen ion secretion along the upper surface of the root (Plate II B). Generally this region extended further toward the apex than is observed during straight growth of vertically-oriented roots. This region of asymmetric acid efflux continued to intensify and expand as gravitropic
Plate II.

Sequence of Changes in the Surface pH Patterns of the Primary Root of Maize Exposed to a Gravitropic Stimulus.

Yellow regions on the plate indicate acidic regions from pH 3.5 to 4.8. Orange regions on the plate indicate regions from pH 4.8 to approximately pH 5.5. The red regions are areas in which the pH is greater than pH 5.5. Times elapsed after placing the root on the agar-dye in the horizontal position were (A) 2 min, (B) 20 min, and (C) 120 min. When the root was initially placed in a horizontal position there was symmetric acid (yellow) efflux in the elongation zone (A). After 10 to 20 min the acidification of the medium along the upper surface of the root intensifies and extended toward the tip along the upper root surface, but diminished and withdrew from the tip along the lower surface (B). This increased acidification on the top of the root and decreased acidification on the bottom of the root was followed by gravitropic curvature.
Plate 11.
curvature progressed (Plate II C). The pattern of hydrogen ion efflux extended along the entire upper surface of the curve by 120 min. Secretion along the lower surface of the root was considerably reduced from that along the upper surface and was somewhat more restricted than that occurring from the elongation zone of a vertically-oriented root. After curvature was completed, a symmetric secretion pattern redeveloped around the vertically-aligned root tip (not shown).

Time lapse movies clearly illustrated the sequence of agar-dye color shifts which occurred along the root surface in association with both the development of the asymmetric hydrogen ion efflux during gravitropic curvature and the reestablishment of symmetric hydrogen ion efflux upon the completion of the gravitropic reorientation.

**Effects of Auxin Transport Inhibitors on Secretion Patterns.** Plate III shows the effect of the auxin transport inhibitors 9-fluorene carboxylic acid (9-FCA), DPX 1840, TIBA, and NPA on the development of gravitropic curvature and hydrogen ion efflux in gravi-stimulated roots. Untreated control roots (Plate III A) developed strong gravitropic curvature within 2 hr with a typical asymmetric hydrogen ion efflux pattern. The light areas in the black and white plates indicate areas in which greater
Plate III.

**Effect of Auxin Transport Inhibitors on Gravitropism and Hydrogen Ion Secretion Patterns in Intact Gravistimulated Primary Roots of Maize.**

The normally yellow zones of acidification are represented by the light areas, while the dark areas in the plate indicate red or alkaline regions (acid uptake). A: Control at 2 hr. B: $10^{-5}$M 9-fluorene carboxylic acid at 2 hr. C: $10^{-5}$M DPX-1840 at 18 hr. D: 1mM TIBA at 18 hr. E: 4.86 mM NPA at 18 hr. The times indicated represent the elapsed time between orienting the seedling horizontally and photographing the plate.
hydrogen efflux occurred, i.e. on the upper surface of the root in Plate 3A. The dark areas indicate regions of acid uptake. Due to the limitations of illustrating color pattern changes in shades of grey, it is difficult to see some of the observations which will be discussed. In the presence of the morphactin, 9-FCA (Plate III B, inset Figure 4) or 9-HFCA (inset Figure 5), no asymmetry in the hydrogen ion secretion pattern developed and no gravitropic curvature occurred in roots placed horizontally. These effects apparently were not due to the effect of morphactin on overall growth rate. The morphactin 9-FCA did not inhibit elongation as determined by auxanometer studies (Figure 4). Only a slight transitory inhibition of growth rate occurred during the first 30 min after application of 9-FCA, after which the rate returned to the pre-treatment rate. The morphactin 9-HFCA had no effect on the rate of elongation (Figure 5).

The auxin transport inhibitor DPX-1840 also prevented gravitropic curvature and asymmetric hydrogen ion efflux even when the root was held horizontally for 18 hr (Plate III C). Although DPX-1840 inhibited straight growth by 60% within 120 min (Fig. 6) it is unlikely that this reduction in growth rate accounted for the inhibition of gravitropism. The root shown in Plate III C was held horizontally for 18 hr during which time there was more than enough elongation to allow expression of gravitropic
Effect of the Morphactin 9-Fluorene Carboxylic Acid on Growth and Acid Secretion Patterns in Intact Primary Roots of Maize.

The effect of application of $10^{-5}$ M 9-FCA to the medium surrounding intact roots of maize was depicted. There is a slight transitory inhibition of growth rate within the first 45 min after the application of the morphactin. The inset illustrates the effect of 9-FCA on secretion patterns around the root placed on $10^{-5}$ M 9-FCA/agar-dye medium. A: Secretion pattern 5 min after the root was placed on the agar-dye medium. B: Secretion pattern 120 min after the root was placed on the agar-dye medium. The XXXX represents regions of acid efflux and ///// depicts areas of acid uptake.
Figure 5.

Effect of the Morphactin 9-Hydroxy-9-Fluorene Carboxylic Acid on Growth and Acid Secretion Patterns in Roots of Maize.

The effect of the addition of $10^{-5}$ M 9-HFCA to the medium surrounding intact roots of maize growing in the auxanometer is illustrated. The morphactin had no effect on growth rate. The inset illustrates the effect on the hydrogen ion secretion pattern around the root when placed on $10^{-5}$ M 9-HFCA/agar-dye medium. Inset A depicts the secretion pattern 5 min after the intact root was placed on the agar-dye medium. Inset B illustrates the secretion pattern 120 min after the root was placed on the medium. The XXXX represents regions of acid efflux (lower pH) while /////// depicts areas of acid uptake (higher pH).
Effect of the Auxin Transport Inhibitor DPX-1840 on Growth and Acid Secretion Patterns in Roots of Maize.

The effect of addition of $10^{-5}$ M DPX-1840 to the medium surrounding intact primary roots of maize growing in the auxanometer is illustrated. DPX-1840 inhibited the growth of roots over the initial 15 min with subsequent recovery and promotion of growth for 10 to 15 min. After this promotion the auxin transport inhibitor inhibited growth by 60% within 120 min. The inset illustrates the effect of DPX-1840 at $10^{-5}$ M on the hydrogen ion secretion pattern around the root place on agar-dye medium. Diagrams indicate the hydrogen ion secretion patterns 5 min (A) and 2 hr (B) after placing the seedling on the agar-dye/DPX-1840 medium. The XXXX represents regions of acid efflux (lower pH) and /// represents regions of acid uptake (higher pH).
curvature if it were possible in the presence of DPX-1840. It was also noted that upon application of DPX-1840 there was a transitory inhibition of growth rate followed by a promotion of elongation for 15 to 20 min. The possible significance of this will be discussed later.

DPX-1840 had another striking effect on the gravitropic response of maize roots. Early in the time course, the root developed a definite upward curvature (Figure 6, inset). This upward curvature began approximately 90 min after the root was placed on the agar-dye/DPX-1840 medium. The upward curvature continued for 3 to 4 hr before the root resumed rectilinear growth.

TIBA prevented the development of asymmetric hydrogen ion secretion and curvature of gravistimulated roots: beginning about 15 to 20 min after addition of TIBA to roots growing vertically in an auxanometer the growth rate of the root increased. The growth rate remained high for about 40 min and then began to decrease. By two hours after the addition of the TIBA the elongation rate had declined to about 60% of the control rate (Fig. 7). The root photographed in Plate III D shows that there was no gravitropic curvature of TIBA treated roots even after remaining in a horizontal position for 18 hr.

The auxin transport inhibitor NPA also prevented gravitropic curvature and asymmetric hydrogen ion efflux even when the root was held horizontally for 18 hr.
Figure 7.

Effect of the Auxin Transport Inhibitor TIBA on Growth and Acid secretion Patterns in Intact Primary Roots of Maize.

The effect of $10^{-5}$ M TIBA on the growth of intact roots of maize is illustrated. TIBA promoted growth by about 40% during the first 45 min after application and then inhibition began. After 2 hr, the growth rate had declined to about 60% of the original rate. The inset illustrates the effect of TIBA on the hydrogen ion secretion pattern of the root placed on $10^{-5}$ M TIBA/agar-dye medium. Diagrams indicate the hydrogen ion secretion patterns 5 min (A) and 18 hrs (B) after placing the seedling on the agar dye/TIBA medium. The XXXX indicates areas of acid efflux (lower pH) and ////
indicates areas of acid uptake (higher pH).
Figure 7.

ELONGATION RATE (mm/hr)

TIME IN MIN

10^{-5} TBA

180

300
(Plate III E). Although NPA inhibited the elongation rate of the root by 70% within 90 min (Figure 8) it is unlikely that this reduction in growth rate accounted for the inhibition of gravitropism. The root shown in Plate III E was held horizontally for 18 hr, during which time there was more than enough elongation to allow expression of gravitropic curvature if it were possible in the presence of NPA. In NPA treated roots, there was no apparent asymmetry in acid efflux and no curvature.

Effects of Vanadate on Secretion Patterns. Sodium orthovanadate, which has been shown to inhibit auxin-induced hydrogen ion secretion and growth in pea stem tissue, was found to alter the hydrogen ion secretion pattern of maize roots. As illustrated in Plate IV C, 1 mM sodium orthovanadate caused an extension of the region of hydrogen ion efflux apically to the tip of the root and basally further into the mature region of the root. Although the zone of secretion was extended, the magnitude of the secretion appeared reduced in comparison with control roots. This reduced secretion was apparent 15 min after placing the root on the vanadate/agar dye plate. Vanadate also reduced the growth rate by 80% within 3.5 hr (Figure 9), thus 18 hr were allowed before the photograph in Plate IV C was made in order to allow for growth comparable with that of the control. No asymmetry in the
Figure 8.

Effect of the Auxin Transport Inhibitor NPA on Growth and Acid Efflux Patterns in Intact Primary Roots of Maize.

The effect of $10^{-5}$ M NPA on the growth of maize roots is illustrated. NPA inhibited the elongation rate of the root by 70% within 90 min. The inset illustrates the effect of NPA on acid secretion patterns around roots placed on $10^{-5}$ M NPA/agar-dye medium for 5 min (A) and 18 hr (B). The XXXX indicate regions of acid efflux (lower pH) and //// indicate regions of acid uptake (higher pH).
Figure 8.
Plate IV.

**Effect of 1 mM Vanadate on Gravitropism and Acid Secretion Patterns in Intact Primary Roots of Maize.**

The lighter areas of the black and white photograph indicate areas in which the agar-dye medium became acidified while the dark areas are regions where acid uptake occurred. A: Control at 15 min. B: Control at 2 hr. C: 1mM vanadate at 18 hr. The hydrogen ion secretion pattern of vanadate treated root at 15 min (not shown) was identical to that of the control at 15 min (A).
Plate IV.

A

B

C
Figure 9.

Effect of Sodium Orthovanadate on the Growth and Acid Secretion Patterns of Intact Primary Roots of Maize.

Sodium orthovanadate at 1 mM inhibited growth of maize roots by 80% within 3 hr. The inset illustrates the acid secretion pattern around the root at 5 min (A) and 18 hr (B) after the root was placed on 1 mM sodium orthovanadate/agar-dye medium. The XXXX represents regions of acid efflux (lower pH). No significant alteration of the agar dye pH was seen in 5 min (A).
Figure 9.

Elongation rate (mm/hr) vs. time in min. The graph shows the elongation rate of tissues treated with 1 mM Sodium Orthovanadate at pH 6.0 over time. The elongation rate decreases as time increases from 60 to 300 minutes.

A and B indicate different stages of elongation, with A showing a higher elongation rate than B.

1 cm scale for reference.
acid secretion pattern in the vanadate-treated roots and no gravitropic curvature occurred. This suggests that asymmetric hydrogen ion efflux may be necessary for curvature to occur.

**Effects of PCIB on Hydrogen Ion Secretion Patterns.** The "anti-auxin" PCIB inhibited gravitropic curvature less effectively than vanadate or the auxin transport inhibitors. Low concentrations of PCIB (less than or equal to $10^{-6}$ M) had no effect or a slightly promotive effect on growth. At these low concentrations, curvature developed at a normal or at a slightly accelerated rate. A higher concentration of PCIB ($10^{-5}$ M) reduced the rate of gravitropic curvature (Plate V C and D). PCIB at $10^{-5}$ M promoted growth by about 10 % during the first 30 min of exposure to the "anti-auxin" (Figure 10). After the initial period of stimulation, the rate of elongation returned to the pre-treatment rate. This higher concentration of PCIB reduced the rate of gravitropic curvature and caused a parallel reduction in the rate of development of asymmetry in the acid efflux pattern across the root. Although the rate of curvature and development of acid efflux asymmetry were retarded by PCIB, the magnitude of curvature and degree of acid efflux asymmetry eventually (after 18 hr) attained values comparable to that of control roots.
Plate V.

Effect of PCIB on Gravitropism and Acid Secretion Patterns in Gravi-Stimulated, Intact Primary Roots of Maize.

The light areas in the black and white plate indicate areas of acid secretion while the dark areas represent areas of acid uptake along the root surface. A: Control root at 15 min. B: Control at 2 hr. C: 10^{-5} M PCIB treated root at 2 hr. D: 10^{-5} M PCIB treated root at 18 hr. The 10^{-5} M PCIB treated root at 15 min (not shown) was identical to the control root at 15 min (A).
Effect of the "Anti-Auxin" PCIB on Growth and Acid Secretion Patterns in Intact Primary Roots of Maize.

PCIB at $10^{-5}$ M promoted growth about 10% during the first 30 min of exposure to the "anti-auxin". After the initial stimulation, the rate of elongation returned to the pre-treatment rate. The inset illustrates the effect of PCIB on the hydrogen ion secretion pattern around roots placed on $10^{-5}$M PCIB/agar-dye medium. Diagrams indicate the hydrogen ion secretion patterns 5 min (A) and 6 hr (B) after placing the seedling on the agar-dye/PCIB medium. The XXXX represents regions of acid efflux (decreased pH) and ///// represents regions of acid uptake (increased pH).
Figure 10.
Discussion

The agar-dye method provides a simple means of detecting, on a qualitative basis, regions of acid efflux and influx in growing intact roots. The pattern of hydrogen ion efflux from intact roots may be significant to the acid growth hypothesis and the question of the involvement of hydrogen ion efflux in determining growth patterns in roots. The findings reported in this chapter indicate that hydrogen ion efflux patterns are closely related to growth patterns as would be predicted by the acid growth hypothesis.

Intact seedlings secrete acid into the surrounding medium from rapidly growing regions of the root such as the elongation zone. Non-growing regions do not appear to secrete acid. This could be due to a decreased hydrogen ions secretion or a reversed flux of hydrogen ions from these regions. Indole-3-acetic acid at concentrations which inhibit growth (2μM) inhibits hydrogen ion efflux into the agar-dye medium. These findings indicate that a role for hydrogen ion movement in the growth of relatively undisturbed tissue (no excision or abrasion, minimal handling).

Roots undergoing gravitropic curvature exhibit hydrogen ion secretion patterns which correlate with the alterations in growth patterns which are observed. Prior
to observable curvature, an increase in hydrogen ion efflux is observed in the elongation zone along the upper surface of the intact root. A decrease in hydrogen ion secretion occurs along the lower surface of the root. The magnitude of the asymmetry in acid efflux increases as the root begins to bend in response to gravity. Subsequent to curvature, a symmetric secretion pattern is re-established around the vertically-oriented root tip. Alteration of the hydrogen ion secretion pattern surrounding the root during environmentally-induced alteration of the growth of the intact root precedes visible growth changes. These observations support the acid-growth hypothesis and the proposed link between hydrogen ion secretion and growth.

Compounds which inhibit gravitropic curvature of roots block the development of asymmetric hydrogen ion efflux patterns. Auxin transport inhibitors such as TIBA, NPA, DPX-1840 and morphactins inhibit auxin movement, gravitropic curvature, and the development of asymmetric acid efflux. The auxin antagonist PCIB is effective in inhibiting gravitropic curvature and the development of asymmetric acid efflux. Vanadate inhibits hydrogen ion efflux from plant tissue (Jacobs and Taiz, 1980). I found that vanadate inhibits hydrogen ion efflux in roots and inhibits gravitropic curvature. The effects of these compounds are independent of their effects on growth.
When the growth modifying effects of the compounds are accounted for, these compounds remain effective in inhibiting gravitropic curvature and asymmetric hydrogen ion efflux.

The effects of the auxin transport inhibitors and auxin antagonists on gravitropism support the Cholodny-Went model of gravitropism in roots. The Cholodny-Went model proposes that auxin moves laterally in rectilinear roots and the accumulation of auxin on the lower side of the root inhibits growth in that region, resulting in downward curvature. These studies on the effect on root growth and gravitropism of compounds which block auxin movement or action indicate that auxin is involved in root gravitropism. Differential acid efflux is consistent with the Cholodny-Went hypothesis since the rate of hydrogen ion efflux has been shown to be dependent on auxin concentration (Rayle, 1973; also see Chapter 5). The accumulation of auxin on the lower side of the root at the expense of auxin concentrations on the upper side would lead to greater hydrogen ion efflux on the upper side of the root since the increase in auxin on the lower side of the root would inhibit hydrogen ion secretion and decrease elongation. The alteration of hydrogen ion secretion during gravitropic curvature in a manner consistent with the predictions of the Cholodny-Went hypothesis and the effect of auxin transport inhibitors
and antagonists on hydrogen ion secretion patterns and growth provide strong though indirect evidence that auxin redistribution is necessary for the development of gravitropic curvature in roots as proposed by the Cholodny-Went hypothesis.

In summary, hydrogen ion secretion appears not to be simply a non-growth related anomaly which occurs in the elongation zone. Regions of roots which are undergoing elongation, i.e. the elongation zone, the root cap, and root hairs, exhibit hydrogen ion efflux. Inhibitors of growth such as 2 μM IAA reverse the movement of hydrogen ions. Environmentally-induced changes in growth such as gravicurvature cause predictable changes in the pattern of hydrogen ion efflux, suggesting a strong correlation between growth and hydrogen ion secretion. These findings link growth to hydrogen ion secretion and to the role of auxin in root growth. The ability of auxin transport inhibitors to alter the hydrogen ion secretion pattern around georesponding roots and inhibit gravicurvature supports the role of auxin in root gravitropism as proposed by the Cholodny-Went hypothesis.
Chapter V. **Auxin-Ethylene Interaction in Auxin Regulation of Root Growth in Maize.**

**Introduction**

Since Thimann's (1936, 1937) report of auxin promotion of growth in root tissue, there have been many attempts to demonstrate promotion of growth by auxin in both intact roots and in isolated segments of roots (Chapter I). A factor which complicates the study of auxin action is that auxin rapidly increases ethylene levels in plant tissue (Abeles and Hubenstein, 1964; Sakai and Imaseki, 1971; Kang et al 1971). Indole-3-acetic acid acts upon the enzymatic control of ethylene biosynthesis (Jones and Kende, 1979; Yu and Yang, 1979). Since ethylene is a potent inhibitor of root growth (Zimmerman and Wilcoxon, 1935; Crocker et al, 1935), several groups have proposed that the potential ability of auxin to promote root growth is masked by auxin-induced production of inhibitory levels of ethylene (Chadwick and Burg, 1967 and 1970; Burg and Burg, 1968; Sankhla and Shykla, 1970).
Recently a number of potent inhibitors of ethylene biosynthesis have been reported. The fungal product aminoethoxyvinylglycine (AVG) at low concentrations has been reported to inhibit ethylene production strongly (Owens et al, 1971; Lieberman et al, 1975; Yu and Yang, 1979; Boller and Kende, 1979). At high concentrations, AVG is less specific and it inhibits numerous pyridoxyl phosphate-dependent enzyme reactions (Owens and Wright, 1965; Giovanelli et al, 1971 and 1972). The inhibitory action of AVG on ethylene production results from its interference with the formation of 1-aminocyclopropane 1-carboxylic acid (ACC), the immediate precursor to ethylene (Yu and Yang, 1979; Yu et al, 1979; Yoshii et al, 1980).

Cobalt nitrate also inhibits ethylene biosynthesis (Lau and Yang, 1976; Lau et al, 1977). Cobalt is thought to inhibit the conversion of ACC to ethylene. Since cobalt is a divalent cation which is known to block certain calcium related processes (Schatzmann, 1982), cobalt may also regulate the production of ACC from S-adenosyl methionine (SAM), an enzymatic step which is influenced by calcium (Lieberman and Wang, 1982).

Silver ions are known to inhibit ethylene action (Beyer, 1976 and 1979; Atsmon and Tabbak, 1979). This antagonism was thought to be a result of silver ion action.
at the site of ethylene action (Beyer, 1976). Recently Veen (1982) reported that silver thiosulphate decreases the content of ACC in carnation petal tissue. This suggests that silver may act through the inhibition of the conversion of SAM to ACC as well as at the site of ethylene action.

A variety of other substances have also been reported to inhibit ethylene biosynthesis. Numerous pyridoxyl phosphate enzyme reaction inhibitors, free radical scavengers and uncoupling agents such as l-canaline, mimosine, dinitrophenol and cycloleucine, have been found to inhibit ethylene production (Apelbaum et al, 1981a). Similar effects have been reported for synthetic growth regulators such as ioxynil and bromoxymil (Apelbaum et al, 1981a) and PCIB (Stenlid, 1982). Polyamines such as spermine, spermidine, and putrecine have been found to be potent anti-ethylene agents (Apelbaum et al, 1981b).

The ever expanding variety of ethylene inhibitory agents allows blocking ethylene biosynthesis and/or action at numerous sites prior and subsequent to the action of auxin on ethylene production. If ethylene is involved in auxin-inhibition of growth, inhibition of ethylene biosynthesis should allow determination of the ethylene-independent effects of auxin on root growth. If this allows expression of the growth-promoting action of
auxin on roots, it should then be possible to examine the relationship between hydrogen ion secretion and auxin-induced growth in roots.

In this chapter I will present evidence for auxin-ethylene interaction in the growth of intact roots. I will show that auxin promotes ethylene production in roots and that it is this auxin-induced ethylene which masks the promotive affect of IAA on growth and the associated hydrogen ion secretion.

**Materials and Methods**

Consult Chapter II for a detailed description of the materials and methods.

**Results**

**Effects of Cobalt and AVG on Growth of Intact Roots of Maize.** The effect of IAA on the growth of intact roots was described in Chapter I (see Figure 1). At very low concentrations IAA had no effect on the rate of elongation of intact roots. Higher concentrations of IAA (greater than $10^{-9}$ M) inhibited growth. In order to determine whether IAA-induced ethylene production was responsible
for the lack of growth promotion by low to moderate concentrations of auxin, concentrations of cobalt and AVG were tested to determine levels which would inhibit ethylene production without inhibiting growth.

Figure 11 illustrates the effect of three different concentrations of cobalt on growth of intact primary roots of maize. At high (greater than or equal to $10^{-3}$ M) concentrations, cobalt was found to be inhibitory to growth. After 4 hr, these high concentrations inhibited growth by greater than 90%. At $10^{-4}$ M, cobalt promoted growth slightly (about 10%) beginning within 15 min. Lower concentrations (less than or equal to $10^{-5}$ M) had no significant effect on growth. Cobalt nitrate at $10^{-4}$ M was chosen as the optimal, non-phytotoxic concentration.

The ethylene inhibitor AVG was also tested for its effect on growth (Figure 12). At low concentrations (less than $10^{-7}$ M), AVG had little effect on growth. At $10^{-7}$ M there was a slight promotion of growth (10 to 13%). Concentrations less than $10^{-7}$ M showed no effect. At $10^{-6}$ M, AVG promoted growth of intact roots of maize by 24 to 30% beginning within 15 min. Concentrations of AVG above $10^{-6}$ M inhibited growth. After an initial promotion of 8 to 10%, $10^{-5}$ M AVG slowly inhibited the growth rate until after 4 hr the rate was 15% below that of the control. The concentration of AVG which could be applied to roots without phytotoxic effects was determined to be $10^{-6}$ M.
Figure 11.

Effect of Cobalt on Growth of Intact Primary Roots of Maize.

The effect of $10^{-3}$ to $10^{-5}$ M cobalt nitrate on the growth of intact roots of maize is illustrated. Concentrations of cobalt greater than $10^{-4}$ M were found to be inhibitory to growth. Concentrations of $10^{-4}$ M or less were found to be promotive or to have no effect.
Figure 11

GROWTH RATE (mm/h)

TIME (min)

$\text{Co}^{2+}$

$10^4 M$

$10^5 M$

$10^{-3} M$
Figure 12.

**Effect of AVG on Growth of Intact Primary Roots of Maize.**

The effect of $10^{-5}$ to $10^{-7}$ M AVG on the growth of intact roots of maize is illustrated. Concentrations greater than $10^{-6}$ M were found to be inhibitory to growth after an initial promotion of the growth rate. Concentrations of $10^{-8}$ M or less were found to be promotive or to have no effect on growth.
Figure 12.
Figure 13 illustrates the effect of treating roots with the optimal concentrations of cobalt nitrate (10^{-4} M) and AVG (10^{-6} M) in combination. There was a synergistic effect of the compounds. Cobalt promoted growth by 10% and AVG promoted growth by 24 to 30%. When applied in combination there was a 60% promotion of growth rate.

**Effect of Co/AVG on Auxin-Induced Growth and Hydrogen Ion Secretion.** The effect of IAA on growth of intact roots of maize with and without Co/AVG pretreatment is shown in Figure 14. In non-pretreated roots there was no promotion of growth by any concentration of auxin and inhibition of growth by concentrations greater than 10^{-9} M. When roots were pretreated with Co/AVG for 1 hr prior to exposure to auxin, concentrations of IAA less than 10^{-7} M were found to promote growth above the rate observed in non-pretreated controls. At low concentrations of IAA (e.g. 10^{-10} to 10^{-9} M), root growth was promoted by over 230%.

Since pretreatment of roots with 10^{-4} M cobalt nitrate and 10^{-6} M AVG allowed for promotion of root growth by concentrations of indole-3-acetic acid at concentrations less than 10^{-9} M, this gave an opportunity to test for the involvement of hydrogen ion efflux in auxin-induced elongation of roots. When 1-cm apical root
Figure 13.

**Effect of Cobalt and AVG on Growth of Intact Primary Roots of Maize.**

A comparison of the effect of treating roots with $10^{-4} \text{ M cobalt nitrate, } 10^{-6} \text{ M AVG, or } 10^{-1} \text{ M cobalt nitrate plus } 10^{-6} \text{ AVG is illustrated. The combination of Co and AVG produced a synergistic effect on growth.}
Figure 14.

Concentration Dependence of IAA Action on the Elongation of Intact Roots of Maize With and Without Pretreatment with Co/AVG.

The upper line indicates the effect of IAA on growth of roots pretreated with $10^{-4}$ M cobalt nitrate plus $10^{-8}$ M AVG for 1 hr and then transferred to the indicated concentration of IAA (plus Co/AVG). The elongation rate plotted is that occurring 2 hr after transfer to the IAA-containing medium. The lower line indicates the effect of IAA on the growth of control roots which were held in buffer for 1 hr and then treated with IAA. Growth rates were measured 2 hr after transfer to IAA. The standard error is indicated for a minimum of 6 roots per treatment.
Figure 1.4

IAA CONCENTRATION (M)

ELONGATION RATE AS % CONTROL
segments were pretreated with Co/AVG for 3 hr and then exposed to $10^{-10}$ M IAA, a concentration that strongly promotes growth (Figure 15), strong hydrogen ion secretion occurred. Secretion was observable within 8 to 10 min. Six hours after the addition of IAA the pH had dropped from 6.3 to 3.4. At $10^{-9}$ M IAA, growth and hydrogen ion secretion were promoted as strongly as observed with $10^{-10}$ M IAA (Figure 16, Table 2), but the equilibrium pH reached after 6 hr was higher (pH 4.2 vs 3.4).

At $10^{-8}$ M IAA, growth of Co/AVG pretreated roots was stimulated by 210% and this was accompanied by strong hydrogen ion secretion. There was significantly less growth promotion observed when $10^{-7}$ M IAA was applied to Co/AVG treated roots. At this higher concentration growth was promoted by only 15 to 20% (Figures 17 and 18). The kinetics of the growth response elicited by this auxin concentration ($10^{-7}$ M) revealed an initial inhibition of growth after 12 to 14 min with subsequent adaptation and recovery to a slightly promoted growth rate. The time course of hydrogen ion secretion by Co/AVG pretreated apical root segments exposed to $10^{-7}$ M IAA correlated with the peculiar time course of the growth response observed. Hydrogen ion uptake was observed for 45 to 50 min after the IAA was added. This corresponded closely to the period during which IAA inhibited growth. Similarly, the
Figure 15.

Effect of $10^{-10}$ M IAA on Growth and Hydrogen Ion Secretion of Co/AVG Pretreated Primary Roots of Maize.

Eighty 1-cm apical root segments were cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus $10^{-2}$ M cobalt nitrate and $10^{-8}$ M AVG for 3 hr before the addition of auxin (arrow on the dashed line). The dashed line indicates hydrogen ion secretion from the cut root segments. The solid line indicates the growth rate of a typical intact root pretreated with Co/AVG for 1 hr beginning at the first arrow. IAA ($10^{-10}$ M) was added to the pretreatment solution at the second arrow.
Figure 15.
Figure 16.

Effect of $10^{-9}$ M IAA on Growth and Hydrogen Ion Secretion of Co/AVG Pretreated Primary Roots of Maize.

Eighty 1-cm apical root segments were cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus $10^{-4}$ M cobalt nitrate and $10^{-8}$ M AVG for 3 hr before the addition of auxin (arrow on the dashed line). The dashed line indicates hydrogen ion secretion from the cut root segments. The solid line indicates the growth rate of a typical intact root. The root was pretreated with Co/AVG beginning at the first arrow. IAA ($10^{-9}$ M) was added to the pretreatment solution at the second arrow.
Figure 16.
Table 2.

Concentration Dependence of IAA Action on Growth and Hydrogen Ion Movement in Roots Pretreated with Cobalt and AVG.

- The control rate of the roots is taken as that of roots receiving neither IAA nor Co/AVG pretreatment. The calculation is based on the rate of elongation 2 hr after addition of IAA to pretreated roots compared to the steady rate of elongation prior to the addition of IAA and Co/AVG.

- The initial effect of the addition of $10^{-7}$ M IAA to pretreated roots was to cause an inhibition of growth. The inhibition reached a maximum after approximately 40 min. The growth rate then recovered during the subsequent 45 min and reached a steady rate about 22% higher than the rate in control roots receiving no Co/AVG pretreatment or IAA.

- The pH of the medium surrounding pretreated root section to which $10^{-7}$ M IAA was added increased during the first hr and then decreased. The first value in the pH shift column indicates the rate of pH rise during the initial phase. The second value indicates the rate of pH drop during the second phase.
Table 2.

<table>
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<th>Log of IAA Concentration</th>
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Effect of $10^{-8}$ M IAA on Growth and Hydrogen Ion Secretion of Co/AVG Pretreated Primary Roots of Maize.

Eighty 1-cm apical root segments were cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus $10^{-4}$ M cobalt nitrate and $10^{-8}$ M AVG for 3 hr before the addition of auxin (arrow on the dashed line). The dashed line indicates hydrogen ion secretion from the cut root segments. The solid line indicates the growth rate of a typical intact root. The root was pretreated with Co/AVG beginning at the first arrow. IAA ($10^{-8}$ M) was added to the pretreatment solution at the second arrow.
Figure 17

GROWTH RATE [mm/h] [\text{\text{-}}]

TIME [min]

\begin{align*}
\text{\text{\text{\text{10}}^{14} \text{C}_{\text{O}}^{2+}}} \quad \text{\text{\text{\text{10}}^{16} \text{AVG}}} \\
\text{\text{\text{\text{10}}^{11} \text{IAA}}}
\end{align*}
Effect of $10^{-7}$ M IAA on Growth and Hydrogen Ion Secretion of Co/AVG Pretreated Primary Roots of Maize.

Eighty 1-cm apical root segments were cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus $10^{-4}$ M cobalt nitrate and $10^{-6}$ M AVG for 3 hr before the addition of auxin (arrow on the dashed line). The dashed line indicates changes in the pH of the medium containing the cut root segments. The solid line indicates the growth rate of a typical intact root. The root was pretreated with Co/AVG beginning at the first arrow. IAA ($10^{-7}$ M) was added to the pretreatment solution at the second arrow.
Figure 18.
period during which adaptation and resumption of growth occurred corresponded to the period when hydrogen ion secretion from the apical root segments resumed.

Concentrations of auxin greater than $10^{-6}$ M inhibited root growth and induced proton uptake into apical root segments without an initial period of enhanced growth and hydrogen ion efflux. Indole-3-acetic acid at $10^{-6}$ M (Figure 19) inhibited growth within 12 to 14 min and induced hydrogen ion uptake within 10 to 12 min. There appeared to be a slight adaptation of the roots to this concentration of IAA after 2 hr. With $10^{-5}$ M IAA there was severe inhibition of growth with no significant adaptation by the roots to the high auxin level (Figure 20). The induction of hydrogen ion uptake by high concentrations of IAA occurred in 8 to 10 min and was extremely strong (Table 2).

Effect of Ethylene Biosynthesis Inhibitors on IAA-Induced Ethylene Production. Figure 21 illustrates the effect of Co/AVG pretreatment of intact maize roots on the auxin-induced biosynthesis of ethylene. Roots of intact seedlings were treated with $10^{-4}$ M cobalt nitrate and $10^{-6}$ M AVG for 1 hour and then transferred to solutions containing indole-3-acetic acid concentrations from zero to $10^{-6}$ M. After 2 hr in the auxin solution, the apical 2-cm of the roots was excised and subjected to vacuum
Eighty 1-cm apical root sections were cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus $10^{-4}$ M cobalt nitrate and $10^{-6}$ M AVG for 3 hr before the addition of auxin (arrow on the dashed line). The dashed line indicates hydrogen ion movement from the cut root segments. The solid line indicates the growth rate of a typical, single intact root. The root was pretreated with Co/AVG beginning at the first arrow. IAA ($10^{-6}$ M) was added to the pretreatment solution at the second arrow.
Figure 19
Figure 20.

Effect of $10^{-5}$ M IAA on Growth and Hydrogen Ion Secretion of Co/AVG Pretreated Primary Roots of Maize.

Eighty 1-cm apical root sections were cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus $10^{-4}$ M cobalt nitrate and $10^{-6}$ M AVG for 3 hr before the addition of auxin (arrow on the dashed line). The dashed line indicates hydrogen ion movement from the cut root segments. The solid line indicates the growth rate of a typical intact root. The root was pretreated with Co/AVG beginning at the first arrow. IAA ($10^{-5}$ M) was added to the pretreatment solution at the second arrow.
Figure 20.
Figure 21.

Effect of Pretreatment with Co/AVG on the Endogenous Ethylene Content of Primary Roots in the Presence or Absence of Various Concentrations of IAA.

Roots of intact seedlings were treated with $10^{-4}$ M cobalt nitrate plus $10^{-8}$ M AVG for 1 hr before transferring them to solutions containing IAA at concentrations from zero to $10^{-6}$ M. Two hr after transfer, the apical 2-cm of each root was excised and the segments were pooled. The segments were then vacuum extracted for collection and determination of ethylene by gas chromatography. The experiment was repeated 4 times using 400 roots per sample treatment. Means +/- standard error are shown.
Figure 21.

Comparison of ethylene and IAA concentrations with and without pretreatment.
extracted (see Chapter II). The internal gases from the root tissue were collected and the ethylene content was determined by gas chromatography. Pretreatment of intact roots with Co/AVG reduced apparent ethylene biosynthesis in comparison with that of the control. Indole-3-acetic acid at $10^{-6}$ M doubled the measurable ethylene within roots but this effect of IAA was completely suppressed in roots pretreated with Co/AVG. Surprisingly, roots treated with low levels of IAA ($10^{-10}$ M and $10^{-9}$ M) and not exposed to Co/AVG exhibited slightly lower internal ethylene content than control roots. According to the currently accepted pathway for ethylene biosynthesis, auxin promotes ethylene production. The reduction in ethylene levels in root tissue treated with low concentrations of IAA suggest auxin may affect ethylene biosynthesis through more than one mechanism. This auxin-induced decrease in ethylene production may possibly contribute to the ability of auxin to promote growth. In the presence of Co/AVG, the roots treated with concentrations of IAA which promoted growth exhibited greatly reduced ethylene levels. Pretreatment of roots with Co/AVG reduced auxin-induced ethylene production by concentrations of IAA below $10^{-6}$ M by more than 65%. These concentrations of IAA promoted root growth. The Co/AVG pretreatment of roots reduced auxin-induced
ethylene production in roots treated with $10^{-6}$ M IAA by 15 to 20%. This high concentration of auxin inhibited growth. The break-point in the ability of Co/AVG to substantially inhibit ethylene production in the presence of auxin and in the ability of auxin to promote growth in Co/AVG pretreated roots occurred between $10^{-7}$ and $10^{-6}$ M IAA. A similar break-point was found for auxin-induced ethylene inhibition of pea roots. Chadwick and Burg (1967, 1970) determined that auxin-induced ethylene was not responsible for inhibition of pea root elongation at concentrations of IAA greater than or equal to $10^{-6}$ M. They found a close correlation between auxin-induced ethylene production and inhibition of root elongation at auxin concentrations lower than $10^{-6}$ M.

**Effects of Other Inhibitors of Ethylene Biosynthesis and Action on IAA Induced Growth.** Figure 22 illustrates the effect of six different inhibitors of ethylene biosynthesis on the growth of intact maize roots with and without treatment with $10^{-10}$ M IAA. The ethylene biosynthesis inhibitor L-canaline at $10^{-9}$ M stimulated growth to the greatest extent. It stimulated growth by 95% and allowed an additional 120% stimulation upon addition of auxin.
Figure 22.

**Effect of Ethylene-Inhibiting Compounds on IAA-Induced Promotion of Maize Root Elongation.**

Eight compounds which inhibit ethylene-biosynthesis or ethylene action were examined. The action of each compound on elongation and IAA-induced promotion of elongation in intact primary roots of maize is illustrated. The optimal concentration of each compound is shown. Higher concentrations of these compounds were found to inhibit elongation. The open bars signify the elongation rate elicited by the compound when applied alone to intact roots. The hatched bars illustrate the elongation rate of intact roots pretreated with the test compounds for 1 hr and then exposed to 10^{-9} M IAA in the presence of the test compound. Elongation rate is expressed as a percent of the control rate. Elongation rates were determined 2 hr after exposure of the roots to the test compound (1 hr after exposure to IAA). Each bar represents the average of a minimum of 6 roots.
Figure 22.
Silver thiosulphate at $10^{-3}$ M was the second most effective compound in this series, with 65% promotion by silver thiosulfate alone and an addition 75% promotion upon addition of $10^{-10}$ M auxin. Cycloleucine ($10^{-8}$ M) promoted growth by 30% with a 40% increase upon auxin application. Bromoxynil promoted by 40% with an addition 40% increase in growth rate when auxin was applied. Ioxynil promoted growth by 25% with a slight (15%) stimulation by auxin. Mimosine was found to be the least effective ethylene inhibitor of this series as measured by its ability to allow auxin stimulation of root growth. The compound promoted growth by 10% with an additional 15 to 20% promotion by IAA.

The Ability of Ethylene to Inhibit Auxin Induced Growth in Maize Roots Pretreated With Ethylene Biosynthesis Inhibitors. As a final indication that the ability of ethylene biosynthesis inhibitors to allow auxin promotion of root growth results from their ability to suppress ethylene biosynthesis, the ability of applied ethylene to suppress auxin-induced growth in AVG pretreated roots was tested. Figure 23 illustrates the effect on auxin-induced root growth of applying ethylene in the form of its precursor ACC. The roots were pretreated with AVG to block auxin-induced biosynthesis. Cobalt treatment was eliminated since cobalt blocks the conversion of ACC.
Effect of Ethylene on IAA-Induced Elongation of Maize Roots Pretreated With Ethylene-Biosynthesis Inhibitors.

Intact primary roots of maize were pretreated with 10⁻⁶ M AVG for 1 hr and then exposed to 10⁻⁹ M IAA. After 90 min, the ethylene precursor 1-aminocyclopropane 1-carboxylic acid was added at a concentration of 10⁻⁶ M. After an initial transitory promotion of elongation, the growth rate returned to the pre-IAA treatment level. The curve is a representative curve chosen from 10 experiments.
Figure 23
to ethylene. Roots were treated with $10^{-9}$ M IAA. Upon establishment of a steady growth rate, $10^{-6}$ M ACC was added. Suprisingly, the ethylene precursor induced an immediate, transient increase in growth rate. This may have resulted from the uptake of the acidic tricyclic compound since uptake of a dissociable acid is expected to lead to subsequent hydrogen ion pumping as part of the homeostatic mechanism which maintains a constant cytoplasmic pH. Within 20 min, however, inhibition of growth began. Application of $10^{-6}$ M ACC returned the auxin-stimulated growth rate to the pre-auxin treatment rate within 80 min. This indicates that ethylene released from its precursor ACC reverses the promotion of growth by auxin in AVG pretreated roots. The ability of ethylene to inhibit growth to a similar degree as auxin promotes growth in pretreated roots suggests that the promotion of growth by auxin in AVG pretreated roots is due to the inhibition of auxin-induced ethylene biosynthesis by AVG.

**Discussion**

It is commonly suggested that applied indole-3-acetic acid fails to promote growth in intact roots because the internal level of auxin is optimal or supraoptimal so that addition of auxin raises the internal auxin level to a
point which causes inhibition of growth. An alternative explanation which is not mutually exclusive with the previous explanation is that the applied auxin may induce rapid ethylene synthesis. The auxin-induced ethylene can be produced within 20 min of auxin application (Rauser and Horton, 1975) and ethylene is known to be a potent inhibitor of growth. Thus, in some cases the action of auxin-induced ethylene and may account for the failure of auxin to promote growth in root.

The auxin level in root tissue appears to be such that the addition of auxin to intact roots stimulates auxin-induced ethylene production. Therefore even low levels of applied auxin inhibit root elongation. When ethylene biosynthesis is suppressed, $10^{-7}$ auxin concentrations (less than or equal to $10^{-7}$ M) promote root growth. Because, under these special conditions applied auxin can stimulate root growth, the auxin level in normal intact roots appears to be sub-optimal. However, since even low concentrations of auxin appear to induce inhibitory levels of ethylene, roots may be viewed as optimal to supraoptimal in regards to auxin effect on ethylene production.

Pretreating roots with ethylene-biosynthesis inhibitors allows the growth promoting potential of auxin to be expressed. Auxin action on growth in Co/AVG pretreated roots is closely paralleled by auxin effects on
hydrogen ion efflux or uptake. In pretreated roots, low concentrations of auxin (e.g. $10^{-10}$ M) promote growth and cause very strong hydrogen ion efflux. In non-pretreated roots low concentrations of IAA promote neither growth nor hydrogen ion efflux. High concentrations of auxin (e.g. $10^{-6}$ M) inhibit growth and stimulate hydrogen ion uptake in both pretreated and non-pretreated (Chapter III) roots. This implies a direct (non-ethylene dependent) inhibition of growth by high concentrations of IAA. An ethylene-independent inhibition of growth by high concentrations (greater than or equal to $10^{-8}$ M) of IAA was found by Chadwick and Burg (1967, 1970).

The eight ethylene-inhibitors examined here were all effective to varying degrees in allowing auxin promotion of root growth. These compounds were effective at blocking ethylene biosynthesis and reducing the endogenous ethylene content.

The data obtained from measurements of ethylene concentrations in auxin-treated versus control root tissue is puzzling in one respect. The data suggests that low levels of auxin (e.g. $10^{-10}$ M) may actually decrease endogenous ethylene levels. This is unexpected since auxin is known to promote ethylene biosynthesis. The decrease in ethylene production suggests that there may be another site of auxin action which may decrease ethylene biosynthesis. This phenomena may account for the slight
promotion in elongation that occurs occasionally when low concentrations of auxin are applied to intact roots. However, it may be that in order for auxin to promote growth strongly, the ethylene concentration in the root must be below a certain low threshold. Once the ethylene concentration of the root tissue is below the threshold, maximal stimulation of elongation (approximately 250 to 300%) and hydrogen ion secretion occurs.

These observations, together with evidence that acidic buffers can promote root elongation (Edwards and Scott, 1974) and neutral buffers inhibit root growth (Moloney et al., 1981) suggest that the initial, rapid effect of auxin action in intact roots may be mediated by hydrogen ion secretion as proposed by the acid growth hypothesis. This plus the similarity in the latent period of auxin promotion of root (8 to 12 min) and stem (6 to 15 min) growth (Evans, 1976) suggests that auxin may be acting in a similar fashion in both stems and roots.

The fact that, under special conditions, indole-3-acetic acid can consistently promote intact root elongation does not conflict with the Cholodny/Went hypothesis of auxin mediated root gravitropism. The Cholodny-Went hypothesis of root gravitropism proposes that auxin moves laterally across the root and accumulates along the lower surface where the higher auxin concentration inhibits growth. Roots are not normally
exposed to compounds which inhibit ethylene biosynthesis, so it is possible that auxin mediation of gravitropic curvature results from auxin-induced ethylene formation on the lower surface of the rectilinear root. This idea was suggested by Chadwick and Burg (1969) after their study of auxin-induced ethylene formation and the growth of pea roots. They noted that auxin rapidly induced the formation of ethylene in pea roots and that carbon dioxide, which is an ethylene antagonist, decreased gravitropic curvature in pea roots.

Since asymmetric acid efflux plays a role in initiating gravitropic curvature in roots (Chapter IV), one might speculate that auxin redistribution combined with auxin-induced establishment of unequal ethylene concentrations across a horizontal, rectilinear root, mediates the development of differential acid efflux across the root. This in turn may cause the asymmetric growth which results in gravitropic curvature. This would be consistent with the observations that: (a) low levels of auxin stimulate hydrogen ion efflux from roots pretreated with ethylene inhibitors; (b) high levels of IAA inhibit and/or reverse hydrogen ion movement in roots; (c) asymmetric acid efflux, which is most intense on the upper root surface, precedes gravitropic curvature; (d) treatment of the roots with auxin transport inhibitors and antagonists inhibits gravitropism (Chapter IV) and
(e) treatment of roots with acidic solution accelerates their growth and greatly delays their gravitropic response (Strugger, 1932).

The results presented in this chapter provide strong though indirect evidence for a role for auxin and ethylene in the establishment of the asymmetric growth causing gravitropic curvature. However, in much of the recent literature dealing with root gravitropism, emphasis is placed on the potential role of abscisic acid as an inhibitor mediating the gravitropic response of roots. In order to investigate the potential role of AbA in gravitropism, I have studied the action of AbA on growth, gravitropism, and acid efflux in roots. The results are described in the following chapter.
Chapter VI. Kinetics of Abscisic Acid Action on Root Growth and Gravitropism.

Introduction

There is disagreement concerning the hormonal factors involved in the regulation of root growth and gravitropism. According to the Cholodny/Went hypothesis, root growth is under the control of auxin and downward curvature of gravi-stimulated roots results from the accumulation of auxin at growth-inhibiting concentrations on the lower side of rectilinear roots. In 1953, Bennet-Clark and Kefferd suggested that the b-inhibitor complex controlled root growth. Audus and Brownbridge (1957, A and B) proposed the "special inhibitor" model for root gravitropism. This model evolved into the "root cap inhibitor" model which proposes that the inhibitor controlling gravitropism of roots is a component of the b-inhibitor complex.

According to the "root cap inhibitor" model, a growth inhibitor moves from the root cap to the elongation zone where it accumulates on the lower side (Keeble et al., 125)
1931; Wilkins et al, 1970; Shaw and Wilkins, 1973; Pilet, 1974, 1976). Abscisic acid has been suggested to act as the root cap inhibitor involved in this model of root gravitropism (Pilet and Rivier, 1980).

The controversy over the involvement of indole-3-acetic acid and abscisic acid in the control of root growth and gravitropic curvature is complicated by conflicting evidence concerning the effects of these hormones on root elongation. It is generally accepted that indole-3-acetic acid at concentrations greater than 0.1 uM strongly inhibits root growth (Thimann, 1937; Chapter III). However, the evidence for abscisic acid acting as an inhibitor of root elongation is inconsistent (Jackson and Barlow, 1981). Abscisic acid has been reported to inhibit root elongation (Pilet and Chanson, 1981), to have no effect on root elongation (de la Torre et al, 1972) or to promote root elongation (Yamaguchi and Street, 1977; Gaither et al, 1975; Abou-Mandour and Hartung, 1980; Smith and Ho, 1982).

The variability in the reported effects of abscisic acid on root growth weakens the credibility of the "root cap inhibitor" model. On examination of the various reports of abscisic acid action on root growth, one finds considerable differences in the precision, accuracy, and time period of growth measurements. These discrepancies instill bias into the reported effects of abscisic acid.
The effect of abscisic acid and indole-3-acetic acid on the kinetics of growth must be accurately determined. Gravitropic curvature in roots occurs within 2 to 4 hours in normal roots. Hormonal control of the gravitropic curvature must be expressed within this time frame. Accurate kinetics of hormonal action have never been measured over this time period. Most measurements of hormonal effects on elongation have been made using periods of two to several hours between individual measurements of elongation rate.

In this chapter I will present the results of high precision auxanometer studies and cinematography showing the effect of AbA on root elongation and gravitropism. This data demonstrates that AbA promotes root elongation during the time period over which hormonal-induced gravitropic curvature occurs. I will also present evidence showing that promotion of root growth by abscisic acid is accompanied by promotion of hydrogen ion secretion comparable to the hydrogen ion secretion observed during indole-3-acetic acid promotion of growth. The results of the cinematographic examination of the kinetics of gravitropic curvature will indicate that when abscisic acid is placed on the lower surface of a rectilinear root, the response of the root is opposite the response predicted by the "root cap inhibitor" model of root gravitropism.
Materials and Methods

For a indepth description of the methods and materials used in the following experiments, consult Chapter II.

Results

Time Course of AbA Action on Root Elongation. The effect of $10^{-4}$ M AbA on elongation of intact primary roots of maize (cv WF9x38MS) is shown in Figure 24. There was an initial 50% increase in the rate of elongation. After 1-2 hr the promotion slowly began to decay. The elongation rate began to drop sharply approximately 10 hr after exposure to AbA. After 12 hr the rate declined to the initial growth rate and continued to decrease until the rate was less than 50% of the initial rate. Twenty-two to twenty-four hours after AbA treatment the rate began to increase and by 36 hr it had returned to within 20% of the original rate.

Low concentrations of AbA (0.1 uM) induced transitory promotion similar to that induced by the higher level of AbA (Figure 24). A similar decay of the promoted rate occurred with the low AbA concentration. The rate of decay in growth promotion increased approximately 10 hr
Time Course of Abscisic Acid Effects on Root Elongation in Intact Seedlings of Bear-Hybrid Maize.

The effect of two concentrations of abscisic acid (10^{-7} M and 10^{-4} M) on root elongation is illustrated. The indicated concentrations of AbA were added at the arrow to the solution surrounding the intact primary roots. Both concentrations initially stimulated root growth with subsequent inhibition. Each curve is a representative curve for AbA treatment of a single intact root chosen from 8 experiments at each concentration.
Figure 24.

The graph shows the elongation rate (mm/h) over time (h) for two different concentrations of ABA: 10^-7 M ABA and 10^-4 M ABA. The elongation rate decreases significantly upon the addition of ABA, with the 10^-4 M ABA curve showing a more pronounced decrease compared to the 10^-7 M ABA curve.
after AbA was added. This inhibition was less severe (in the rate at which the inhibition developed and the magnitude of the inhibition) than that caused by $10^{-4}$ M AbA. After 22 hr the rate had declined to the initial rate. The triphasic effect of high concentrations of AbA and the biphasic effect of low concentrations of AbA occurred in roots of the maize cultivars Orla and LH11 and in both pea and okra roots (Table 3).

**Concentration Dependence of AbA Action on Root Elongation.**

Figure 25 illustrates the concentration dependence of the transient promotive phase of AbA induced root elongation over the range of $10^{-7}$ to $10^{-3}$ M AbA. All concentrations of AbA promoted growth. There was increasing promotion by AbA concentrations from $10^{-7}$ M to $10^{-4}$ M. In most causes, very high concentrations ($10^{-3}$ M) resulted in less promotion.

**Role of Ethylene in AbA Action on Root Elongation.** The possible involvement of ethylene in AbA action was examined. Ethylene is a potent inhibitor of root growth and is involved in the inhibition of growth in roots exposed to IAA. Figure 25 illustrates the effect of pretreating roots with the ethylene inhibitors cobalt nitrate and AVG on the concentration dependence of AbA action on root growth during the initial promotive phase
Table 3.

Time Dependent Promotion and Inhibition of Root Elongation by AbA.

The concentration and time dependence of AbA action on the growth of intact primary roots of three cultivars of maize and roots of pea and okra are illustrated. The percent promotion or inhibition of elongation in relation to untreated control roots is shown at 4 hr and 24 hr over the range of AbA concentrations from $10^{-7}$ M to $10^{-3}$ M. Each value is the average of a minimum of 6 experiments, each on a single intact primary root.
<table>
<thead>
<tr>
<th>Log concn. ABA (M)</th>
<th>% Promotion (+) or inhibition (−)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maïe, WF 9 x 38</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
</tr>
<tr>
<td>−3</td>
<td>+ 80</td>
</tr>
<tr>
<td>−4</td>
<td>+ 55</td>
</tr>
<tr>
<td>−5</td>
<td>+ 33</td>
</tr>
<tr>
<td>−6</td>
<td>+ 25</td>
</tr>
<tr>
<td>−7</td>
<td>+ 20</td>
</tr>
</tbody>
</table>

*Note: The table shows the percentage of promotion or inhibition at different concentrations and times.
Figure 25.

Concentration Dependence of the Promotive Effect of AbA on Root Elongation in Intact Seedlings of Bear-Hybrid Maize.

Elongation rate is the rate measured 4 hr after application of AbA to the solution surrounding intact primary roots of Bear-hybrid maize. The lower curve (open circles) represents control roots to which AbA was added. The upper curve (closed circles) represents roots pretreated for 2 hr with 0.1 μM aminoethoxyvinylglycine plus 0.1 mM cobalt nitrate (AVG/Co) prior to the addition of AbA to the AVG/Co solution. Each point on the curves represents an average (plus standard error) of a minimum of six experiments, each on a separate intact primary root.
Figure 25.
of AbA action. Roots were pretreated with $10^{-4}$ M cobalt nitrate plus $10^{-6}$ M AVG for 1 hr prior to the addition of AbA to the medium surrounding the root. The Co/AVG pretreatment shifted the curve to slightly higher growth rate values due to the promotion of growth by the pretreatment solution. There was no significant alteration of the ability of AbA to promote growth.

Table 4 shows the ethylene content of vacuum extracted gas samples from roots treated with $10^{-7}$ to $10^{-3}$ M AbA. As the AbA concentration was increased, ethylene content in gas samples extracted from the tissue increased whether measured both 4 hr or 20 hr after the addition of AbA. The increase in ethylene content was extremely large after 20 hr exposure to high concentrations of AbA. Ethylene content was about 5 times that found in untreated root tissue. Although treatment of the roots with Co/AVG decreased the extractable ethylene in AbA-treated roots compared with root not treated with Co/AVG, the ethylene level was still higher (about double) than that of untreated controls.

In addition to ethylene, several other gaseous constituents of the vacuum extracted gas samples were observed to increase under the influence of AbA. Figure 26 illustrates gas chromatograms of roots treated with $10^{-4}$ M AbA for 0 hr, 4 hr, and 20 hr. Numerous peaks
Table 4.

Suppression of AbA-Induced Ethylene Biosynthesis in Maize Roots Treated with Cobalt and AVG.

The concentration and time dependence of AbA effects on ethylene content of roots are illustrated. The ethylene content of roots (nl 1⁻¹) was measured at 4 hr and 24 hrs after exposure to AbA (10⁻⁷ M to 10⁻³ M). The effect of pretreating roots with 10⁻⁴ M cobalt nitrate plus 10⁻⁶ M AVG for 1 hr prior to exposure to AbA is also shown. The values are means and standard errors for 3 experiments with 400 roots per treatment per experiment.
### Table 4.

<table>
<thead>
<tr>
<th>AbA Conc</th>
<th>4 hrs treatments</th>
<th>20 hrs treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>AVG + Co&lt;sup&gt;2+&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;-3&lt;/sup&gt; M</td>
<td>80.3 ± 1.3</td>
<td>47.4 ± 1.7</td>
</tr>
<tr>
<td>10&lt;sup&gt;-4&lt;/sup&gt; M</td>
<td>67.2 ± 1.8</td>
<td>37.0 ± 1.4</td>
</tr>
<tr>
<td>10&lt;sup&gt;-5&lt;/sup&gt; M</td>
<td>64.4 ± 1.5</td>
<td>28.1 ± 1.6</td>
</tr>
<tr>
<td>10&lt;sup&gt;-6&lt;/sup&gt; M</td>
<td>59.3 ± 1.0</td>
<td>21.8 ± 1.4</td>
</tr>
<tr>
<td>10&lt;sup&gt;-7&lt;/sup&gt; M</td>
<td>50.6 ± 1.1</td>
<td>20.6 ± 0.8</td>
</tr>
<tr>
<td>0 M</td>
<td>39.7 ± 1.0</td>
<td>10.0 ± 1.1</td>
</tr>
</tbody>
</table>

**ETHYLENE PRODUCTION (nl/l)**
Figure 26.

Effect of AbA on Volatile Compounds Extractable From Roots of Bear-Hybrid Maize.

Sample gas-chromatography tracings are shown for vacuum extracted gas samples from roots treated with $10^{-4}$ M AbA for 4 hr or 20 hr. The peak labelled (A) is the ethylene peak. Numerous gaseous components were observed after 4 hr of exposure to AbA (middle curve) and after 20 hr of exposure to AbA (top curve). The gas sample from the control measured at 4 hr (not shown) was identical to the sample made at 20 hr (lower curve). The chemical compositions of the non-ethylene peaks have not been identified.
Figure 26

- ABA - 20 hrs
- ABA - 4 hrs
- CONTROL - 20 hrs

Alumina column 6 ft x 1/8 in
FID: Nitrogen carrier gas
Deflector lamp - 170°C
Injection lamp - 170°C
Column lamp - 100°C
developed after exposure to AbA. These peaks represented sizable components of the gas sample but were not identified.

The effect of AbA on ethylene production was reflected in increased lateral swelling of the elongation zone of roots treated with high levels of AbA (Plate VI). Pretreatment of the roots with Co/AVG prior to exposure to high levels of AbA reduced the lateral swelling.

The ethylene-biosynthesis inhibitors AVG and cobalt suppressed AbA-induced ethylene biosynthesis in roots but did not prevent long-term growth inhibition by AbA (Figure 27). This is in contrast to the results obtained with IAA (Chapter V) since suppression of ethylene biosynthesis inhibited the action of moderate levels of the hormone.

Inhibition of ethylene biosynthesis and action extended the initial period of growth promotion induced by AbA slightly and increased the rate of recovery from the inhibitory phase of AbA action. In Co/AVG pretreated roots, the final growth rate in the presence of AbA was the same as the initial pre-AbA treatment rate instead of being 20% less as in non-pretreated roots (Figure 24).

Effect of AbA on Hydrogen Ion Secretion in Apical Root Segments. The effect of AbA on the pH of the medium surrounding 1-cm apical root segments of maize is illustrated in Figure 28. In roots pretreated with Co/AVG
Plate VI.

Interaction of AbA and Inhibitors of Ethylene Biosynthesis on Root Elongation and Swelling in Bear-Hybrid Maize Seedlings.

Intact primary roots (initial length 1.5 cm) were treated for 18 hr in the following solutions: A) 0.1 mM cobalt nitrate plus 0.1 μM AVG; B) control; C) 1 mM AbA plus 0.1 mM cobalt nitrate and 0.1 μM AVG; D) 1 mM AbA. The inset at the bottom of the plate illustrates the root tips aligned to allow comparison of tip swelling 28 h after treatment.
Plate VI.
Effect of 0.1 mM AbA on Root Elongation in Intact Primary Roots of Bear-Hybrid Maize Pretreated With Co/AVG.

At the first arrow both roots were treated with 0.1 uM AVG and 0.1 mM cobalt nitrate. At the second arrow, 0.1 mM AbA was added to one root (solid line). The other root (dashed line) remained in the AVG/Co solution only. Each curve represents treatment of a single intact primary root chosen from a minimum of 6 roots given each treatment.
ELONGATION RATE (mm/h)

Figure 27,
Figure 28.

Promotion of Hydrogen Ion Efflux From Apical Sections of Bear-Hybrid Maize Roots Treated With AbA.

Sixty 1-cm apical root sections were cut and pretreated for 3 hr with 0.1 uM AVG plus 0.1 mM cobalt nitrate. Abscisic acid was added and hydrogen ion movement was monitored. The effect of two concentrations of AbA is illustrated. The solid line shows the effect of 10^-7 M AbA on hydrogen ion efflux. The dashed line shows the effect of 10^-3 M AbA on hydrogen ion efflux. The curves are representative traces of single experiments.
Figure 28.

- ABA

Time in min

pH

330 360 390 420 450 480 520

$10^{-7}$M

$10^{-4}$M
for 3 hr, AbA stimulated hydrogen ion secretion within 5 to 8 min after exposure to exogenously applied AbA. This is prior to promotion of growth (10-14 min). Concentrations of AbA which caused greater increases in growth rate caused stronger hydrogen ion secretion.

**Comparative Effects of Unilateral Application of AbA and IAA on Root Gravitropism.** Using the cinemagraphic techniques described in Chapter II, the timing of gravitropic responses in 3 cultivars of maize, in peas, and in okra roots was compared. Agar blocks containing AbA or IAA were applied to the upper, the lower, or to both surfaces of horizontally oriented roots. Representative results are illustrated in Plate VII. When AbA at $10^{-4}$ M was applied to the upper surface of the maize hybrid WF9x38MS (Plate VII, A1), gravitropism was enhanced relative to the control (Plate VII, A3). These roots exhibited gravitropic curvature which exceeded 90°. This is consistent with the evidence presented indicating that AbA initially promotes root growth. Similar results were obtained with the maize hybrids Orla (Plate VII, B1) and LH11 (Plate VII, C1) as well as with pea (Plate VII, D1) and okra (Plate VII, E1).

When the agar block containing AbA was placed on the lower surface of the root, gravitropic curvature of the root was severely retarded in the three maize hybrids, and
Plate VII.

Comparative effects of Unilateral Application of AbA and IAA on Gravitropism in Intact Primary Roots.

Rows A to C are Maize cultivars, WF9x38MS, Orla, and LH11 respectively. Row D is pea cv. Alaska and row E is okra cv. Clemson spineless. Column 1 illustrates the effect of applying an agar block containing 0.1 mM AbA to the upper surface of the root and a blank agar block to the lower surface of the root. In column 2, the block containing 0.1 mM AbA was applied to the lower surface while a blank agar block was applied to the upper surface. Roots in column 3 received blank agar blocks on both the upper and lower surface of the root. In column 4, agar blocks containing 0.01 mM IAA were placed on the upper surface of the root and blank agar blocks were placed on the lower root surface. Column 5 received blank agar blocks on the upper surface of the root and blocks containing 0.01 mM IAA on the lower root surface. The roots were oriented horizontally immediately after applying the agar (1%) blocks. The photographs are frames from Super-8mm movies of the gravitropic response of the roots at 1 hr after the roots were oriented horizontally.
Plate VII.
also in pea and okra (Plate VII, A2 through E2). In the case of pea roots, treatment of the lower surface of a rectilinear root with AbA induced a substantial upward curvature (Plate VII, D2).

A small upward curvature often occurred in control roots (Plate VIII). This normal pattern of gravitropic curvature occurred in approximately 30% of the roots of the maize hybrid WF9x38MS. This pattern will be designated a Type I pattern (Plate IIIV). A second pattern of curvature, Type II, occurred in about 70% of the roots observed (Plate IX). Type I roots exhibited an initial upward curvature prior to the downward bending of the root. The final curvature of Type I roots was always less than 90°. By comparison, Type II roots exhibited no upward curvature. Type II roots consistently overshoot 90° curvature prior to the completion of the gravitropic response. These two patterns of curvature which are observed in control roots do not account for the initial upward curvature observed in pea roots treated with AbA on the lower root surface or the overshoot of roots past 90° in roots treated with AbA on the upper surface. The abscisic acid-induced responses were more pronounced than the mild upward curvature and the overshoot of 90° observed in control roots. The AbA-induced responses occurred in all roots, not the percentage of roots associated with each type of root response.
Plate VIII.

**Kinetics of Gravitropic Curvature of Roots: Type I.**

A cinematographic time-lapse depiction of the kinetics of curvature of intact primary roots of Bear-hybrid maize seedlings is illustrated. The type I response was signified by an initial upward curvature of the root. This was evident 9 min after the root was placed horizontal. The root then began an oscillatory downward curvature. Roots in this category did not achieve complete 90° curvature. This type of curvature pattern occurred in approximately 30% of the roots observed.
Plate 8.
Plate IX.

**Kinetics of Gravitropic Curvature of Roots: Type II**

A cinemagraphic time-lapse depiction of the kinetics of curvature of intact primary roots of Bear-hybrid maize seedlings is illustrated. The type II response was signified by a downward curvature which occurred more rapidly than observed in Type I roots which responded initially by curving upward. This pattern of root curvature resulted in an over-shoot past $90^\circ$ (85 min) followed by an oscillatory readjustment to $75^\circ$ to $90^\circ$ curvature. This type of response (Type II) occurred in approximately 70% of the roots.
Plate 9.
Abscisic acid promoted growth and increased the rate of gravitropic curvature when placed on the upper surface of the root. Indole-3-acetic acid (10^-5 M) placed on the upper surface of the root strongly retarded gravitropism (Plate VII, A4 to E4). When IAA was placed on the lower surface of the root, strong gravitropism occurred, but the magnitude of the curvature relative to the controls was variable (Plate VII, A5 to E5).

Discussion

This data indicates that the effect of AbA on intact root elongation is concentration dependent and time dependent. Short term experiments demonstrate that both low (e.g. 0.1 uM) and high (e.g. 0.1mM) concentrations of AbA promote root growth. In long-term experiments, the net effect of high concentrations of AbA was inhibitory due to a period of inhibition of root elongation following the initial transient promotion. Lower concentrations elicited only the transient stimulation of elongation with no inhibitory phase. This resulted in a net promotion of root elongation.

A similar bimodal concentration-dependent effect of AbA on root growth was reported by Smith and Ho (1982). They found that low concentrations of AbA promoted root
growth in barley and in maize while high concentrations were inhibitory. My findings differ slightly from theirs since I was able to measure the kinetics of AbA action. The influence of abscisic acid on root growth occurred in three phases: 1) a stimulation of elongation lasting about 12 hr, 2) progressive inhibition of the rate of elongation to a maximum at 20 to 24 hr after treatment with AbA, 3) gradual recovery of the growth rate to the original rate (at low concentrations of AbA) or to a rate slightly less than the original rate (at high concentrations of AbA).

The fact that high concentrations of abscisic acid initially promote and then inhibit root elongation may account for the discrepancies in the effects of AbA on root growth reported in the literature. Gaither et al (1975) reported that AbA stimulated root growth in peas, and I found that the initial promotive phase of AbA action on root elongation is particularly strong in pea roots. In reports of AbA inhibition of root elongation in maize, the measurements were made after 24 hr or more (e.g. Pilet and Chanson, 1981). These time periods would include both the initial promotive phase and the inhibitory phase of AbA action. In Milborrow's (1980) study of short-term (e.g. induction of stomatal closure) and long-term (inhibition of protein synthesis) effects of AbA,
different molecular bases for short- and long-term action of AbA were proposed. This temporal difference in AbA action may also apply to root elongation.

These results confirm other reports of long-term inhibition of root growth by high concentrations of abscisic acid. The maximum inhibition found in this study (approximately 70%) was stronger than reported in most previous publications. This probably resulted from my ability to separate the promotive and inhibitory phases of AbA action. In the long-term measurements used in most previous publications, the inhibition reported was most likely partially offset by the initial enhancement of root elongation by abscisic acid. Using a less sensitive method this initial period of stimulation could not be measured as a separate effect.

The long-term inhibition of root growth by AbA observed in this study does not support the role for AbA proposed by the "root cap inhibitor" model for root gravitropism. Very high concentrations of AbA were required to obtain net inhibition and it is unlikely that concentrations of abscisic acid as high as 0.1 mM are encountered in intact root tissue. The AbA content of maize root tips has been reported to be in the range of 30-60 ug Kg^{-1} fresh weight (Rivier et al, 1977). This is roughly equivalent to 0.2 mM if uniformly distributed.
Asymmetry of abscisic acid distribution during gravitropism has not been reported to be strong (Hartung, 1976; Pilet and Rivier, 1981). Additionally, even very high concentrations of AbA are promotive to root growth during the time period over which gravitropic curvature occurs.

The results from experiments in which AbA was applied asymmetrically to roots placed horizontally also fail to support the proposal that AbA plays a major role as a growth inhibitor governing gravitropism. Application of abscisic acid to the lower surface of rectilinear roots delayed gravitropic curvature while application of AbA to the upper surface of the roots promoted gravitropic curvature. These results are opposite from those which would be predicted by the "root cap inhibitor" model. Since the initial effect of AbA is to promote root growth, abscisic acid movement to the upper surface of horizontally oriented roots would be required to account for gravitropic curvature. This possibility cannot be ruled out. Suzuki et al (1979) reported accumulation of AbA on the upper side of graviresponding maize roots. Similarly, Hartung (1976) found a preferential short-term upward movement of radio-labelled abscisic acid in gravistimulated roots.

Unlike AbA, the action of indole-3-acetic acid on root growth is consistent with its proposed role as a
hormone mediating gravitropism. Indole-3-acetic acid is a potent inhibitor of root growth, possibly through its interaction with ethylene. There is evidence that IAA occurs in root tips (Rivier and Pilet, 1974; Feldman, 1980), and that radioactivity from radio-labeled IAA can move basipetally from the tip of the root (Davies et al, 1976; Pernet and Pilet, 1979) and may accumulate along the lower side of horizontal roots (Konings, 1967).

The data presented in this chapter does not invalidate the "root cap inhibitor" model. Though the evidence indicates that AbA does not play a role as a cap produced inhibitor mediating gravitropism, the possibility remains that some other inhibitor from the cap plays a central role in gravitropism. Suzuki et al (1979) and Feldman (1981) reported an unidentified inhibitor in roots which does not co-chromatograph with AbA. This inhibitor is light induced and is found in roots which require light for gravitropic sensitivity. Since IAA strongly inhibits root growth even at low concentrations, indole-3-acetic acid is a candidate for the root cap inhibitor. Alternatively, a hormone other than AbA or IAA (e.g. ethylene) may be the growth regulating hormone involved in gravitropic responses of roots. Wheeler and Salisbury (1980) presented evidence that establishment of ethylene gradients is important in the gravitropic response of sunflower hypocotyls. In view of the auxin-ethylene
interaction outlined in Chapter IV, ethylene may have a similar role in root gravitropism. Alternatively, gravitropism may be mediated by gravity-induced gradients in sensitivity to one or more endogenous growth regulators and/or hormones (Trewavas, 1981) or by direct effects of cell orientation on elongation as proposed by Digby and Firn (1980).
Chapter VII. Summary

Root growth and graviresponse have been proposed to be mediated by a number of hormonal and non-hormonal factors, e.g. indole-3-acetic acid, ethylene, abscisic acid, calcium. The work presented in this thesis examined the effect three of the plant hormones (indole-3-acetic acid, abscisic acid and ethylene) on the growth and graviresponse roots. High resolution auxanometer and cinemagraphic techniques applied in these studies allowed precise measurement of hormonal effects on both elongation and the graviresponse of roots.

Indole-3-acetic acid was identified as a plant hormone in the late 1930's and was considered to be the hormonal factor mediating root growth and gravitropism. In recent years the role of IAA in root elongation and gravitropism has been discounted largely due to the inability of researchers to demonstrate promotion of root growth by IAA. Using very high resolution automated growth measuring technique, I re-examined the effect of IAA on root growth and found in agreement with much of the earlier work, that indole-3-acetic acid inhibited the
elongation of intact maize roots at concentrations greater than $10^{-9}$ M. Low concentrations of IAA (less than $10^{-9}$ M) had no effect on root elongation. However, in experiments designed to test the potential role of auxin-induced ethylene in the action of auxin on root growth, I found that the inhibition of root growth by IAA apparently results primarily from IAA promotion of the biosynthesis of ethylene, a potent inhibitor of root growth. When roots were pretreated with ethylene biosynthesis inhibitors to block IAA-induced ethylene formation, low concentrations of IAA (less than $10^{-7}$ M) strongly promoted root growth (up to 250%). The IAA-induced promotion of elongation in roots pretreated with ethylene biosynthesis inhibitors was found to be reversible by ethylene. Using this methodology, I was able, for the first time, to obtain consistent promotion of root growth by IAA and therefore to study the various aspects of the promotive action of auxin on root growth.

The promotion of root elongation by IAA was accompanied by strong hydrogen ion secretion from the elongation zone of the root. In fact, I found that the rate of IAA-induced hydrogen ion secretion in pretreated apical root segments was even greater than the rate of IAA-induced hydrogen ion secretion from coleoptile segments reported by other researchers. In related experiments, I found that concentrations of IAA high
enough to inhibit the growth of intact roots promoted hydrogen ion uptake by root segments. The fact concentrations of IAA which stimulate root growth induce hydrogen ion secretion from root segments while growth-inhibitory concentrations of the hormone inhibit or reverse hydrogen ion efflux suggests that the acid growth hypothesis which has been proposed for coleoptiles and hypocotyls is also applicable to roots.

The occurrence of hydrogen ion secretion from roots is not limited to IAA-treated root segments pretreated with ethylene biosynthesis inhibitors. Using a pH indicator dye/agar medium, I found that intact roots secreted hydrogen ions from actively growing regions. The elongation zone and the region in which root hairs were developing secreted acid. Non-growing regions appeared to take up acid from the surrounding agar-dye medium.

Environmentally-induced modification of the growth pattern was found to alter the acid secretion pattern of intact roots growing on the agar-dye medium. When roots were oriented horizontally, the elongation zone along the upper surface of the roots exhibited greater hydrogen ion efflux than the elongation zone along the lower root surface during the ensuing graviresponse. This alteration of the hydrogen ion secretion pattern correlated with changes in the growth rate of the upper and lower root surface. The gravitropically-induced alteration of the
hydrogen ion secretion pattern was blocked by auxin transport inhibitors (NPA, TIBA, DPX-1840 and TIBA) and by the auxin antagonists (PCIB). Both PCIB and the transport inhibitors were found to inhibit gravitropic curvature and the development of the asymmetric hydrogen ion secretion pattern. This suggests that auxin is the hormone involved in graviresponse of roots.

Abscisic acid has been proposed as an inhibitory plant hormone controlling root growth and gravitropism. I found that the influence of abscisic acid on root growth occurred in three phases: 1) a stimulation of elongation lasting about 12 hr, 2) progressive inhibition of the rate of elongation to a maximum at 20 to 24 hr after treatment with AbA, 3) gradual recovery of the growth rate to the original rate (at low AbA concentrations) or to a rate slightly less than the original rate (at high concentrations of AbA). I found that apical root segments pretreated with ethylene biosynthesis inhibitors secreted hydrogen ions into the surrounding medium upon exposure to AbA.

Cinemagraphic studies of horizontally placed roots to which AbA had been asymmetrically applied also failed to support the proposal that AbA plays a major role as a growth inhibitor of roots. Application of abscisic acid to the lower surface of horizontal roots promoted growth of the lower root surface and delayed gravitropic
curvature. When abscisic acid was applied to the upper surface of horizontal roots, gravitropic curvature occurred at an accelerated rate apparently as a result of promotion of elongation along the upper root surface.

My findings argue against the proposal that abscisic acid mediates gravitropism by acting as an inhibitor asymmetrically moving back from the cap of gravistimulated roots (the "root cap inhibitor" model). Inhibition of root elongation occurs only with high concentrations of AbA and only after a substantial lag. This indicates that if AbA is involved in gravitropism it must be acting as a growth promotor in the establishment of asymmetric growth rather than a growth inhibitor.

In summary, I found that low concentrations of indole-3-acetic acid inhibited growth apparently by promotion of ethylene biosynthesis. If auxin-induced ethylene biosynthesis was inhibited, IAA strongly promoted elongation. Abscisic acid influenced root growth in three sequential phases; 1)promotion, 2)inhibition, 3) recovery. The promotion of root elongation by either IAA or AbA was accompanied by the stimulation of hydrogen ion secretion. Since IAA at low concentrations strongly inhibited root growth and since compounds which block auxin action were also found to block gravitropic curvature, indole-3-acetic acid remains as the strongest candidate for the hormonal inhibitor involved in gravitropism.
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