KINETICS OF AUXIN TRANSLOCATION
IN INTACT BEAN SEEDLINGS

By

John W. Long

Bachelor of Science
McPherson College
McPherson, Kansas
1966

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1969

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1971

OKLAHOMA
STATE UNIVERSITY
LIBRARY
DEC 31 1971

KINETICS OF AUXIN TRANSLOCATION

IN INTACT BEAN SEEDLINGS

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

803940

ACKNOWLEDGEMENTS

I wish to express special gratitude to Dr. Eddie Basler, under whom I have received my graduate training. His ability to provide the right amount of guidance at the right time was highly valuable during the conduct of this research and is greatly appreciated.

Special acknowledgement is due my wife, Bonnie, whose patience and moral support have prevailed unwaveringly. This is valued far beyond the typing of this manuscript.

I am also indebted to the other members of my committee, Drs. J. K. McPherson, P. E. Richardson, G. W. Todd and P. W. Santlemann for guidance in their individual specialties, for suggestions concerning my research and for critical review and suggestions concerning this manuscript. Many other members and former members of the Department of Botany and Plant Pathology have provided encouragement during my training.

I also acknowledge the aid of Dr. R. D. Morrison of the Department of Mathematics and Statistics in the statistical evaluation of this research.

I am indebted to Harold Spiegel for the preparation of two of the illustrations and to Mrs. Sandra Reisbeck for assistance in the laboratory.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	Auxin Translocation in Plants	3
	Factors Affecting Auxin Translocation	4
	Auxin and Assimilate Interactions	8
	Protein Synthesis and Auxin Translocation	9
III.	METHODS AND MATERIALS	12
	Source of Plants	12
	Method of Treatment	12
	Method of Assay	13
	Steam Girdling	16
IV.	RESULTS AND DISCUSSION	18
	Time-Course Studies	18
	Effect of Auxin Concentration on Translocation	31
	Effect of Steam Girdling on 2,4,5-T Translocation	36
	Effect of Antiauxin on 2,4,5-T Translocation	39
	2,4-D/2,4,5-T Interactions in Translocation	42
	2,4,5-T Effects on Sugar Translocation	47
	Effect of Cycloheximide on 2,4,5-T Translocation	53
V.	SUMMARY AND CONCLUSIONS	60
LITERATURE CITED		64

LIST OF TABLES

Table		Page
I.	^{14}C-Labeled Auxin Accumulated in Plant Parts and Nutrient Solution After Injection of ^{14}C-Labeled and Unlabeled 2,4-D and 2,4,5-T into the Stem of Bean Seedlings	44

LIST OF FIGURES

Figure		Page
1.	Line Drawing of 10-Day-Old Bean Seedling Showing Points of Dissection	14
2.	Apparatus Used to Apply a Steam Girdle to Various Points on the Stem of Bean Seedlings	17
3.	Influence of Treatment Time on Accumulation of 2,4,5-T in Nutrient Solution, Roots, Primary Leaves and Growing Points	20
4.	Accumulation of 2,4-D and 2,4,5-T in Primary Leaves and Growing Points Throughout a 72-Hour Treatment Period	21
5.	Accumulation of 2,4-D and 2,4,5-T in Nutrient Solution and Roots Throughout a 72-Hour Treatment Period	22
6.	Accumulation of 2,4,5-T in Nutrient Solution and Roots During a 24-Hour Treatment Period	25
7.	Accumulation of 2,4,5-T in Primary Leaves and Growing Points During a 24-Hour Treatment Period	26
8.	Accumulation of 2,4,5-T in Nutrient Solution and Roots During a 6-Hour Treatment Period	28
9.	Accumulation of 2,4,5-T in Primary Leaves and Growing Points During 6 Hours of Treatment	29
10.	Influence of 2,4,5-T Concentration on the Accumulation of 2,4,5-T in Roots and Nutrient Solution	32
11.	Influence of 2,4,5-T Concentration on the Accumulation of 2,4,5-T in the Growing Points	34
12.	Influence of Concentration of Auxin on Accumulation of Auxin in the Growing Points	35
13.	Influence of a Steam Girdle Located at Various Positions Along the Stem on 2,4,5-T Accumulation in Growing Points, Primary Leaves and Roots	38
14.	Influence of PCIB on Accumulation of 2,4,5-T in Nutrient Solution and Roots	40

Figure		Page

15. Influence of PCIB on Accumulation of 2,4,5-T in Primary Leaves and Growing Points 41

16. Accumulation of ^{14}C-Sucrose and ^{3}H-Glucose in Growing Points and Primary Leaves With or Without Simultaneous Injection of 2,4,5-T. 48

17. Influence of 2,4,5-T on Accumulation of Sugars in Roots and Loss of Sugars From the Stem After Stem-Injection of Labeled Sugars in Bean Seedlings. 50

18. Recovery of ^{14}C of Sucrose and ^{3}H of Glucose From all Plant Parts After Injection in the Stem of Bean Seedlings 52

19. Influence of Cycloheximide on the Accumulation of 2,4,5-T in Nutrient Solution and Roots 54

20. Influence of Cycloheximide on the Accumulation of 2,4,5-T in Primary Leaves and Growing Points 55

21. Influence of Concentration of Cycloheximide on Accumulation of 2,4,5-T in Various Plant Parts After 4 Hours of Treatment . 57

CHAPTER I

INTRODUCTION[1]

Auxins are a major hormonal component of plant development. In many instances the site of auxin synthesis is distal from the site at which auxin exerts its influence, so that the translocation properties of auxin constitute a major factor in the regulation of plant developmental processes. Likewise, the herbicidal activity of systemic herbicides such as the phenoxy compounds is highly correlated with the ease with which the compound moves from the point of application to the site of highest herbicidal activity.

The strong basipetal polarity of transport of auxins and auxin-like compounds in isolated segments of stems, petioles and coleoptiles is well documented in the literature. It is generally conceded, however, that in isolated segments the vascular tissues are rendered inactive and polar auxin movement is predominantly through parenchymatous tissue. Acropetal transport is generally considered to be of little physiological significance or non-existent.

Since the phytotoxicity of any systemic herbicide depends upon its

[1]The following abbreviations are used: 2,4-D (2,4-dichlorophenoxyacetic acid); IAA (indole-3-acetic acid); NAA (naphthaleneacetic acid); PCIB (p-chlorophenoxyisobutyric acid); 2,4,5-T (2,4,5-trichlorophenoxyacetic acid); TIBA (2,3,5-triiodobenzoic acid).

movement from the point of application to the area in which the chemical produces its highest herbicidal activity, it is important to study the processes which regulate the translocation patterns of the auxin-like herbicides. Studies have consistently shown that small changes in the molecular structure of the herbicide result in marked changes in the mobility and polarity of translocation of the molecule. Recently it has been noted that using mixtures of two auxin-like herbicides enhanced their effectiveness as herbicides, suggesting that properties inherent in one herbicide may enhance the mobility of the other herbicide.

The present study was designed to explore the kinetics of translocation of auxins utilizing intact bean seedlings.

CHAPTER II

REVIEW OF LITERATURE

Auxin Translocation in Plants

Early plant physiologists discussed auxin movement primarily in terms of its characteristic basipetal polar transport (Went and Thimann, 1937). Experiments involving both intact plants and plant parts demonstrated that IAA moved in the plant via three separate mechanisms: 1) both endogenous and externally applied IAA underwent basipetal polar transport in isolated stem segments; 2) auxin applied in the nutrient medium moved upward in the transpiration stream and; 3) translocation of auxin applied to decapitated plants moved downward via the phloem (Skoog, 1938).

Later more attention was given to the auxin-like phenoxy herbicides. Day (1952) carried out a rather thorough series of experiments involving the velocity and direction of movement of 2,4-D applied to the primary leaf of bean. Work involving foliar applications, such as this, tended to support the hypothesis that these growth regulators moved with the stream of assimilates (Rice, 1948; Rohrbaugh and Rice, 1949; Hay and Thimann, 1956). However, the meristimatic regions of the stem apex and the young leaf primordia appeared to be the primary sites of auxin biosynthesis, while fully expanded leaves exported little or no auxin (Scott and Briggs, 1960). Consequently translocation patterns of foliarly applied herbicides did not necessarily reflect the distribution

mechanisms for growth regulators in the plant. In general, growth regulators applied to roots or shoots of intact plants appeared to move in both the phloem and xylem (Skoog, 1958; Little and Blackman, 1963). The extent of interaction between xylem and phloem in regulating the movements of auxins in plants is not well understood.

Recently, two papers have elucidated several details involving the pattern and mechanism of auxin translocation. Eschrich (1968) quantitatively followed the distribution of label in *Vicia faba* following the application of IAA-2-^{14}C to fully expanded primary leaves. He recovered ^{14}C-labeled compounds from the stem, roots and young expanding leaves, but not from mature leaves. This suggested that both acropetal and basipetal movement of label from the treated leaf occurred. Morris, et al., (1969) followed the movement of ^{14}C-labeled IAA which had been applied to the apical bud of intact dwarf pea seedlings. The label of IAA was traced through the stem and roots where it was readily converted to indoleacetyl aspartate and indole-3-aldehyde. The indole-3-aldehyde appeared to be readily transported in both stems and roots while the IAAsp represented an immobilized form of the auxin. It is not clear from these studies whether retranslocation of IAA or its metabolites occurred in these plants.

Factors Affecting Auxin Translocation

Auxin Concentration

The movement of auxins through isolated stem segments and coleoptile sections is proportional to IAA concentration in the donor block over a limited range. (Goldsmith and Thimann, 1962; Gillespie and Thimann, 1963; Scott and Jacobs, 1963). However, the ratio of the

amount in receivers to the amount in the donors steadily decreased with increasing dosage. A logarithmic plot of the radioactivity received as a percentage of the net loss from the donor shows a linear decrease in transport with an increase in concentration (McCready and Jacobs, 1963). There is relatively little information concerning the effect of concentration on translocation of auxins in intact plants. In one such study, as amount of 2,4-D or 2,4,5-T applied to the leaves increased from 1 to 20 μgrams/plant the time taken to initiate curveture of the stems decreased (Little and Blackman, 1963). This seems to suggest only that the amount of auxin required to initiate bending of the stems reaches the stems more rapidly at the higher concentrations.

Steam Girdling

Regardless of the site of application of exogenous auxins the question of whether movement is via the xylem or the phloem remains. From the distribution patterns determined by autoradiographic studies Crafts and Yamaguchi (1958) concluded that the auxins 2,4-D and 2,4,5-T moved out of the treated leaves via the phloem and movement occurred almost exclusively in the phloem. A number of workers have concluded that the movement of auxin-like herbicides out of the leaves is dependent upon the translocation of assimilates (Mitchell and Brown, 1946; Rohrbaugh and Rice, 1949; Jaworski, et al., 1955) and Little and Blackman (1963) have shown that ringing the petiole before treatment with 2,4-D completely inhibited initiation of stem bending suggesting that movement was via the phloem in the assimilate stream.

Skoog (1938) applied auxin to tomato plants above a steam girdle on the stem and noted that auxin collected in the stem directly above

the girdle. However IAA applied to the culture solution in which the plants were growing moved upward and moved freely through the killed portion of the stem. This suggested to Skoog that downward movement was via the phloem while upward movement was in the xylem.

Antiauxins

Antiauxins, as a general rule, are auxin-like molecules without the typical auxin properties of polar basipetal transport in isolated segments and without pronounced promotional effect on growth of isolated stem and coleoptile tissue. Furthermore, antiauxins such as TIBA are known to inhibit both the transport of IAA (Zwar and Rijven, 1956) and the growth promoting activities of auxins (de la Fuente and Leopold, 1970).

It has been noted that TIBA affects the uptake differently from export of IAA in coleoptile sections (Christie and Leopold, 1965a). By increasing the concentration of IAA, the effect of TIBA on IAA entry into the cell could be overcome. High concentrations of IAA did not overcome the inhibition of export of IAA by TIBA. Christie and Leopold (1965b) consider TIBA to be a "sulfhydryl poison" and that TIBA may modify secondary structural features related to the export of IAA such as some constituent of the cell membrane.

Hagar and Schmidt (1968) noted the production of an oxidation product of IAA upon illumination of corn coleoptile segments. They were further able to identify the compound as 3-methylene oxindole (3-M). This compound inhibited the export of IAA from the cells as well as the phototropic response to unilateral light source. This suggests a regulatory role for naturally occurring antiauxins.

Information regarding the effects of antiauxins on natural auxin responses in intact plants is sparse. Mullins (1970) showed that TIBA at concentrations which inhibit polar auxin transport in stem segments augment the IAA-effect on import of ^{14}C-labeled photosynthates into the internodes of decapitated bean seedlings.

Auxin-Auxin Interactions

The study of auxin-auxin interactions has thus far been primarily concerned with the effects of other auxin-like molecules on the polar transport of IAA and growth in coleoptile and stem segments (Niedergang-Kamien and Leopold, 1959). These workers noted that the extent of inhibition of IAA transport in sunflower stem segments varied with the number of substituted chlorines on the phenoxy ring and their position. Further studies showed that these substituted chlorophenoxyacetic acids had little effect on the uptake of IAA by the segments but markedly inhibited the export of IAA from the basal end of the segments. As was discussed earlier, much the same situation is described for the anti-auxin TIBA (Christie and Leopold, 1965a).

Evans and Hokanson (1969) observed that some of the auxins which inhibit IAA transport (e.g. 2,4-D and NAA) also are themselves exported from coleoptile tissue more slowly than IAA. A study of the auxin activities of a series of substituted benzoic acids and their effect on polar auxin transport led Keitt and Baker (1966) to the conclusion that the auxins with highest growth promotive activity were the least inhibitory to IAA transport and that the most effective inhibitors of IAA transport were the least growth promotive.

2,4-D is one of the auxins which inhibits the transport of IAA.

Hertel's group (Hertel and Flory, 1968; Rayle, et al., 1969) reported that IAA enhanced the basipetal transport of 2,4-D in corn coleoptiles several fold. Thus we see a situation in which there is an interaction between two structurally related molecules which, depending on the molecule, may be promotive or inhibitory to transport.

Auxin and Assimilate Interactions

Sugar translocation and auxin translocation appear to interact in several ways. It was pointed out earlier that the translocation of foliarly applied auxins and auxin-like herbicides from the leaf depends upon the active export of photosynthetic assimilate from the leaf (Mitchel and Brown, 1946; Rohrbaugh and Rice, 1949; Jaworski, et al., 1955).

Besides the widely recognized effects of source-sink relationships on the translocation of organic materials (Wardlaw, 1968), it has been suggested that there is also a hormone-directed translocation of assimilates in plants (Went, 1939). Booth, et al. (1962) showed that replacement of the apex of decapitated pea seedlings with IAA stimulated the movement of ^{14}C-photosynthate to the site of auxin application within 6 to 11 hours. Davies and Wareing (1965) noted that the application of IAA to the decapitated stems of peas enhanced the accumulation of ^{32}P to the auxin-treated portion of the stem in 6 to 12 hours. TIBA applied to the surface of the stem midway between the IAA source and the ^{32}P-treated leaf considerably reduced the translocation of ^{32}P.

Crafts and Yamaguchi (1958) and Khan and Sagar (1969) considered the role of auxins applied to developing shoots and fruits to be the establishment or the enhancement of metabolic sinks which in turn stim-

ulates the movement of nutrients into the sinks.

Hew, et al.,(1967) have conducted experiments with durations as short as one hour in which they showed that IAA applied to the tip of decapitated soybean seedlings enhanced the basipetal translocation of photosynthate in the stem. This seems irreconcilable with the hypothesis that IAA enhances movement of assimilate toward the site of IAA treatment unless it is considered in the light of vascular anatomy of bean assuming that the stem anatomy of soybean is similar to *Phaseolus*. Doutt (1932) and Mullins (1970a) pointed out that all vascular bundles that originate from the primary leaf node descend the stem to anastomose with ascending vascular bundles at the base of the stem. Mullins (1970a) noted that the pulse of ^{14}C-photosynthate applied to the primary leaf descended the stem from the primary leaf to the base of the stem and then ascended the stem into the first trifoliolate leaf.

Lepp and Peel (1970) reported that IAA increased sucrose loading into the sieve elements of isolated segments of willow bark. IAA also enhanced the polar transport of sucrose in isolated segments of willow bark (Lepp and Peel, 1971a) suggesting a direct effect of IAA on assimilate movement localized in the stem. Hew, et al.,(1967) found that when IAA-^{14}C was applied to the decapitated soybean seedlings the label remained almost exclusively in the stem, further suggesting that auxins exert a regulatory influence on the vascular system within the stem.

Protein Synthesis and Auxin Translocation

Auxins promote elongation and fresh weight increases in isolated stem and coleoptile tissue suggesting that they are accompanied by *de novo* protein synthesis. IAA and 2,4-D promote the increase in fresh

weight of tissue and the ^{14}C-glycine incorporation into protein in excised pea stem segments (Fang and Yu, 1965). Chloramphenicol, an inhibitor of protein synthesis, has been shown to inhibit the cell expansion induced by IAA and the synthetic auxins NAA and 2,4-D in pea stem sections, oat coleoptiles and artichoke tuber sections (Nooden and Thimann, 1965). The same concentrations of chloramphenicol which inhibited auxin-induced growth also inhibited the incorporation of ^{14}C-leucine into both soluble and insoluble protein fractions.

Abeles (1966) noted that the 2,4-D and IAA stimulation of ethylene production could be blocked by a number of inhibitors of RNA and protein synthesis. Abeles concluded that de novo protein synthesis was necessary for ethylene biogenesis.

There is considerable controversy in the literature as to whether IAA is effective at the translational or the transcriptional level in protein synthesis. It seems well established that auxins modify the metabolism of nucleic acids, particularly RNA (Basler and Nakazawa, 1961; Key and Shannon, 1964). Fites, et al.,(1969) reported that m-RNA levels in etiolated soybean tissue were enhanced by 2,4-D. At high concentrations of 2,4-D m-RNA synthesis was inhibited. From experiments on the timing of auxin responses in etiolated pea seedlings, Warner and Leopold (1969) concluded that the latent periods for IAA induction of elongation were exceptionally short in comparison to the nucleic acid half-life in higher plants suggesting that the initial response to auxin was at a site other than nucleic acid metabolism. Nelson and Reinhold (1969) have studied the effects of protein and nucleic acid synthesis inhibitors on auxin-induced elongation of sunflower hypocotyl segments. They noted a 45-60 minute lag in the inhibition of auxin-induced growth

in the presence of the RNA synthesis inhibitors 8-azaguanine or actinomycin D, whereas no lag could be detected in the depression of auxin-induced growth by cycloheximide. Thus it appears that although RNA levels are modified by auxins and increases in RNA levels are coincident with continued auxin-induced growth, the involvement of auxins with protein synthesis appears to precede that of auxins with nucleic acid metabolism.

The non-covalent bonding of auxins with proteins has been suggested a number of times (Niedergang-Kamein and Leopold, 1959; Hagar and Schmidt, 1968b; Hertal and Flory, 1968). Osborne (1968) suggested that polar transport of auxins in plants might be associated with a specific protein carrier like the ones demonstrated in bacterial and animal systems. She suggested that the binding of the auxin to the carrier molecule was a prerequisite to transport across the cell membrane. Further studies (Osborne and Mullins, 1969) led her to modify her model to one which had two sites; one specific for auxin and one specific for ethylene. Filling one site mutually excluded filling of the other site. When ethylene occupied its site the binding and subsequent transport of auxin was blocked. Thus IAA modifies protein synthesis and is capable of non-covalent binding at highly stereo-specific sites. From this it seems reasonable to suggest that auxin may induce the synthesis of its own carrier molecule since very short pretreatments (20 min.) enhance the transport of IAA in coleoptile tissue (Rayle, et al., 1969).

CHAPTER III

METHODS AND MATERIALS

Source of Plants

Bush bean (Phaseolus vulgaris L. cv. stringless GreenPod) seeds were germinated in perlite moistened with Hoagland's nutrient solution (Hoagland and Arnon, 1950) for 5 days at 29 C under continuous fluorescent light of 500 ft-c. The seedlings were then transplanted to 500 ml amber jars containing 400 ml of one-half strength Hoagland's nutrient solution, placed in a controlled environment chamber, and the roots provided with aeration. The plants were grown for an additional 3 days at 33 C day temperature, 29 C night temperature and relative humidity ranging from 20 to 30%. The plants were provided with a 14-hour photoperiod with light intensities of about 2100 ft-c of cool-white fluorescent and incandescent light.

Approximately 12 hours prior to treatment the plants were transferred to fresh one-fourth strength Hoagland's nutrient solution and returned to the growth chamber. Thus at the time of treatment the plants were generally 10 days old.

Method of Treatment

The plants were treated by injecting the compound being studied into the pith area of the stem with a 1 μl syringe. The cotyledons were removed and the syringe needle was inserted at the cotyledonary node

and forced down the center of the stem to about one centimeter below the cotyledonary node where the treatment solution was deposited.

Method of Assay

The plants were harvested after the designated treatment time and separated into growing points, primary leaves including petioles, stems and roots (see Figure 1). The plant parts were quickly frozen at -40 C and freeze-dried. The dried plant parts were then weighed and homogenized in 10 milliliters of 95% ethanol in a Virtis high-speed homogenizer.

Small portions of the homogenates were pipetted into counting vials containing scintillation cocktail consisting of xylene:dioxane:ethanol (5:5:3,v/v/v) containing 80g; naphthalene and 5g 2,5-diphenyloxazole (PPO) per liter. These samples were assayed for radioactivity using liquid scintillation techniques. Background values were corrected for quenching through the use of standard quench curves for external standards. All computations and statistical analyses were performed using an IBM 360, model 65 computer and programs prepared by the author.

The nutrient solution in which the treated plants were growing were monitored by taking 5 milliliter samples at designated time intervals. The samples were assayed for radioactivity again using liquid scintillation techniques.

^{14}C-Labeled Auxins

The experiments represented by Figures 2 and 3 utilized 2,4-D-1-^{14}C (sp. act. 31.6 mCi/mM) and 2,4,5-T-1-^{14}C (sp. act. 30.0 mCi/mM) as the labeled molecule. All other experiments conducted in this study uti-

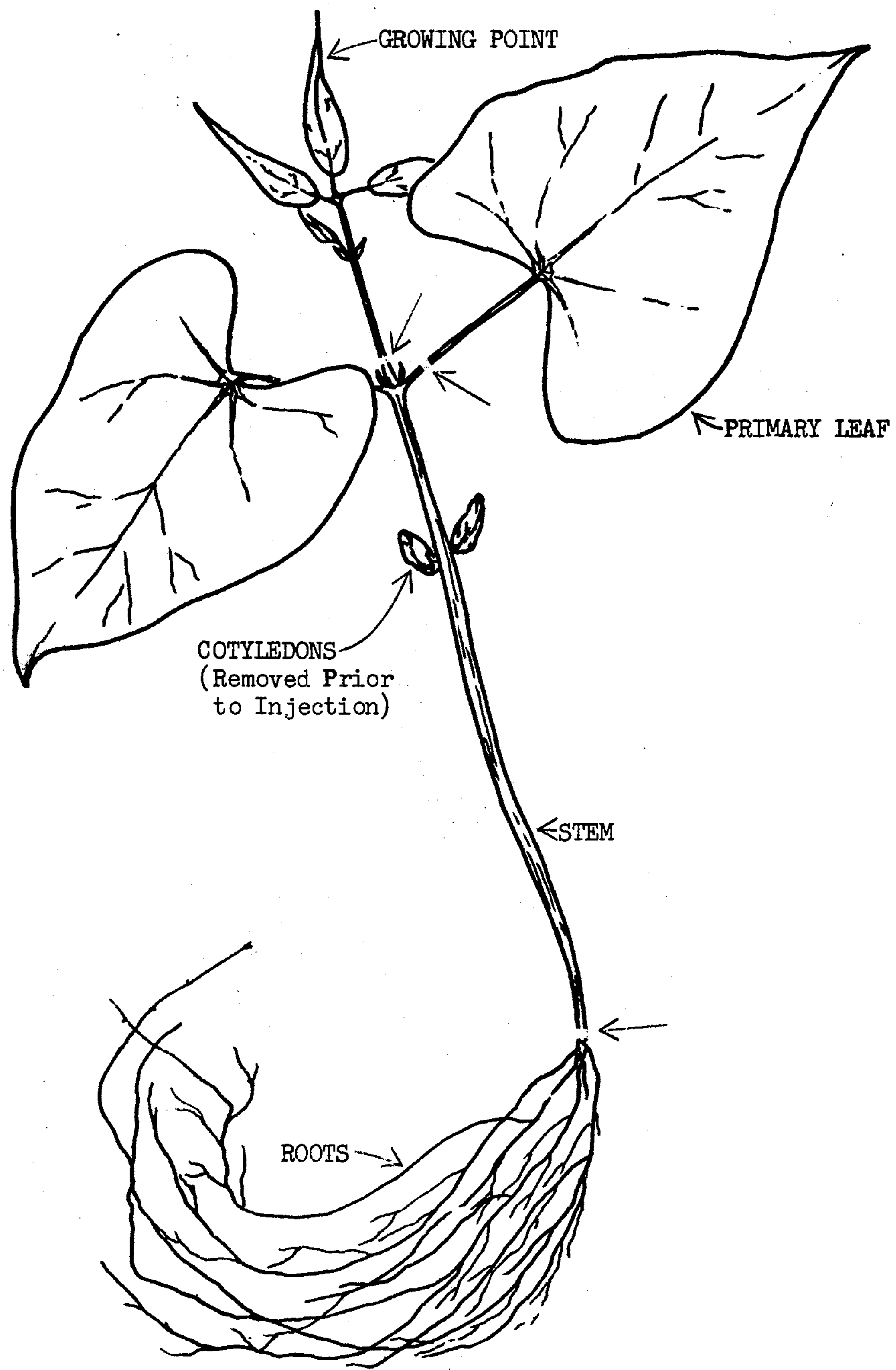

Figure 1. Line drawing of 10-day-old bean seedling showing points of dissection. 4/5 scale.

lized 2,4,5-T-1-^{14}C (sp. act. 12.0 mCi/mM), 2,4-D-1-^{14}C (sp. act. 28.4 mCi/mM) and IAA-1-^{14}C (sp. act. 57.0 mCi/mM). Radiochemical purity was determined by ascending paper strip chromatography with a solvent composed of n-butanol:acetone:water (5:3:3,v/v/v). The chromatograms were assayed in a Picker strip-chromatograph scanner. Purity always exceeded 95%. The unlabeled 2,4-D and 2,4,5-T were purified by repeated recrystallization, first from hot benzene and final recrystallization from boiling 50% ethanol. Unlabeled IAA was purified by recrystallization from 30% ethanol.

Generally, 1 µg of labeled compound was injected into each plant in the experiment. In those experiments in which the total amount of compound injected in the plants exceeded 1 µg, the difference was made up by the addition of unlabeled compound to the injection mixture. For instance, if the treatment was 10 µg of 2,4,5-T, 1 µg of 2,4,5-T-1-^{14}C plus 9 µg of unlabeled 2,4,5-T were injected per plant. In those instances in which the total amount of compound was less than 1 µg, the injection solution consisted solely of labeled material. In terms of auxin activity, 1 µg of 2,4-D or 2,4,5-T generally promoted growth and increases in dry weight whereas levels of 3 µg or more became increasingly growth inhibitory and caused strong epinastic responses.

In the experiments in which the influence of other compounds (specifically PCIB and cycloheximide) on the translocation of 2,4,5-T was determined, these compounds were injected into the plant simultaneously with 2,4,5-T in 1 µl of 95% ethanol.

<u>^{14}C- and ^{3}H-labeled Sugars</u>

In the experiments designed to determine the effect of 2,4,5-T on

translocation of sugars injected into the stem along with 2,4,5-T, 1.46×10^{-10} moles of sucrose-^{14}C(UL) (sp. act. 360.0 mCi/mM), 6.82×10^{-10} moles of D-glucose-3-^{3}H(n) (sp. act. 1100.0 mCi/mM) and 3 μg of unlabeled 2,4,5-T were injected into the stem in 1 μl of 80% ethanol. Radioactivity which accumulated in the plant parts was measured by use of Packard Tri-Carb 3220 Liquid Scintillation Detector calibrated for simultaneous dual-label detection. External standard values were recorded for each sample in the third channel and these values were used to correct the ^{14}C and ^{3}H counts/minute for quenching from a standard curve.

Steam Girdling

Steam girdles were applied to plants in three locations on the stem (1. 2 cm below the cotyledonary node; 2. 1 cm above the cotyledonary node; 3. 1 cm above the primary leaf node) by applying steam to a short segment of the stem for about 15 seconds duration using a device made for the purpose (Figure 2). This apparatus consisted of a piece of tygon tubing approximately 6" long. A small cork stopper was placed in one end of the tubing and near this a 4 mm hole was bored in the tubing perpendicular to its longitudinal axis. A slit was made on one side of the tubing into the hole so that the hole in the tubing could be opened up and wrapped around the stem. This procedure killed about a 1 cm portion of the stem. The killed portions of the stem shriveled very rapidly and became incapable of supporting the upper portion of the stem. A ring support made of pipe cleaners, the lower end of which was attached to the nutrient solution bottles were constructed to support the upper portion of the plant after girdling.

Preliminary experiments indicated that the leaves remained turgid and maintained a normal appearance for at least 2-3 days after the stems were girdled suggesting that transpirational flow remained adequate after the girdling process.

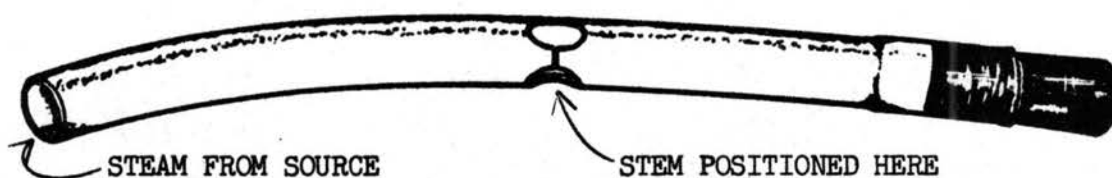

Figure 2. Apparatus used to apply a steam girdle to various points on the stem of bean seedlings.

CHAPTER IV

RESULTS AND DISCUSSION

Time-Course Studies

Effect of Time-of-Day on Treatment Response

The influence of environmental factors on the translocation of growth regulators is widely recognized (cf. Little and Blackman, 1963). Although the effects of all environmental factors are not well documented, several workers have shown that moisture stress severely retards the movement of auxin-like herbicides out of treated leaves (Basler, et al., 1961; Merkle and Davis, 1967; Davis, et al., 1968b). A study of the absorption and translocation of 2,4,5-T in blackjack oak suggested that translocation of 2,4,5-T from a treated leaf paralleled trends in foliar respiration rate throughout the growing season but appeared to be independent of soil moisture levels (Dalrymple and Basler, 1963). Low relative humidities caused marked increases in the acropetal movement of 2,4,5-T injected into the stems of bean seedlings and decreased the amount of 2,4,5-T moving to the roots (Basler, et al., 1970). High relative humidities reversed these trends. Thus it is important that the conditions under which translocation experiments are performed are well defined and precisely controlled.

One variable which it seemed important to control was the time of day when the treatment was administered. In other words, it was recog-

nized that the physiological status, (which fluctuates with diurnal changes in the environment) was likely to produce changes in the response of the plant to the treatment. In order to test this, a preliminary experiment was devised in which identical treatments were administered to sets of 10 plants at 4 hour intervals over a 24-hour period. It appears that there was strong basipetal translocation of 2,4,5-T-^{14}C (Figure 3) between 12 noon and 4 AM, but little movement of label in the acropetal direction. While at the 4 AM, 8 AM and to a lesser extent the 12 noon treatment times acropetal translocation was stronger with somewhat less basipetal movement. Little and Blackman (1963) noted that movement of 2,4-D from a treated leaf was maximum during the morning and decreased as the day progressed. Therefore, treatment times in all subsequent experiments between 9 AM and 11 AM was selected.

72 Hour Translocation Studies

A study of the translocation of 2,4-D and 2,4,5-T applied to mature leaves of wild and cultivated cucumber plants showed that for these two plants 2,4-D entered the plant rather rapidly and was freely mobile throughout the first 24 hours after which very little movement of labeled 2,4-D occurred (Slife, et al., 1962). They also noted that 2,4,5-T moved into the plant rather slowly as compared to 2,4-D but remained mobile in the plant for up to eight days. When 2,4-D and 2,4,5-T were injected into the stems of bean seedlings, both herbicides appeared to move quite rapidly both acropetally and basipetally during the first 24 hours (Figures 4 and 5).

Slife, et al.,(1962) suggested that the difference in translocation

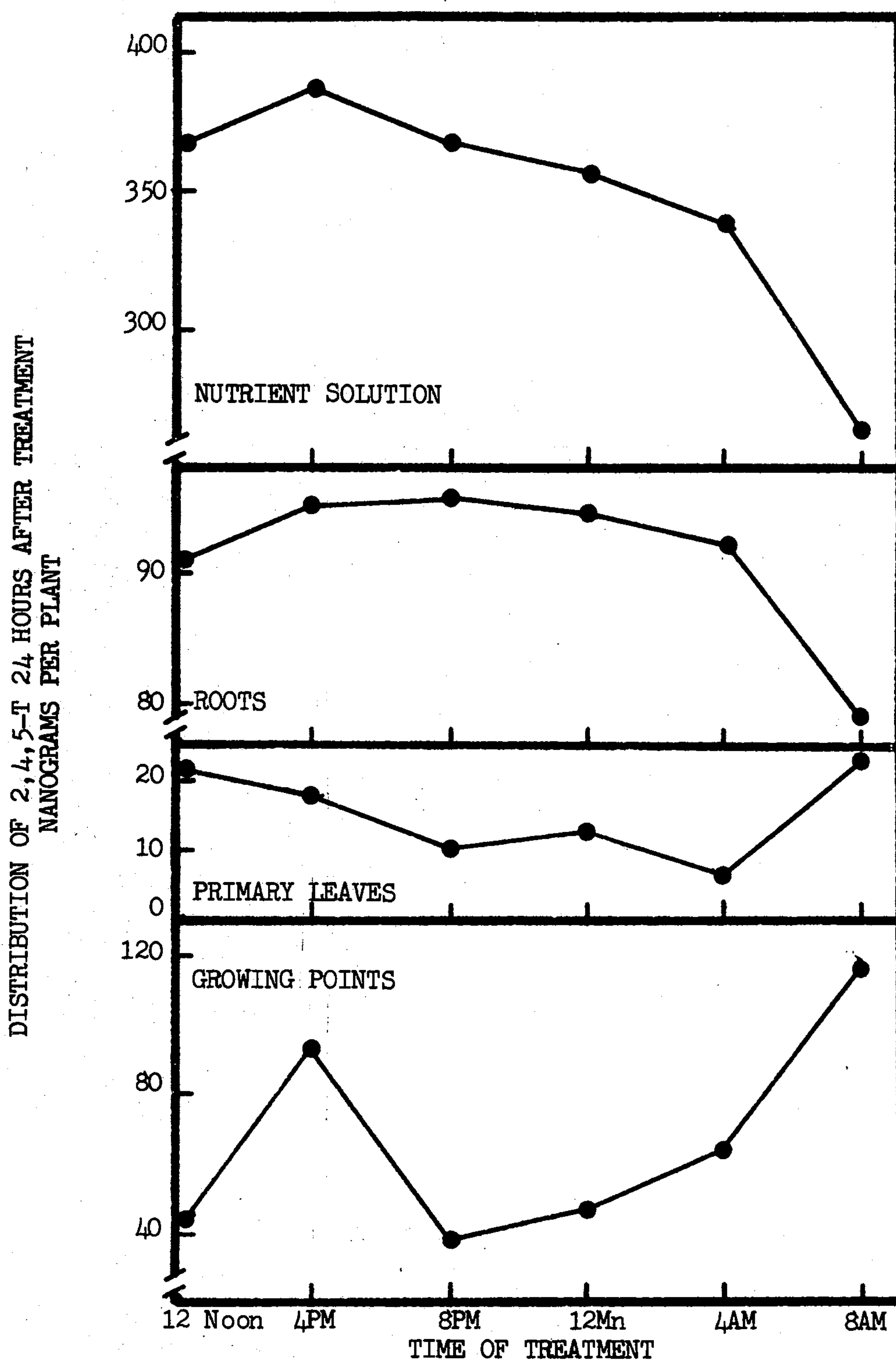

Figure 3. Influence of treatment time on the accumulation of 2,4,5-T in nutrient solution, roots, primary leaves and growing points 24 hours after treatment. The treatment amount was 0.75 µg/ plant.

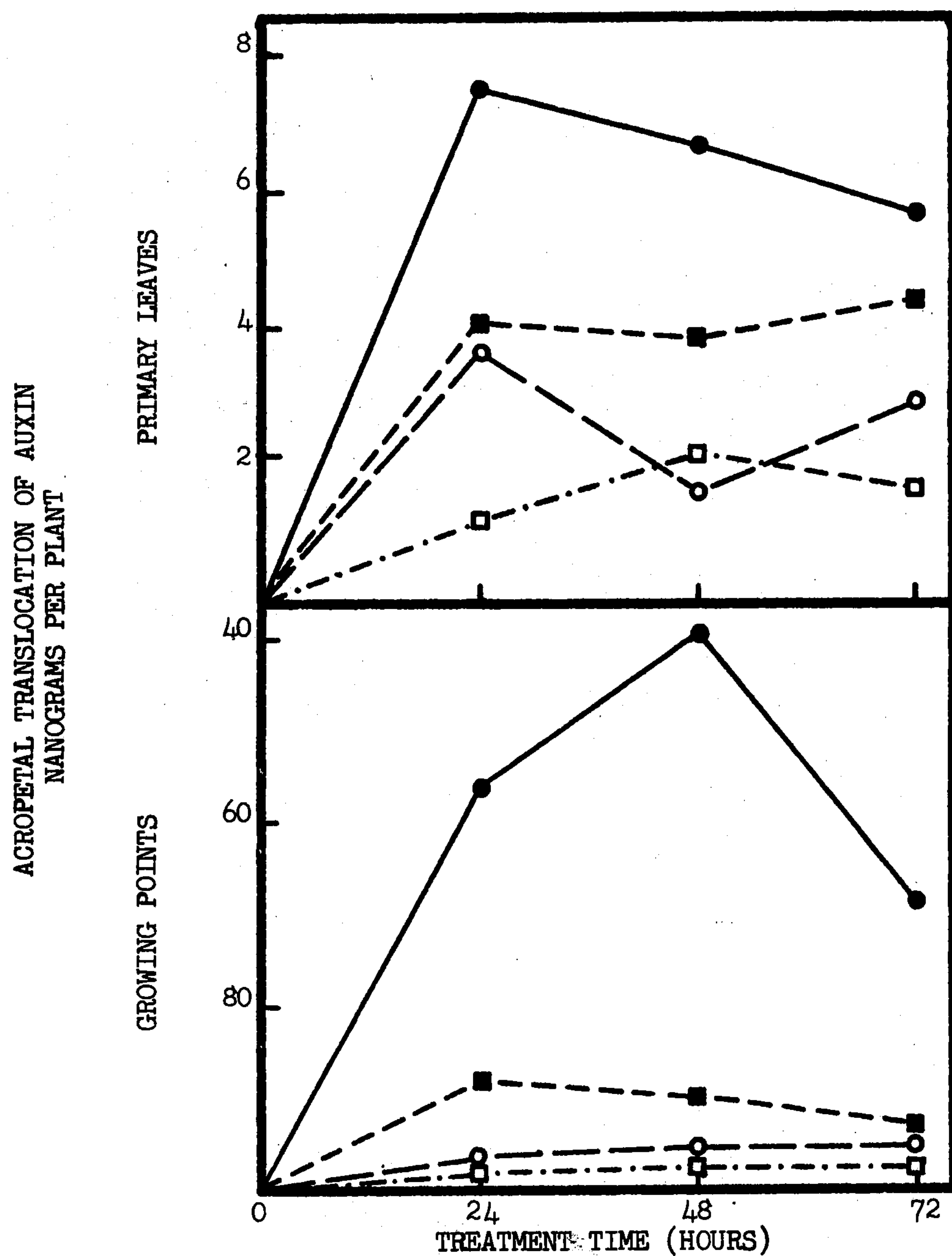

Figure 4. Accumulation of 2,4-D and 2,4,5-T in primary leaves and growing points throughout a 72-hour treatment period. Auxins applied in the following amounts: 0.75 µg 2,4-D□ - · □; 2.0 µg 2,4-D■ - ■; 0.75 µg 2,4,5-T○— —○; 2.0 µg 2,4,5-T.●——●

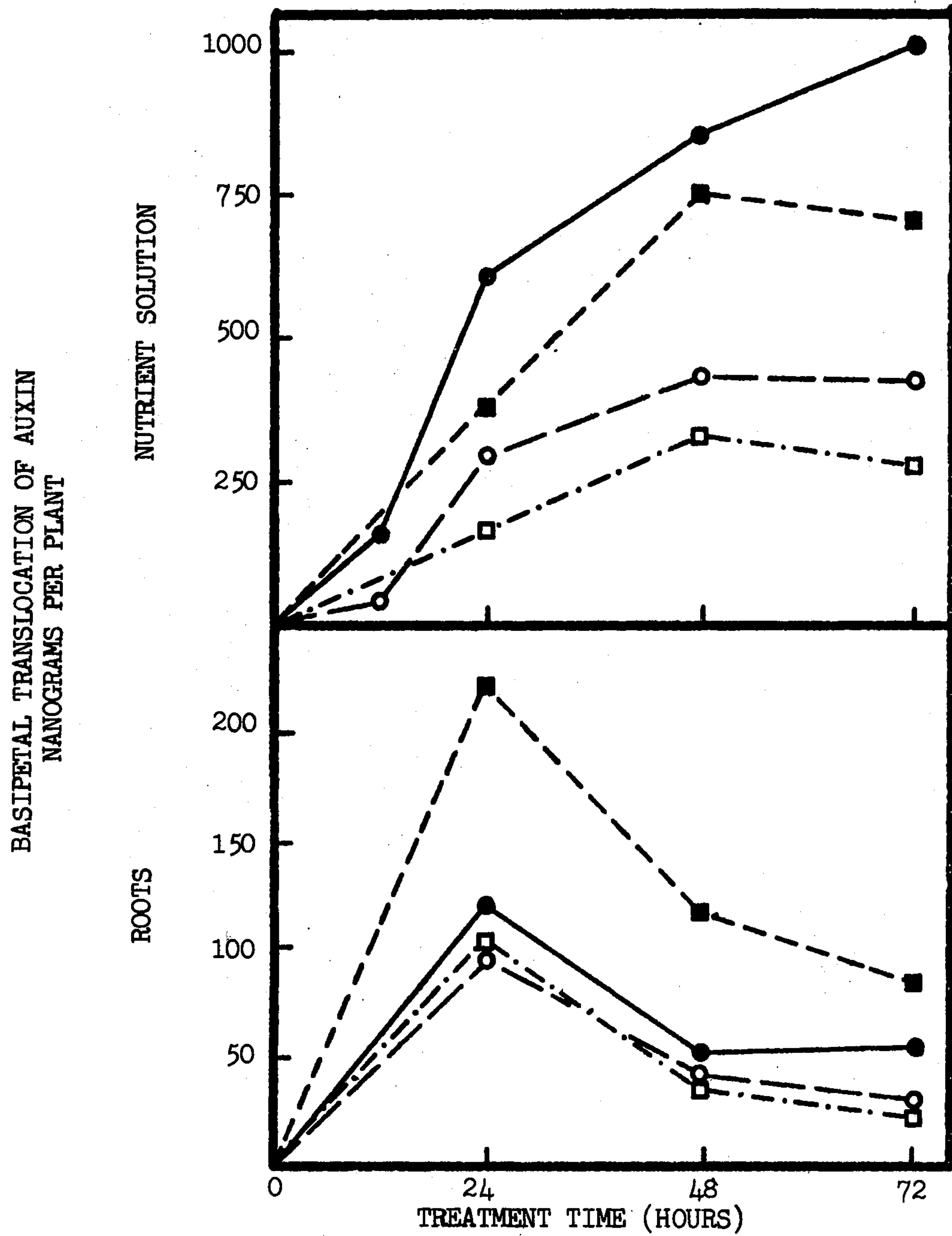

Figure 5. Accumulation of 2,4-D and 2,4,5-T in the nutrient solution and roots throughout a 72-hour treatment period. Auxin applied in the following amounts: 0.75 µg 2,4-D□–·□; 2.0 µg 2,4-D■– –■; 0.75 µg 2,4-T○——○; 2.0 µg 2,4,5-T●——●.

patterns of 2,4-D and 2,4,5-T during the first 24 hours after treatment was due to the fact that 2,4,5-T penetrated the leaf surface slower than 2,4-D. The similarity of translocation patterns when 2,4-D and 2,4,5-T are injected into the stems (Figures 4 and 5) seems to support this view even though the relative amounts of the two herbicides that accumulated in the various plant parts were somewhat different. There was little or no accumulation of label in any of the plant organs beyond 24 hours with the exception of the levels in the growing points at the 2.0 µg 2,4,5-T treatment level. There was a striking effect of concentration on acropetal movement of 2,4,5-T but not 2,4-D to the growing points. There was a 10-fold increase in accumulation of label in the growing points with a 2.5-fold increase in concentration of 2,4,5-T. Translocation to the primary leaves was more nearly proportional to concentration although the pattern of accumulation was somewhat similar to accumulation in the growing points. There was a rapid loss of label from the roots after 24 hours which in part may have been responsible for the continued accumulation in the nutrient solution. The higher accumulation of 2,4-D in the roots at 24 hours as compared to 2,4,5-T was also observed by Slife, et al, (1962). A greater affinity of roots for 2,4-D than 2,4,5-T also is implied by the fact that slightly more 2,4,5-T than 2,4-D accumulated in the nutrient solution at both the 0.75 µg and 2.0 µg levels.

It should be noted at this point that the data for 2,4-D and 2,4,5-T were plotted on the same graphs for purpose of comparison only. The experiment for the data on 2,4-D was run several days after the experiment using 2,4,5-T so that they are separated by time. Therefore they are not directly comparable statistically. However, experiments con-

ducted subsequent to these confirmed that while the absolute amounts of translocation varied with time the patterns of movement of label were very similar from one experiment to the next.

24 Hour Translocation Studies

When basipetal translocation of 2,4,5-T was monitored at frequent intervals over a 24-hour treatment period (Figure 6) the label was accumulated in the roots for the first 12 hours after which levels in the roots remained static at the 1 µg treatment level or actually showed some loss as at the 3 µg level. Accumulation in the nutrient solution continued at a nearly linear rate throughout the latter three-fourths of the 24-hour treatment period. This suggests that the roots were saturated after 12 hours of treatment and that any translocation into the roots after this was equalled by exudation of label into the nutrient solution.

Acropetal movement of 2,4,5-T to the growing points and primary leaves is represented in Figure 7. Approximately one-fourth of the 2,4,5-T in the 3 µg treatment level moved acropetally and two-thirds of that was recovered from the growing points. A similarly substantial acropetal translocation also was observed in studies of the movement of foliarly applied auxins by Eschrich (1968) and Agbakoba and Goodin (1970). In the present studies, net accumulation in the growing points had ceased sometime prior to the 6 hour-harvest time. Although the pattern of accumulation in the growing point was very similar in the 1 µg and 3 µg treatments, there was roughly a 20-fold increase in label present in the growing point at the 3 µg level as compared to the 1 µg level. This represents a marked enhancement of acropetal translocation.

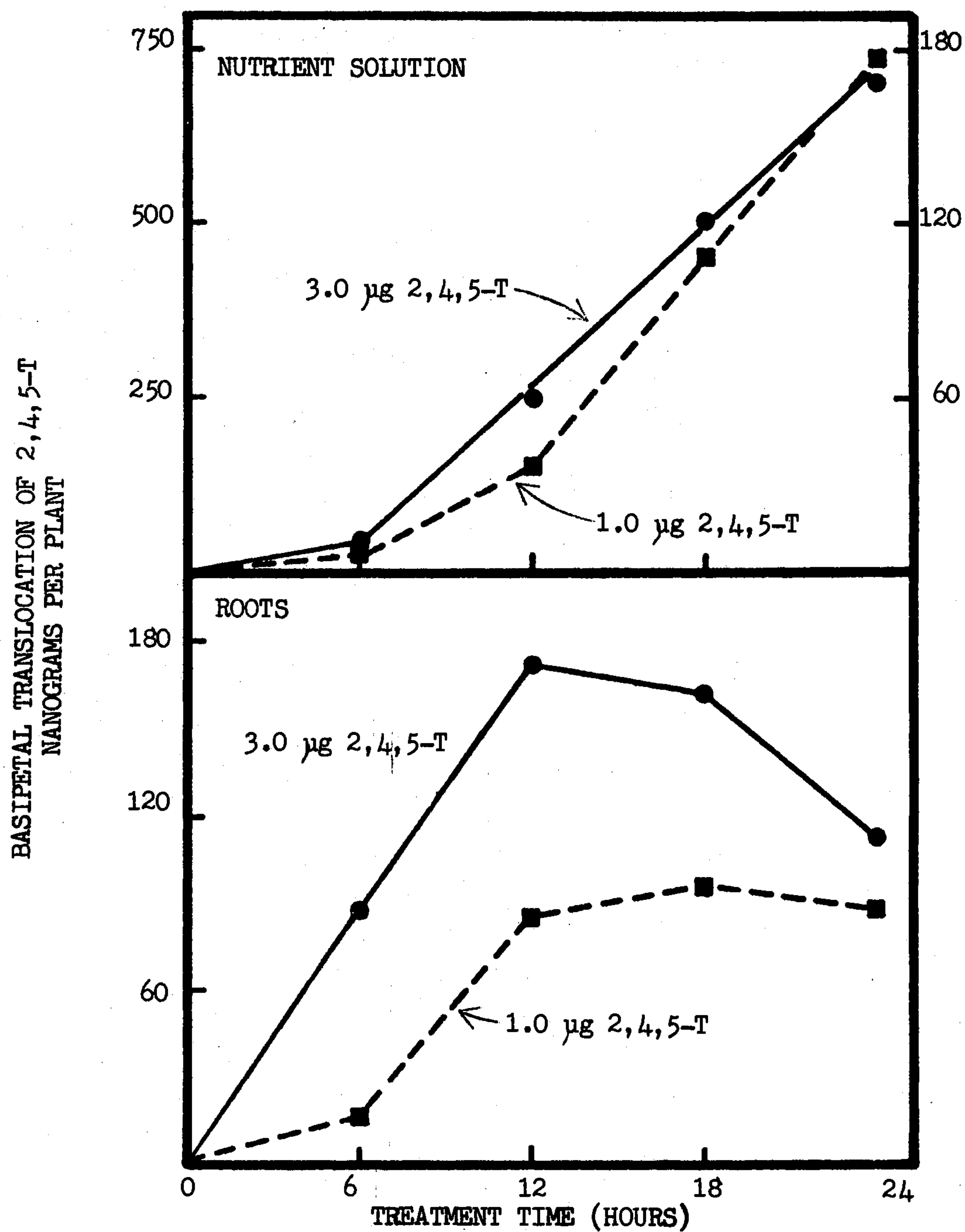

Figure 6. Accumulation of 2,4,5-T in the nutrient solution and roots during a 24-hour treatment period. Right ordinate is for nutrient solution for plants with 1 µg 2,4,5-T treatment only, all other values refer to the left ordinate.

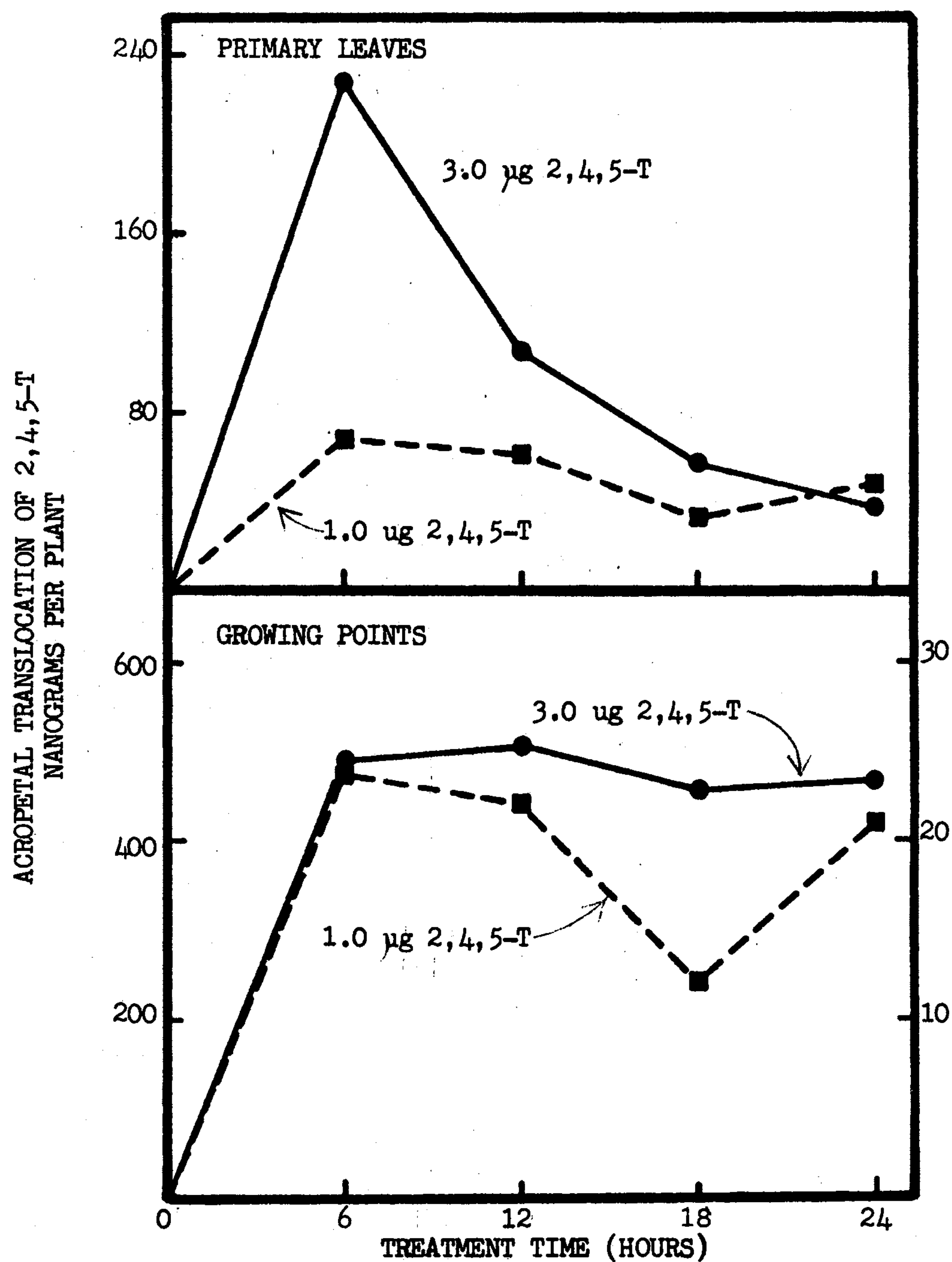

Figure 7. Accumulation of 2,4,5-T in primary leaves and growing points during a 24-hour treatment period. Right ordinate is for the growing points on plants with 1 µg 2,4,5-T treatment, all other values refer to the left ordinate.

The loss of label from the primary leaves after the lapse of more than 6 hours after treatment was particularly pronounced at the 3 µg treatment level. This provides at least a partial explanation as to why so little label was recovered from the primary leaves after 24 hours in Figure 4. This loss of label might have been due to decarboxylation and subsequent loss of the ^{14}C or possibly to export of 2,4,5-T from the leaf to other plant parts.

6 Hour Translocation Studies

In order to approximate initial velocities of translocation it became apparent that sampling during the first 6 hours after treatment was necessary. These studies showed that translocation of 2,4,5-T to the roots and nutrient solution (Figure 8) at the 3 µg treatment level appeared to proceed in two phases. Accumulation in the roots was near maximal, or reached saturation levels, at 2 hours at which time exudation into the nutrient solution commenced. At the 1 µg treatment level, however, translocation to the roots proceeded at a low rate in a linear fashion throughout the 6-hour treatment period. Consequently saturation did not occur and loss of label into the nutrient solution remained minimal.

Acropetal translocation is illustrated in Figure 9. As in Figure 7, approximately one-fourth of the 3 µg of 2,4,5-T injected into the plant was recovered in the growing points and primary leaves. Accumulation of 2,4,5-T in the growing points occurred in two distinct phases with a 2-hour period of little or no net accumulation interpolated between the two translocation phases. In subsequent experiments in which plants were harvested at 1,2,4 and 6 hours, no such biphasic

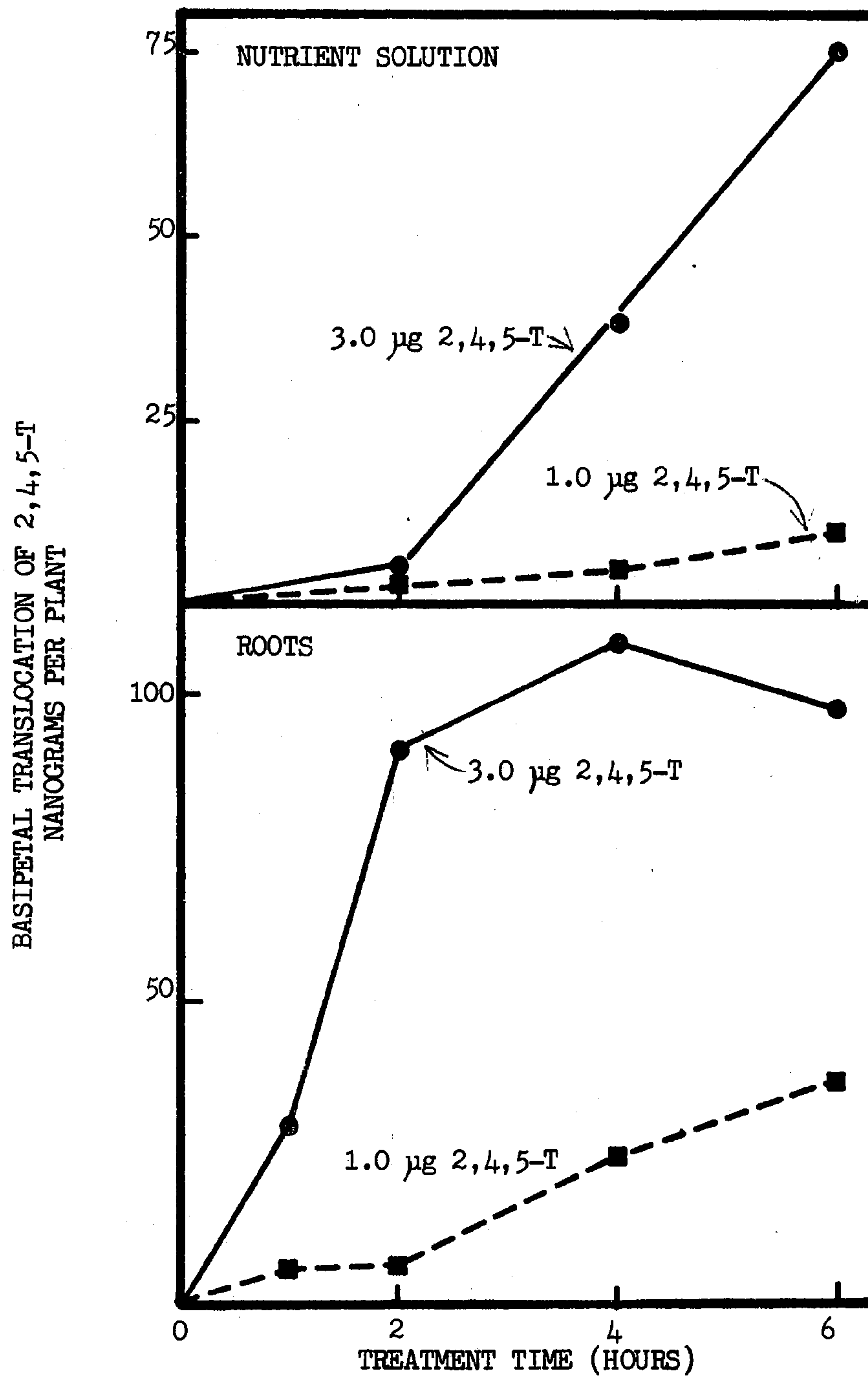

Figure 8. Accumulation of 2,4,5-T in nutrient solution and roots during a 6-hour treatment period.

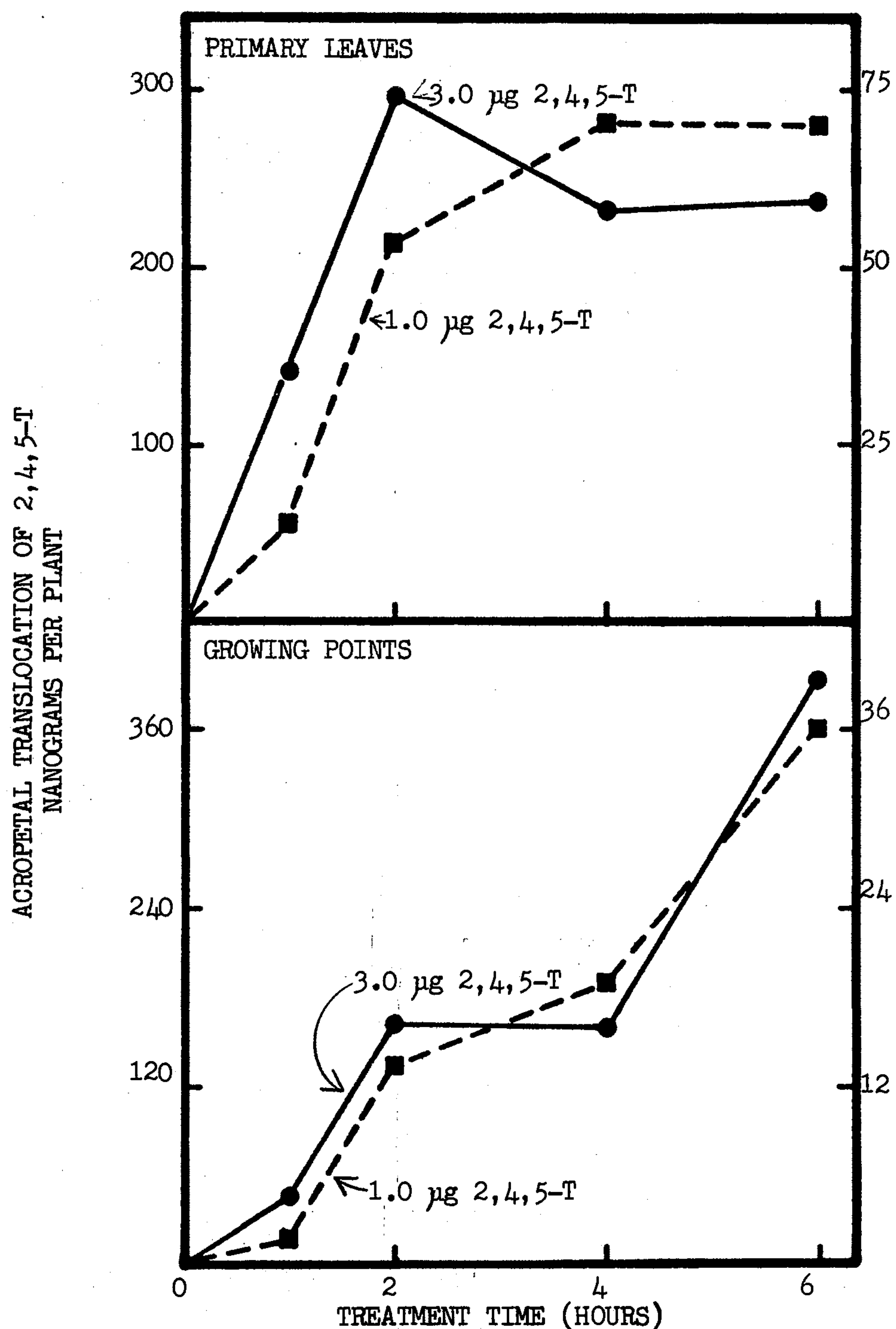

Figure 9. Accumulation of 2,4,5-T in primary leaves and growing points during 6 hours of treatment. Right ordinates are for primary leaves and growing points from plants treated with 1 µg 2,4,5-T, all other values refer to left ordinate.

curve was noticed. As in previous experiments the translocation patterns for the 1 µg and 3 µg treatment levels were very similar, but more than 10 times as much 2,4,5-T accumulated in the growing points of plants treated with 3 µg 2,4,5-T.

Translocation to the primary leaves proceeded linearly and roughly proportional to the amount of 2,4,5-T injected into the plant during the first 2 hours only, after which no net accumulation occurred.

It is clear from Figures 8 and 9 and also from Figures 6 and 7 that translocation of 2,4,5-T was primarily acropetal for the first 6 hours after which acropetal translocation ceased and subsequent translocation was basipetal. It will be noticed in Figure 9 also that accumulation in the primary leaves ceased as early as 2 hours after treatment while translocation beyond the primary leaves and into the growing points continued as long as 6 hours past the initiation of treatment. One speculation is that there is a formation of a metabolite(s) of 2,4,5-T, the translocation of which would be strictly basipetal. In this connection, Veen (1966) has shown that when ^{14}C-labeled NAA is applied to _Coleus_ explants within 6 hours almost all of the parent compound has been metabolized. In _Coleus_ the primary metabolites of NAA are NAA-aspartic acid and a glucoside of NAA. However there is no evidence to suggest that 2,4,5-T is rapidly metabolized in bean seedlings, although the formation of at least 10 metabolites of 2,4-D has been shown to occur in isolated bean stem segments (Bach, 1961).

It is possible to estimate the translocation rates of 2,4,5-T by extrapolation of the linear portion of the curve to the time baseline. For a criticism of the methods used in determining translocation rate see McCready (1966). In studies of transport through stem segments of

bean the velocity of basipetal movement has been reported to range from 1 mm/hr for 2,4-D to 6 mm/hr for IAA (McCready and Jacobs, 1963). The velocity of translocation of auxins applied to leaves ranges from 10-12 cm/hr for 2,4-D and 2,4,5-T to 20-24 cm/hr for IAA (Little and Blackman, 1963). Day (1952) has even reported values ranging from 10-100 cm/hr for 2,4-D. However, the improved techniques make the values of Little and Blackman (1963) more reliable. This author was able to determine velocities ranging from 13-15 cm/hr for basipetal translocation to the roots and translocation to the growing point averaged about 12 cm/hr. ^{14}C-Labeled 2,4,5-T appeared in the primary leaves very quickly after treatment.

Effect of Auxin Concentration on Translocation

Accumulation of 2,4,5-T in the roots 5 hours after treatment was initiated increased linearly with increases of 2,4,5-T concentration injected (Figure 10). This suggests that the basipetal translocation of 2,4,5-T was not inhibited at relatively high auxin levels in bean seedlings as is polar basipetal transport in stem segments (cf McCready and Jacobs, 1963). Exudation of 2,4,5-T into the nutrient solution increased very rapidly as amount of 2,4,5-T applied increased at low 2,4,5-T levels. At levels above 5 µg, movement of label into the nutrient solution proceeded in a more linear manner. Earlier, it was noted that there was an apparent threshold effect on the exudation of 2,4,5-T into the nutrient solution (see Figure 8). These data also suggest some sort of relationship between 2,4,5-T concentration in the roots and movement of label into the nutrient solution at treatment concentrations up to 5 µg.

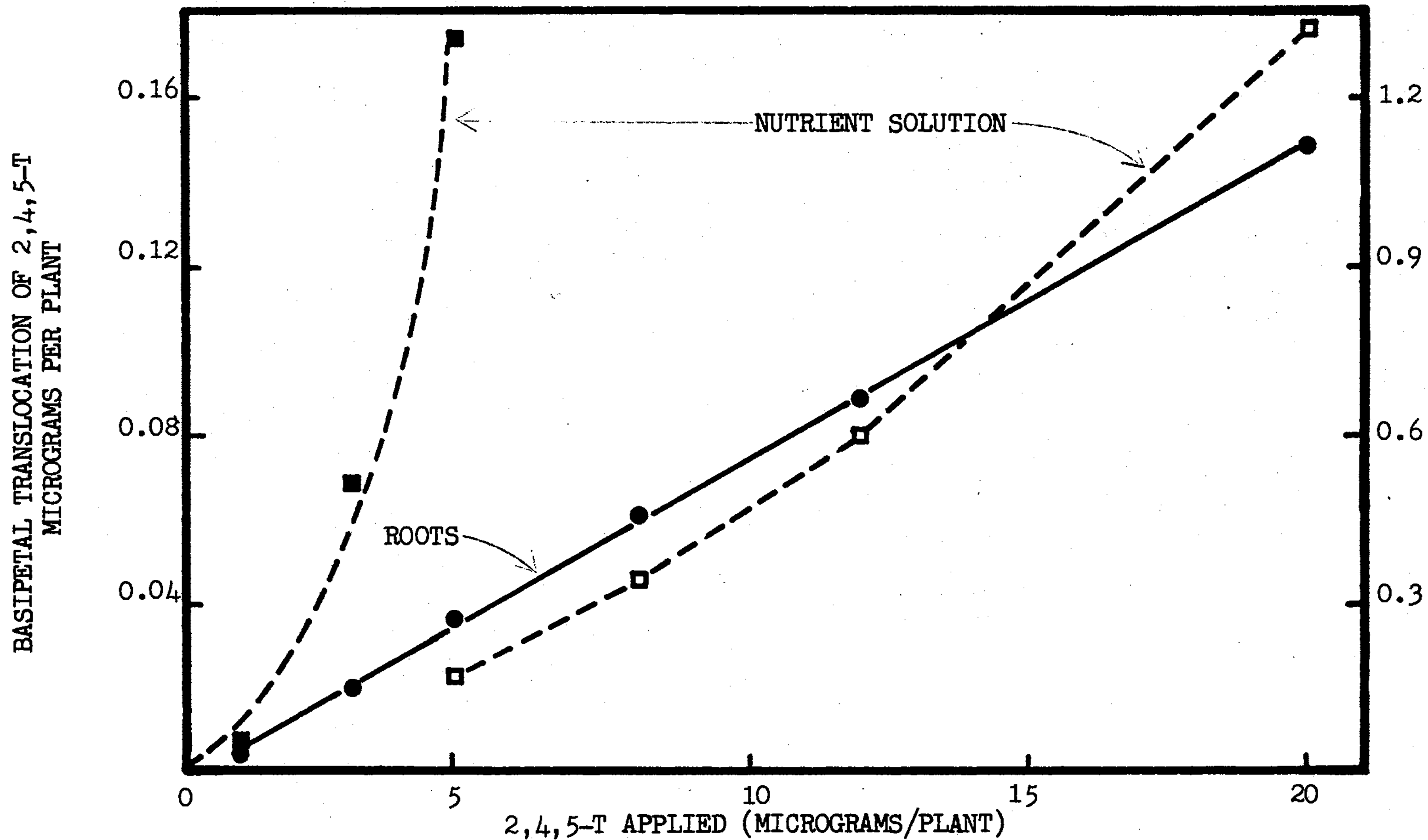

Figure 10. Influence of 2,4,5-T concentration on the accumulation of 2,4,5-T in roots and nutrient solution. Plants harvested 5 hours after treatment. Left ordinate is a magnified scale for 2,4,5-T concentrations between 1-5 µg per plant for nutrient solution values only. All other values refer to right ordinate.

Acropetal translocation to the growing point (Figure 11A) also increased in a geometrical fashion very rapidly as the amount of 2,4,5-T injected was increased up to 5 µg/plant. At treatment levels higher than 5 µg/plant there was an essentially linear relationship between amount applied and translocation to the growing point. The relationship between the amount of 2,4,5-T applied and amount translocated to the growing points is illustrated in Figure 11B in which the ratio of 2,4,5-T translocated to 2,4,5-T applied is plotted against injection concentration. The ratio increased very rapidly up to 5 µg treatment level and then leveled off at the higher treatment concentrations.

To be considered an auxin response this stimulation of acropetal movement must be a generalized phenomenon common to all auxins and not a response unique to 2,4,5-T. In Figure 12 it is apparent that a geometric increase in acropetal translocation was induced by 2,4-D and IAA as well as 2,4,5-T. However, higher treatment levels of 2,4-D and IAA are necessary to induce the geometric response.

The geometric increases in acropetal movement with increases in treatment levels argues against strictly diffusional processes involved in acropetal movement. There are at least two hypotheses presently in the literature which might help explain this type of response. Veen (1966) has suggested the occurrence of an inducible auxin-conjugating enzyme. He has shown that the induction of this enzyme is not only time-dependent but also concentration-dependent (Veen, 1967). It is possible, therefore, that the presence of high concentrations of auxin promotes the formation of a diffusible metabolite of the auxin, or an acropetally mobile metabolite of auxin. This would explain very nicely the marked enhancement of translocation of 2,4,5-T to the growing

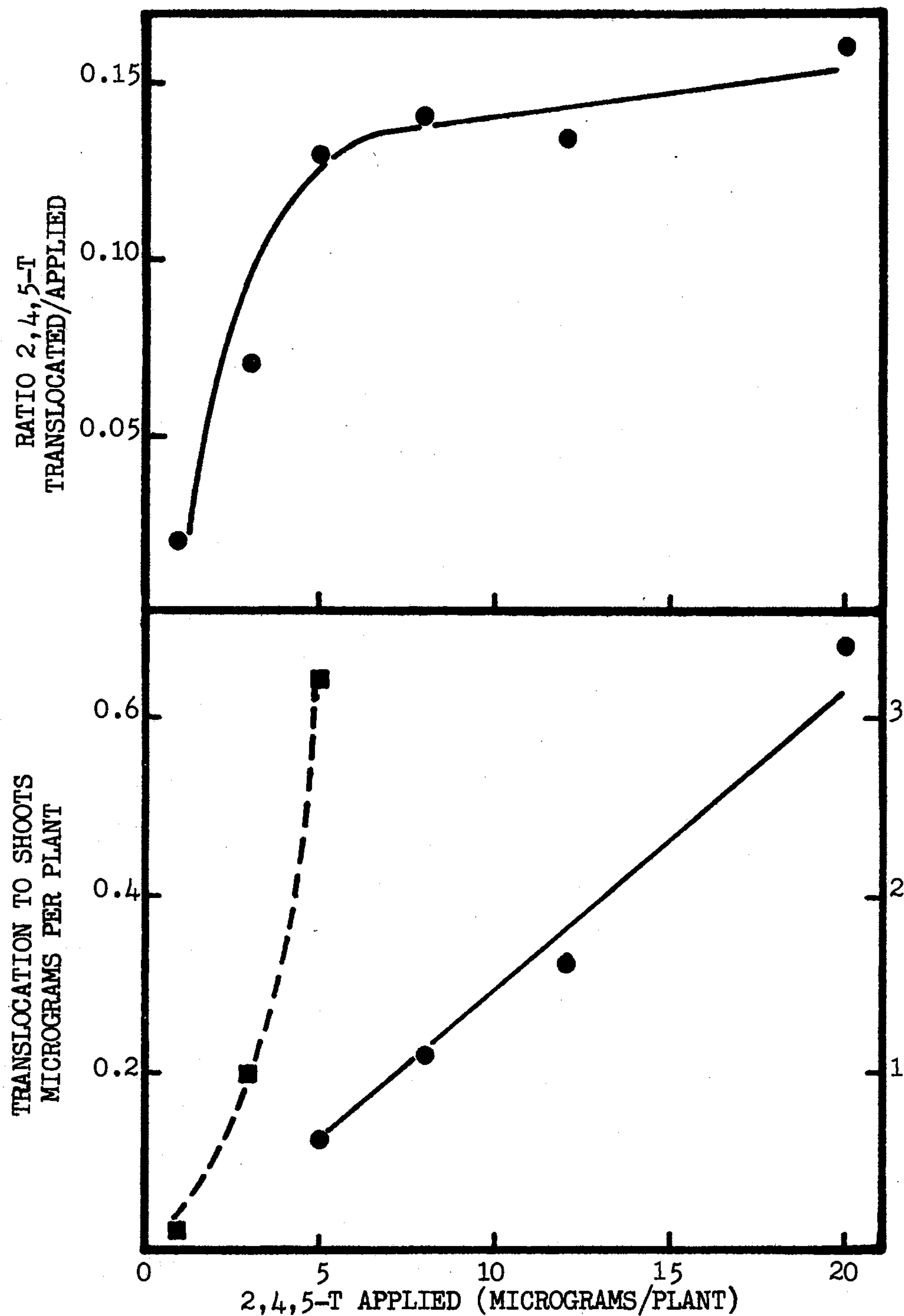

Figure 11. A)Influence of 2,4,5-T concentration on accumulation of 2,4,5-T in the growing points. B)Ratio of the amount of 2,4 5-T translocated to the growing points to the amount of 2,4,5-T applied The left ordinate for growing points is an expanded scale for 2,4,5-T concentrations from 1 to 5 μg per plant. All other values refer to right scale.

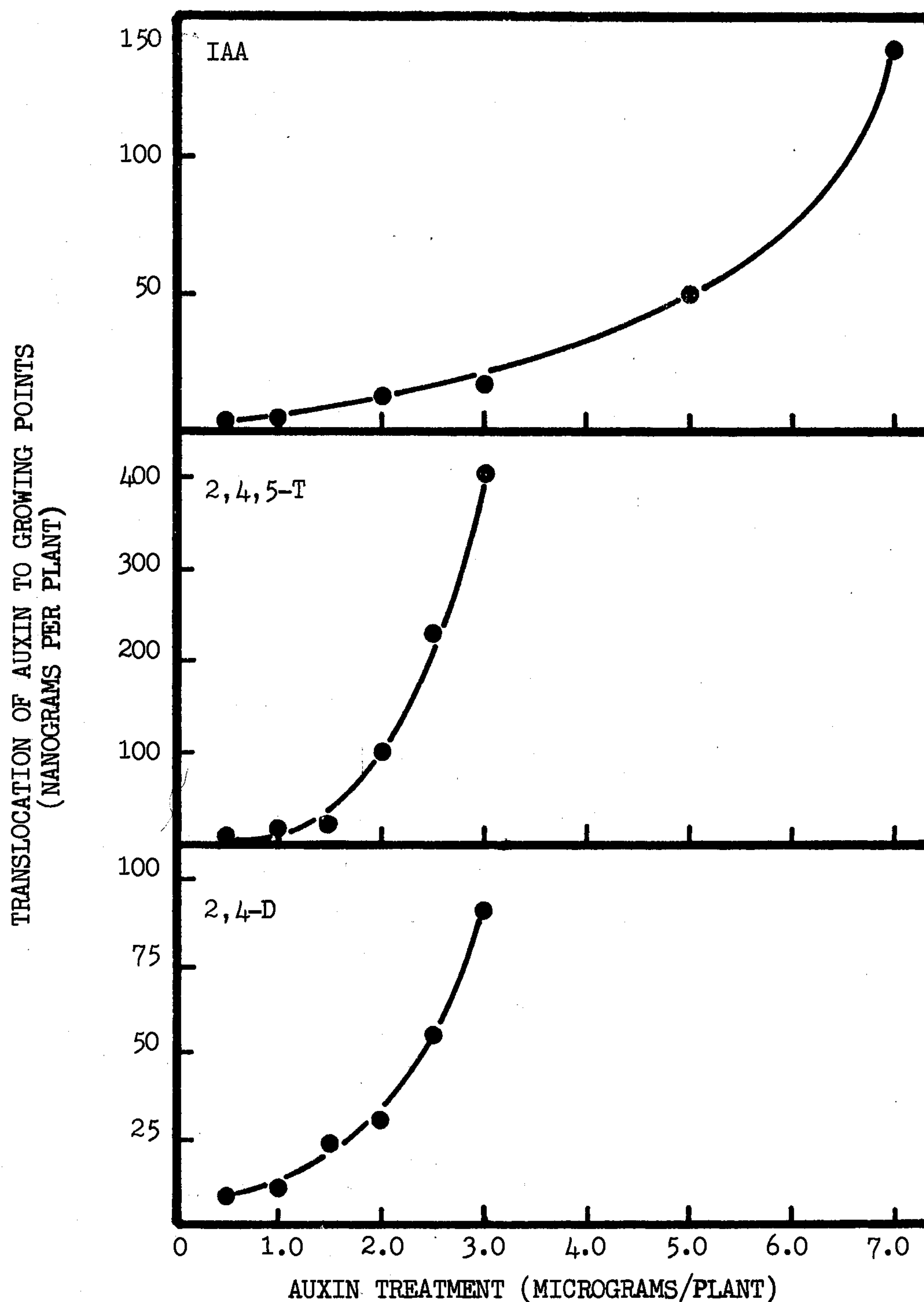

Figure 12. Influence of concentration of auxin on accumulation of auxin in the growing points. Treatment time was 6 hours for 2,4-D and 2,4,5-T and 24 hours for IAA.

points at the 3 µg treatment level in Figure 7. However, if the induction of the enzyme were time-dependent, the 1 µg treatment should have induced a response by the end of the 24-hour treatment period.

Another possible explanation was provided by Hertel and Flory (1968). They suggest the operation of monovalent interactions with a protein moiety possibly at the plasmalemma. This system would rely on cooperativity in membranes and in auxin molecules. In other words, auxins binding non-covalently to the transport site (protein) would lead to an increased number of auxin binding sites. Filling of these sites would lead to excretion or secretion of auxin. This hypothesis could easily be expanded to include binding to effect a growth response, induce or activate an auxin-conjugating enzyme or directional loading into xylem or phloem.

Effect of Steam Girdling on 2,4,5-T Translocation

The relatively slow translocation velocities for 2,4,5-T mentioned earlier suggested that movement both basipetally and acropetally occurs via the phloem. Translocation velocities in the range of 10-50 cm/hr are often considered to be indicative of movement in the phloem (cf. review by Zimmerman, 1960). In order to determine the extent of movement of 2,4,5-T in the xylem and the phloem the stems of the bean seedlings were girdled in one of three places; 1) a girdle covering about a 1 cm segment of the stem was placed 3 cm below the cotyledonary node which is about 2 cm below the site of injection; 2) a girdle was placed about 1 cm above the cotyledonary node or midway between the primary leaf node and the cotyledonary node; or 3) a girdle was placed between the primary leaf node and the young expanding first trifoliolate

leaf.

The results illustrated in Figure 13 suggest that translocation to the growing point was increased 2-or 3-fold over controls when the stem was girdled below the cotyledonary node. Translocation to the primary leaves was increased only slightly (3 µg/plant) or not at all (1 µg/plant). Translocation to the roots was almost completely inhibited by a girdle placed between them and the site of injection. When the stem was girdled above the cotyledonary node translocation to the growing points was again enhanced 2-or 3-fold. Movement of label to the primary leaves was also significantly increased. Surprisingly, once again translocation to the roots was completely inhibited. This suggests that basipetal translocation from the point of injection is dependent upon the flow of assimilates in the phloem. Steam girdling above the primary leaves almost completely inhibited the accumulation of 2,4,5-T in the young growing points, but had little or no effect on translocation to the primary leaves or flow of label basipetally to the roots. This overall pattern suggests that translocation of 2,4,5-T from the site of injection to the primary leaves was via the xylem while translocation beyond the primary leaves into the growing points was apparently via the phloem. This is inexplicable in terms of what is known of the vascular anatomy of Phaseolus vulgaris seedlings (Doutt, 1932; and Mullins, 1970). Both of these authors indicate that all vascular bundles originating at the primary leaf descend the stem and that connections to the trifoliolate leaves are by anastomoses at the base of the stem. The present data suggest the ready exchange of 2,4,5-T between the xylem and phloem at some site in the vicinity of the primary leaf node.

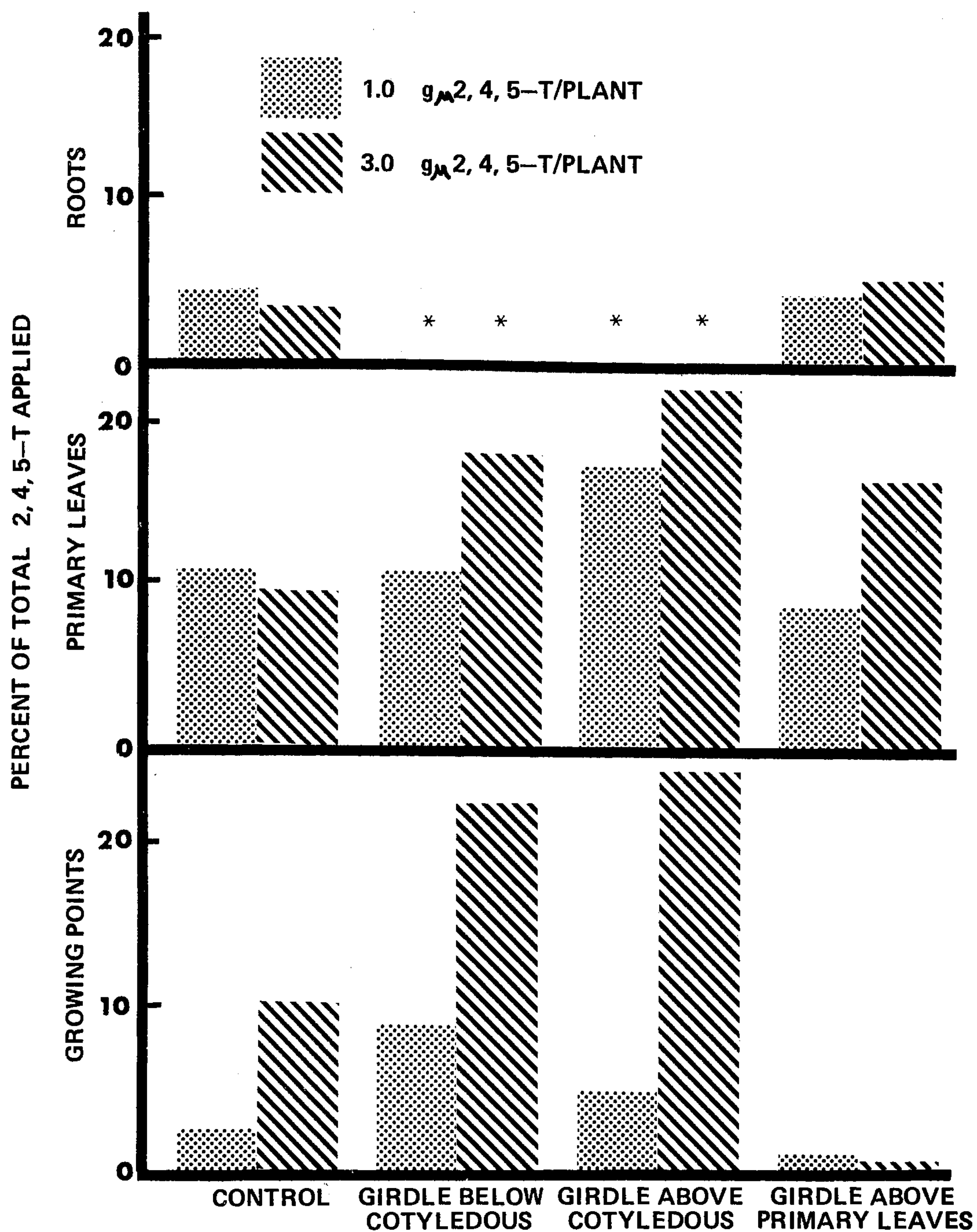

Figure 13. Influence of a steam girdle located at various positions along the stem on 2,4,5-T accumulation in growing points, primary leaves and roots. *Indicates trace amounts detected.

Effect of Antiauxin on 2,4,5-T Translocation

Recently, the effects of the antiauxin p-chlorophenoxyisobutyric acid (PCIB) on basipetal transport of IAA and growth in corn coleoptile segments have been characterized (Hertel, et al., 1969). These workers showed that PCIB inhibited auxin transport just as it inhibited auxin-induced growth. They suggested that the parallel action on these two processes indicated that auxin transport was linked closely to or identical with auxin-effects on growth.

The experiments illustrated in Figures 14 and 15 were designed to test the influence of PCIB on the translocation of stem-injected 2,4,5-T in bean seedlings. 10^{-9} moles PCIB/plant is equivalent on a molar basis to 1 μg 2,4,5-T/plant so that concentrations of PCIB applied to the plants were in the same concentration range as the 2,4,5-T applications. Since PCIB is known to inhibit the basipetal polar transport of auxin in isolated stem segments, the inhibition of basipetal translocation in intact plants might also be expected. However, accumulation of 2,4,5-T in the roots was not inhibited by a range of 10^{-10} to 10^{-8} moles PCIB/plant (Figure 14). At the higher concentrations of PCIB the movement of 2,4,5-T into the nutrient solution was enhanced slightly. At the 1 μg 2,4,5-T/plant level, however, the response to increasing concentrations of PCIB was not statistically significant while at the 3 μg 2,4,5-T treatment level there were significant differences in treatment response.

Acropetal translocation was more sensitive to PCIB than basipetal translocation. Movement to the growing points was enhanced by the highest concentrations of PCIB, particularly at the 3 μg 2,4,5-T/plant treatment level (Figure 15). There also was a significant enhancement

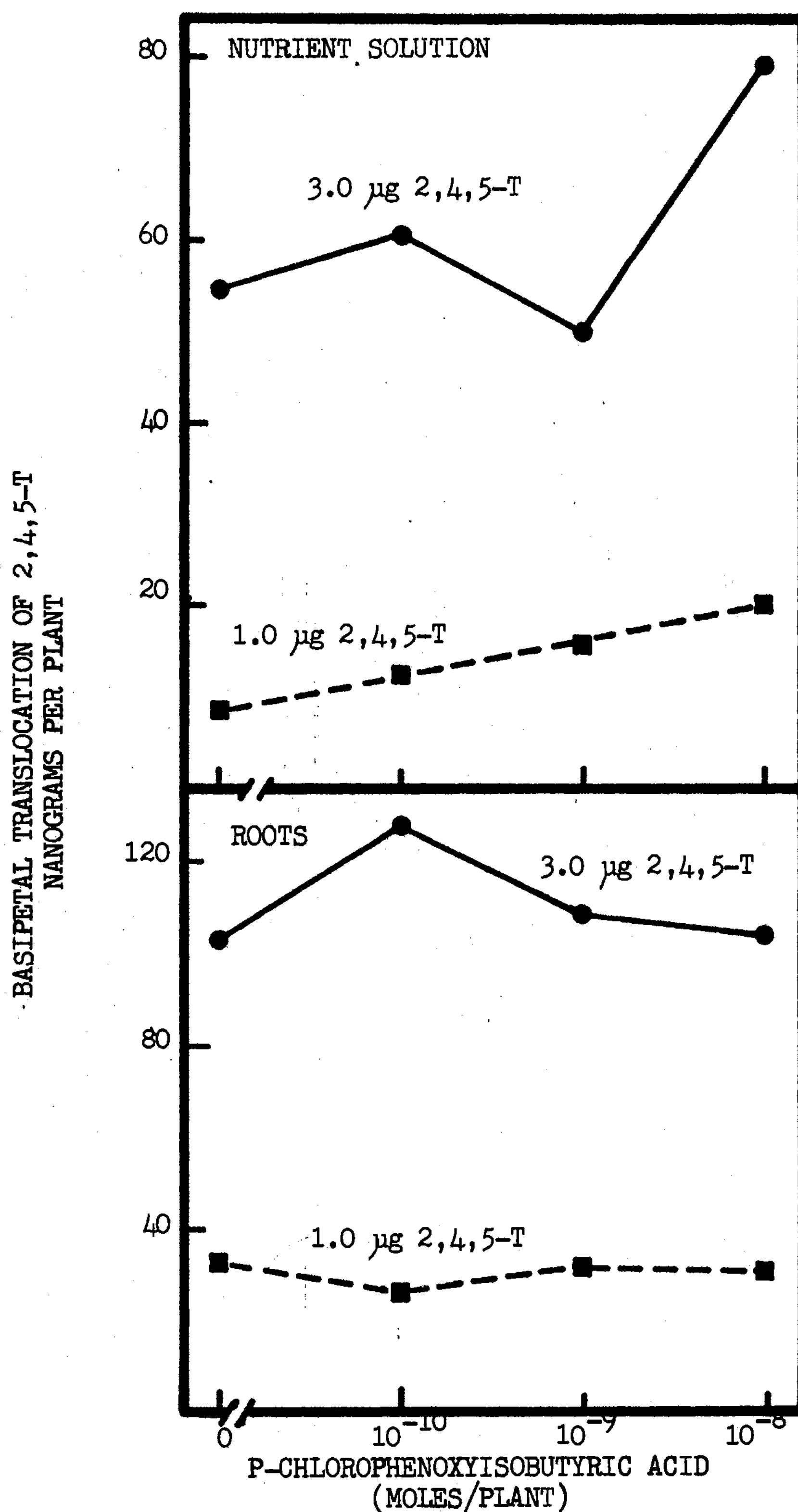

Figure 14. Influence of PCIB on accumulation of 2,4,5-T in nutrient solution and roots. Plants harvested 5 hours after treatment.

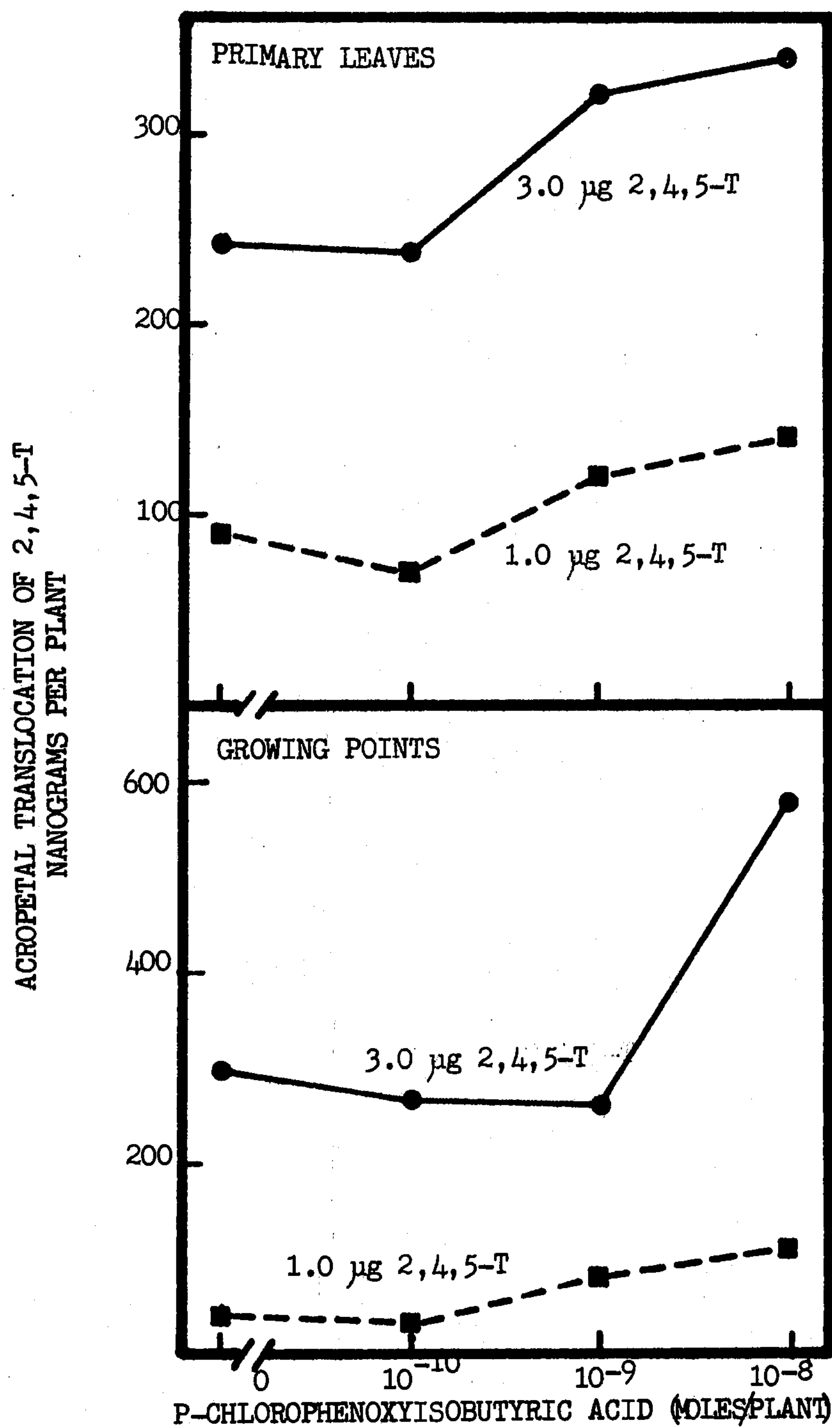

Figure 15. Influence of PCIB on accumulation of 2,4,5-T in primary leaves and growing points. Plants were harvested 5 hours after treatment.

of accumulation of 2,4,5-T in the primary leaves by PCIB.

These patterns of PCIB influence on translocation do not correspond to the effects of antiauxins on auxin transport in isolated stem segments. At this point it seems appropriate to make a distinction between auxin translocation which occurs in intact plants in which the xylem and phloem are fully functional, and auxin transport in isolated segments of stems or coleoptiles. Sieve tubes are notoriously sensitive to perturbations of any kind and it is questionable that the phloem is fully active in segments in which the phloem elements have been severed at both ends. Also all transpirational movement in the xylem would be interrupted in isolated segments of stems and coleoptiles. The polar basipetal movement (transport) of auxins in stem and coleoptile segments occurs predominantly in parenchymatous tissue whereas the movement of auxins in intact plants is primarily vascular in nature (translocation) as the results of the present study have indicated. Therefore, comparisons between transport and translocation are exceedingly risky.

2,4-D/2,4,5-T Interactions in Translocation

It has been pointed out several times in the literature that the transport of one auxin may be modified by the presence of a second type of auxin (Niedergang-Kamein and Leopold, 1959; Keitt and Baker, 1966; Rayle, et al., 1969). It was also pointed out earlier in this study that high concentrations of auxins appeared to stimulate their own acropetal translocation (Figures 7 and 9). Thus it was of interest to determine the effect of 2,4-D on 2,4,5-T translocation in intact plants and vice versa.

The interactions of 2,4-D and 2,4,5-T in translocation are

expressed in Table I which shows the amounts (in nanograms) of labeled auxins accumulated in the various plant parts and nutrient solution. These data do not reflect total amounts of auxin or unlabeled auxin which might accompany the movement of labeled auxin and be recovered in each plant part. The experiments showed that high concentrations of 2,4-D are not as effective in promoting acropetal auxin translocation as 2,4,5-T. When 0.75 µg 2,4-D-1-^{14}C was applied in the stems of the plants, 10 nanograms of 2,4-D-1-^{14}C were recovered from the growing points. When 0.75 µg 2,4-D-1-^{14}C + 1.25 µg unlabeled 2,4-D were injected in the stems, 11 ng 2,4-D-1-^{14}C were recovered from the growing point. Thus although there was a 2.67-fold increase in total auxin injected into the stem there was no net increase of 2,4-D-1-^{14}C accumulation in the growing point. The accumulation of labeled compound in the roots was essentially unchanged by the addition of unlabeled 2,4-D to the treatment, but the exudation into the nutrient solution was curtailed. When 1.25 µg 2,4,5-T (unlabeled) was added to the 0.75 µg 2,4-D-1-^{14}C injection, accumulation of label in the growing point was stimulated 3-fold. Accumulation in the roots remained unchanged from the 2,4-D-1-^{14}C treatment while again the exudation into the nutrient solution was reduced by the addition of 2,4,5-T.

When a 0.75 µg 2,4,5-T-1-^{14}C treatment was administered, translocation to the growing points was quite low as compared to the 0.75 µg 2,4-D treatment. Accumulation of label in the roots was relatively unchanged from the 0.75 µg 2,4-D treatment, however; exudation into the nutrient solution was considerably greater for the 2,4,5-T-1-^{14}C. The addition of 1.25 µg of unlabeled 2,4,5-T enhanced acropetal movement to the growing points some 10-fold just as unlabeled 2,4,5-T also enhanced

TABLE I

^{14}C-LABELED AUXIN ACCUMULATED IN PLANT PARTS AND NUTRIENT SOLUTION 48 HOURS AFTER INJECTION OF ^{14}C-LABELED AND UNLABELED 2,4-D AND 2,4,5-T INTO THE STEM OF BEAN SEEDLINGS. EXPRESSED AS NANOGRAMS OF ^{14}C-LABELED MATERIAL ONLY. THE UNLABELED AUXIN IS NOT ACCOUNTED FOR.

	Growing Points	Primary Leaves	Stems	Roots	Nutrient Solution
0.75 µg 2,4-D-1-^{14}C	10.3 ± 4.3	0.9 ± 0.7	200.0 ± 18.8	45.0 ± 4.9	386.1 ± 30.2
0.75 µg 2,4-D-1-^{14}C + 1.25 µg 2,4-D	11.0 ± 5.4	1.9 ± 2.4	257.7 ± 54.7	52.5 ± 6.7	283.8 ± 50.5
0.75 µg 2,4-D-1-C + 1.25 µg 2,4,5-T	30.4 ± 24.8	3.4 ± 3.7	261.8 ± 33.7	47.9 ± 5.6	271.8 ± 61.2
0.75 µg 2,4,5-T-1-^{14}C	3.4 ± 2.6	8.0 ± 7.2	211.5 ± 49.8	50.8 ± 2.9	460.3 ± 93.1
0.75 µg 2,4,5-T-1-^{14}C + 1.25 µg 2,4,5-T	36.6 ± 35.4	1.6 ± 2.5	306.8 ± 47.8	15.4 ± 3.6	356.3 ± 68.8
0.75 µg 2,4,5-T-1-^{14}C + 1.25 µg 2,4-D	2.8 ± 1.3	3.9 ± 5.0	297.6 ± 66.0	28.7 ± 6.7	390.5 ± 79.3

accumulation of 2,4-D-1-^{14}C in the growing points. Accumulation in the roots, however, was decreased nearly 3-fold. Exudation into the nutrient solution was also inhibited, although not as much as accumulation in the roots. The addition of 1.25 µg 2,4-D to the 0.75 µg 2,4,5-T-1-^{14}C resulted in acropetal translocation to the growing point that was not significantly different from that of the 0.75 µg 2,4,5-T-1-^{14}C treatment. The 1.25 µg of 2,4-D increased the translocation to the roots as compared to the 1.25 µg 2,4,5-T treatment while exudation into the nutrient solution was only slightly higher than the 1.25 µg 2,4,5-T treatment.

Thus there were auxin-auxin interactions in intact plant systems just as there are in transport systems of stem and coleoptile segments. However, in intact plants acropetal translocation appeared to be more sensitive to molecular differences of the auxin molecule, although the accumulation of 2,4,5-T in roots and nutrient solution also showed considerable sensitivity to molecular structure. This type of response also has been reported by Davis, et al., (1968a) who showed an interaction between foliarly applied 2,4,5-T and Picloram on uptake and translocation in mesquite seedlings. As the ratio of 2,4,5-T:Picloram increased, the uptake and translocation of Picloram also increased. Conversely, when the ratio of Picloram:2,4,5-T was increased the translocation of 2,4,5-T was depressed. These data support the "cooperativity" concept of Hertel and Flory (1968), namely, that binding of one auxin molecule alters the binding affinity for auxins at other sites. It also suggests the existence of a system for auxin transport or translocation in which one or more sites are catalytic and others are allosteric or regulatory in nature. A system such as this would explain

the data of Hagar and Schmidt (1968a) who reported that an oxidation product of IAA inhibited the transport of IAA and growth responses to IAA. A number of oxidation products of IAA are formed during illumination but only methyleneoxindol (3-M) was active in inhibition of transport and auxin-induced elongation growth. They also noted that 3-M inhibits the active excretion of IAA from coleoptile tissue (Hagar and Schmidt, 1968b). Furthermore, 3-M or TIBA inhibits the export of NAA-^{14}C. They have suggested on the basis of these data that this represents an auxin excreting system in the border layers of the cell cytoplasm, possibly the cell membrane.

Several workers have suggested the operation of some component of the cell membrane in auxin responses (Hertel and Flory, 1968; Rayle, et al., 1969; Burstrom, et al., 1970). Veen (1966) has provided microautoradiographic evidence that NAA-1-^{14}C is preferentially localized at the cell membrane. Lepp and Peel (1971b) have reported that in bark strips of willow there is polar movement of IAA only when the isolated segments of willow are oriented in the vertical position with the morphological base downward. Furthermore, the conversion of IAA to IAA-aspartate occurred only when the segment is oriented vertically. The compound, morphactin (methyl-2-chloro-9-hydroxyflourene-(9)-carboxylic acid) which inhibits geo- and phototropism in plants has been shown by Parups (1970) to inhibit the transport of IAA in corn coleoptiles. He also noted that in this tissue the starch grains become immobile and were evenly distributed in the cytoplasm after treatment with morphactin. Thus there are many data indicating that the plasmalemma may play a very important role in the transport of auxin molecules and possibly in the translocation of auxin as well.

2,4,5-T Effects on Sugar Translocation

Went(1939) has suggested that metabolic sinks such as apical meristems and rapidly maturing fruits maintain the dormancy of lateral buds by directing the flow of metabolites to the active growth centers. Both the loading of sugars into the sieve elements (Lepp and Peel, 1970) and the polar transport of sugars (Lepp and Peel, 1971a) was enhanced by IAA treatment in willow stem segments suggesting that IAA has a direct effect on assimilate translocation. If this is true, 2,4,5-T injected into the stem of bean seedlings along with isotopically labeled sugars should alter the distribution pattern of the sugars as compared to treatment without 2,4,5-T. The movement of auxin-like phenoxyacetic acids from treated leaves is considered to be via the assimilate stream (Mitchell and Brown, 1946; Rohrbaugh and Rice, 1949; Little and Blackman, 1963). We have noted that small increases in the amount of auxin injected into the stem of bean seedlings markedly enhanced the acropetal translocation of the auxin (Figures 7 and 9). If the movement of auxin is via the assimilate stream, then the translocation of sugars should also be enhanced.

To test this possibility bean seedlings were treated by simultaneously injecting 0.146 nanomoles of sucrose-^{14}C(UL) and 0.722 nanomoles of D-glucose-3-^{3}H(n) into the stem. In the treatments so indicated 3 μg of unlabeled 2,4,5-T was injected along with the two labeled sugars.

The movement of sucrose-^{14}C and glucose-3-^{3}H to the growing point is illustrated in Figure 16A. The peak times for both sucrose and glucose were delayed by about 2 hours by the addition of 2,4,5-T to the labeled sugars in the injection mixture. Although the total amount of

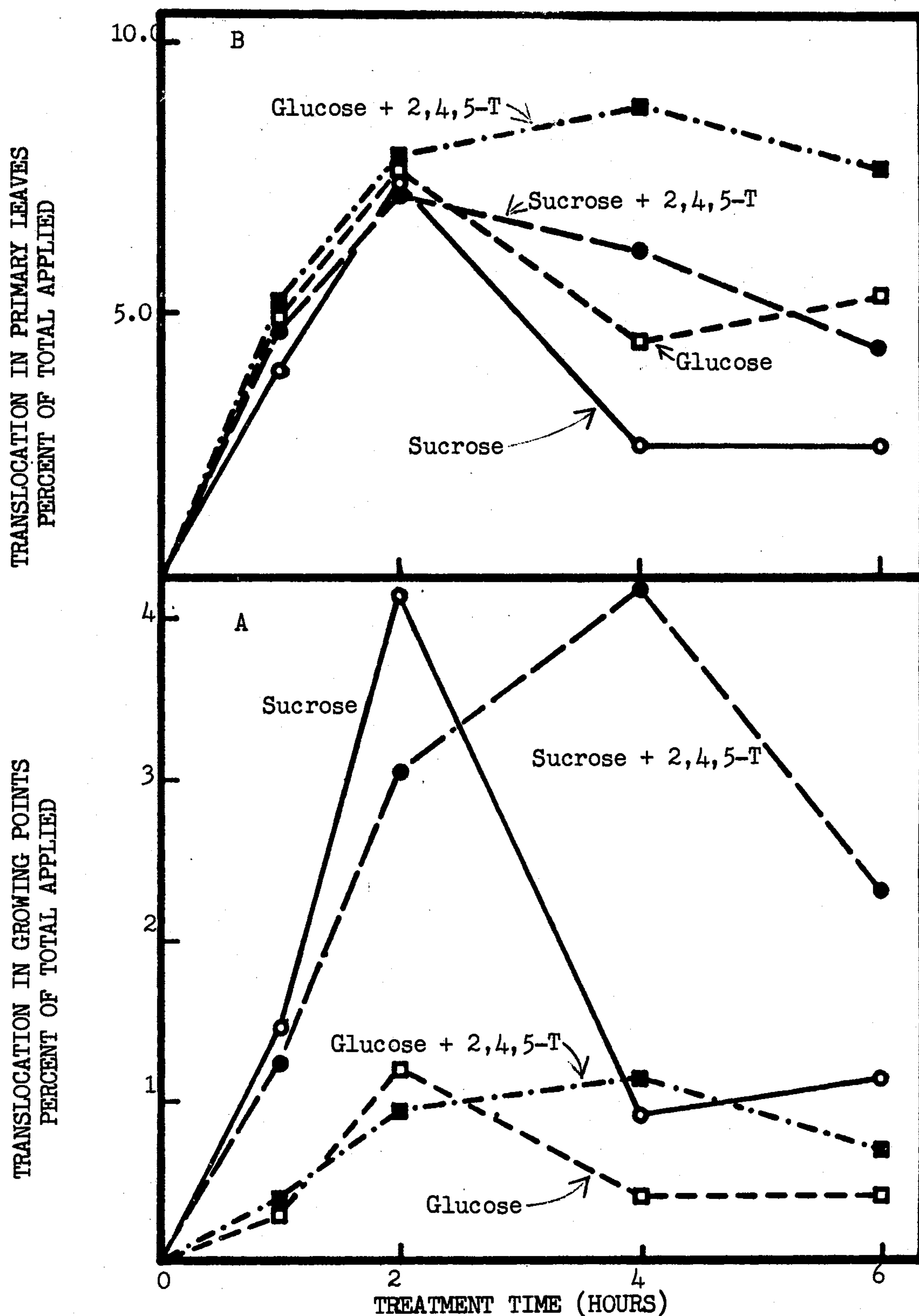

Figure 16. Accumulation of ^{14}C-sucrose and ^{3}H-glucose in A) growing points and B) primary leaves with or without simultaneous injection of 3 µg 2,4,5-T per plant.

glucose-label translocated to the growing points was much less than that of sucrose-label, the patterns of translocation of both sugars were quite similar. Movement to the growing point of stem-injected sugars is not enhanced by 2,4,5-T and thus the previously noted 2,4,5-T translocation response is not a generalized mobilization of nutrients in the stem with a concomitant movement of 2,4,5-T but rather is specific for auxin molecules.

The pattern of accumulation in the primary leaves (Figure 16B) was somewhat different from that in the growing points although the peak times in both plant parts were similar and similarly affected by the addition of 2,4,5-T. Whereas 4% of the sucrose applied moved to the growing points, only 1% of the total glucose applied moved to the growing points while essentially the same amounts of sucrose and glucose moved into the primary leaves both in the presence and in the absence of 2,4,5-T. This may suggest that both sugars reached the primary leaves via the same pathway (possibly the xylem) while translocation to the growing points seemed to show a preferential selectivity for sucrose. 2,4,5-T apparently delayed the disappearance of ^{14}C and ^{3}H from both the primary leaves and the growing points, possibly by enhancing the incorporation of the label into a more stable metabolic pool.

The amount of basipetal movement of ^{14}C and ^{3}H to the roots was very small even when compared to growing points and primary leaves (Figure 17A). As in the growing points there seemed to be a preference for sucrose in basipetal translocation of label to the roots. 2,4,5-T appeared to cause a slight increase in the accumulation of label in the roots. Analysis of the nutrient solutions in which the plants were

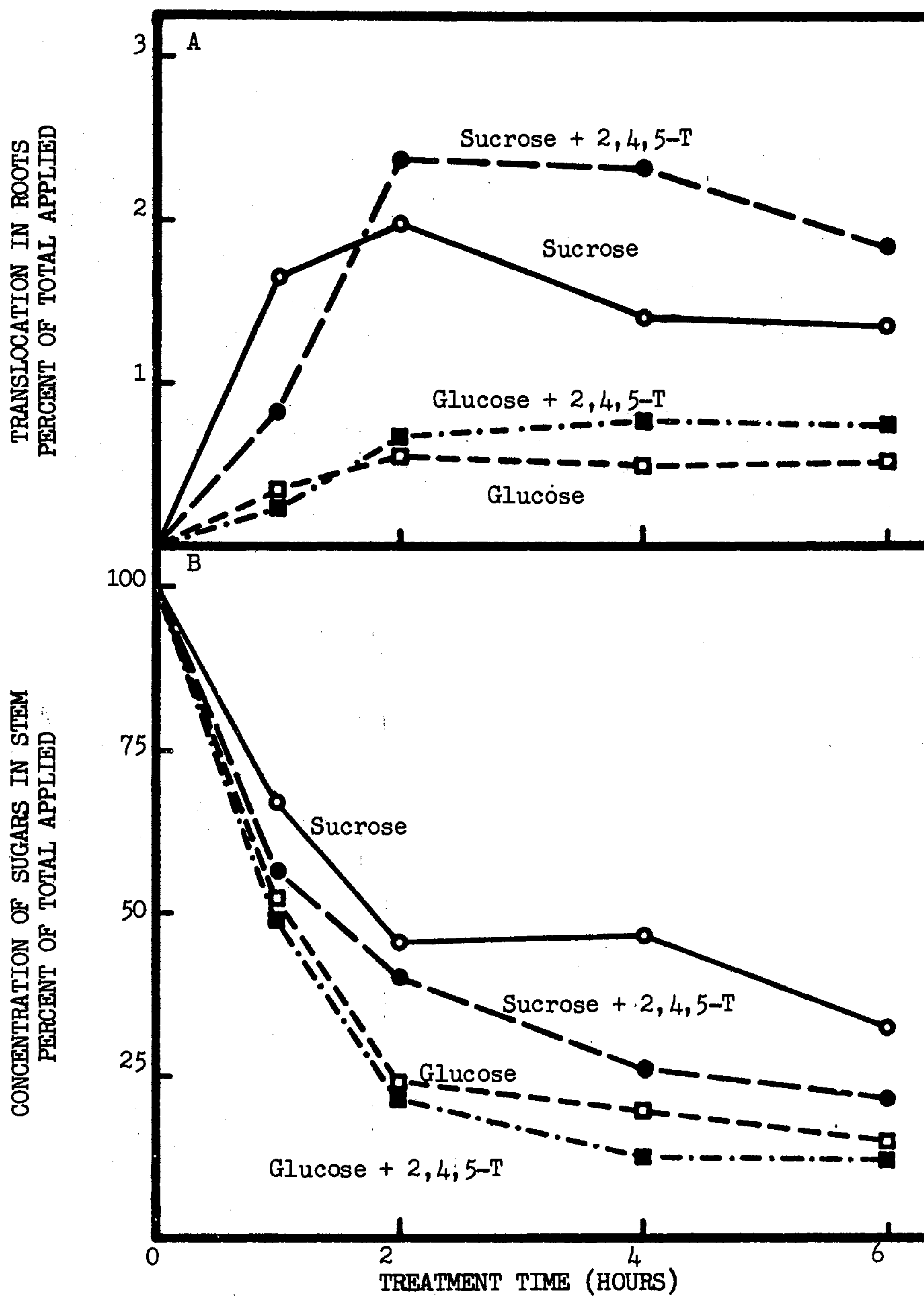

Figure 17. Influence of 2,4,5-T on A)accumulation of sugars in roots and B)loss of sugars from the stem after stem-injection of labeled sugars in bean seedlings.

growing indicated that there was essentially no accumulation of label in the nutrient solution during the course of the treatment.

Only 10-15% of the label applied can be accounted for in the growing points, primary leaves, and roots. However, 50% of the ^{14}C of sucrose and 70% of the ^{3}H of glucose disappeared from the stem by 2 hours after treatment (Figure 17B). 2,4,5-T slightly enhanced the disappearance of ^{14}C and ^{3}H from the stem. If the total of ^{14}C and ^{3}H recovered from all plant parts is plotted against time (Figure 18) it is clear that the label disappeared from the plant very rapidly. The pattern of disappearance of label from the total of all plant parts very closely paralleled the pattern of disappearance of label from the stem suggesting that a considerable portion of the label is lost due to metabolic activities of the stem, probably as $^{14}CO_2$ and $^{3}H_2O$ evolution. This pattern might be expected on the basis of several papers showing that respiration in the stem is quite high (Coulson and Peel, 1968; Whittle, 1970). It is interesting to speculate that this rapid loss of label in the stem may help explain the log front so often observed in translocation studies. This view has been suggested by Whittle (1970) on the basis of her work on the translocation of ^{14}C in Helianthus seedlings. She showed that ^{14}C from leaf assimilates accumulated very quickly in the alcohol insoluble fractions in the stem during translocation. In the present experiment the loss of label appears to be due to respiration which might reflect a wound reaction brought about by the injection of sugars.

Thus, although auxins modify their own acropetal transport it is clear that any effect on sugar transport is minimal. The effect of 2,4,5-T on sugars injected into the stem seems to be on the incorpora-

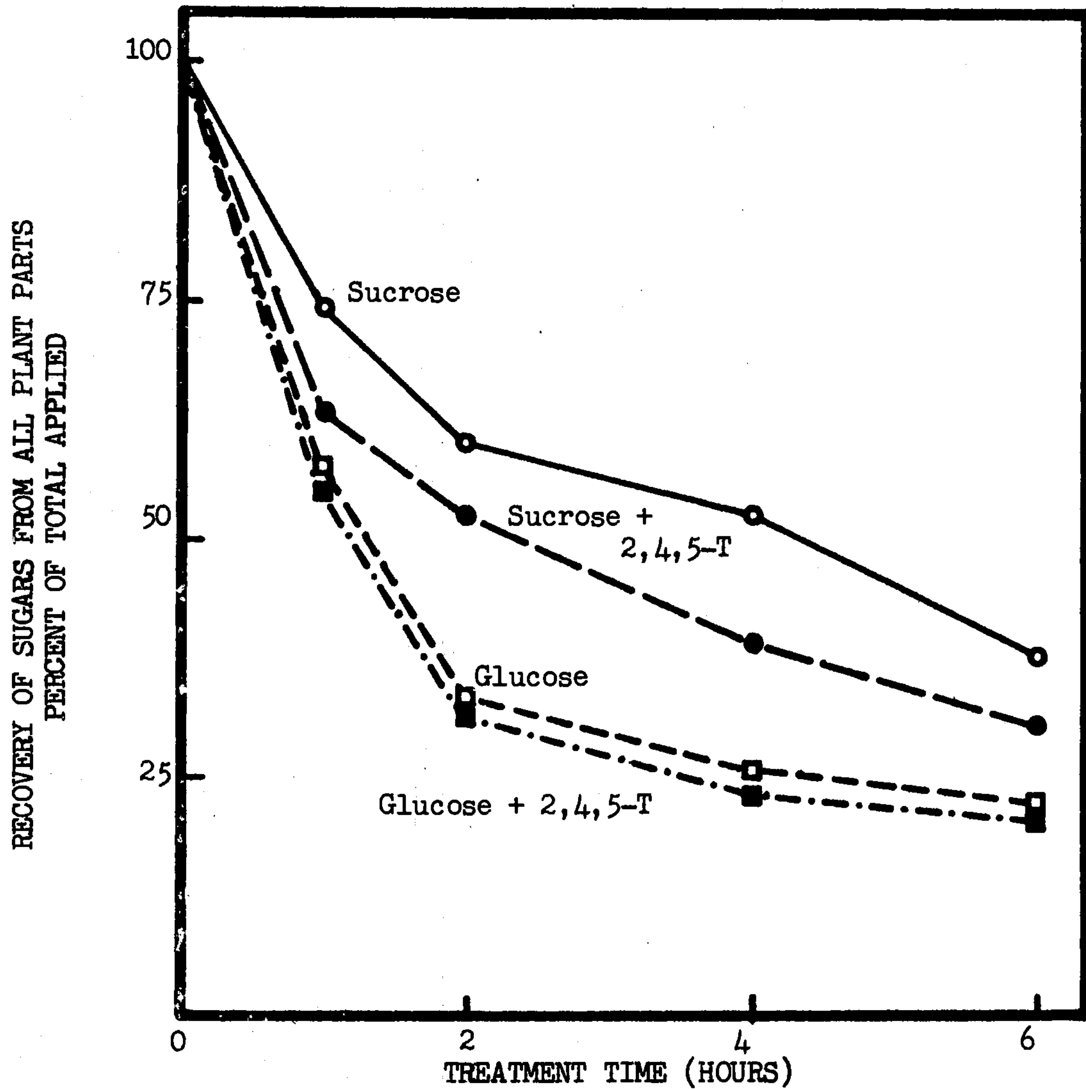

Figure 18. Recovery of ^{14}C of sucrose and ^{3}H of glucose from all plant parts after injection in the stem of bean seedlings.

tion or accumulation of label in the more stable metabolic pools and to some extent on disappearance of label from the plant.

Effect of Cycloheximide on 2,4,5-T Translocation

Protein synthesis has been shown by different workers to accompany the elongation responses to IAA treatment in isolated plant tissue (Key, 1964; Nooden and Thimann, 1965; Fang and Yu, 1965). Protein and nucleic acid synthesis inhibitors have been shown to inhibit both the incorporation of ^{14}C-labeled amino acids into proteins and the auxin-induced elongation of isolated segments (Nooden and Thimann, 1965; Abeles, 1966). Cycloheximide is a potent inhibitor of protein synthesis (Ellis and McDonald, 1970; Viau and Davis, 1970) which has been shown to markedly reduce the auxin-induced elongation responses in etiolated pea stem segments (Barkley and Evans, 1970). Sudi (1964) reported that IAA and other active auxins will induce the enzyme which conjugates IAA with aspartate in pea stem tissue. Cycloheximide inhibits the conjugation of IAA with aspartic acid and markedly increases the free (methanol soluble) IAA in pea stem tissue (Kang, et al., 1971).

The present series of experiments was conducted to determine the influence of a protein inhibitor, cycloheximide, on the translocation patterns of 2,4,5-T in intact bean seedlings. Cycloheximide (5µg/plant) severely depressed basipetal translocation of 2,4,5-T applied at a rate of 3 µg/plant (Figure 19). The amounts of 2,4,5-T accumulated in both the roots and nutrient solutions were markedly inhibited by cycloheximide and the effect is already obvious just one hour after treatment.

The effect of cycloheximide on acropetal translocation is illustrated in Figure 20. The influence of cycloheximide on accumulation of

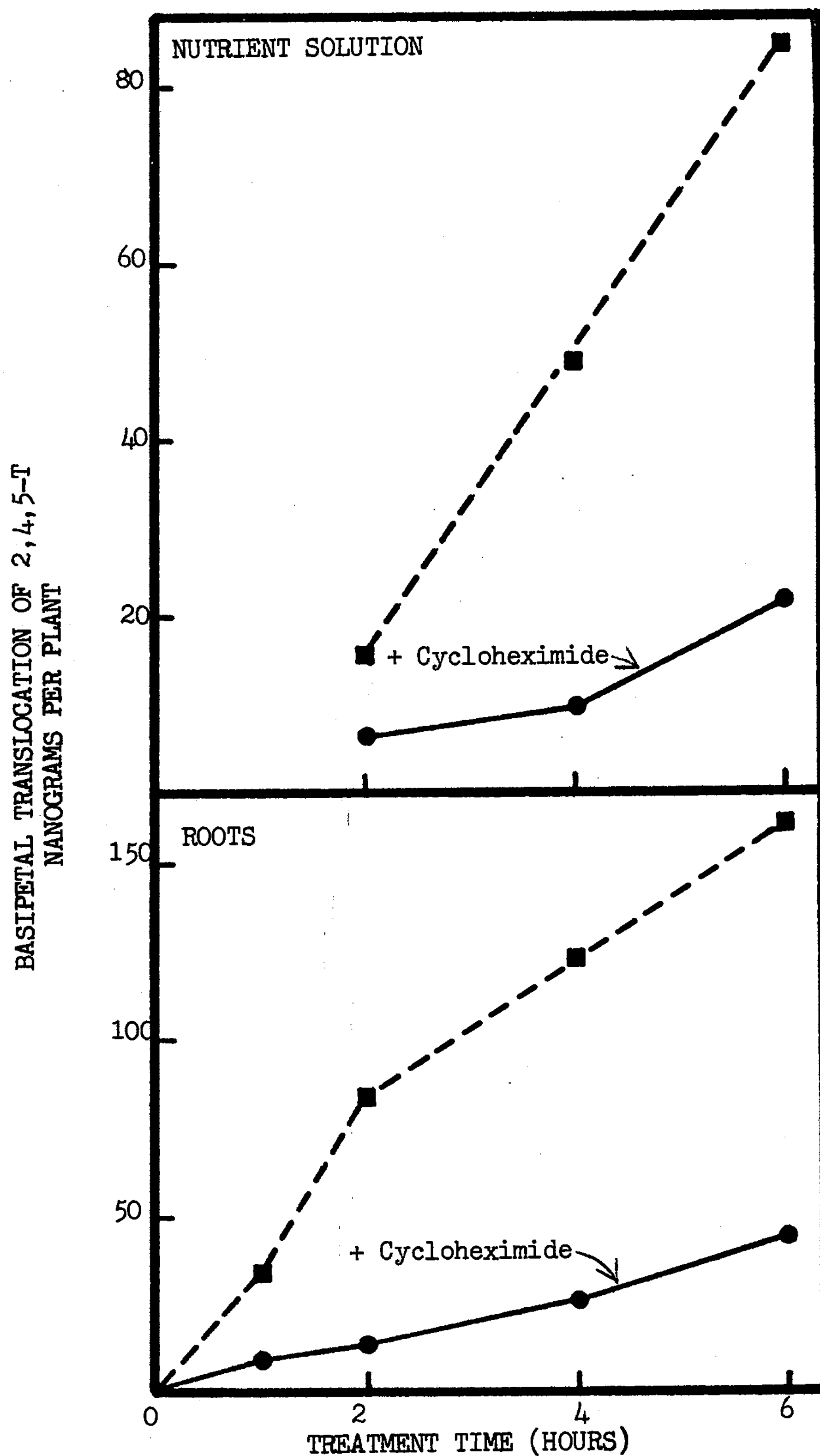

Figure 19. Influence of cycloheximide (5 µg/plant) on the accumulation of 2,4,5-T (3 µg/plant) in nutrient solution and roots.

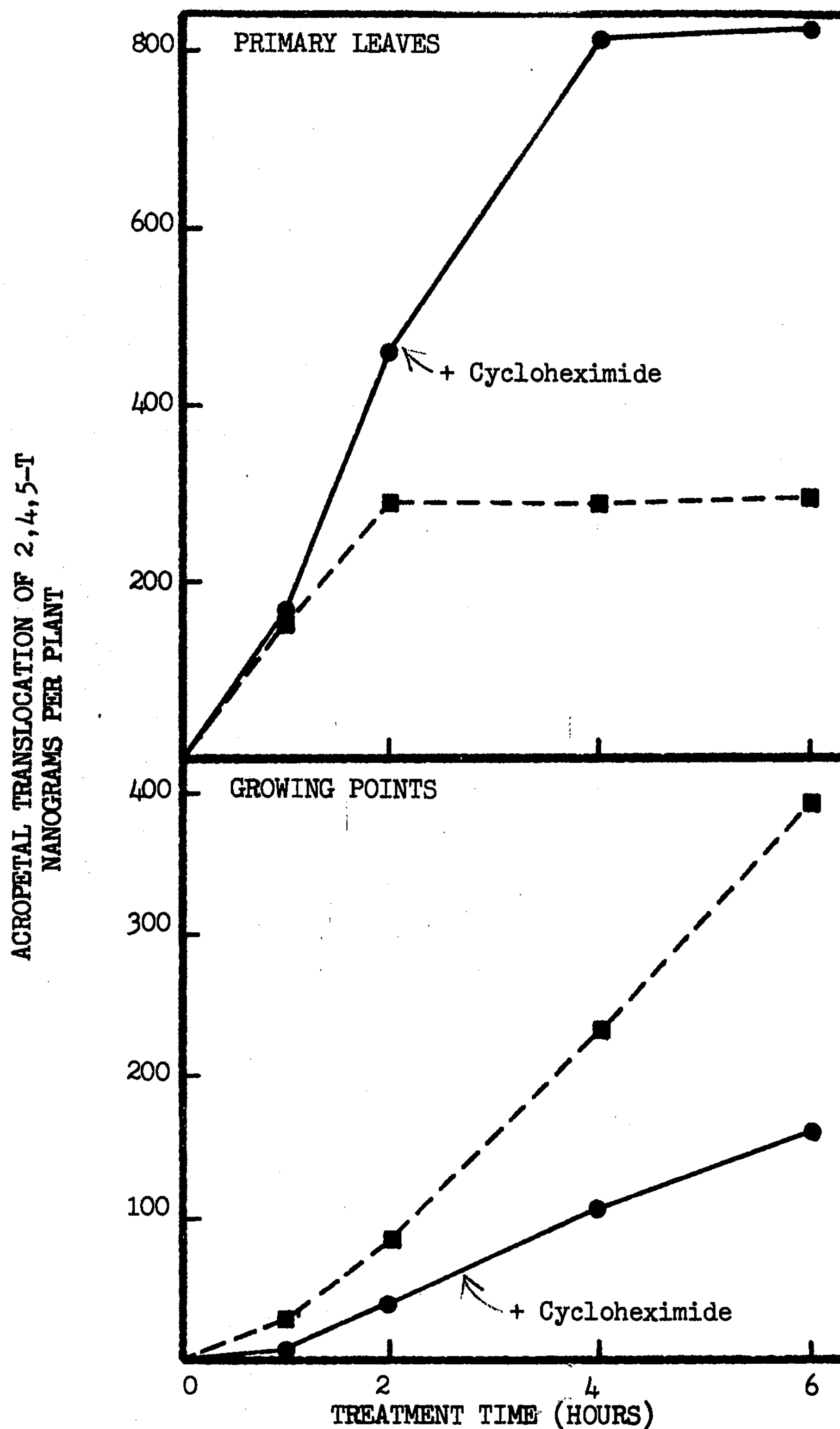

Figure 20. The influence of cycloheximide (5 µg/plant) on the accumulation of 2,4,5-T (3 µg/plant) in primary leaves and growing points.

2,4,5-T in the growing points follows much the same pattern as accumulation of 2,4,5-T in the roots. Cycloheximide is strongly inhibitory to accumulation in the growing points and its effects are present at 1 hour after treatment. Surprisingly, cycloheximide enhanced the accumulation of 2,4,5-T in the primary leaves drastically and maintained 2,4,5-T movement into the leaves 2 hours longer than the control treatment. The effects of cycloheximide on translocation did not become distinct until 2 hours after treatment began. Thus while translocation of 2,4,5-T to the roots, nutrient solution and growing points are markedly inhibited by cycloheximide, translocation to the primary leaves was increased some 3-fold. The results of the steam girdling experiments which were discussed earlier suggested that translocation to the roots and to the growing point were predominently via the phloem while translocation to the primary leaves was via the xylem. This suggests that auxin translocation through the phloem is dependent on protein synthesis. The increase in translocation to the primary leaves in the presence of cycloheximide might possibly have been due to the increased levels of auxin available for translocation in the xylem in the absence of protein synthesis.

Cycloheximide has been shown to stimulate respiration in red beet storage tissue disks at concentrations that normally inhibit protein synthesis (Ellis and McDonald, 1970). For this reason the response of 2,4,5-T translocation to a series of cycloheximide concentrations was tested (Figure 21). A 50% inhibition of translocation of 2,4,5-T (15 µg/plant) to the growing points was achieved at rates as low as 0.5 µg cycloheximide/plant (Figure 21A) while 2.0 µg cycloheximide/plant resulted in a 2-fold increase in translocation to the primary

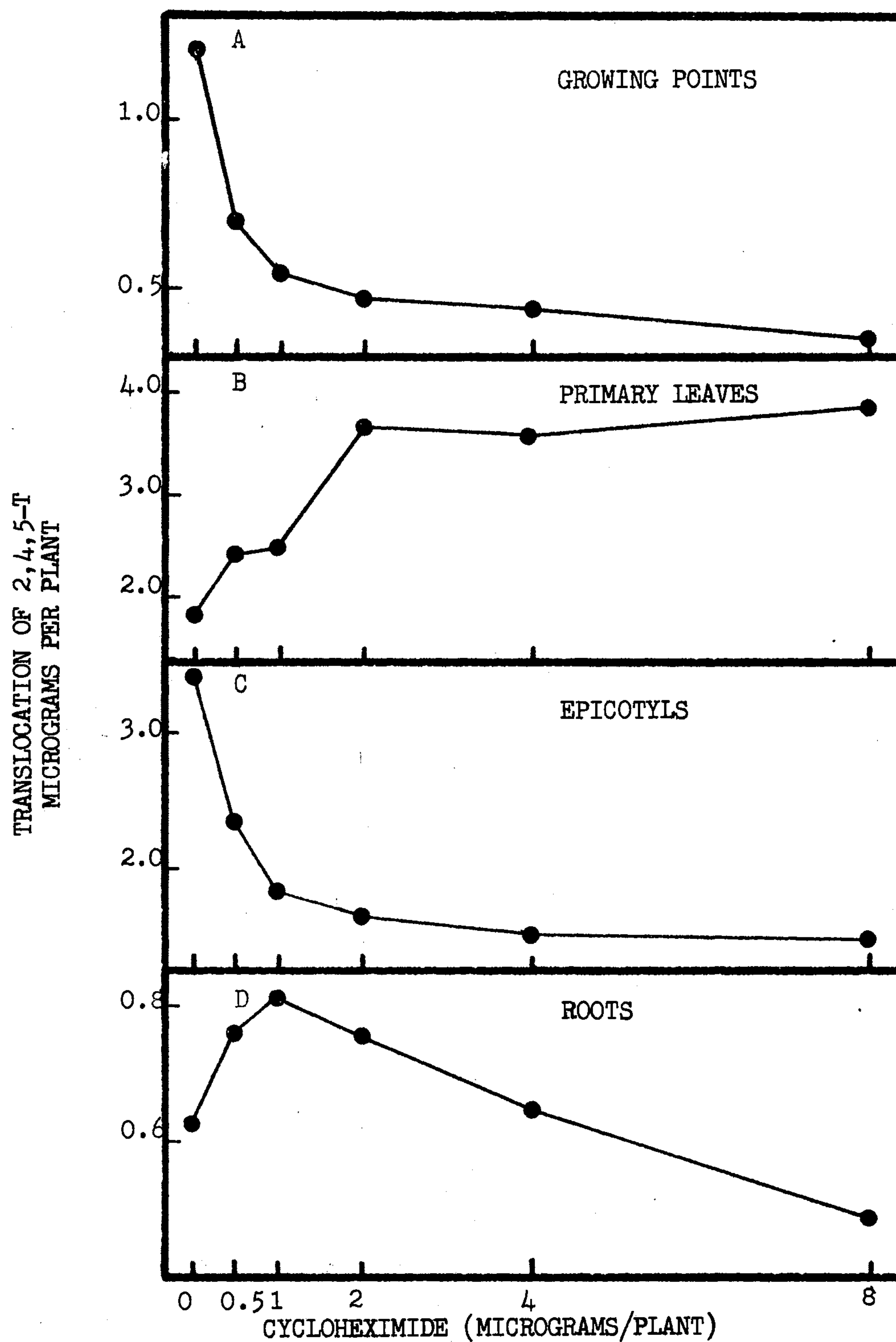

Figure 21. Influence of concentration of cycloheximide on accumulation of 2,4,5-T (15 µg/plant) in various plant parts after 4 hours of treatment.

leaves (Figure 21B). The pattern of inhibition of accumulation of 2,4,5-T in epicotyls was very similar to the pattern of accumulation in the growing point suggesting that in these two tissues the effects of cycloheximide were very similar. Eschrich (1968) has observed that insoluble forms of IAA accumulate in the stem of Vicia faba. Extraction from the stem and hydrolysis in HCl yielded IAA and aspartic acid suggesting that the immobile residue of IAA in stems is IAA-aspartate. Kang (1971) noted that the formation of IAA-aspartate was markedly inhibited by cycloheximide also suggesting that accumulation in the epicotyls was due to the conjugation of IAA and aspartate in the stem.

It was observed during the course of this experiment that within 2 hours after treatment strong nastic responses occurred in the stem above the primary leaves in the control plants, i.e., those which had no cycloheximide added. Bending of the stem became less pronounced as the amount of cycloheximide increased so that at the concentration of 4 and 8 µg cycloheximide/plant no bending was apparent suggesting once again that protein synthesis is a prerequisite of auxin-induced cell elongation. It is interesting in this regard to note that Little and Blackman (1963) reported that accumulations of 2,4,5-T as small as 8 nanograms in the stem of Phaseolus vulgaris was sufficient to induce bending. In the presence of cycloheximide as much as 1500 nanograms of 2,4,5-T accumulated in the epicotyl without inducing nastic responses (Figure 21C).

The response of 2,4,5-T translocation to the roots in the presence of cycloheximide was mixed (Figure 21D). Cycloheximide enhanced 2,4,5-T translocation to the roots at concentrations up to 1 µg cycloheximide per plant. At concentrations higher than this there was a gradual in-

crease in the inhibition of 2,4,5-T accumulation in the roots. It would appear that at high levels of 2,4,5-T (15µg/plant) basipetal translocation was not as sensitive to cycloheximide as at relatively low 2,4,5-T concentrations (3 µg/plant). A high concentration of 2,4,5-T (15 µg/plant) appeared to partially overcome the inhibition of basipetal translocation by cycloheximide (compare Figure 19). At 3 µg 2,4,5-T/plant cycloheximide at 5 µg/plant caused a 3-fold inhibition of 2,4,5-T accumulation in the roots, while at 15 µg 2,4,5-T/plant 8 µg cycloxehimide resulted in only a 30% decrease in translocation to the roots.

Thus it is clear that accumulation of auxin in all plant parts was notably sensitive to cycloheximide and presumably to protein synthesis. This may reflect the inhibition of the synthesis of a carrier molecule (Osborne and Mullins, 1969) or the inhibition of the synthesis of an auxin conjugating enzyme (Kang, 1971) or possibly both. In any event it seems clear that protein synthesis plays a vital role in auxin translocation in intact bean seedlings.

CHAPTER V

SUMMARY AND CONCLUSIONS

Determinations of the translocation patterns of stem-injected IAA and the synthetic auxins 2,4-D and 2,4,5-T were made in intact bean seedlings. It was found that the time of day at which the auxin was applied was a strong determinant of the direction of translocation of exogenously supplied auxin. For this reason all the experiments reported in this study were initiated between 9 A.M. and 11 A.M.

The translocation of 2,4-D and 2,4,5-T was remarkably similar throughout a 72-hour period with one notable exception: 2,4,5-T markedly enhanced its own acropetal translocation to the growing point. The translocation of 2,4,5-T into the nutrient solution continued unimpeded throughout 72 hours while net accumulation of 2,4,5-T in the roots reached a maximum at 2 hours after treatment after which time levels in the roots gradually decreased. Acropetal translocation of 2,4,5-T to the primary leaves reached quite high levels after only 2 hours of treatment after which time it very rapidly decreased to barely detectable levels. Accumulation of 2,4,5-T in the growing points continued for the first 6 hours of treatment. Levels of 2,4,5-T in the growing point remained essentially unchanged thereafter. Roughly one-fourth of 2,4,5-T originally injected into the stem was translocated acropetally during the first 6 hours after treatment so that during this time translocation of 2,4,5-T was essentially all in the acropetal direction.

Basipetal translocation of 2,4,5-T proceeded in a linear fashion throughout a wide range of 2,4,5-T injection levels. Acropetal translocation to the growing point was markedly enhanced by increases in 2,4,5-T treatment at low levels.

The placement of a steam girdle either above or below the site of 2,4,5-T injection in the stem resulted in data which suggested that all basipetal translocation of 2,4,5-T and translocation to the growing point was via the phloem, while translocation to the primary leaves occurred predominantly in the xylem.

PCIB, an antiauxin which is a potent inhibitor of polar basipetal transport of auxins in isolated stem and coleoptile tissue, had no effect on the basipetal translocation of 2,4,5-T to the roots while it slightly enhanced accumulation of 2,4,5-T in the nutrient solution. Acropetal translocation as evidenced by the accumulation of 2,4,5-T in the primary leaves and growing points was increased by relatively high levels of PCIB.

Not only do auxins enhace their own acropetal translocation to the growing point, but the auxins 2,4-D and 2,4,5-T each modify the translocation of the other. 2,4,5-T markedly enhanced the accumulation of 2,4-D-1-^{14}C in the growing points of bean seedlings, while 2,4-D depressed the accumulation of 2,4,5-T-1-^{14}C in growing points.

2,4,5-T had relatively little effect on the translocation of labeled sugars other than to delay the peak times of acropetal translocation of the sugars. 2,4,5-T also caused a slight increase in the disappearance of label from the plants.

The protein synthesis inhibitor cycloheximide strongly depressed the basipetal translocation of 2,4,5-T when simultaneously injected

with 2,4,5-T in the stem of bean seedlings. While cycloheximide stimulated the movement of 2,4,5-T into the primary leaves it inhibited the accumulation of 2,4,5-T in the growing point. Cycloheximide also markedly inhibited the accumulation of 2,4,5-T in the epicotyl of the treated plants.

It would appear from the data presented in this study that there are factors within the stems of bean seedlings regulating the distribution of auxins in the plant. The fact that 2,4,5-T enhanced the acropetal translocation of 2,4-D while conversely 2,4-D depressed the acropetal movement of 2,4,5-T suggests the presence of two stereospecific sites, possibly one for auxins which would activate the translocation mechanism and one for auxin-analogs which, when filled would depress the translocation mechanism.

The fact that 2,4,5-T did not stimulate the translocation of sugars from the stems suggests that 2,4,5-T treatment in the stem did not increase the rate of flow in the general assimilate stream. It is conceivable that 2,4,5-T enhanced its own rate of loading into the phloem. For instance, both steam girdling and cycloheximide inhibited what appeared to be the translocation of 2,4,5-T in phloem. This suggests that there is a relationship between protein synthesis and translocation of auxins in the phloem.

No attempt has been made during the course of this study to identify the compound to which the ^{14}C was attached after the label was recovered from the plant. Information of this sort could conceivably help provide a better general understanding of the mechanisms involved in the regulation of auxin translocation in intact plants.

Finally, it must be admitted that although there are artificial

components in this method of study just as there are in other methods of studying the regulation of auxin movements in plants, it is hoped that the limitations on this system are less severe than those imposed on other current methods and that this information will provide a better general understanding of mechanisms involved in auxin translocation in plants.

Most of the literature pertaining to the movement of auxins in plants is restricted to basipetal polar transport in isolated coleoptile and stem segments. While translocation of auxins in intact bean seedlings is sensitive to many of the same stimuli as auxin transport, the response of auxin translocation in intact plants to these stimuli is often vastly different from the response of auxin transport in stem and coleoptile tissue. For instance acropetal movement of auxins in stem and coleoptile segments is considered to be diffusional in nature. Acropetal translocation in intact seedlings, on the other hand, occurs at velocities exceeding those for strictly diffusional processes. Acropetal translocation is also remarkably sensitive to the concentration of auxin applied, antiauxins, auxin-auxin interactions and protein synthesis inhibitors, as well as disruption of the phloem by girdling. Acropetal transport in segments is not affected by any of these factors.

Basipetal polar transport in isolated segments is strongly inhibited by antiauxins but basipetal translocation of auxins in intact plants is unaffected by antiauxins while acropetal translocation is markedly enhanced by antiauxins. The data presented in this study suggest that the patterns and mechanisms illustrated by the study of auxin transport do not always represent the mechanisms for auxin movement which function in intact plants.

LITERATURE CITED

Abeles, F. B. 1966. Auxin stimulation of ethylene evolution. Plant Physiol. 41:585-587.

Agbakoba, C. S. O. and J. R. Goodin. 1970. Absorption and translocation of ^{14}C-labeled 2,4-D and picloram in field bindweed. Weed Sci. 18:168-170.

Bach, M. K. 1961. Metabolites of 2,4-dichlorophenoxyacetic acid from bean stems. Plant Physiol. 36:558-565.

Barkley, G. M. and M. L. Evans. 1970. Timing of the auxin response in etiolated pea stem sections. Plant Physiol. 45:143-147.

Basler, E. and K. Nakazawa. 1961. Some effects of 2,4-D on nucleic acids of cotton cotyledon tissue. Bot. Gaz. 122:228-232.

__________, F. W. Slife and J. W. Long. 1970. Some effects of humidity on the translocation of 2,4,5-T in bean plants. Weed Sci. 18(3):396-398.

__________, G. W. Todd and R. E. Meyer. 1961. Effects of moisture stress on absorption, translocation and distribution of 2,4-D in bean plants. Plant Physiol. 36:573-576.

Booth, A., J. Moorby, C. R. Davies, H. Jones and P. F. Wareing. 1962. Effects of indole-3-acetic acid on the movements of nutrients within plants. Nature. 194:204-205.

Burstrom, H. G., I. Uhrstrom and B. Olausson. 1970. Influence of auxin on Young's modulus in stems and roots of Pisum and the theory of changing the modulus in tissues. Physiol. Plant. 23:1223-1233.

Christie, A. E. and A. C. Leopold. 1965a. On the manner of triiodobenzoic acid inhibition of auxin transport. Plant Cell Physiol. 6:337-345.

__________ and __________. 1965b. Entry and exit of indoleacetic acid in corn coleoptiles. Plant Cell Physiol. 6:453-465.

Coulson, C. L. and A. J. Peel. 1968. Respiration of ^{14}C-labelled assimilates in stems of willow. Ann. Bot. 32:867-876.

Crafts, A. S. and S. Yamaguchi. 1958. Comparative tests on the uptake and distribution of labeled herbicides by _Zebrina pendula_ and _Tradescantia fluminensis_. Hilgardia. 27:421-454.

Dalrymple, A. V. and E. Basler. 1963. Seasonal variation in absorption and translocation of 2,4,5-T and respiration rates in blackjack oak Weeds. 11:41-45.

Day, B. E. 1952. The absorption and translocation of 2,4-dichlorophenoxyacetic acid by bean plants. Plant Physiol. 27:143-152.

Davies, C. R. and P. F. Wareing. 1965. Auxin-directed transport of radio-phosphorus in stems. Planta. 65:136-156.

Davis, F. S., R. W. Bovey and M. G. Merkle. 1968a. Effect of paraquat and 2,4,5-T on the uptake and transport of picloram in woody plants Weed Sci. 16:336-339.

____________, M. G. Merkle and R. W. Bovey. 1968b. Effect of moisture stress on the absorption and translocation of herbicides in woody plants. Bot. Gaz. 129:183-189.

de la Fuente, R. K. and A. C. Leopold. 1970. Time course of auxin stimulations of growth. Plant. Physiol. 46:186-189.

Doutt, M. T. 1932. The anatomy of *Phaseolus vulgaris* L. var. black valentine. Mich. Agr. Expt. Sta. Tech. Bul. No. 128:1-33.

Ellis, R. J. and I. R. McDonald. 1970. Specificity of cycloheximide in higher plant systems. Plant Physiol. 46:227-232.

Eschrich, W. 1968. Translokation radioaktiv markierter Indolyl-3-Essigsaure in Siebrohren von *Vicia Faba*. Planta. 78:144-157.

Evans, M. L. and R. Hokanson. 1969. Timing of the response of coleoptiles to the application and withdrawal of various auxins. Planta. 85:85-95.

Fang, S. C. and T-C Yu. 1965. Influence of auxins on *in vitro* incorporation of glycine in pea shoot proteins. Plant Physiol. 40: 299-303.

Fites, R. C., J. B. Hanson and F. W. Slife. 1969. Alteration of m-RNA and ribosome synthesis in soybean hypotocotyl by 2,4-D. Bot. Gaz. 130:118-126.

Gillespie, B. and K. V. Thimann. 1963. Transport and distribution of auxin during tropistic response. I. The lateral migration of auxin in geotropism. Plant Physiol. 38:214-225.

Goldsmith, M. H. M. and K. V. Thimann. 1962. Some characteristics of movement of indoleacetic acid in coleoptiles of *Avena*. I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. Plant Physiol. 37:492-505.

Hagar, A. and R. Schmidt. 1968a. Auxintransport and Phototropismus. I. Die lichtbedingte Bildung eines Hemmstoffes für den Transport von Wuchsstoffen in Koleoptilen. Planta. 83:347-371.

__________ and __________. 1968b. Auxintransport and Phototropismus. II. Der Hemmechanismus des aus IES gebildeten Photosoxidationsproduktes 3-Methylenoxindol beim transport von Wuchsstoffen. Planta. 83:372-386.

Hay, J. R. and K. V. Thimann. 1956. The fate of 2,4-dichlorophenoxyacetic acid in bean seedlings. II. Translocation. Plant Physiol. 31:446-451.

Hertel, R., M. L. Evans, A. C. Leopold and H. M. Sell. 1969. The specificity of auxin transport system. Planta. 85:238-249.

__________ and R. Flory. 1968. Auxin movement in corn coleoptiles. Planta. 82:123-144.

Hew, C. S., C. D. Nelson and G. Krotkov. 1967. Hormonal control of translocation of photosynthetically assimilated ^{14}C in young soybean plants. Amer. J. Bot. 54:252-256.

Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Agri. Exp. Sta. Calif. Circ. 347.

Jaworski, E. G., S. C. Fang and V. H. Freed. 1955. Studies in plant metabolism. V. The metabolism of radioactive 2,4-D in etiolated bean plants. Plant Physiol. 30:272-275.

Kang, B. G., W. Newcomb and S. P. Burg. 1971. Mechanism of auxin-induced ethylene production. Plant Physiol. 47:504-509.

Keitt, G. W. and R. A. Baker. 1966. Auxin activity of substituted benzoic acids and their effect on polar auxin transport. Plant Physiol. 41:1561-1569.

Key, J. L. 1964. Ribonucleic acid and protein synthesis as essential processes for cell elongation. Plant Physiol. 39:365-370.

__________ and J. C. Shannon. 1964. Enhancement by auxin of ribonucleic acid synthesis in excised soybean hypocotyl tissue. Plant Physiol. 39:360-374.

Khan, A. and G. R. Sagar. 1969. Alteration of the pattern of distribution of photosynthetic products in the tomato by manipulation of the plant. Ann. Bot. 33:753-762.

Lepp, N. W. and A. J. Peel. 1970. Some effects of IAA and kinetin upon the movement and sugars in the phloem of willow. Planta. 90: 230-235.

__________ and __________. 1971a. Influence of IAA upon the longitudinal and tangential movement of labelled sugars in the phloem of willow. Planta. 97:50-61.

__________ and __________. 1971b. Patterns of translocation and metabolism of ^{14}C-labelled IAA in the phloem of willow. Planta. 96:62-73.

Little, E. C. S. and G. E. Blackman. 1963. The movement of growth regulators in plants. III. Comparative studies of transport in Phaseolus vulgaris. New Phytol. 62:173-197.

McCready, C. C. 1966. Translocation of growth regulators. Ann. Rev. Plant Physiol. 17:283-294.

__________ and W. P. Jacobs. 1963. Movement of growth regulators in plants. II. Polar transport of radioactivity from IAA-^{14}C and 2,4-D-^{14}C in petioles of Phaseolus vulgaris. New Phytol. 62:19-34.

Merkle, M. G. and F. S. Davis. 1967. Effect of moisture stress on absorption and movement of picloram and 2,4,5-T in beans. Weeds. 15:10-12.

Mitchell, J. W. and J. W. Brown. 1946. Movement of 2,4-dichorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. Bot. Gaz. 107:393-407.

Morris, D. A., R. E. Briant and P. G. Thomson. 1969. The transport and metabolism of ^{14}C-labelled indoleacetic acid in intact pea seedlings. Planta. 89:178-197.

Mullins, M. G. 1970a. Transport of ^{14}C-assimilates in seedlings of Phaseolus vulgaris in relation to vascular anatomy. Ann. Bot. 34:889-896.

__________. 1970b. Hormone-directed transport of assimilates in decapitated internodes of Phaseolus vulgaris. L. Ann. Bot. 34:897-910.

Nelson, H., I. Ilan and L. Reinhold. 1969. The effect of inhibitors of protein and RNA synthesis on auxin induced growth. Isr. J. Bot. 18:129-134.

Niedergang-Kamien, E. and A. C. Leopold. 1959. The inhibition of transport of IAA by phenoxyacetic acids. Physiol. Plant. 12:776-785.

Nooden, L. D. and K. V. Thimann. 1965. Inhibition of protein synthesis and auxin induced growth by chloramphenicol. Plant Physiol. 40:193-201.

Osborne, D. J. 1968. A theoretical model for polar auxin transport. In: The transport of plant hormones (Ed. Y. Vardar). p. 97. Amsterdam.

__________ and M. G. Mullins. 1969. Auxin, ethylene and kinetin in a carrier-protein model system for the polar transport of auxins

in petiole segments of Phaseolus vulgaris. New Phytol. 68:977-991.

Parups, E. V. 1970. Effect of morphactin on the gravimorphism and the uptake, translocation and spatial distribution of indol-3yl-acetic acid in plant tissues in relation to light and gravity. Physiol. Plant. 23:1176-1186.

Rayle, D. L., R. Ouitrakul and R. Hertel. 1969. Effect of auxins on the auxin transport system in coleoptiles. Planta. 87:49-53.

Rice, E. L. 1948. Absorption of ammonium-2,4-dichlorophenoxyacetate by bean plants. Bot. Gaz. 109:301-314.

Rohrbaugh, L. M. and E. L. Rice. 1949. Effect of application of sugar on the translocation of sodium-2,4-dichlorophenoxyacetate by bean plants in the dark. Bot. Gaz. 111:85-89.

Scott, T. K. and W. R. Briggs. 1960. Auxin relationships in the Alaska pea. Amer. J. Bot. 47:492-499.

__________ and W. P. Jacobs. 1963. Auxin in Coleus stems: limitation of transport at higher concentrations. Sci. 139:589-590.

Skoog, F. 1938. Absorption and translocation of auxin. Amer. J. Bot. 25:361-372.

Slife, F. W., J. L. Key, S. Yamaguchi and A. S. Crafts. 1962. Penetration, translocation and metabolism of 2,4-D and 2,4,5-T in wild and cultivated cucumber plants. Weeds. 10:29-35.

Sudi, J. 1964. Induction of the formation of complexes between aspartic acid and indole-3-acetic acid or 1-naphthaleneacetic acid by other carboxylic acids. Nature. 201:1009-1010.

Veen, H. 1966. Transport, immobilization and localization of Naphthylacetic acid-1-^{14}C in Coleus explants. Acta Bot. Neerl. 15:419-433.

__________. 1967. On the relation between auxin transport and auxin metabolism in explants of Coleus. Planta. 73:281-295.

Vian, J. P. and F. F. Davis. 1970. Effect of cycloheximide on the synthesis and modification of ribosomal RNA in Neurospora crassa. Biochim. Biophys. Acta. 209:190-195.

Wardlaw, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-104.

Warner, H. L. and A. C. Leopold. 1969. Timing of plant hormone responses in etiolated pea seedlings. Plant Physiol. 44(Suppl.):28.

Went, F. W. 1939. Some experiments on bud growth. Amer. J. Bot. 26:109-117.

____________ and K. V. Thimann. Phytohormones. New York:Macmillan. 1937. 294 pp.

Whittle, C. M. 1970. Lateral movement out of the sieve tubes and its effect on the ^{14}C translocation profile in Helianthus seedlings. Planta. 95:247-263.

Zimmerman, M. H. 1960. Transport in the phloem. Ann. Rev. Plant. Physiol. 11:167-190.

Zwar, J. R. and A. H. G. C. Rijven. 1956. Inhibition of transport of indoleacetic acid in the etiolated hypocotyl of Phaseolus vulgaris. Aust. J. Biol. Sci. 9:528-538.

VITA

John W. Long

Candidate for the Degree of

Doctor of Philosophy

Thesis: KINETICS OF AUXIN TRANSLOCATION IN INTACT BEAN SEEDLINGS

Major Field: Botany

Biographical:

Personal Data: Born in Hays, Kansas, February 22, 1944, the son of Mr. and Mrs. Jesse W. Long.

Education: Graduated from Quinter Rural High School, Quinter, Kansas in 1962; received the Bachelor of Science Degree from McPherson College, McPherson, Kansas in 1966; completed requirements for the Master of Science Degree from Oklahoma State University in 1969; fulfilled the requirements for the Ph.D. Degree in Botany in July, 1971.

Experience: Graduate teaching assistant in the Department of Botany and Plant Pathology 1966-1967, Oklahoma State University; graduate research assistant in the Department of Botany and Plant Pathology, 1967-1971, Oklahoma State University; Recipient of the Summer Research Assistantship Award, 1969.

Professional Organizations: Member of the American Society of Plant Physiologists, Sigma Xi.