THE REVERSAL OF GIBBERELLIC ACID ENHANCEMENT OF LETTUCE SEEDLING HYPOCOTYL ELONGATION

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THE REVERSAL OF GIBBERELLIC ACID ENHANCEMENT OF LETTUCE SEEDLING HYPOCOTYL ELONGATION

An Abstract

Presented to the

Graduate and Research Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Lisa A. Stokes

August 1985

ABSTRACT

Lettuce seedlings (*Lactuca sativa* var. "Grand Rapids") were studied to determine the effects of antibiotics, glucosamine and vanadate upon the gibberellin enhancement of hypocotyl elongation and upon root growth. The effects of dolichyl phosphate upon root elongation and upon bacitracin reversal of GA stimulation of lettuce seedling hypocotyl growth were also examined.

Bacitracin, tunicamycin, monensin, glucosamine and vanadate all reversed the hormone-enhanced elongation of lettuce hypocotyls. They also significantly inhibited root growth. Bacitracin was most effective in inhibiting root elongation and in reversing the gibberellin stimulation of lettuce hypocotyl growth.

Dolichyl phosphate reversed the bacitracin negation of gibberellin enhancement of lettuce hypocotyl growth. This phosphorylated polyprenol also significantly inhibited lettuce seedling root elongation.

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August 1985

To the Graduate Council:

I am submitting herewith a Thesis written by Lisa Ann Stokes entitled "The Reversal of Gibberellic Acid Enhancement of Lettuce Seedling Hypocotyl Elongation." I have examined the final copy of this paper for form and content, and I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biology.

Benjamin P. Stone
Major Professor

We have read this thesis and recommend its acceptance.

A. Floyd Scott
Second Committee Member

Charles N. Boehms
Third Committee Member

Accepted for the
Graduate Council:

Linda B. Ludwig
Dean of the Graduate School

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Chapter I

INTRODUCTION

Interest in the physiology of lettuce seedling elongation has been concerned mainly with the action of promoters and inhibitors upon both hypocotyls and roots. The promotion of hypocotyl elongation by gibberellic acid (GA) in Grand Rapids lettuce seedlings has been demonstrated by Frankland and Wareing (1960); however GA has been observed to have little or no effect upon root growth (Cleland, 1969). Three specific modes of action of GA have been proposed in recent years: the control of enzyme synthesis through the control of the synthesis of specific species of messenger RNA (Higgins et al., 1976), the stimulation of the activity of glycosylation reactions in plant tissues (Schwaiger and Tanner, 1979) and the activation of ion pumps within the cell membrane (Cleland, 1971; Fisher and Albersheim, 1974; Hager et al., 1971).

A few exogenous inhibitors affecting gibberellin stimulation of lettuce hypocotyl elongation have also been discovered. Stone (1981) observed that certain concentrations of exogenously applied bacitracin were sufficient to reverse hormone-stimulated growth in the tissue. Limited work has been performed on the effects of

various exogenously applied inhibitors upon root elongation. Malca et al. (1967) reported that glucosamine (GlcN), galactose and mannose are toxic above certain concentrations and will inhibit root growth; they also noted that this inhibition could be reversed with the application of glucose to the tissue.

The objectives of this study were to determine the: (1) effects of five inhibitors of various metabolic pathways upon the gibberellin stimulation of hypocotyl elongation in Grand Rapids lettuce seedlings, (2) effects of these inhibitors upon lettuce seedling root elongation and (3) effects of dolichyl phosphate (Dol-P) upon the bacitracin reversal of gibberellin stimulation of lettuce hypocotyl growth and upon lettuce seedling root elongation.

Chapter II

REVIEW OF THE LITERATURE

Gibberellic acid is a natural plant growth substance which was identified by Kurosawa (1926) and was first isolated from a Fusarium heterosporum culture by Brian et al. in 1955. Phinney et al. (1957) demonstrated that gibberellins occur in higher plants and that they play an important role in the control of plant growth and development. Gibberellic acid has been observed to promote stem elongation, especially in dwarf plants (Brian and Hemming, 1955; Arney and Mancinelli, 1966; Kende and Lang, 1964; Sachs et al., 1959), and to reverse some of the inhibitory effects of light (Lockhart, 1956). Cell division and cell enlargement in leaves have been noted to increase upon application of GA (Cleland, 1964; Wheeler, 1960); however this substance rarely has any significant effect upon root growth (Mertz, 1966; Cleland, 1969; Torrey, 1976). Eagles and Wareing (1964) reported that GA is capable of substituting for cold or light treatments resulting in restored growth capacity in buds of birch and sycamore. Gibberellins also restore growth capacity in dormant seeds (Amen, 1968; Black and Naylor, 1959), may cause certain types of plants to flower (Lang, 1957; Lang and Reinhard, 1961) and can modify

the sex of flowers (Galun, 1959). Another growth effect of GA is its ability to cause parthenocarpic growth of fruits (Crane, 1964).

It has been reported that the particular biochemical action of GA involves its control of the synthesis of certain enzymes, the most thoroughly investigated case being the secretion of α-amylase in barley endosperm (Paleg, 1960; Yomo, 1960; Moll and Jones, 1983). Gibberellic acid has also been noted to control the synthesis of β-1,3-glucanase (Taiz and Jones, 1970), protease (Jacobsen and Varner, 1967) and ribonuclease (Chrispeels and Varner, 1967). Through further experimentation it has been observed that GA controls enzyme levels by regulating the synthesis of specific species of mRNA (Higgins et al., 1976). Therefore substances which inhibit RNA and protein synthesis have been noted to prevent the production of enzymes in plants (Key, 1964; Varner and Chandra, 1964). It has been postulated that the control of the synthesis of particular enzymes is the universal mode of action of gibberellins in plant growth regulation (Moore, 1981).

Gibberellic acid also stimulates the activity of glycosylation reactions in plant tissue, therefore affecting the production of glycoproteins (Schwaiger and Tanner, 1979). Glycoproteins are widely distributed in both plant and animal cells (Sharon and Lis, 1979). It

has recently been observed that those glycoproteins which have an N-acetylglucosamine-asparagine linked oligosaccharide chain are synthesized by means of lipid-linked saccharide intermediates (Elbein, 1979; Ericson and Delmer, 1977; Forsee and Elbein, 1975). Since plants contain various lectins and storage proteins, some of which are glycoproteins, it is likely that the synthesis of some of these molecules involves lipid-linked saccharide intermediates (Hori and Elbein, 1981).

Hormone stimulation of elongation growth results from a temporary weakening or relaxing of the cell wall (Cleland, 1971). There is considerable evidence that pH 5 catalyzes the relaxation of the wall in a manner similar to that catalyzed by hormones (Adams et al., 1973; Rayle, 1973; Nitsch and Nitsch, 1956; Rayle and Cleland, 1970). Gibberellins may activate ion pumps within the cell membrane, thereby lowering the pH of the wall (Cleland, 1971; Fisher and Albersheim, 1974; Hager et al., 1971). It is possible that the direct action of plant hormones is on the cell membrane and that the reactions within the cell wall which permit elongation take place most efficiently at pH 5. However it is more likely that elongation is catalyzed by wall loosening enzymes which are most effective at pH 5 (Albersheim, 1976). Albersheim (1976) has also suggested that the enzyme in the plant cell wall which catalyzes cell growth is likely to be an endotransglycolase, transferring a

portion of a polysaccharide to itself.

Bacitracin is a polypeptide antibiotic produced by strains of Bacillus licheniformis (Johnson et al., 1945). The commercial form is a mixture of at least nine bacitracins, the main component of which is bacitracin A (Stone and Strominger, 1971). It is an inhibitor of the glycosyl transfers of phosphorylated polyprenols involved in cell wall synthesis in bacteria (Siewert and Strominger, 1967), as well as in plants (Ericson et al., 1978; Montezinos and Delmer, 1980). In experiments by Stone (1980 and 1981), this antibiotic was observed to reverse the hormone-stimulated elongation of pea epicotyls, oat coleoptiles and lettuce seedling hypocotyls. Bacitracin inhibition of mannose incorporation into lipid-linked saccharides has been reversed at high concentrations of dolichyl phosphate; however an unexplained stimulation was observed in the tissue (Spencer et al., 1978).

Tunicamycin is a glucosamine-containing antibiotic which was isolated from a Streptomyces bacterium (Takatsuki et al., 1971). It is an inhibitor of the glycosylation of asparagine-linked glycoproteins and it specifically blocks the first step in the lipid-linked sugar pathway (Hori et al., 1985). This antibiotic has been shown to inhibit the formation of N-acetylglucosamine-lipid in particulate enzyme preparations from mung bean

seedlings (James and Elbein, 1980), cotton bolls (Ericson et al., 1977), cultured soybean cells (Hori and Elbein, 1981) and barley (Schwaiger and Tanner, 1979).

Monensin is a carboxylic ionophore that is known to slow down the proteolytic processing of lectin in developing rice embryos and the glycolytic processing of phytohemagglutinin in developing bean cotyledons (Stinissen et al., 1985). It disrupts the normal structure of the Golgi apparatus of plant cells (Mollenhauer et al., 1982; Morre' et al., 1983), resulting in an inhibition of protein transport from the cisternae to the transport vesicles (Chrispeels, 1983; Stinissen et al., 1984).

Glucosamine is a specific precursor of amino sugar components of glycoproteins (Roberts, 1970) and arises naturally in germinating seedlings. Above certain concentrations exogenous GlcN is toxic and inhibits growth in plant tissues, including roots (Malca et al., 1967) and coleoptiles (Roberts et al., 1971). It has been postulated that the cytotoxic effects of GlcN may be due to the inhibition of enzymes of carbohydrate metabolism by its phosphate esters (Bernheim and Dobrogosz, 1970), or to the fact that the monosaccharides themselves may be toxic agents (Roberts et al., 1971).

Sodium orthovanadate (vanadate) is an inhibitor of cation-stimulated ATPase associated with corn leaf plasma

membranes (Perlin and Spanswick, 1981) and rhythmic leaf movement in Albizzia julibrissin (Saxe and Satter, 1979). It specifically blocks the (Na^+,K^+) ATPase which functions as a proton pump, the H^+ gradient driving the co-transport of sugars, amino acids and inorganic ions (Bowman et al., 1978). Millimolar concentrations of this substance are known to inhibit both mitochondrial and glycolytic energy metabolism (DeMasters and Mitchell, 1973; Velours et al., 1975).

Polyprenyl phosphates are sugar carriers known to be involved in the synthesis of bacterial wall polymers (Hemming, 1974) and are thought to serve as intermediates in the formation of glycoproteins in yeast (Bretthauer and Wu, 1975) and higher plants (Forsee and Elbein, 1975; Lehle and Tanner, 1975). Dolichyl phosphate is a polyprenyl phosphate that has been found in many eukaryotic cells, including higher plants (Daleo and Pont Lezica, 1977; Delmer and Ericson, 1978). It has been determined that the glycosyl transfer reactions in the endoplasmic reticulum of castor bean endosperm is dependent upon Dol-P (Marriott and Tanner, 1979).

Chapter III

MATERIALS AND METHODS

Seedling Preparation

Lettuce seeds (*Lactuca sativa* L. var. "Grand Rapids") were obtained from the Ferry-Morse Seed Company, Mountain View, California and were stored at 9.0°C until utilized in the study. Seeds were imbibed in distilled water for two hours before being sown under white light in 100x15 mm Petri dishes lined with Fisher 9.0 cm coarse filter paper moistened with 5.0 ml of distilled water. The Petri plates were then wrapped in aluminum foil and the seeds allowed to germinate on the shelves of a General Electric Precision Scientific Model 805 growth chamber maintained at 25±1°C for 20 h. After this incubation period, seedlings with approximately 1.0 mm of the radicle protruding from the seed coat were removed from the growth chamber and immediately placed in the respective test solutions.-

Seedling Growth

Approximately 12 to 15 seedlings were removed from the first germination dishes using a small paintbrush to reduce damage to the plants. These were then scattered into 100x25 mm Petri dishes lined with coarse filter

paper which had been saturated with 5.0 ml of either the control solution (doubly distilled water) or the appropriate concentration of experimental solution. All series of experiments were performed in duplicate with either two or three runs being executed for each portion of the study. The dishes were placed in a Precision Scientific growth chamber, 15.2 cm below two 15-watt fluorescent lights, and maintained at 25±1°C for 48 h. The intensity of incident light at shelf level was 290 foot-candles. After incubation the hypocotyl and root of each plant were measured to the nearest 0.5 mm with the aid of a standard centimeter ruler.

The following hormone and chemicals were obtained from the Sigma Chemical Company, Saint Louis, Missouri: bacitracin, dolichyl phosphate, gibberellic acid, glucosamine, monensin, sodium orthovanadate and tunicamycin. Except for monensin, vanadate and Dol-P, all were diluted to the appropriate concentrations with doubly distilled water. The monensin and vanadate were first dissolved in 1.0 ml of 95% ethanol before the proper amounts of distilled water were added. The Dol-P was mixed with 2.0 ml of methanol and was heated to steaming before the appropriate amount of distilled water was added.

Duncan's multiple-range test (Steele and Torrie, 1960) was used to determine significant differences among experimental treatments at the 5% level. Treatment means

that are not significantly different at the 5% level are denoted by an asterisk in the tables.

Chapter IV

RESULTS

Effects of Bacitracin and Gibberellic Acid

Treatments of Grand Rapids lettuce seedlings with varying concentrations of bacitracin, alone and with GA, had separate effects upon hypocotyl and root growth (Table I and Figure 1). All experimental treatments utilizing GA were at a concentration of 10 µg/ml. Gibberellic acid alone promoted hypocotyl growth 358% above that of the control (Table I). Treatments of 5 to 50 µM bacitracin had no significant effect upon lettuce seedling hypocotyl elongation at the 5% level as determined using Duncan's multiple-range test. However these concentrations of bacitracin in the presence of GA reversed the gibberellin enhancement of hypocotyl elongation. A treatment of 25 µM reversed the hormone stimulation of hypocotyl growth to the level of the control.

Gibberellic acid had no effect upon lettuce seedling root growth (Figure 1). However bacitracin concentrations of 5 to 50 µM significantly inhibited root growth both in the presence and absence of GA.

Effects of Tunicamycin and Gibberellic Acid

The effects of tunicamycin and GA upon lettuce

Table I. Effects of varying concentrations of bacitracin upon gibberellin stimulation of lettuce seedling hypocotyls after 48 hours growth. Data are averages of three sets of replicate experiments.

Treatment	Percent of Control
10 µg/ml GA	358.0
5 µM bacitracin*	117.3
10 µM bacitracin*	112.3
25 µM bacitracin*	95.1
50 µM bacitracin*	86.4
10 µg/ml GA + 5 µM bacitracin	246.9
10 µg/ml GA + 10 µM bacitracin	159.3
10 µg/ml GA + 25 µM bacitracin*	101.2
10 µg/ml GA + 50 µM bacitracin*	86.4

* Denotes treatments not significantly different from control at the 5% level as determined using Duncan's multiple-range test.

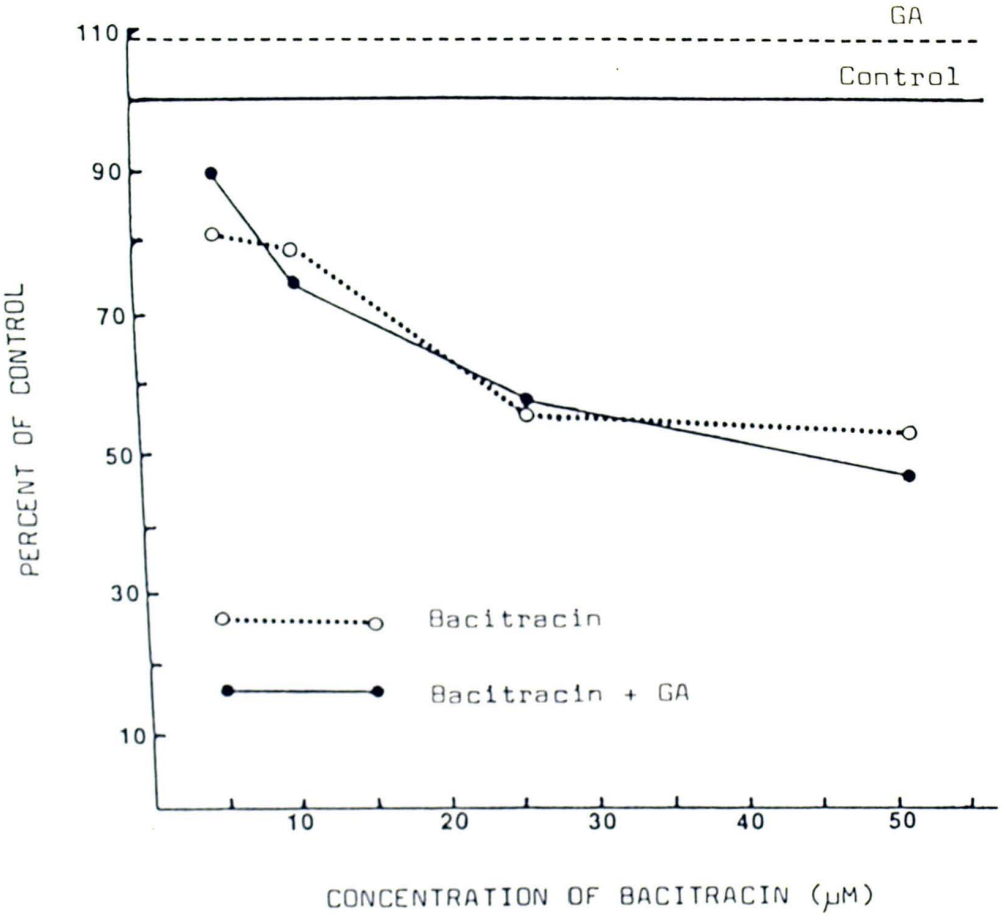

Figure 1. Effects of bacitracin and gibberellic acid upon lettuce seedling roots after 48 hours growth. Data are averages of three sets of replicate experiments.

seedling hypocotyls, both with and without GA, are depicted in Figure 2. In these experiments the effects of varying concentrations of tunicamycin alone differed little from the control; no significant differences were found among any of these values. Tunicamycin in the presence of GA reversed the hormone stimulation at concentrations of 5 ug/ml and greater. The gibberellin enhancement was reversed to the level of the control at a concentration of 50 ug/ml.

Tunicamycin also had a pronounced inhibitory effect upon root growth (Figure 3). Although concentrations of 5 ug/ml tunicamycin in the presence and absence of hormone did not significantly reduce growth as compared to the control, concentrations of 25 to 50 ug/ml did significantly inhibit elongation.

Effects of Monensin and Gibberellic Acid

Monensin and GA effects upon Grand Rapids lettuce seedling hypocotyl growth appear in Figure 4. Experimental treatments containing only antibiotic had no significant effect upon hypocotyl elongation at concentrations less than 20 ug/ml. At a concentration of 20 ug/ml, it was determined that monensin significantly inhibited hypocotyl growth. In these experiments monensin at concentrations of 5 ug/ml and higher negated the stimulatory effect of the GA. Lettuce hypocotyls treated

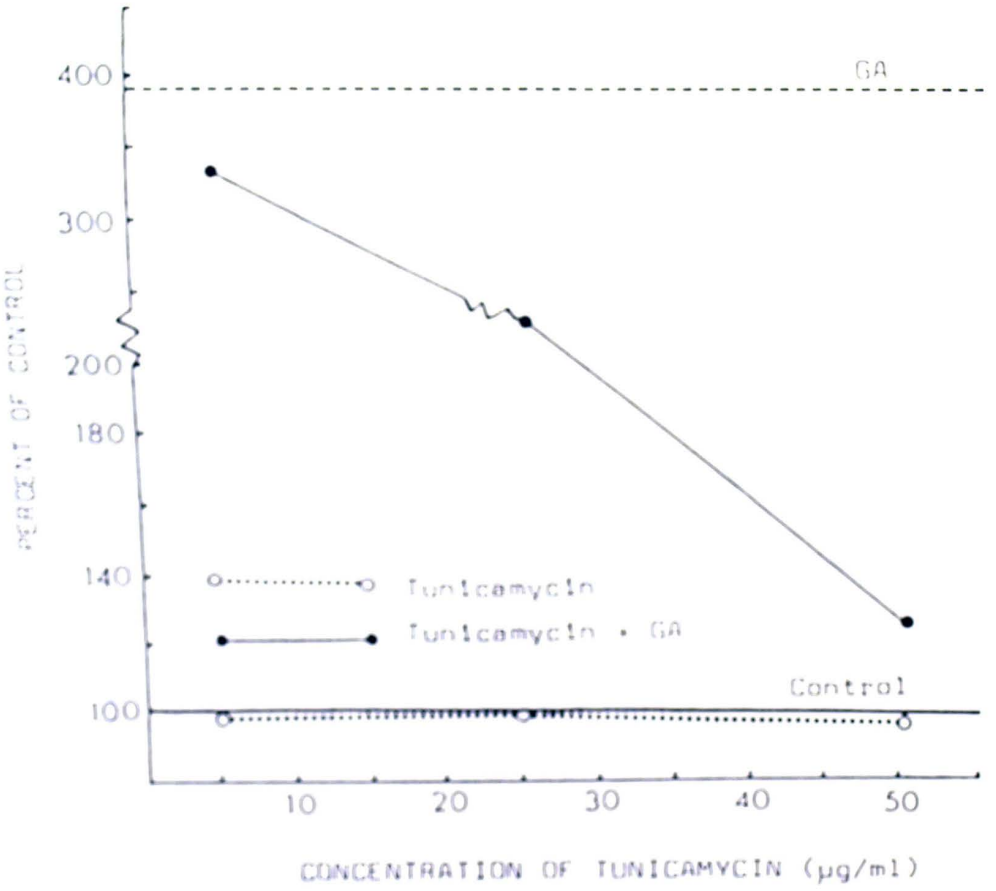

Figure 2. Effect of tunicamycin upon gibberellin stimulation of lettuce seedling hypocotyls after 48 hours growth. Data are averages of three sets of replicate experiments.

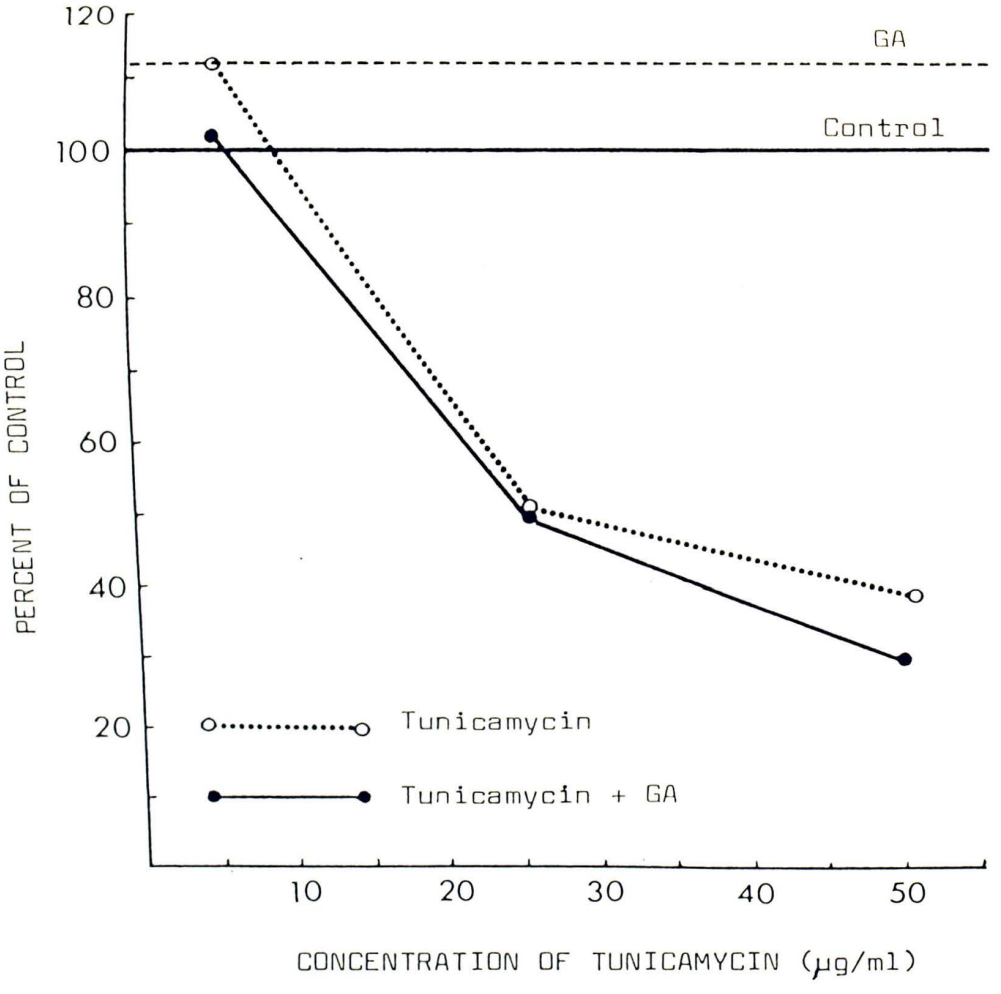

Figure 3. Effects of tunicamycin and gibberellic acid upon lettuce seedling roots after 48 hours growth. Data are averages of three sets of replicate experiments.

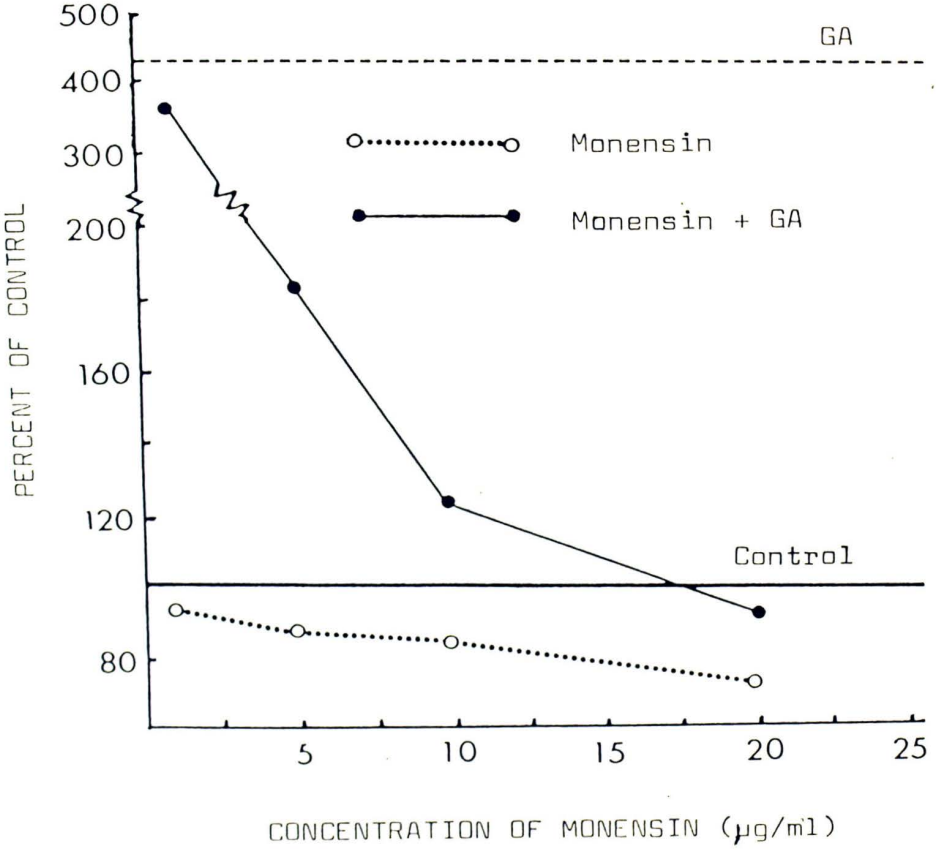

Figure 4. Effect of monensin upon gibberellin stimulation of lettuce seedling hypocotyls after 48 hours growth. Data are averages of three sets of replicate experiments.

with 20 µg/ml of this ionophore reversed the hormone enhancement of elongation to that of control level.

Concentrations less than 20 µg/ml monensin had no significant effect upon lettuce seedling root growth (Figure 5). At 20 µg/ml this antibiotic significantly inhibited root elongation in the presence and absence of GA.

Effects of Glucosamine and Gibberellic Acid

The effects of GlcN and GA upon the growth of intact lettuce seedlings are listed in Tables II and III. No significant differences were observed among treatments of 10 to 200 µg/ml GlcN alone and the control. Experimental treatments of GlcN at or above 20 µg/ml were sufficient to negate the gibberellin stimulation of lettuce hypocotyl elongation. Treatments of 75 to 200 µg/ml GlcN and GA were not significantly different from the control. All treatments of GlcN (10 to 200 µg/ml) were observed to significantly inhibit root elongation with and without GA.

Effects of Vanadate and Gibberellic Acid

The effects of vanadate upon the gibberellin stimulation of lettuce seedling hypocotyl elongation after 48 hours growth are shown in Figure 6. Vanadate alone had no significant effect upon hypocotyl growth; however

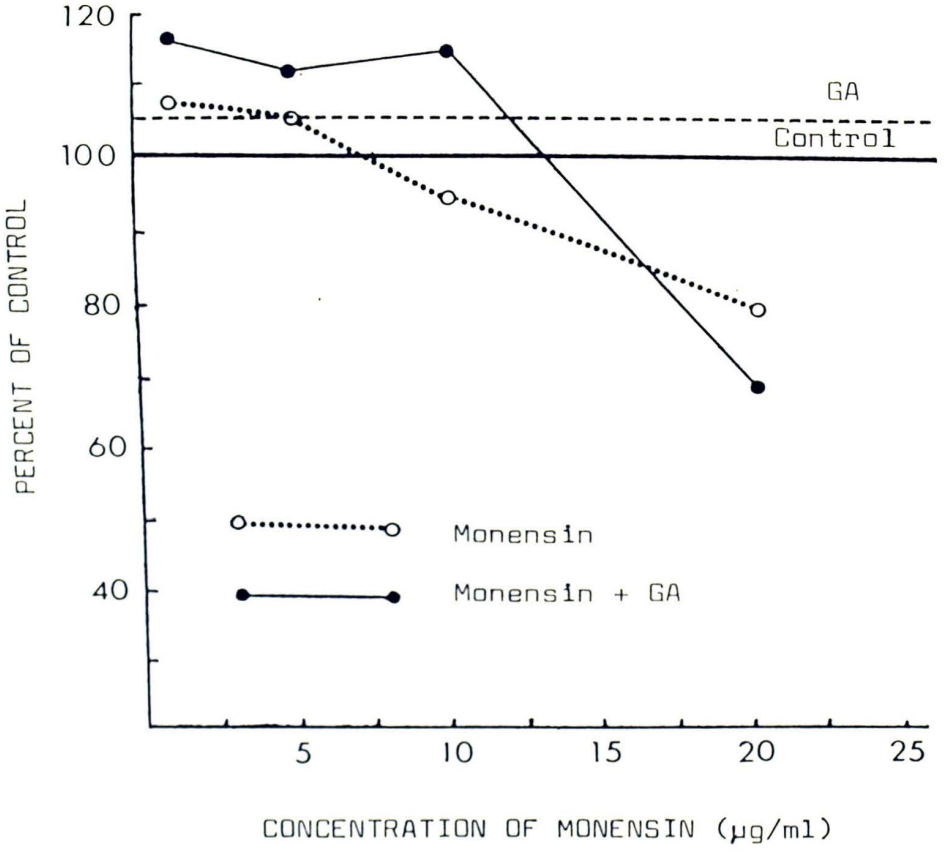

Figure 5. Effects of monensin and gibberellic acid upon lettuce seedling roots after 48 hours growth. Data are averages of three sets of replicate experiments.

Table II. Effects of varying concentrations of glucosamine upon gibberellin stimulation of lettuce seedling hypocotyl growth after 48 hours incubation. Data are averages of three sets of replicate experiments.

Treatment	Percent of Control
10 µg/ml GA	324.2
10 µg/ml glucosamine*	99.0
20 µg/ml glucosamine*	96.8
50 µg/ml glucosamine*	80.0
75 µg/ml glucosamine*	76.8
100 µg/ml glucosamine*	93.7
200 µg/ml glucosamine*	80.0
10 µg/ml GA + 10 µg/ml glucosamine	315.3
10 µg/ml GA + 20 µg/ml glucosamine	269.8
10 µg/ml GA + 50 µg/ml glucosamine	229.5
10 µg/ml GA + 75 µg/ml glucosamine*	139.0
10 µg/ml GA + 100 µg/ml glucosamine*	113.7
10 µg/ml GA + 200 µg/ml glucosamine*	87.4

* Denotes treatments not significantly different from the control at the 5% level as determined using Duncan's multiple-range test.

Table III. Effects of glucosamine and gibberellic acid upon lettuce seedling roots after 48 hours growth. Data are averages of three sets of replicate experiments.

Treatment	Percent of Control
10 µg/ml GA*	104.1
10 µg/ml glucosamine	36.5
20 µg/ml glucosamine	17.9
50 µg/ml glucosamine	12.3
75 µg/ml glucosamine	9.2
100 µg/ml glucosamine	5.9
200 µg/ml glucosamine	5.1
10 µg/ml GA + 10 µg/ml glucosamine	40.7
10 µg/ml GA + 20 µg/ml glucosamine	18.1
10 µg/ml GA + 50 µg/ml glucosamine	13.1
10 µg/ml GA + 75 µg/ml glucosamine	13.1
10 µg/ml GA + 100 µg/ml glucosamine	8.2
10 µg/ml GA + 200 µg/ml glucosamine	5.7

* Denotes treatments not significantly different from the control at the 5% level as determined using Duncan's multiple-range test.

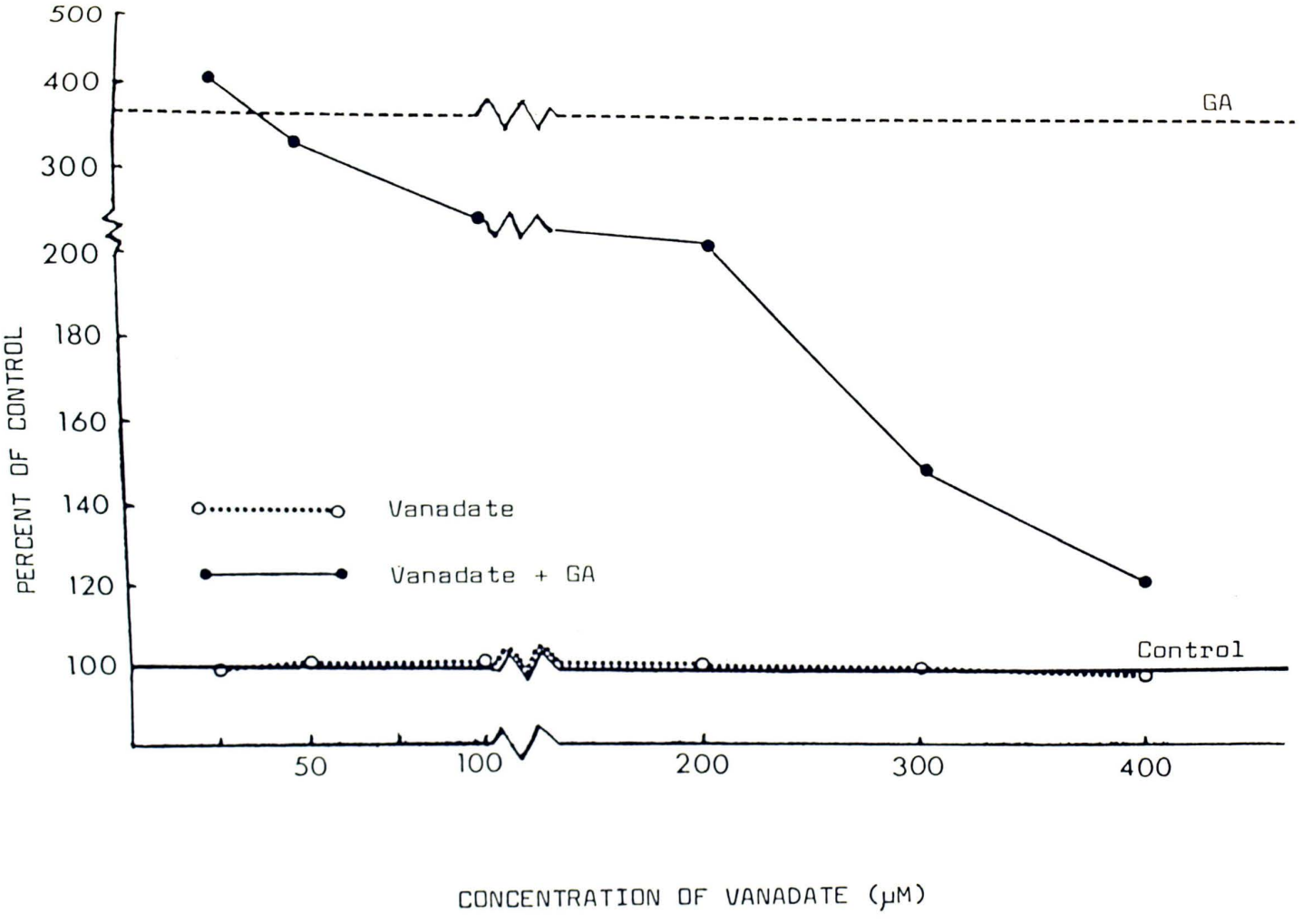

Figure 6. Effect of vanadate upon the gibberellin stimulation of lettuce seedling hypocotyls after 48 hours growth. Data are averages of three sets of replicate experiments.

it negated the hormone stimulation of this tissue at a minimum concentration of 50 μM. A concentration of 400 μM vanadate reversed the GA enhancement of hypocotyl elongation to the level of the control.

Lettuce seedling root growth was inhibited by treatments containing 200 to 400 μM vanadate (Figure 7). Treatments with 100 μM vanadate or less did not differ significantly from the control.

Effects of Dolichyl Phosphate, Bacitracin and Gibberellic Acid

Dolichyl phosphate negated the bacitracin reversal of gibberellin enhancement of lettuce hypocotyl elongation (Table IV). A concentration of 50 μg/ml Dol-P was required to reverse the bacitracin negation of hormone-stimulated elongation; treatments containing 100 μg/ml Dol-P significantly inhibited hypocotyl growth, except when 10 μg/ml GA and 10 μM bacitracin were present in the treatment. A concentration of 50 μg/ml Dol-P alone had no significant effect upon hypocotyl elongation; however a treatment of 50 μg/ml Dol-P and 10 μM bacitracin was observed to significantly inhibit hypocotyl growth.

Dolichyl phosphate inhibited root elongation at concentrations of 50 and 100 μg/ml, both in the presence and absence of 10 μg/ml GA and/or 10 μM bacitracin (Table V). Treatments containing 100 μg/ml Dol-P were observed to be

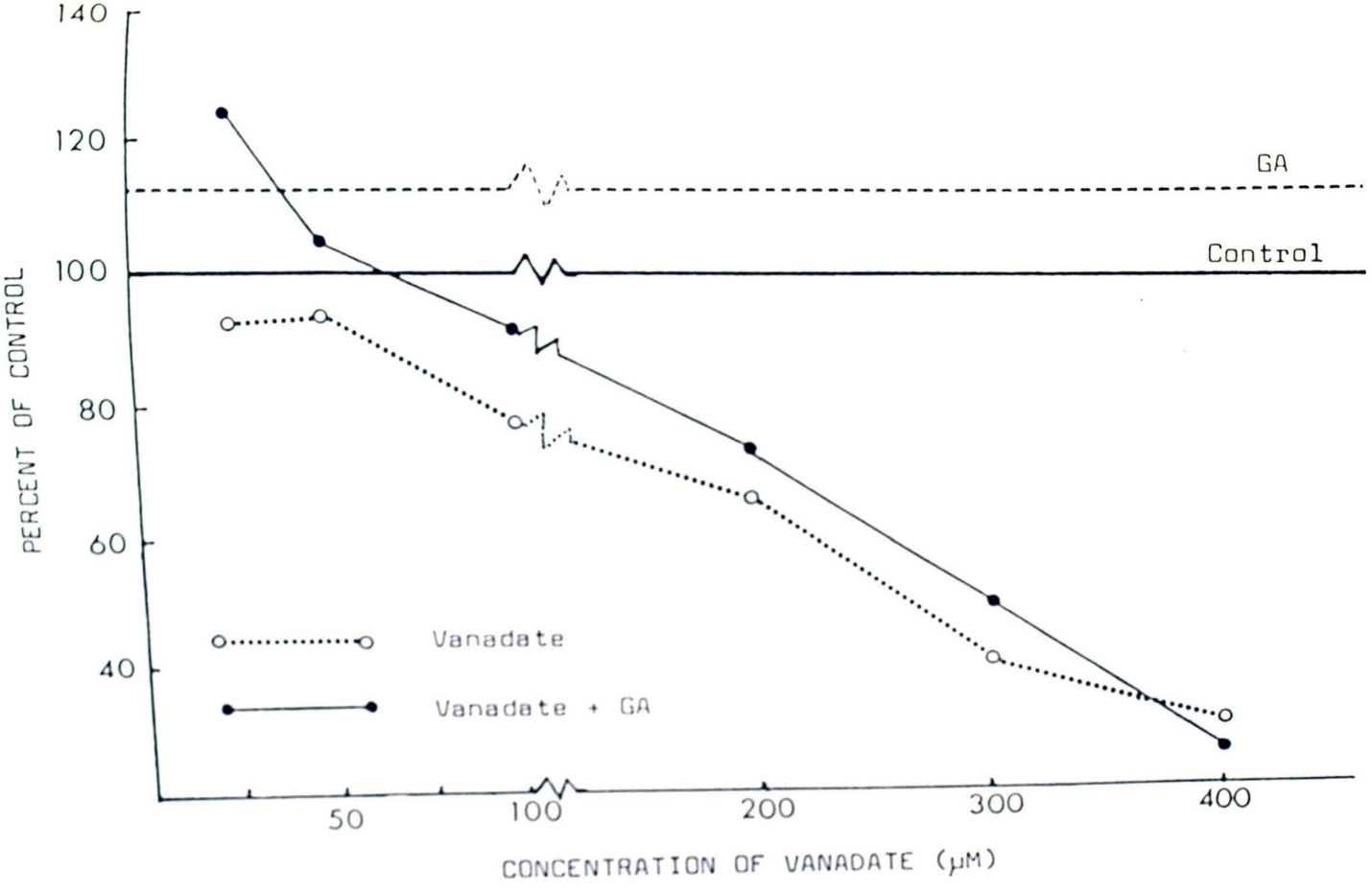

Figure 7. Effects of vanadate and gibberellic acid upon lettuce seedling roots after 48 hours growth. Data are averages of three sets of replicate experiments.

Table IV. Effect of dolichyl phosphate upon the bacitracin negation of gibberellin enhancement of lettuce hypocotyl elongation after 48 hours growth. Data are averages of two sets of replicate experiments.

Treatment	Percent of Control
10 µg/ml GA	280.0
10 µM bacitracin*	88.3
50 µg/ml Dol-P*	90.0
100 µg/ml Dol-P	50.0
10 µg/ml GA + 10 µM bacitracin*	94.0
10 µg/ml GA + 50 µg/ml Dol-P	152.0
10 µg/ml GA + 100 µg/ml Dol-P	54.7
10 µM bacitracin + 50 µg/ml Dol-P	76.7
10 µM bacitracin + 100 µg/ml Dol-P	34.3
10 µg/ml GA + 10 µM bacitracin + 50 µg/ml Dol-P	118.0
10 µg/ml GA + 10 µM bacitracin + 100 µg/ml Dol-P*	100.7

* Denotes treatments not significantly different from the control at the 5% level as determined using Duncan's multiple-range test. Dol-P= dolichyl phosphate and GA= gibberellic acid.

Table V. Effects of dolichyl phosphate, bacitracin and gibberellic acid upon lettuce seedling roots after 48 hours growth. Data are averages of two sets of replicate experiments.

Treatment	Percent of Control
10 µg/ml GA*	105.0
10 µM bacitracin*	88.5
50 µg/ml Dol-P	48.0
100 µg/ml Dol-P	16.3
10 µg/ml GA + 10 µM bacitracin*	89.0
10 µg/ml GA + 50 µg/ml Dol-P	45.0
10 µg/ml GA + 100 µg/ml Dol-P	13.5
10 µM bacitracin + 50 µg/ml Dol-P	42.6
10 µM bacitracin + 100 µg/ml Dol-P	15.0
10 µg/ml GA + 10 µM bacitracin + 50 µg/ml Dol-P	41.7
10 µg/ml GA + 10 µM bacitracin + 100 µg/ml Dol-P	16.1

* Denotes treatments not significantly different from the control at the 5% level as determined using Duncan's multiple-range test. Dol-P= dolichyl phosphate and GA= gibberellic acid.

more inhibitory than treatments containing 50 µg/ml Dol-P.

Chapter V

DISCUSSION

The data obtained in this study indicate that 10 µg/ml GA stimulates elongation of Grand Rapids lettuce seedling hypocotyls incubated under white light at 25°C for 48 hours. This has been observed by many other investigators, although the experimental parameters varied with respect to incubation time, temperature, amount of light and particular variety of lettuce used (Frankland and Wareing, 1960; Silk and Jones, 1975; Stone, 1981).

There are conflicting reports as to the significance of gibberellin affecting the growth of roots (Mertz, 1966; Torrey, 1976). In this study a treatment of 10 µg/ml GA had no significant effect upon root elongation when the plants were grown under white light at 25°C for 48 hours. Cleland (1969) suggests that evidence supporting the fact that GA does stimulate root elongation is limited.

A concentration of 25 µM bacitracin reversed the gibberellin enhancement of lettuce seedling hypocotyl growth to the level of the control (Table I). Stone (1981) also demonstrated that this antibiotic is effective in negating hormone stimulation of lettuce hypocotyl

elongation. Since bacitracin is an inhibitor of the glycosyl transfers of phosphorylated polyprenols involved in cell wall synthesis (Siewert and Strominger, 1967), it is possible that its mode of action is to affect the GA stimulation of glycosylation reactions in plant tissue (Hori and Elbein, 1981; Schwaiger and Tanner, 1979). In this way bacitracin could limit cell wall elongation and hypocotyl growth through the reversal of GA enhancement of glycoprotein synthesis.

Bacitracin alone had no significant effect upon hypocotyl elongation, yet it significantly inhibited root growth at concentrations between 5 and 50 μM (Figure 1). It is known that GA has no effect upon root elongation (Torrey, 1976; Cleland, 1969). The separate effects of bacitracin upon lettuce hypocotyls and roots could be due to some physiological mechanism which controls either enzyme synthesis or protein glycosylation only in roots, thus inhibiting cell wall elongation in the tissue.

Tunicamycin alone had no effect upon hypocotyl elongation, however at a minimum concentration of 50 μg/ml it significantly reversed the hormone stimulation of lettuce seedling hypocotyl growth to the level of the control (Figure 2). Since tunicamycin has been reported to inhibit the formation of N-acetylglucosamine-lipid in several types of plants (James and Elbein, 1980; Ericson et al., 1977; Schwaiger and Tanner, 1979), it is probable

that this action may contribute to the negation of gibberellin stimulation of hypocotyl growth in some way. Further biochemical research on the effects of this antibiotic on glycoprotein synthesis is needed before any conclusions can be drawn.

Tunicamycin also inhibited Grand Rapids lettuce seedling root elongation at concentrations of 25 to 50 µg/ml (Figure 3). This may be a result of the inhibition of some aspect of protein glycosylation and therefore cell wall elongation (Hori et al., 1985), or a result of the inhibition of root growth by the glucosamine component of tunicamycin (Malca et al., 1967).

Monensin alone had no effect upon hypocotyl elongation at concentrations less than 20 µg/ml (Figure 4). At a concentration of 20 µg/ml, this antibiotic significantly inhibited hypocotyl growth. Gibberellin enhancement of hypocotyl elongation was also reversed to the level of the control with 20 µg/ml monensin. It is probable that its action upon the Golgi apparatus accounts for the inhibition of hypocotyl growth at high concentrations (Mollenhauer et al., 1982; Morre' et al., 1983). Its effect upon proteolytic processing as described by Stinissen et al. (1985) most likely is the mechanism by which monensin acts to negate gibberellin stimulation. A concentration of 20 µg/ml monensin was also required to significantly inhibit root elongation (Figure 5). As in

the case of its effect upon hypocotyl growth, the action of this ionophore upon the Golgi apparatus may result in growth inhibition at concentrations of at least 20 µg/ml.

Treatments of 10 to 200 µg/ml GlcN had no significant effect upon lettuce hypocotyl elongation (Table II). A minimum concentration of 75 µg/ml GlcN reversed hormone enhancement of hypocotyl growth to control level. Roberts et al. (1971) reported that GlcN has cytotoxic effects in plant tissues. The effects of GlcN upon gibberellin stimulation of elongation in lettuce may be a result of the inhibition of the enzymes of carbohydrate metabolism by its phosphate esters (Bernheim and Dobrogosz, 1970), or a result of the toxicity of the monosaccharides themselves (Roberts et al., 1971). Glucosamine was also observed to inhibit root elongation at concentrations of at least 10 µg/ml (Table III). This observation is supported by the work of Walca et al. (1967) in which exogenously applied GlcN inhibited root growth in plants.

Vanadate negated the gibberellin stimulation of hypocotyl growth to control level at a minimum concentration of 50 µM, but alone it had no significant effect upon hypocotyl elongation (Figure 6). It has been reported that vanadate is an inhibitor of cation-stimulated ATPase synthesis (Perlin and Spanswick, 1981; Saxe and Satter, 1979), specifically blocking that ATPase which

functions as a proton pump. Hormone-stimulated elongation of hypocotyls has been proposed to be a result of a change in pH due to stimulation of an ion pump (Cleland, 1971; Fisher and Albersheim, 1974; Hager et al., 1971). Therefore it is possible that the action of vanadate could block a specific ion pump that may be catalyzed by GA. It has also been observed that vanadate inhibits mitochondrial and glycolytic energy metabolism (DeMasters and Mitchell, 1973; Velours et al., 1975). This may also contribute to the negation of GA enhancement of lettuce hypocotyl elongation.

Vanadate was observed to inhibit lettuce seedling root growth with treatments containing 200 to 400 μM of this substance (Figure 7). Again this may be caused by the inhibition of both mitochondrial and glycolytic energy metabolism, thereby inhibiting cell wall elongation by blocking glycoprotein synthesis.

Molar effectiveness of the various experimental treatments were compared by determining the minimum concentration required to reverse gibberellin stimulation of hypocotyl elongation to the level of the control. Bacitracin at a concentration of 25 μM was most effective in reversing hormone-enhanced growth in lettuce hypocotyls; GlcN (75 μg/ml or 348 μM) was least effective in negating gibberellin stimulation of the tissue. Minimum concentrations of the other substances noted to reverse

the hormone enhancement of growth were: 20 µg/ml (29 µM) monensin, 50 µM vanadate and 50 µg/ml (60 µM) tunicamycin.

Bacitracin was also observed to be most effective in inhibiting root elongation of lettuce seedlings. A concentration of only 5 µM significantly inhibited root growth as compared to the control. Concentrations of 20 µg/ml (29 µM) monensin, 25 µg/ml (30 µM) tunicamycin and 10 µg/ml (46 µM) GlcN were moderately effective in inhibiting root growth. Vanadate at a concentration of 200 µM was least effective in inhibiting lettuce seedling root elongation.

Dolichyl phosphate was observed to reverse the bacitracin negation of gibberellin stimulation of lettuce hypocotyl elongation at a concentration of 50 µg/ml (Table IV). Ericson et al. (1978) and Montezinos and Delmer (1980) have reported that bacitracin is an inhibitor of the glycosyl transfers of phosphorylated polyprenols involved in cell wall synthesis in plants. Dolichyl phosphate is a phosphorylated polyprenol known to be involved in the synthesis of glycoproteins in higher plants (Forsee and Elbein, 1975; Lehle and Tanner, 1975). Glycosyl transfer reactions in castor beans are also known to be dependent upon Dol-P (Marriott and Tanner, 1979). It is very likely that bacitracin may be involved in the inhibition of the glycosyl transfer of this

particular phosphorylated polyprenol. Addition of Dol-P to the GA/bacitracin treatment may allow the glycosylation reactions to continue and therefore allow cell wall elongation to proceed as usual. A concentration of 100 ug/ml Dol-P accompanied by 10 uM bacitracin and 10 ug/ml GA did not reverse the bacitracin negation of hormone-stimulated hypocotyl growth. It is possible that this high concentration was slightly toxic to the tissue, since other treatments containing 100 ug/ml Dol-P significantly inhibited hypocotyl elongation. There is a possibility that the accumulation of this lipid in the cell membrane may contribute to the growth suppression. A treatment containing only 50 ug/ml Dol-P had no significant effect upon hypocotyl elongation. It is postulated that this concentration did not contain enough lipid to significantly inhibit cell wall elongation in the lettuce hypocotyl.

Dolichyl phosphate was also observed to significantly inhibit lettuce seedling root elongation in all treatments containing 50 or 100 ug/ml (Table V). It is possible that lettuce roots are more sensitive to concentrations of Dol-P than are lettuce hypocotyls. The data in this study indicate that treatments of only 50 ug/ml Dol-P significantly inhibited root elongation, but had no effect upon hypocotyl growth.

Chapter VI

SUMMARY

This study was undertaken to determine the: (1) effects of bacitracin, tunicamycin, monensin, glucosamine and vanadate upon the gibberellin stimulation of Grand Rapids lettuce seedling hypocotyl elongation, (2) effects of these inhibitors of various metabolic pathways upon lettuce seedling root elongation and (3) effects of dolichyl phosphate upon the bacitracin reversal of gibberellin stimulation of lettuce hypocotyl growth and upon lettuce seedling root elongation.

Bacitracin, tunicamycin, monensin, GlcN and Vanadate all reversed the gibberellin enhancement of Grand Rapids lettuce hypocotyl elongation. Minimum concentrations required to reverse hormone stimulation of the hypocotyls to control level were: 25 μM bacitracin, 60 μM tunicamycin, 29 μM monensin, 348 μM glucosamine and 50 μM vanadate. These chemicals also inhibited lettuce seedling root elongation. Minimum concentrations required to significantly inhibit root growth were: 5 μM bacitracin, 30 μM tunicamycin, 29 μM monensin, 46 μM glucosamine and 200 μM vanadate. Bacitracin was most effective in reversing the gibberellin-stimulated hypocotyl elongation and in inhibiting root growth.

Dolichyl phosphate was observed to reverse the bacitracin negation of GA enhancement of lettuce hypocotyl growth at a concentration of 50 µg/ml. This phosphorylated polyprenol also inhibited lettuce seedling root elongation at concentrations of both 50 and 100 µg/ml; roots treated with 100 µg/ml Dol-P were significantly shorter than those treated with 50 µg/ml Dol-P after 48 hours growth.

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