THE EFFECTS OF VALINOMYCIN, POTASSIUM, AND GIBBERELLIN A_3 ON THE TRANSLOCATION OF VARIOUS AUXINS

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Bachelor of Science

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Stillwater, Oklahoma

1976

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1980

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ACKNOWLEDGMENTS

I would like to express my thanks to Dr. Eddie Basler, who so willingly gave his time and many valuable contributions to this study. I am also grateful for the contributions of my committee, Dr. Jim Ownby and Dr. Paul Richardson.

I would like to thank Marisa Bunning, Ruth Richardson, and Janice Green for all the assistance they gave me in the course of my studies at OSU.

I am especially indebted to my husband, Rusty, for his support throughout graduate school and his active participation in the completion of my thesis.

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ABBREVIATIONS

ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
DCCD	N,N--dicyclohexylcarbodiimide
2,4-D	2,4-dichlorophenoxyacetic acid
GA	gibberellin A_3
IAA	indole-3-acetic acid
NAA	naphthaleneacetic acid
PCIB	p-chlorophenoxyisobutyric acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
Val	valinomycin

CHAPTER I

INTRODUCTION

The actions of auxin have been studied intensively from the aspects of synthesis, transport, translocation, and mode of action. The latest findings seem to indicate that some of the functions of auxin center around the plasma membrane.

In an extensive review, Sheldrake (1973) determined that the most likely sites of auxin synthesis are areas undergoing cytolysis, such as senescent leaves, differentiating xylem cells, differentiating sieve tubes, and young expanding leaves. Here, the normally small cellular pool of tryptophan (the precursor to IAA) is augmented by the breakdown of proteins, and thus allows more substrate for IAA synthesis. These production sites may lie far from the areas where IAA is needed, such as the meristematic areas. Therefore, the movement of auxin becomes an important factor in the overall growth and development of the plant.

Traditionally, auxin movement was thought to be via a slow, energy-dependent, polar system oriented from the plant apex downward (Cande et al., 1973; Goldsmith and Ray, 1973). The most recent theories on polar transport hold that IAA diffuses passively into cells in its lipid-soluble, undissociated state; thus, its absorption is promoted by low pH's. Once inside the cells, where the pH is higher, IAA ionizes. This establishes an inward concentration gradient for unionized IAA and an outward one for the ion (Goldsmith, 1977), which may move passively out

of the cell via a carrier (Rubery and Sheldrake, 1974). The polarity of this transport system depends on polarization of the auxin carriers in the cell membrane so that undissociated auxin is taken up at the apex-end of the cell and the auxin ion exits at the basipetal end via a carrier. The system depends on metabolic energy to maintain the pH gradient and the polar arrangement of auxin carriers (Goldsmith, 1977; Rubery and Sheldrake, 1974).

The polar movement of auxin is centered around the phloem, cambium, and its immediate derivatives without actually traveling through the vascular system (Goldsmith, 1977). But auxins have been found in the phloem sap of both woody and herbacious plants (Baker, 1978; Goldsmith, 1977), moving at velocities of 10-24 cm per hr (Goldsmith, 1977). Since polar transport velocities have been measured at approximately 1 cm per hr (Cande et al., 1973), this indicates that there are two different systems for endogenous auxin movement. Trewavas (1979) postulates that auxin circulates freely in the vascular system and is sequestered by tissues as needed. If the control mechanisms of this long-distance translocation system could be elucidated, then the growth and development of the plant could be modified. To this end, studies utilizing exogenous auxin in intact plants were initiated to begin a new phase in the study of auxin translocation.

When ^{14}C-IAA and tritiated sugar are applied to mature leaves, the $^{14}C/^{3}H$ ratio is similar throughout the plant except at the shoot apex (Goldsmith, 1977). Here, the ratio decreases sharply, indicating that less ^{14}C-IAA is present, probably due to export out of the apex via the polar transport system (Goldsmith, 1977). This implies an interaction between the two translocation systems whereby auxin may leave sink areas

where no basipetal assimilate flow exists.

Hew et al. (1967) found that both IAA and GA applied to shoot tips changed the rate and pattern of sucrose-^{14}C translocation. Although the total amount of sucrose translocated also increased, there was no increase in $^{14}CO_2$ fixation. Therefore, Hew et al. (1967) concluded that the action of IAA and GA was on longitudinal translocation in the stem and not on leaf photosynthate production. Mullins (1970) found that ^{14}C-sucrose accumulated in regions of high IAA concentrations, and that this effect was enhanced by GA. If, as the previous experiments indicate, hormones do control translocation of assimilates, then they may also control their own translocation. Indeed, auxins have been shown to enhance their own movement (Goldsmith, 1977; Long and Basler, 1974). Therefore, the mechanisms involved in sucrose loading and translocation may also apply to auxin translocation. Giaquinta (1977, 1976) and Malek and Baker (1977) described a model for phloem loading of sucrose that involved a proton gradient formed across the plasma membrane of sieve tube elements. Sucrose escaped into the apoplast of the vascular tissue near the phloem (Giaquinta, 1977). Here sucrose entered the sieve tube elements at the expense of the proton gradient. Potassium and low pH enhanced phloem loading of ^{14}C-sucrose, indicating the operation of a H^+-efflux/K^+-influx pump (Malek and Baker, 1977). Since IAA affects sugar transport, it may utilize the same mechanism to control its own transport.

The use of metabolic inhibitors with exogenously applied auxin can provide a great deal of information about the mechanisms involved in auxin translocation. Long and Basler (1973) found that the antiauxin PCIB enhanced upward translocation of 2,4,5-T-1-^{14}C and IAA-1-^{14}C.

DCCD, an APTase inhibitor (Katsumi and Kazama, 1978; Corbett, 1977), abscisic acid, a growth inhibitor (Basler and McBride, 1977), and cycloheximide (Long and Basler, 1973), a protein synthesis inhibitor, also inhibit the translocation of auxin. Environmental factors such as temperature (Pallas, 1960), humidity (Basler et al., 1970), and time of day of treatment (Long and Basler, 1974) all affected auxin translocation. Stem girdling of bean stems showed that auxins can exchange readily between the xylem and phloem (Long and Basler, 1973). Gibberellic acid enhanced upward movement of auxin and overcame the inhibition of DCCD (Basler, 1974; Corbett, 1977). In summary, long-distance translocation of auxin occurred in the xylem and phloem and may be regulated by a protein synthesized at the time of movement and/or by the ATPase. Metabolic energy or specific enzymes were apparently important in some aspect of translocation since increasing the temperature also increased translocation (Pallas, 1960).

Auxins are known to increase cell elongation and much research has been done to determine the mechanism by which they act. These mechanisms for auxin action may also be involved in auxin translocation. Several researchers found that IAA caused H^+ excretion from cells (Cleland and Lomax, 1977; Hager et al., 1971; Marre, 1977; Cleland, 1976). Hager (1971) showed that ATP, Mg^{++}, and K^+ enhanced auxin-stimulated elongation by increasing H^+ extrusion. He explained that a lower cell wall pH activated wall-loosening in some manner and allowed elongation. Marre (1977) also found that IAA enhanced K^+ uptake and that 10mM KCl increased H^+ extrusion. Cleland and Lomax (1977) reported that IAA caused a hyperpolarization of the membrane potential starting about the same time as H^+ excretion, and caused an increase in K^+ uptake

after a long lag. They explained hormone-induced H^+-excretion as an electrogenic H^+-pump, coupled with passive K^+-uptake. Other workers, in salt uptake (Pitman, 1970), pH-regulation of cells (Hill and Bown, 1977), and acidostability of bacteria (Yamazaki et al., 1973) have found evidence for a H^+-K^+, energy-dependent pump at the cell membrane. Since many ion pumps are ATPases, Davies (1977) and Hager et al. (1971) reported that ATP enhanced auxin-stimulated elongation, it seems logical to assume a plasma membrane ATPase is involved with IAA-H^+-extrusion. Hodges et al. (1972) and Leonard et al. (1973) reported isolation of a plasma membrane-bound KCl-stimulated ATPase from oat roots. Erdei et al. (1979) reported that auxin stimulated the Ca^{++}, K^+-ATPase activity of a plasma membrane-rich fraction from roots. They also found that a lipid was necessary for the interaction between auxin and the ATPase. Marre (1977) found that ion exchange across the membrane was partly under hormonal control via a K^+-H^+-ATPase. These data indicate that auxin has a direct or indirect effect on a H^+-K^+-ATPase, thus increasing its activity, and causing H^+ extrusion. Just how auxin stimulates this ATPase is not known. However, the interaction between auxin and membranes and between lipids and ATPase may provide an answer.

Morre and Bracker (1976) found that IAA caused a rapid narrowing of soybean plasma membranes that was temperature-dependent, auxin-specific, and reversible. Smith (1977) reported an auxin-binding to plasma membrane sites and suggested it might cause conformational changes in the membrane. Ray (1977) found that auxins also bind to the endoplasmic reticulum. Such changes could increase permeability of the membrane and result in increased ion flux. Trewavas (1979) and Rayle et al. (1969) both suggested that the initial, or primary, effect of auxin was to

change the permeability of the membrane.

Val is a cyclododecadepsipeptide isolated from Streptomyces fulvissimus (Hunter and Schwartz, 1968). It selectively complexes with K^+, forming a positively charged moiety, and increases the permeability of K^+ in membranes (Hunter and Schwartz, 1968; Pressman, 1968; Tosteson, 1968; Pinkerton et al., 1969). Its selectivity for K^+ was not dependent on its interaction with a membrane, but seemed to be an inherent characteristic of the molecule (Pressman, 1968). Pinkerton et al. (1969) found that K^+ was coordinated with oxygen atoms in the interior of the cyclic compound, while the exterior presented a lipid soluble surface to the environment. Krasne et al. (1971) reported that Val acted as a carrier for K^+, diffusing across the membrane according to a concentration gradient. Several workers have found that Val stimulated oxygen uptake, i.e. it uncoupled oxidative phosphorylation (Moore and Pressman, 1964; Hensley and Hanson, 1975; Smith and Beyer, 1967). Pressman (1968) reported that Val caused K^+ uptake followed by H^+ efflux, and a concomitant swelling of the mitochondria. He also suggested that Val action may be to promote penetration of K^+ to a cation$^+$/H^+ antiporter in the membrane, rather than simply conducting K^+ through the membrane. Hensley and Hanson (1975) concurred in this concept of Val action, and they also noted that there was a difference in its effect on mitochondria at different concentrations. Others have also seen a concentration effect and reported that low Val concentrations did not uncouple oxidative phosphorylation (Christensen, 1975).

Other metabolic activities are effected by Val as well. Poole et al. (1971) reported that Val increased glycolysis in Ehrlich ascites tumor cells. This may be expected, since K^+ is necessary for the

activity of some glycolytic enzymes. However, Poole et al. (1971) claimed to see an enhancement even in K^+ depleted cells. Yocum (1977) found that Val inhibited photosystem II cyclic photophosphorylation whether KCl was added to the medium or not. He stated that Val acts as an electron transport inhibitor rather than as an uncoupler. Voegeli et al. (1977) reported a Val-induced inhibition of noncyclic electron transport and anaerobic, menedione-dependent cyclic phosphorylation of chloroplasts. Both of these systems are thought to include plastoquinone (Voegali et al., 1977). Other parts of the system not containing plastoquinone appeared to be insensitive to Val (Voegali et al., 1977). Plastoquinone is thought to be the mobile component of the electron transport chain in chloroplasts that transfers H^+ across the membrane (Hinkle and McCarty, 1978). With this in mind, Voegali et al. (1977), suggested that one possibility for Val action in cholorplasts is to decrease membrane permeability so that plastoquinone is no longer mobile. Others also have seen Val effects on membranes that are not dependent on K^+. Walz (1977 and 1976) noted that there was an interaction between lecithin (phosphatidylcholine) vesicles and Val so that the aggregational state of the lipid molecules was altered. He (1977) explained that Val probably reduced the motional freedom of the phospholipids around it by a hydrophobic interaction with them. This effect was not related to the amount of KCl present but was a function of the amount of Val dissolved in the membrane (Walz, 1976). Hsu and Chan (1973) found that Val interacted with the polar headgroups of some artificial lipid bilayers while it penetrated the hydrophobic core of others. Haest et al. (1972) found that Val increased the permeability of $^{86}Rb^+$ in a phosphatidylglycerol liposome but not in a

lysylphosphatidylglycerol liposome. Apparently the charges on polar headgroups affect the solubility of K^+-Val. Haest et al. (1972) also found that the total uptake of $^{86}Rb^+$-Val by bacteria cells was reduced in the presence of DCCD, an ATPase inhibitor. This agrees with the experiments of Cereijo-Santalo (1972), who found that ATPase from rat liver mitochondria was stimulated by Val plus K^+. However Haest (1972) stated that Val does not stimulate the K^+-pump in all bacteria. How Val might effect ATPases, or even if it does in higher plants, is not known. Poole et al. (1971) reported that Val did not abolish the pH gradient across the plasma membrane. Val is capable of interconverting gradients of K^+ or the electrical potential of a membrane, but is not thought to directly effect H^+ flux (Christensen, 1975). Most work with Val was done with mitochondria or artificial lipids. However, Willenbrink and Schuster (1978) found that Val caused a localized inhibition of ^{14}C-labeled assimilate translocation in the petiole of Pelargonium zonale (L.) L'Herit ex Ait. They stated, therefore, that compartmentation of K^+ is necessary for the translocation process.

In all the data compiled, the prevailing opinion is that K^+ flux, ATPase, and membrane permeability are important in auxin action. Hartt (1965) also showed that K^+ was necessary for translocation of sugar. This study will look at the effects of valinomycin, a K^+ ionophore (Hinkle and McCarty, 1978; Pressman, 1968), and GA, which may effect the membrane (Katsumi and Kazama, 1978), on auxin translocation. Since valinomycin is known to effect respiration (Murav'eva et al., 1973; Hensley and Hanson, 1975; Moore and Pressman, 1964), its effect on oxygen uptake at the concentration used will be determined.

CHAPTER II

METHODS AND MATERIALS

Bush beans (Phaseolus vulgaris L. cv. Stringless Green-Pod) were germinated in perlite moistened with half-strength Hoagland's solution (Hoagland and Arnon, 1950) for 5 days at 32° C, under continuous fluorescent light of 5.4 klux. The seedlings were then transferred to amber glass jars with 400 ml of aerated, half-strength Hoagland's solution. They were grown four more days in a growth chamber set at 14 hour, 32° C, 22 klux days, and 10 hour, 29° C nights. Approximately 24 hours before treatment, the seedlings were transferred to fresh half-strength Hoagland's solution.

The nine-day-old bean plants were treated at approximately the third hour of daylight. Plants were treated by injection with a syringe inserted into the pith at the cotyledonary node and extended below the node 1 cm. Treatments included 0.5 µg 2,4,5-T-1-^{14}C (54mCi/mmol), 0.5 µg 2,4-D-1-^{14}C (57mCi/mmol), 0.5 µg α-NAA (16mCi/mmol); or 0.5 µg IAA β-[2-^{14}C] (48.7mCi/mmol). These auxins were dissolved in 1 µl of 95% ethanol along with various amount of Val or GA. When KCl was used, it was added to the nutrient solution one hour before treatment time to make a 30mM KCl solution.

Eight replications of each treatment were used and were completely randomized within the growth chamber. The plants were usually left in the growth chamber four hours before harvesting, but treatment times of

2 hrs, 6 hrs, and 8 hrs were also used. The plants were harvested by dividing them into: young shoots, which included all tissues above the primary leaf node; primary leaves, including petioles; epicotyl, from 0.5 cm above the cotyledonary node up to the primary leaf node; treated areas, including tissue 0.5 cm above the cotyledonary node and 2.5 cm below the node; hypocotyl, from 2.5 cm below the cotyledonary node down to the roots; and the roots. The plant parts were lyophilized, weighed, and then homogenized in 5 ml 95% ethanol (10 ml for leaves) with a Brinkmann Polytron homogenizer. One-half ml aliquots of each part (0.2 ml for leaves) were transferred to 15 ml of Budget Solve complete counting cocktail or Beckman Ready-Solv EP Scintillation cocktail and assayed for radioactivity with a packard Tri-Carb liquid scintillation spectrometer. Five ml of the nutrient solution was frozen, lyophilizied, dissolved in 15 ml of counting cocktail, and assayed for radioactivity. Adjustments for quenching were made by the use of standard quench curves.

For the respiration experiment, beans were grown as usual but were not transferred to fresh Hoagland's on the 8th day. The beans were treated with 0.5 µg of unlabeled 2,4,5-T and 16.67 µg of Val by injecting the chemicals into the pith at the cotyledonary node. After average treatment times of 91 minutes and 4.3 hrs, a 1 cm segment of the treated area was excised to measure its rate of oxygen consumption using a YSI Model 53 Biological Oxygen Monitor. Four replicates of each treatment were used. Each treatment flask contained 4 ml distilled-deionized water and one treated area. The water bath was held at 33° C. The procedure for measuring oxygen consumption in Instructions for YSI Model 53 Biological Oxygen Monitor was followed. Oxygen consumption was measured

for at least five minutes and the treatment flasks were covered to prevent the interference of photosynthesis.

Data was analyzed by using standard F tests and Duncan's new multiple range test. Nanograms (ng) of ^{14}C per plant part and ng of ^{14}C per gram of tissue were both used in data analysis. The analysis using ng of ^{14}C per plant part consistently showed greater significant differences and, therefore, was used throughout this study.

Val, unlabeled 2,4,5-T, and GA were purchased from Sigma Chemical Company. The 2,4,5-T-1-^{14}C and 2,4-D-1-^{14}C were purchased from Radiochemical Centre, Amersham, England. The IAA β-[2^{14}C] was purchased from New England Nuclear. Thin-layer chromatography showed that all of the labeled auxins were 80-90% pure and all had some breakdown products.

CHAPTER III

RESULTS

Interaction of GA and Various Levels of Val on 2,4,5-T Translocation

Val was used at three levels: 3.33 µg, 8.33 µg, and 16.67 µg per plant. GA at 16.67 µg per plant was used alone and in combination with the three levels of Val. Val at 8.33 µg and 16.67 µg inhibited movement of 2,4,5-T out of the treated area, while 16.67 µg of Val also inhibited movement of 2,4,5-T into the hypocotyls (Table I). The other levels of Val showed no significant effects on 2,4,5-T translocation. However, there was a possible decrease in 2,4,5-T translocation to the young shoots and epicotyls when 16.67 µg Val was used. This same treatment caused significant differences in translocation to young shoots and epicotyls in other experiments (Table III and IV) . Also, 3.33 µg Val caused a slight (but not significant) increase in translocation to the young shoots, primary leaves, and epicotyls. The sum of this increase in acropetal translocation was equal to that caused by GA alone.

GA alone increased acropetal translocation to the growing points and epicotyls, but had no effect on the amount of 2,4,5-T remaining in the treated area or on basipetal transloction of 2,4,5-T (Table I).

The addition of 3.33 µg Val to the GA treatment did not significantly alter GA's effect on translocation of 2,4,5-T. The addition of 8.33 µg Val to the GA treatment caused a significant decrease in GA's

TABLE I

THE EFFECT OF VARYING CONCENTRATIONS OF VAL AND 16.67 µg GA ON 2,4,5-T TRANSLOCATION FOUR HOURS AFTER TREATRMENT*

Treatment	Young Shoots	Primary Leaves	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-T	6.8a	76.2cd	77.0ac	181.5a	110.1a	4.3ab	0.4a
0.5 µg 2,4,5-T 3.33 µg Val	7.6a	97.5d	86.2cd	195.8ab	87.5abc	2.7b	0.8a
0.5 µg 2,4,5-T 8.33 µg Val	5.3a	67.4acd	71.2ad	235.8b	93.3ab	3.3b	0.8a
0.5 µg 2,4,5-T 16.67 µg Val	4.4a	74.0acd	65.4abe	324.7c	66.8bc	3.3b	0.5a
0.5 µg 2,4,5-T 16.67 µg GA	18.6b	77.6cd	95.6d	182.5a	94.7ab	5.1ab	0.8a
0.5 µg 2,4,5-T 16.67 µg GA 3.33 µg Val	13.7b	67.8acd	93.0d	176.6a	118.5a	7.1a	1.9B
0.5 µg 2,4,5-T 16.67 µg GA 8.33 µg Val	8.1a	55.5abc	72.3abc	242.3b	100.1ab	5.2ab	0.6a

TABLE I (Continued)

Treatment	Young Shoots	Primary Leaves	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-T 16.67 µg GA 16.67 µg Val	7.1a	43.8ab	56.0e	313.0c	58.4c	2.0b	0.1a

*Values are given in ng 2,4,5-T. Values for a single plant part followed by the same letter are not significantly different at the 5% level.

effect on translocation to the growing points and epicotyls. It also caused an increase in the amount of 2,4,5-T remaining in the treated area. Val at 16.67 µg caused a significant decrease in GA's effect on translocation to all acropetal areas and the hypocotyls, and increased the amount of 2,4,5-T remaining in the treated area. This indicates that Val had a much more measurable effect on GA-enhanced 2,4,5-T translocation than on unmodified 2,4,5-T translocation. Or, conversely, GA did not reverse the inhibitory effect of high levels of Val, since the interaction with Val caused an inhibition of 2,4,5-T translocation equal to that caused by Val alone. GA and 3.33 µg Val caused translocation to the young shoots, roots, and nutrient solution to increase compared to the Val control. In this instance Val did not seem to alter the effects of GA.

Interaction of Val and Various Levels of GA_3 on 2,4,5-T Translocation

Three levels of GA per plant were used: 4 µg, 16 µg, and 40 µg. They were mixed with 0.5 µg 2,4,5-T only or with 2,4,5-T and 16.67 µg of Val (see Table II).

The only significant effects caused by GA alone were an increase in 2,4,5-T translocation to the young shoots by 16 µg and 40 µg GA, an increase in 2,4,5-T in the nutrient solution by 4 µg GA, and a decrease in 2,4,5-T translocation to the hypocotyls by 40 µg GA when compared to the 16 µg or 4 µg GA-treated plant.

Val alone caused a significant increase in the amount of 2,4,5-T remaining in the treated area and an apparent decrease in 2,4,5-T in the epicotyls. The addition of Val to the 4 µg GA treatment caused a

TABLE II

THE EFFECT OF VARYING CONCENTRATIONS OF GA AND 16.67 µg VAL ON 2,4,5-T TRANSLOCATION FOUR HOURS AFTER TREATMENT*

Treatment	Young Shoots	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-T	9.1a	119.2ab	172.2ab	112.4ab	5.7abc	1.0a
0.5 µg 2,4,5-T 4 µg GA	16.2abc	136.4a	184.5ab	143.3a	8.5c	5.3b
0.5 µg 2,4,5-T 16 µg GA	23.4c	143.0a	150.4a	141.3a	7.8bc	2.5ab
0.5 µg 2,4,5-T 40 µg GA	18.4bc	133.9a	166.0ab	90.4b	5.8abc	1.6a
0.5 µg 2,4,5-T 16.67 µg Val	9.0a	92.3b	258.0c	100.1b	2.2a	1.0a
0.5 µg 2,4,5-T 16.67 µg Val 4 µg GA	13.3ab	121.0ab	227.1bc	94.1b	2.8ab	0.3a
0.5 µg 2,4,5-T 16.67 µg Val 16 µg GA	18.5bc	125.3ab	215.8bc	99.9b	4.6abc	1.3a

TABLE II (Continued)

Treatment	Young Shoots	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-t 16.67 µg Val 40 µg Ga	17.0abc	125.5ab	200.3abc	115.2ab	6.7abc	1.3a

*Values are given in ng 2,4,5-T. Values for a single plant part followed by the same letter are not significantly different at the 5% level.

significant decrease in basipetal translocation. The addition of Val to the 16 µg GA treatment caused a significant decrease in translocation to the hypocotyls, and an increase in the amount of 2,4,5-T staying in the treated area. Adding Val to 40 µg GA caused no significant differences from the 40 µg GA control, the Val control, or the auxin control. Comparing the Val control to the GA + Val treatments, the only significant difference was an increase in translocation to the growing points caused by Val plus 16 µg GA. For the same treatment in the previous experiment, no increase was seen. So even large amounts of GA do not alter the main effect of Val--to increase the amount of 2,4,5-T remaining in the treated area.

The Effects of Val on the Translocation of Auxins With Different pK Values

Plants were treated with 0.5 µg of one of the auxins; 2,4-D, 2,4,5-T, NAA, or IAA, with the pK values: 3.6, 2.8, 4.2, and 4.8, respectively. Each auxin was also combined with 16.67 µg of Val (see Table III).

Val significantly decreased translocation of 2,4,5-T to the young shoots, primary leaves, and epicotyls, and increased the amount of auxin remaining in the treated area. Val also decreased the amount of 2,4-D translocated to the growing points, epicotyls, roots, and nutrient solution. NAA translocation to the primary leaves was significantly decreased by Val. No significant changes in IAA translocation were caused by Val.

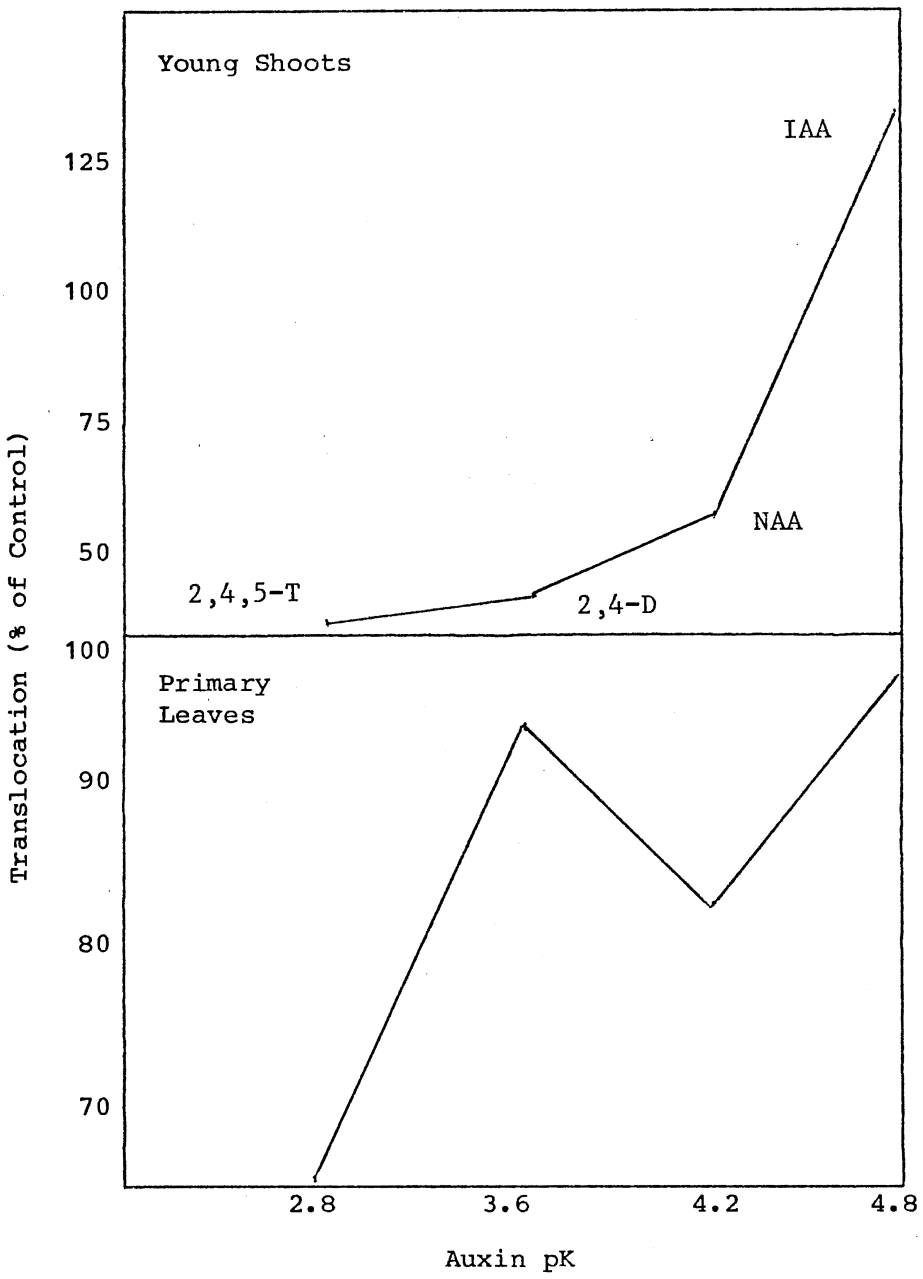
Plotting the data as a percent of the control versus pK of auxin (Figure 1) showed that as auxin pK values increased, Val inhibition of

TABLE III

THE EFFECT OF 16.67 µg OF VAL ON THE TRANSLOCATION OF AUXINS WITH VARIOUS pK VALUES FOUR HOURS AFTER TREATMENT*

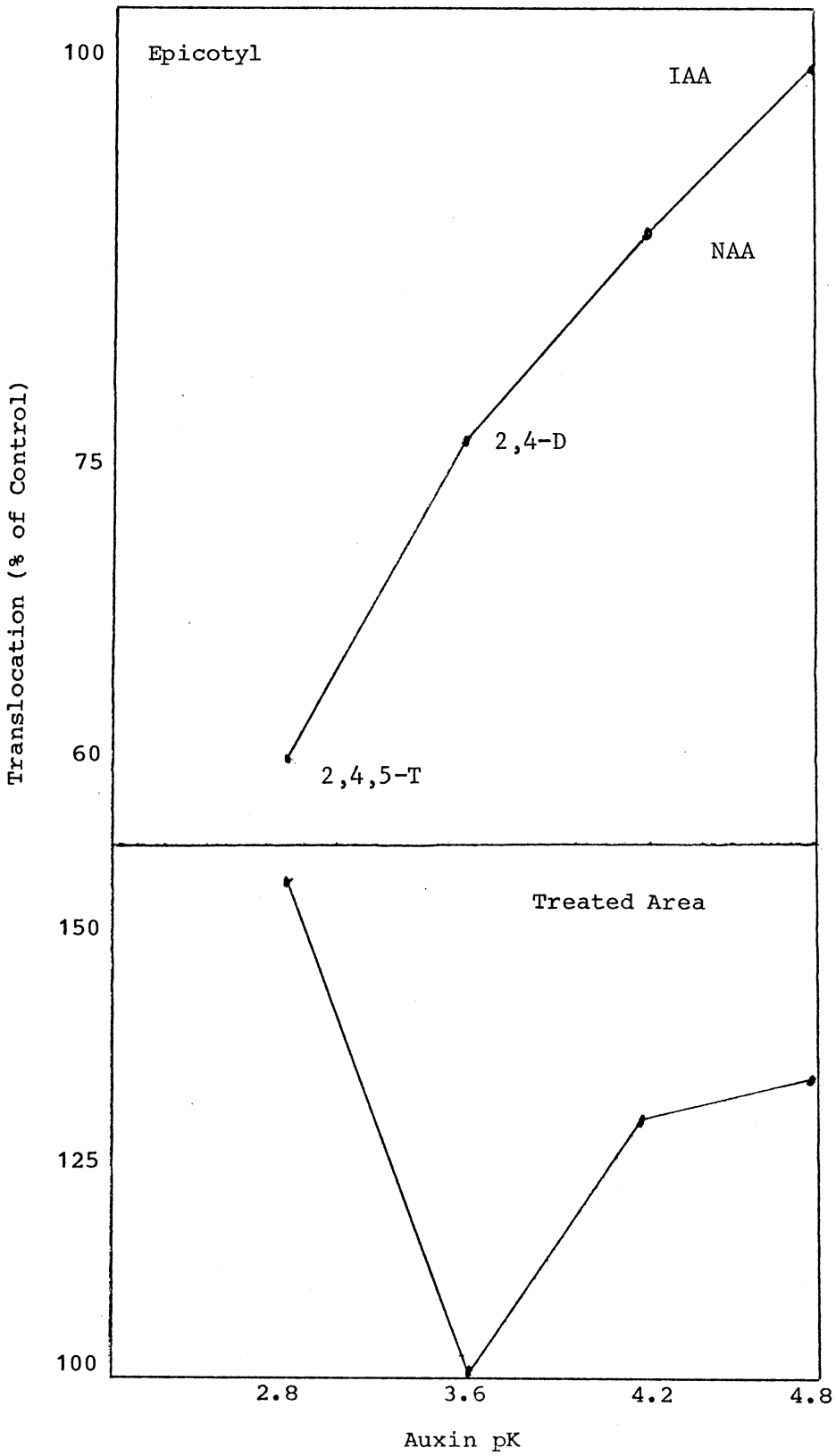
Treatment	Young Shoots	Primary Leaves	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-T	0.6b	23.2d	16.3a	30.3a	14.3bc	2.4cd	0.8bc
0.5 µg 2,4,5-T 16.67 µg Val	0.2c	15.2c	9.6b	45.2b	12.0cd	1.7d	0.4cd
0.5 µg 2,4-D	1.4a	3.7a	9.3b	33.0a	19.3a	6.3a	1.17a
0.5 µg 2,4-D 16.67 µg Val	0.6b	3.4a	7.0c	31.7a	16.5ab	3.9bc	1.1b
0.5 µg NAA	0.6b	20.6d	10.6b	26.3a	9.5de	3.8bc	1.6a
0.5 µg NAA 16.67 µg Val	0.4bc	16.3c	9.3b	32.4a	8.8def	3.8bc	2.0a
0.5 µg IAA	0.3bc	8.3b	4.5d	25.4a	5.7f	4.7b	0.1d
0.5 µg IAA 16.67 µg Val	0.4bc	7.9b	3.3d	31.9a	6.0ef	4.2b	0.1d

*Values are given in percent of total auxin applied. Values for a single plant part followed by the same letter are not significantly different at the 5% level. pK values are as follows: 2,4,5-T is 2.8; 2,4-D is 3.6; NAA is 4.2; IAA is 4.8.



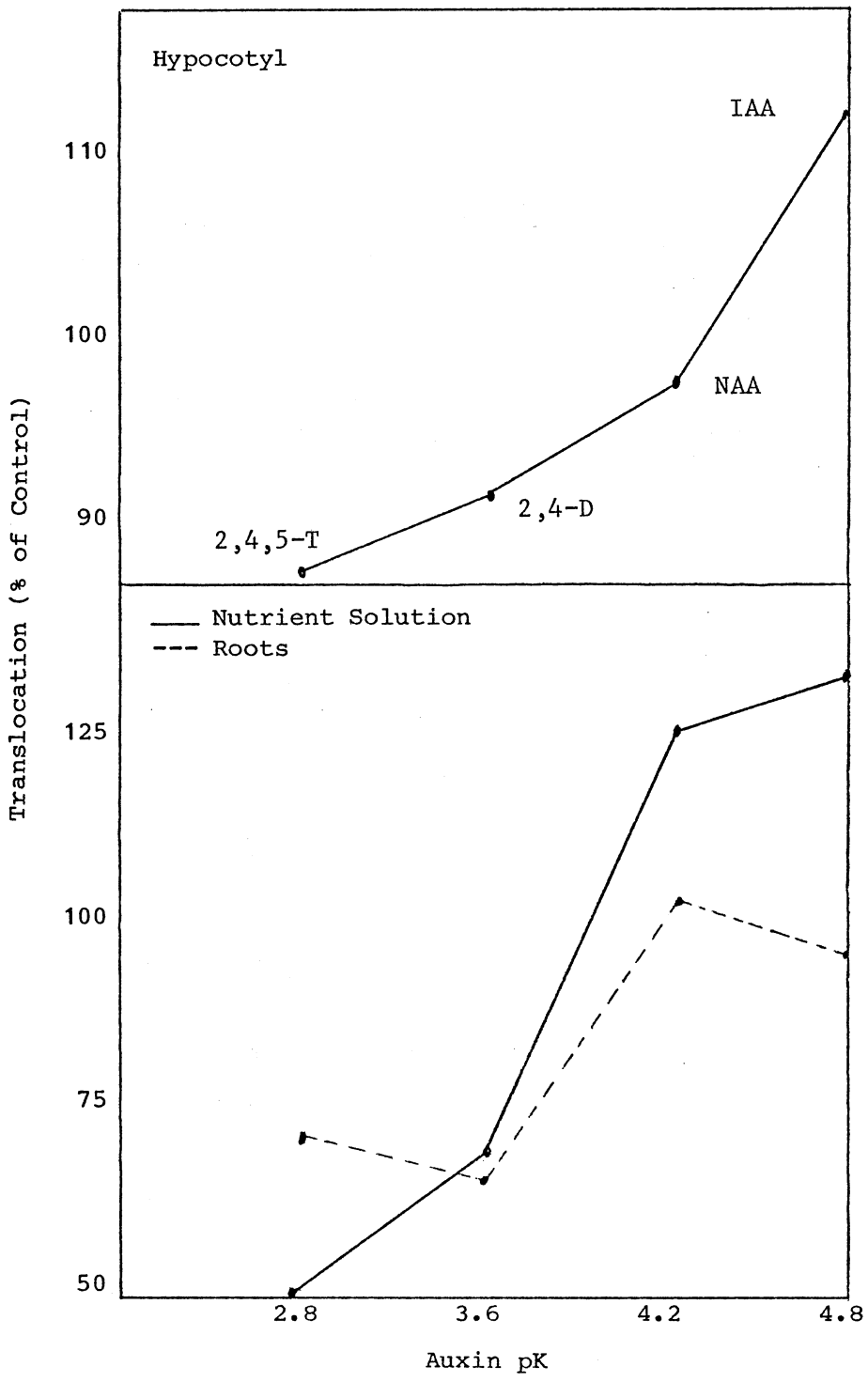
(a) Young Shoots and Primary Leaves

Figure 1. The Effects of Val on the Translocation of Auxins of Increasing pK Values (Val at 16.67 µg per plant and 0.5 µg of auxin per plant was used.)



(b) Epicotyl and Treated Area

Figure 1. (Continued)



(c) Hypocotyl, Nutrient Solution

Figure 1. (Continued)

translocation to acropetal and basipetal areas decreased. The amount of auxin remaining in the treated area also decreased as the pK values increased. Thus, Val inhibition seems to be related in some way to pK values and/or pH values.

The Effects of Nutrient Solution KCl and Val on Translocation of 2,4,5-T

Val again caused a significant decrease in 2,4,5-T translocation to the epicotyls, an apparent decrease to the young shoots, and a significant increase in the amount of 2,4,5-T remaining in the treated area (see Table IV).

The addition of KCl to the nutrient solution caused an increase in translocation of 2,4,5-T to the hypocotyls and roots above the auxin control values. Movement into other areas was not significantly altered by KCl. Compared to the Val treatment, KCl increased movement of auxin into the young shoots, hypocotyls, and roots.

The combination of Val and KCl affected 2,4,5-T translocation in the same manner as Val alone, i.e., decreased movement to the epicotyls and increased the amount staying in the treated area. However, compared to KCl, there was a substantial difference in translocation patterns: movement to the young shoots, epicotyls, hypocotyls, and roots was decreased and more auxin remained in the treated area. Therefore, KCl did not alleviate Val inhibition of auxin translocation when it was added to the Val treatment. But, Val seems to inhibit KCl enhanced translocation more than unmodified translocation.

TABLE IV

THE EFFECTS OF KCl AND VAL ON 2,4,5-T TRANSLOCATION FOUR HOURS AFTER TREATMENT*

	0.5 µg 2,4,5-T			
Plant Part	Control	0.5 µg Val	30mM KCl	0.5 µg Val 30mM KCl
Young Shoots	8.6ab	5.0b	11.9a	3.8b
Primary Leaves	51.3a	57.4a	62.6a	47.3a
Epicotyl	59.9a	43.9bc	55.0ab	41.5c
Treated Area	151.8a	205.8b	148.3a	212.5b
Hypocotyl	75.5a	73.4a	98.3b	75.0a
Roots	5.8a	7.0a	10.2b	6.3a
Nutrient Solution	1.6a	1.6a	4.2a	3.0a

*Values are given in ng 2,4,5-T. Values for a single plant part followed by the same letter are not significantly different at the 5% level.

The Effect of Val on 2,4,5-T Translocation Over Time

At two hours, the only significant differences between the Val and control treatments were a decrease in translocation to the epicotyls and an increase in the amount remaining in the treated area of the Val treatment (Table V). At four hours, Val caused a significant decrease in translocation to the epicotyls and hypocotyls and increased the auxin remaining in the treated area. At six hours, Val had the greatest effect, decreasing transloction to the epicotyls and young shoots and increasing the auxin in the treated area. At eight hours, Val caused a significant decrease in translocation to the epicotyls and nutrient solution and increased the amount of auxin remaining in the treated area.

Auxin translocation to the young shoots increased to a maximum at six hours and decreased to about the original two hour level at eight hours. Addition of Val tended to erase this peak so that there were no significant differences in translocation over time. However, translocation appeared to peak at four hours, two hours earlier than that of auxin alone, and then decreased at six and eight hours. Auxin translocation to the primary leaves did not change significantly over time although it appeared to peak at six and eight hours. Val caused translocation to the primary leaves to increase to a maximum at six and eight hours. Auxin translocation to the epicotyls increased to a peak at six hours and then decreased to the original level at eight hours. Val decreased translocation to the epicotyls at all times, therefore, there was not a peak in Val-effected translocation at any time. Auxin moved out of the treated area steadily and still appeared to be leaving at

TABLE V

THE EFFECT OF 16.67 µg VAL ON 2,4,5-T TRANSLOCATION OF VARYING TREATMENT TIMES*

Treatment	Young Shoots	Primary Leaves	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-T 2 hours	5.9a	67.4abc	68.7a	174.3c	72.8bcd	4.5d	0.5c
0.5 µg 2,4,5-T 16.67 µg Val 2 hours	5.4a	54.8bc	52.7c	252.8e	55.0d	3.9d	2.2bc
0.5 µg 2,4,5-T 4 hours	10.2ab	68.3abc	74.7ab	137.4b	91.0ab	5.6d	2.5bc
0.5 µg 2,4,5-T 16.67 µg Val 4 hours	9.9ab	50.5c	55.0c	202.1d	65.7cd	5.1d	3.2bc
0.5 µg 2,4,5-T 6 hours	16.2b	77.8a	79.9b	111.5a	90.5ab	11.9bc	4.0bc
0.5 µg 2,4,5-T 16.67 µg Val 6 hours	7.3a	69.4ab	49.1c	183.0cd	81.8abc	7.3cd	3.2bc
0.5 µg 2,4,5-T 8 hours	9.8ab	75.2a	65.2a	103.8a	98.6a	20.2a	11.5a

TABLE V (Continued)

Treatment	Young Shoots	Primary Leaves	Epicotyl	Treated Area	Hypocotyl	Roots	Nutrient Solution
0.5 µg 2,4,5-T 16.67 µg Val 8 hours	5.4a	70. 0ab	48.8c	187.9cd	95.2a	16.3ab	5.7b

*Values are given in ng 2,4,5-T. Values for a single plant part followed by the same letter are not significantly different at the 5% level.

eight hours. Val caused more auxin to stay in the treated area at all times when compared to the control at that time, but caused auxin to move out of the treated area at four hours compared to two hours. It remained at a fairly constant level at six and eight hours. Auxin movement into the hypcotyls increased to a maximum at eight hours. Val changed the beginning of the peak of auxin movement to six hours. Auxin movement into the roots showed no real increase until six hours, and increased to the highest levels at eight hours. Val caused translocation to the roots to peak at eight hours. Translocation to the nutrient solution increased to maximum at eight hours. Val caused a significant inhibition of translocation to the nutrient solution at the eight hour treatment.

Val inhibited auxin translocation at all times, but the inhibition did not always affect the same plant parts. When Val did change the time-pattern of translocation to a certain plant part, it did not reverse the pattern, but changed the time of maximum translocation.

Measurement of Oxygen Consumption of Bean Stems at One Hour and Four Hours After Treatment With 2,4,5-T and Val

Analysis of the data in Table VI shows that there were no significant differences among any of the treatments. The amount of oxygen consumed per minute was not changed significantly by 2,4,5-T, Val, or difference in length of treatment. Therefore, it appears that Val does not inhibit oxidative phosphorylation at the concentration used here.

TABLE VI

THE EFFECTS OF 2,4,5-T AND VAL ON THE OXYGEN CONSUMPTION OF TREATED AREAS AT TWO TREATMENT TIMES

Average Treatment Time	µl Oxygen Consumed Per Minute		
	Control	2,4,5-T	2,4,5-T and Val
91 minutes	1.45a	1.29a	1.20a
4 hours, 16 minutes	1.54a	1.67a	1.55a

*Values followed by the same letter are not significantly different at the 5% level.

CHAPTER IV

DISCUSSION

In light of the recent work with Val, its effect on auxin translocation in this study could be via one of several mechanisms:

1. Prevents binding of K^+ to a K^+-H^+ pump.
2. Collapses an electrical or K^+ gradient across the membrane.
3. Decreases the ATP available for translocation by interfering with photosynthesis or oxidative phosphorylation.
4. Changes the membrane status and thus effects ATPase activity or permeability of the membrane to auxin.

The most consistent effect of Val was to inhibit movement of 2,4,5-T out of the treated area. However, 2,4,5-T translocation to all areas but the roots was inhibited by Val in some experiment. A low level of Val (3.33 µg) seemed to increase acropetal translocation as much as 16.67 µg of GA did. However, low Val levels caused most of the extra 2,4,5-T to accumulate in the primary leaves, while GA caused the greatest accumulations in the epicotyl and young shoots. The higher levels of Val (8.33 µg and 16.67 µg) inhibited GA's enhancement of acropetal auxin movement. The lowest level of Val (3.33 µg) interacted with GA to cause an increase in translocation unlike that of Val alone or GA alone. Adding more GA to the Val treatment did not seem to decrease Val action, since the amount of 2,4,5-T remaining in the treated area was not different from that of Val alone. However, the value for 40 µg GA

plus Val also was no different from the 40 µg GA control or the auxin control. So it appears that above 40 µg GA Val inhibition might be overcome. At any rate, the effects of low GA and Val concentrations appear to differ from that of the higher concentrations. An explanation for this may lie in different actions of Val at different concentrations.

Val inhibited 2,4,5-T translocation from the treated area but had no significant effect on 2,4-D, IAA, or NAA movement out of the treated area. Some inhibition of translocation of 2,4-D and NAA to other plant parts was noted . This effect on auxins of different pK values seems to indicate that Val affects the pH gradient. Corbett (1977) showed that DCCD, an ATPase inhibitor, had the same effect as Val on auxins of different pK values. It appears possible that because DCCD inhibits the ATPase and its H^+ gradient, the extracellular pH does not drop below the lower pK values of some of the auxins. Thus, they will be ionized to a greater extent and not able to pass through the membrane. Translocation of auxins with a higher pK will not be inhibited by DCCD since they will tend to remain undissociated and thus lipid soluble. This implies that the initial absorption of auxins may be the limiting step in translocation of auxins in these studies. Val seems to have the same effect as DCCD on these different auxins. However, Poole et al. (1971) reported that Val does not abolish pH gradients across membranes. This data lends credence to the idea that Val could inhibit ATPase and the H^+ gradient it forms by its effect on ATP synthesis or the membrane itself.

Since Val is a K^+ ionophore, it may collapse a K^+ gradient or it may prevent K^+ from interacting with a pump. The addition of extra KCl should alleviate any inhibition of translocation due to its

unavailability of K+ to a K^+-H^+ pump. When KC1 was added to a Val treatment, however, Val inhibition of translocation was not reversed. Additional KC1 increased translocation to the hypocotyls and roots; adding Val prevented this increase as well as decreasing translocation to the young shoots and epicotyls and increasing the amount in the treated area. So, as with GA, Val especially inhibited KC1-enhanced translocation. This also indicated that K^+ does not need to bind with a K^+-H^+ pump, as Val still caused an inhibition of translocation even in the presence of extra K^+. One could argue that the concentration of K^+ was not high enough, but a 30mM solution in the nutrient solution makes 22.9 mmole of K available to the plant. This compares with 0.0148 µmole of val applied to each plant. If only a fraction of the nutrient solution K^+ entered the plant, it should have been enough to override any chelating effects of Val. Even if a K^+ pump is not involved in translocation, it is still possible that a K^+ gradient is important. Cleland and Lomax (1977) described an auxin stimulated H^+ efflux pump coupled with passive K^+ uptake. In such a system, H^+ and K^+ traversed the membrane at different sites. The addition of Val to this sytem would not change the direction of K^+ flux. Additional K^+ in this sytem might actually enhance the H^+ gradient formed by making more positive charges (i.e., K^+) available for exchange with H^+. Eventually the enhancement would halt as the electrochemical potential becomes too high to allow further extrusion. Even without added K^+, Val could enhance translocation by increasing the amount of endogenous K^+ crossing the membrane, and thus the amount of H^+ extrusion. However, a study of Val translocation over time revealed no such enhancement; Val inhibited movement out of the treated area at two hours, four hours, six hours, and eight hours. It is possible that the

enhancement occurred at less than two hours. Another trend shown by the time experiment was the lack of an increase in translocation to the primary leaves over time. This indicated that 2,4,5-T was being exported from the primary leaves after entry at all treatment times. Long and Basler (1973) stated that auxin moved upward to the primary leaves in the xylem and was then exported from the leaves via the phloem in stem-girdled beans. The primary leaves would then act as another source of auxin for the plant. Val did not seem to effect this action since it caused no significant differences in translocation to the leaves. This was expected since the primary action of Val seemed to be an inhibition of translocation out of the treated area. The movement of auxin after it leaves the treated area would remain unchanged.

Another possibility explaining Val inhibition is a decrease in available energy caused by Val uncoupling action on mitochondria. Val may uncouple mitochondria by diverting ATP energy to pumping out K^+ transported in by Val. This would inhibit the formation of a H^+ gradient by a plasma membrane ATPase. Val also interferes with photosynthesis, but this action probably would not play a large part at the treated area where little photosynthesis occurs. The measurement of oxygen consumption of bean stems injected with Val and 2,4,5-T showed no difference among treatments. This indicated that energy supplies were not effected by Val at the concentrations used. Other workers have found that auxins stimulate respiration, yet no enhancement of oxygen uptake by 2,4,5-T was seen (Marre, 1977). It is possible that the injected auxin was diluted within the treated area so that no effect on respiration occurred , or that the effect on respiration occurred within such a small number of cells that the effect was masked by the rest of

the tissue. In this case, Val could have decreased available ATP at the site of auxin uptake without showing a change in respiration of the treated area. Further work needs to be done to ascertain the effect of Val on respiration at the site of auxin uptake.

Val definitely inhibited auxin translocation. It inhibited the upward increase in translocation caused by GA and the downward increase in translocation caused by KCl. It affected the translocation of auxins with different pK values differently but did not seem to decrease the ATP available to form a H^+ gradient. Val inhibition of translocation out of the treated area did not change over time, but low concentrations of it did seem to increase upward translocation. Also, adding K^+ to the plants did not alleviate Val inhibition at higher concentrations. The experiments of Corbett (1977) seemed to show that auxin must be taken up by living cells to be transported out of the treated area and that a H^+ gradient is involved in this uptake. Therefore, the mechanism of Val action could be to inhibit auxin uptake into cells. The known action of Val as a K^+ ionophore would seem to enhance the formation of a H^+ gradient across the plasma membrane by increasing the amount of K^+-H^+ exchange. There is some indication that this happened since low levels of Val seemed to enhance upward movement of auxin. Yet at higher concentrations there was an inhibition of translocation that is hard to explain by the classic role of Val as an ionophore. Val seemed to effect the pH gradient since there was a different effect on auxins with different pK values. This, too, is not explained by the ionophore properties of Val. Therefore, it may be that at higher concentrations, Val effects membrane permeability and thus decreases ion flux, auxin transport across the membrane, or even ATPase activity. This membrane action

would explain the effect of Val on GA-affected translocation. Katsumi and Kazama (1978) suggested that GA interacts with the membrane to somehow enhance ATPase activity. Therefore, Val could inhibit GA-enhanced translocation by decreasing the permeability of the membrane. Erdei et al. (1978) reported that lipids are necessary for the activity of ATPase, so Val could have a direct effect on ATPase activity by changing its lipid environment. This would also explain Val inhibition of KCl enhanced translocation since there would be no H^+ extrusion to drive K^+ uptake.

The almost exclusive use of 2,4,5-T in this study brings up the question of how pertinent these findings are to the endogenous translocation mechanism that functions using IAA. Particularly disturbing in this respect is the data showing that Val inhibited 2,4,5-T translocation but had no significant effect on IAA translocation. Even though it is known that 2,4,5-T has many of the same actions on growth and development as IAA does, it would be erroneous to assume all characteristics of the two chemicals are the same. Therefore, until more is known about the mechanism of translocation it cannot be stated for certain that IAA and 2,4,5-T use the same mechanism for translocation. Because the molecules do have some different characteristics, such as pK values, it would be just as false to assume they do move by separate mechanisms, since the differences in translocation may be due to a different response to the same mechanism.

CHAPTER V

SUMMARY AND CONCLUSIONS

The long distance translocation of auxins was increased by GA and KCl and inhibited by Val. The effects of GA and KCl were in opposite directions, yet Val inhibited their enhancement of translocation. The known functions of Val and auxin allow several possible mechanisms for Val action:

1. Prevents K^+ from binding with and thus stimulating, a H^+ efflux/K^+ influx pump.
2. Collapses an electrical, or K^+, gradient across the membrane.
3. Decreases the energy available for formation of a H^+ gradient by interfering with oxidative phosphorylation or photosynthesis.
4. Alters the fluidity of the membrane and thus inhibits an ATPase and decreases permeability of the membrane.

The addition of K^+ to a Val treatment did not relieve its inhibition, so a specific K^+-H^+ pump must not function in auxin translocation.

No direct evidence was obtained to confirm or deny the supposition that a K^+, or electrical, gradient is needed for auxin translocation. However, the interaction of Val with auxins of different pK values indicated that a H^+ gradient was involved. Corbett's (1977) work with DCCD (an ATPase inhibitor) also indicated the importance of a H^+ gradient in

auxin translocation. KCl enhanced auxin translocation, so, a K^+ gradient could be involved in translocation. However, the involvement of K^+ could be explained by an indirect effect on formation of a H^+ gradient.

Val had no effect on respiration in this study. However, more careful measurements are needed to ascertain the effects on respiration at the site of auxin uptake. Until then, it appears there was sufficient energy present to form a H^+ gradient across the membrane. This gradient is assumed to enhance auxin uptake and its subsequent translocation. This model parallels the theory of sugar uptake from the apoplast and its ensuing translocation (Malek and Baker, 1977).

No direct evidence was obtained for the effect of Val on membranes, but it is an attractive explanation for the diverse actions of this ionophore. Val not only inhibited auxin translocation, but it also inhibited the mechanisms by which GA and KCl enhanced translocation. It also appeared to change the pH gradient involved in translocation. All of these effects could be explained by a Val action on the membrane. Walz (1976) stated that the concentration of Val is the relevant parameter in its interaction with membranes, and that K^+ is not needed for the interaction. It was found that the effect of Val on translocation changed at different concentrations and that additional K^+ did not change the inhibition of Val. So of the possible actions of Val, the best explanation of inhibition lies with its effect on the membranes.

The actions of Val tended to vary among the experiments with respect to inhibition of translocation to the different plant parts. Further work needs to be done to determine which of these inhibitions are reproducible. Also, more work with low Val concentrations needs to

be done to determine whether the slight increase in translocation seen here is a reproducible effect. The use of a K^+ electrode would be invaluable in determining the importance of a K^+ gradient at the membrane. Finally, as stated previously, the measurement of respiration as effected by Val needs to be repeated.

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