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The Use of Cucumber Seedlings to Measure Growth Regulator Activity

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THE USE OF CUCUMBER SEEDLINGS TO MEASURE GROWTH REGULATOR ACTIVITY

BY

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor

Head of the Major Department

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I wish to take this opportunity to express my appreciation to Dr. David J. Holden, and to thank him for his suggestion of the problem and guidance throughout the investigation.

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LIST OF ABBREVIATIONS

Abbreviation	Term
NAA	alpha - naphthaleneacetic acid
NPA	beta - naphthoxy - alpha - propionic acid
CCA	cis-cinnamic acid
4ClN	4-chloro-1-naphthylacetic acid
GA	gibberellic acid
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
MH	maleic hydrazide
OCA	o-chlorophenoxyacetic acid
PCA	p-chlorophenoxyacetic acid
TCA	trans-cinnamic acid
TIBA	2,3,5-triiodobenzoic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4,6-T	2,4,6-trichlorophenoxyacetic acid

INTRODUCTION

At the present time, interest is great in the use of growth regulators for obtaining various effects with plants. Many of these regulators have proven to be quite useful in industry, for weed control, and in investigations into the biochemical and physiological studies of plants.

Growth regulators have a long history of development. In 1758, Duhamel du Monceau proposed a theory of "descending sap" causing root formation in plants (15, 19). But it was not until 1880, when Sachs put forth the first theory of substances controlling plant growth (6, 15, 19) that any real interest was aroused in this study. Work up to 1880 was hampered by lack of analytical procedures, funds, and cooperation between scientists. The investigative studies that had been carried out were mainly concerned with morphological aspects of plants, Sachs (15) felt differentiation of roots and flowers was due to minute amounts of chemicals moving up and down in the plant. These chemicals he considered to be organ forming substances which, in minute amounts, direct development. He also felt there was a polar distribution which may be modified by external forces, such as light and gravity (19). Beijerinck (1888-1897) studied gall formations and came to the conclusion they were formed by "growth enzymes". He stated that "form is determined by liquid substances, which move freely through considerable numbers of cells in growing tissue" (15, 19).

Charles Darwin became interested in this study, and in 1880-1881 he began investigating the responses of plants to light and gravity. He

sectioned off coleoptile tips of canary-grass seedlings, and found the seedlings to have lost their sensitivity to light (6, 15, 19). He placed great importance to this controlling tip, and when he reported his findings, a great deal of controversy was stirred up regarding the actual importance of this tip. This was settled, to some degree, when Rothert, in 1894, confirmed Darwin's work by showing separate plant zones were affected differently by light and gravity (19).

When Fitting found, in Java, in 1907-1908, an extract of orchid pollen would cause swelling of the orchid ovary, interest was further stimulated in this avenue of research. Fitting felt polarity was set up by light stimuli and was spread from cell to cell (6, 14, 15, 19).

The next stride forward was taken by Boysen-Jensen, when, in 1910-1913, in the laboratories of W. Pfeffer in Germany, he cut off the tips of oat coleoptiles, replaced them again with a gelatin layer between the tips and the stumps and observed a curvature of the seedlings (6, 15, 19). Here he showed the phototropic stimulus to be transmitted across a wound gap, and felt this transmission to be a complex chain of reactions. Paal (1914, 1919) took the next step and showed the stimulus was halted when cocoa-butter, mica, or platinum foil, rather than the gelatin was placed in the gap (6, 15, 19). He also replaced the tip on only one side of the stump, thereby producing a curvature, showing a substance or correlation carrier to be present. Soding (1923, 1925) confirmed Paal's conclusions using the straight growth method (19).

Cholodny, in 1927, and Went, 1928, came to the conclusion that phototropism and geotropism are due to a correlation carrier and that all tropisms are mediated by a growth hormone system - "Ohne Wuchsstoff,

kein Wachstum"; without auxin, there is no growth (16).

When Went, again in 1928, finally succeeded in isolating the elusive Wuchsstoff by diffusion into an agar block, the door was opened to the extensive study of plant growth regulators (6). Went proceeded to establish the oat coleoptile curvature as a bio-assay test for growth regulators. Under specified conditions, a unit of measure was established. One AE (Avena Einheit) = amount of material in one block of agar, which caused a 10^{o} curvature in the coleoptile 2 hours after treatment (15). Various technical modifications have been made in the original test by such men as: Van der Weij (1931), Laibach and Kornmann (1933), Heyn (1935), Brecht (1936), Jost and Reiss (1936), Soding (1936), Skoog (1936) and others (19). In 1933, Kogl showed two auxins to be present in the agar block used to obtain the Wuchsstoff, and named them auxin a and auxin b (6). These two auxins proved to be growth stimulators, while up until this time, all extracts and chemicals tested had proven to be inhibitors. Went's Avena coleoptile test, along with other tests, such as the split stem of pea, straight growth test, and the tomato ovary test (6) all allow for quantitative analysis of chemicals, to establish their relative activity as growth regulators. Each of these tests and especially the Avena coleoptile test, requires elaborate manipulations and procedures in order to obtain definite results (18). In reviewing this test and others, and seeing the importance of growth regulators, it was questioned whether or not another, quicker, and easier test could be designed, which could supplement or compare to the oat coleoptile test.

This paper is devoted to the investigation of this problem with the use of the hypocotyl hooks of dark-grown cucumber seedlings, variety Marketeer.

MATERIALS AND METHODS

Cucumber seedlings were chosen for this investigation, because of observations and tests made by some authors which showed the cucumber seedling to be sensitive to growth substances (9, 11). Variety Marketeer was chosen for these tests, and three approaches were made in investigating the problem.

SOLUTION METHOD - A stock solution of TIBA was made up at 100 ppm in water, and a buffer solution of 10 ml M/15 Na_2HPO_4 and 190.4 ml M/15 KH_2PO_4 was added to reach a pH of 5.6 (9). Dilutions were made by a 10:1 ratio to reach concentrations of 10 ppm, 1 ppm, .1 ppm, .01 ppm. The cucumber seeds were placed on filter paper in Petri dishes, and 3 cc of each solution was then placed in the separate dishes, and the seeds allowed to germinate. After five days, measurements of root lengths, and hypocotyl lengths were made, and notations taken on the degree of effect the solutions had on the angle of bending of the hypocotyl, geotropism, and any other abnormalities encountered.

The next two methods made use of the fact that cucumbers grown in the dark, maintain a hook in the hypocotyl, immediately behind the cotyledons. This hook will remain as long as the plant is in darkness. Once light has had sufficient time to act on the hypocotyl, the hook will straighten out normally. By treating this hook with various growth substances, inhibition or stimulation of cell growth is achieved, causing the hook to straighten out or hook tighter.

Cucumber seeds were placed on moist paper towels on vermiculite in

plastic trays. Two plastic trays were then placed in a metal water-proof tray. The metal tray was then placed in a light-proof incubator at 24° C. The seedlings were allowed to germinate and grow to a height of 7-10 cm., at which height they responded most actively to the tests. It took from four to six days after placing the seeds on the moist paper until they reached the proper height.

AGAR METHOD - Agar was prepared with 100 ppm trans-cinnamic acid. The agar was placed in Petri dishes, and allowed to set. The cotyledons of cucumber seedlings were then removed, and the top 3 cm. of the seedlings cut from the remainder of the hypocotyls. These hooked hypocotyl sections were then placed in the Petri dishes, so that the hooks alone were in contact with the agar, and the rest of the hypocotyl propped upright against the sides of the Petri dishes. Measurements of the degree of straightening or closing of the hook angle was then attempted.

LANOLIN PASTE METHOD - When the seedlings reached their optimum height range of 7-10 cm., they were treated with various lanolin paste applications, in some cases on top of the hook and in other cases on the bottom of the hook. The pastes were prepared as follows: the substances were weighed out, dissolved in appropriate solvents, and mixed with melted lanolin, which upon cooling formed the paste. The stock concentrations for this procedure were 1000 ppm, and were arrived at by weighing out 10 mg. of the substance, and adding it, dissolved to 10 gm. of pure anhydrous lanolin. A wetting agent, TWEEN 20 (polyoxyethelene sorbitan monolaurate), was added (1 drop per gm. of lanolin) to insure prompt

penetration of the growth substances into the hypocotyl hook. If any dilutions were desired, 1 gm. of the stock solutions of 1000 ppm was added to 10 gm. of pure lanolin to form 100 ppm. This procedure was followed till, in some cases, concentrations of .01 ppm were arrived at.

The substances used for these experiments were suggested by several authors (1, 11, 14, 15, 17). The substances, solvents, and concentrations used are as follows:

Substances	Solvent	Concentrations (ppm)
TCA	alcohol	1,000; 100; 10; 1; .1; .01
CCA	"	100; 10; 1
TIBA	"	10,000; 1,000; 100; 10; 1
IAA	"	10,000; 1,000; 100; 10; 1
2,4,5-T	"	1,000
2,4,6-T	"	"
coumarin	"	"
PCA	"	"
OCA	"	"
IBA	"	"
4ClN	"	"
NAA	"	"
NPA	"	"
fumaric acid	"	"
pyruvic acid	"	"
succinic acid	"	"
2,4-D	"	"
gibberellic acid	"	"
eosin	"	1000; 100; 10; 1; .1
erythrosin	"	" " " " "
malonic acid	water	"
nicotinic acid	"	"
phenyl lactic acid	"	"
maleic acid	"	"
maleic hydrazide	"	"

Some applications were made using combinations of two of the previous substances. These combinations are as follows, all concentrations being at 1000 ppm:

NPA	+	IBA	Eosin	+	IBA
"	+	pyruvic acid	"	+	2,4,5-T
"	+	2,4,5-T	"	+	2,4,6-T
"	+	2,4,6-T	"	+	NAA
"	+	malonic acid	"	+	TIBA
"	+	4ClN	"	+	IAA
"	+	fumaric acid	"	+	2,4-D
"	+	NAA	"	+	GA
"	+	coumarin			
"	+	nicotinic acid			
"	+	succinic acid			
"	+	phenyl lactic acid			
"	+	PCA			
"	+	OCA			
"	+	TIBA			
"	+	IAA			
"	+	eosin			
"	+	erythrosin			
"	+	maleic hydrazide			
"	+	2,4-D			
"	+	TCA			
"	+	CCA			
"	+	GA			

Application of the paste was made to the top of the hooks, in most cases, with some applications also made on the bottom of the hooks. The flat ends of toothpicks were used as applicators. The seedlings were allowed 24 hours to respond, and then measured with a protractor designed so that the complete straightening of the hook constituted a 180° measurement (fig. 1). Comparison was made in each case with a control group of untreated seedlings.

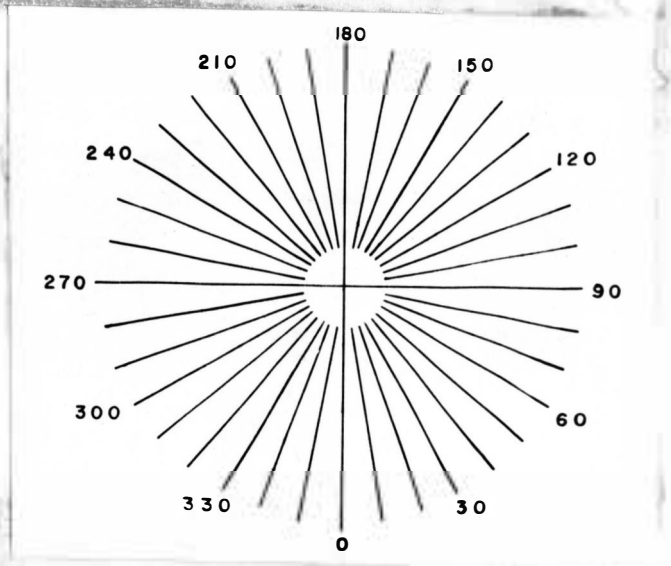

Figure 1. Example of protractor used to measure the cucumber hypocotyl hook angles. The hypocotyl was placed on the 0° line and the point of application on the hook, at the center of the circle. Measurement was made to the angle at which the cotyledons pointed.

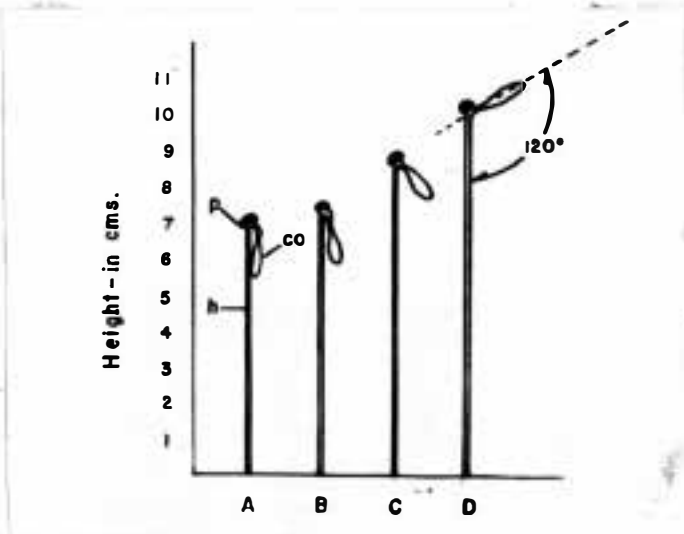

Figure 2. Diagrammatic representation of cucumber hypocotyl and typical reaction to eosin, a growth inhibitor. h, hypocotyl; co, cotyledons; p, point of application on top side of hook. A, height of seedling when treated; B, approximately 2 hours after treatment; C, 5 hours after treatment D, 24 hours after treatment.

RESULTS

Using the solution method, it was found that triiodobenzoic acid (TIBA) inhibited growth of both hypocotyl and root of the cucumber seedlings (fig. 3). At a concentration of .01 ppm, the total growth of the two (hypocotyl and root) was only 60% that of the control group. This was also true of the .1 ppm concentration. The 1 ppm concentration seemed to show a slight stimulus towards increased growth, averaging 74% of the control group. After this, the concentrations again showed increased inhibition of growth, 10 ppm averaging 48%, and 100 ppm averaging 43% of the control group's growth. The angle of bending of the hypocotyl did not seem to indicate any particular trend, though all but one of the concentrations showed less bending than did the control, the 100 ppm concentration showing considerably more bending than the control group. The measure of geotropism in each concentration was also lower than that of the control group.

With the agar method, the hypocotyl sections placed on the agar and propped up against the sides of the petri dishes, dried up long before any effect could be shown by the growth substance (TCA) included in the agar.

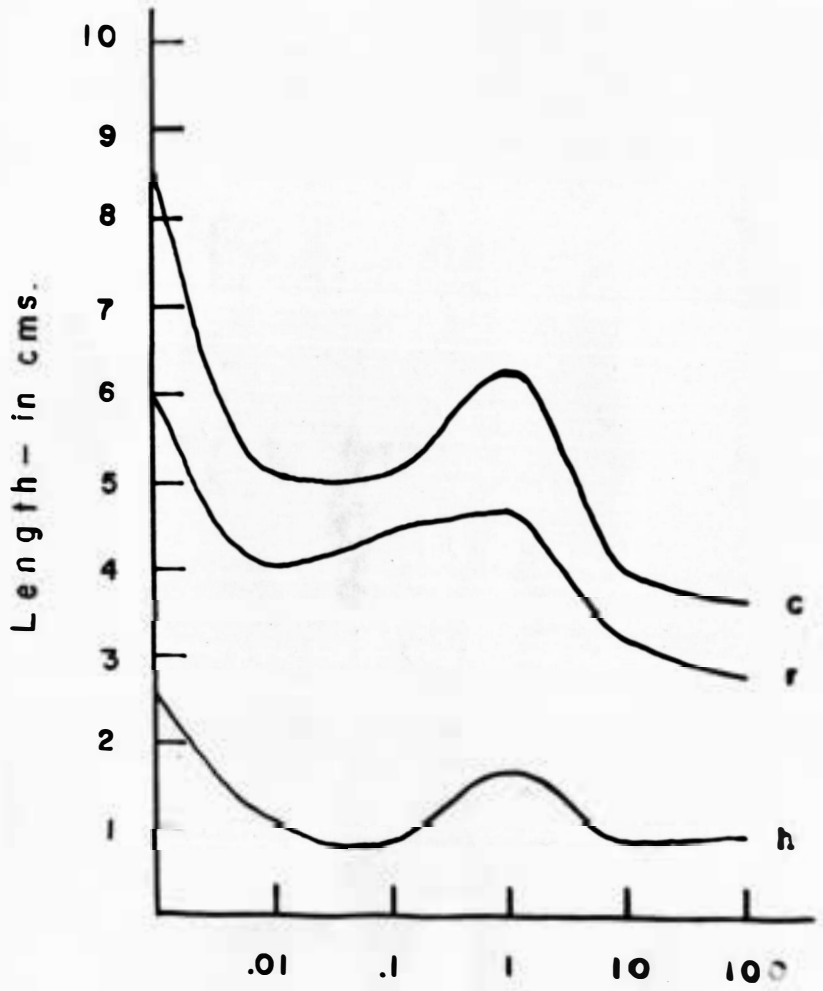

Figure 3. Cucumber seedling development using various concentrations of TIBA in solution. h, hypocotyl length; r, root length; c, length of combination of the hypocotyl and root

In preparing for the treatments with lanolin a growth curve under experimental conditions was established for the cucumber seedlings (fig. 4). Treatment with eosin at 1000 ppm concentration was then applied to cucumber seedlings at various heights to determine at what range of the growth curve the seedlings were most sensitive to growth substances (fig. 5). This range turned out to be 7-10 cm., which is towards the peak of the growth curve, or just at the end of the grand period of growth. This grand period is also called the log phase of the growth curve. This is the point at which maximum cell elongation is occurring and just before the food supply in the dark grown seedlings becomes limiting. If more food would be available the sensitivity range may have been more centrally located in this log phase of the growth.

A time test was also run to establish a reasonable period to let the seedlings react to the growth substances. IBA, at 1000 ppm was used and 24 hours was chosen as the most convenient length of time, though 48 hours, would probably show greater differential responses (fig. 6).

Tests were run to establish sensitivity of the hypocotyl hooks to various substances at different concentrations when applied to the top of the hook. Results are shown in figs. 7 and 8.

Treatment of the underside or bottom of the hypocotyl hooks and comparison with the topside treatment of the hooks showed some of the substances to be translocated, to the bottom of the hook, and others not. Stimulation or inhibition of the translocated ones then took place at the bottom of the hook, rather than the top, while the action of those not translocated was at the point of application at the top of the hook. (table 1)

Table 1. Results obtained by treating cucumber hypocotyl hooks on the underside and topside with various growth substances. Concentrations are all at 1000 ppm. Plus indicates stimulator and translocation, minus indicates inhibitor and non-translocation.

Substance	Underside	Topside	Type	Translocated
IAA	45°	42°	+	+
IBA	70	65	+	+
NPA	78	63	+	+
NAA	70	-73	+	-
Eosin	- 4	136	-	-
Erythrosin	5	103	-	-
2,4,5-T	44	38	+	+
2,4,6-T	-10	-10	-	+
TIBA	10	50	-	-

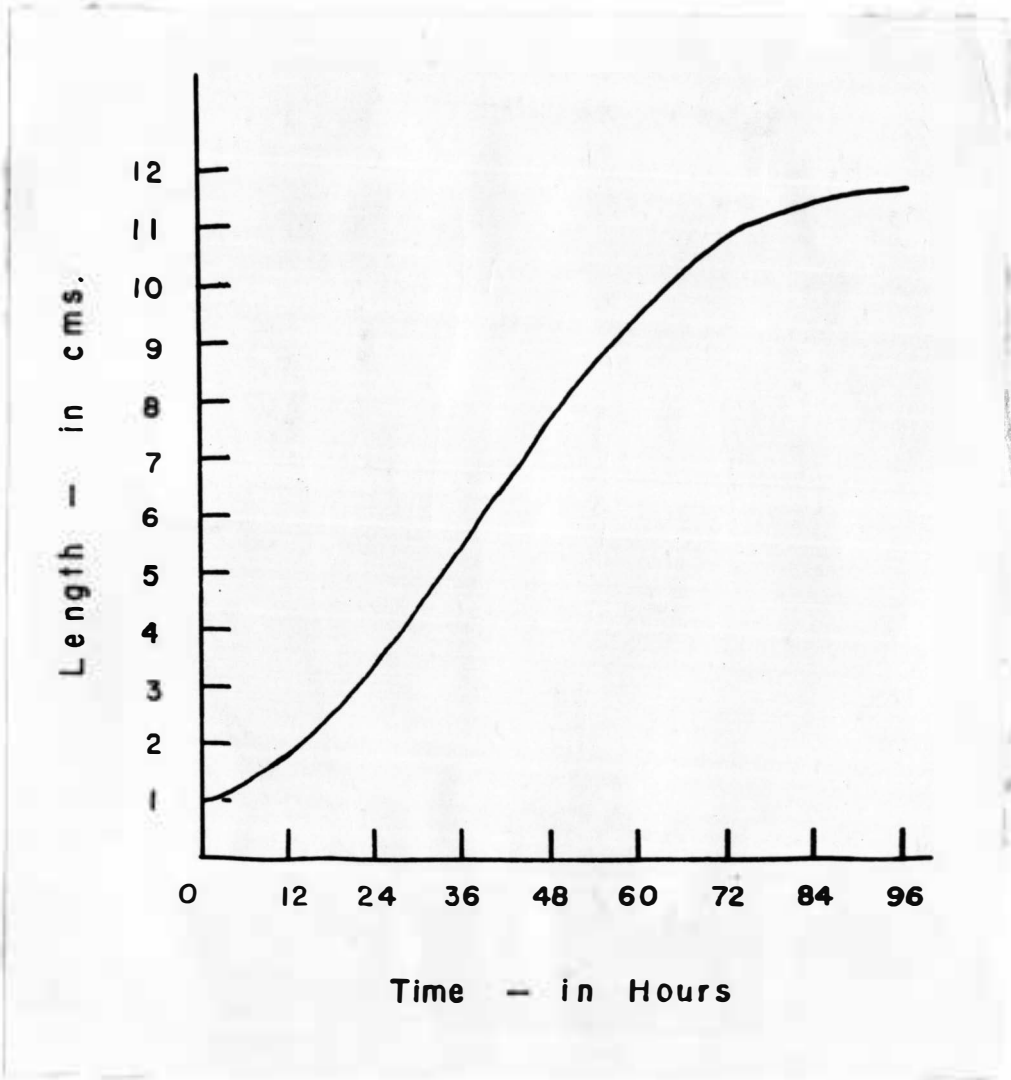

Figure 4. Growth curve of untreated cucumber seedlings, under laboratory conditions

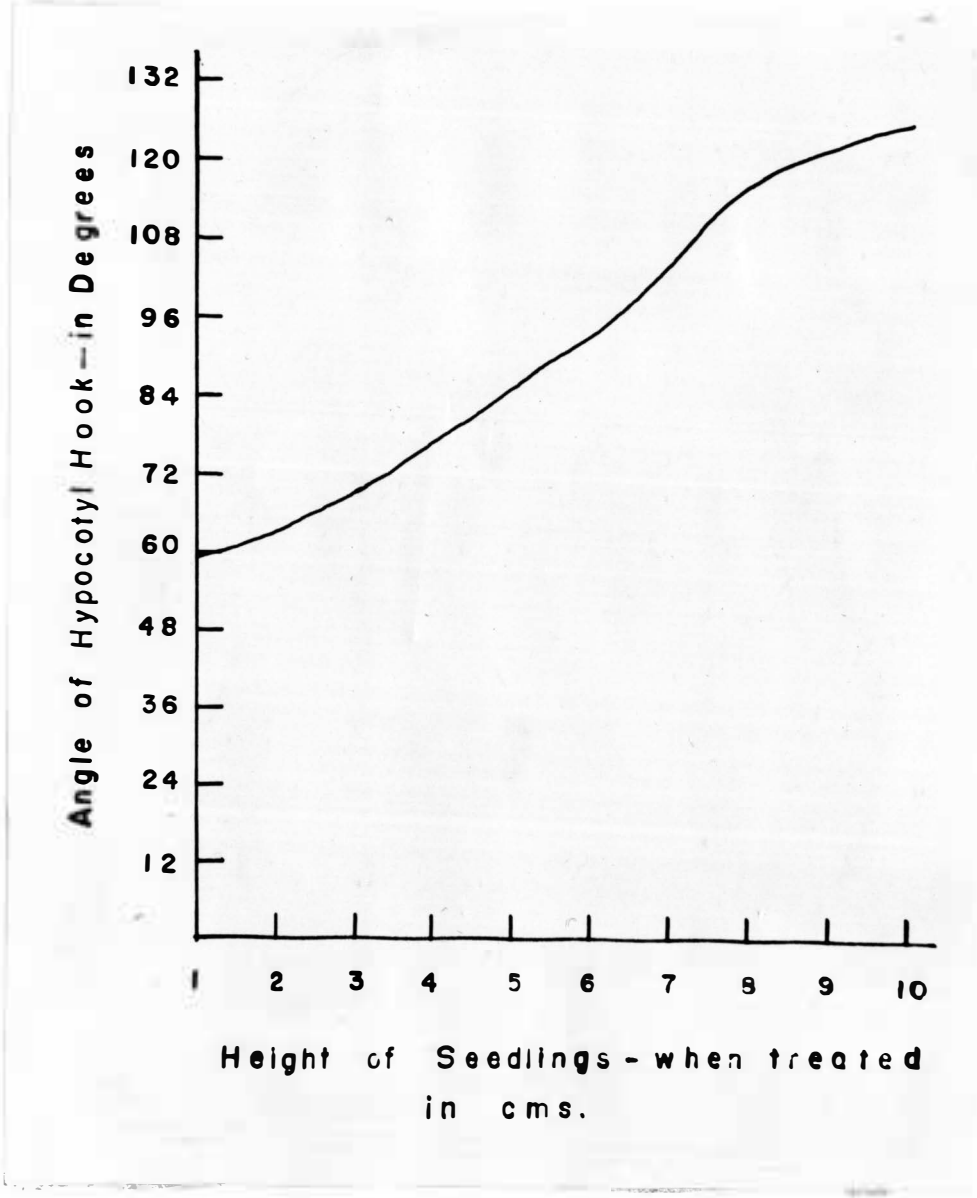

Figure 5. Sensitivity curve of cucumber seedlings when treated with eosin at 1000 ppm, for 24 hours

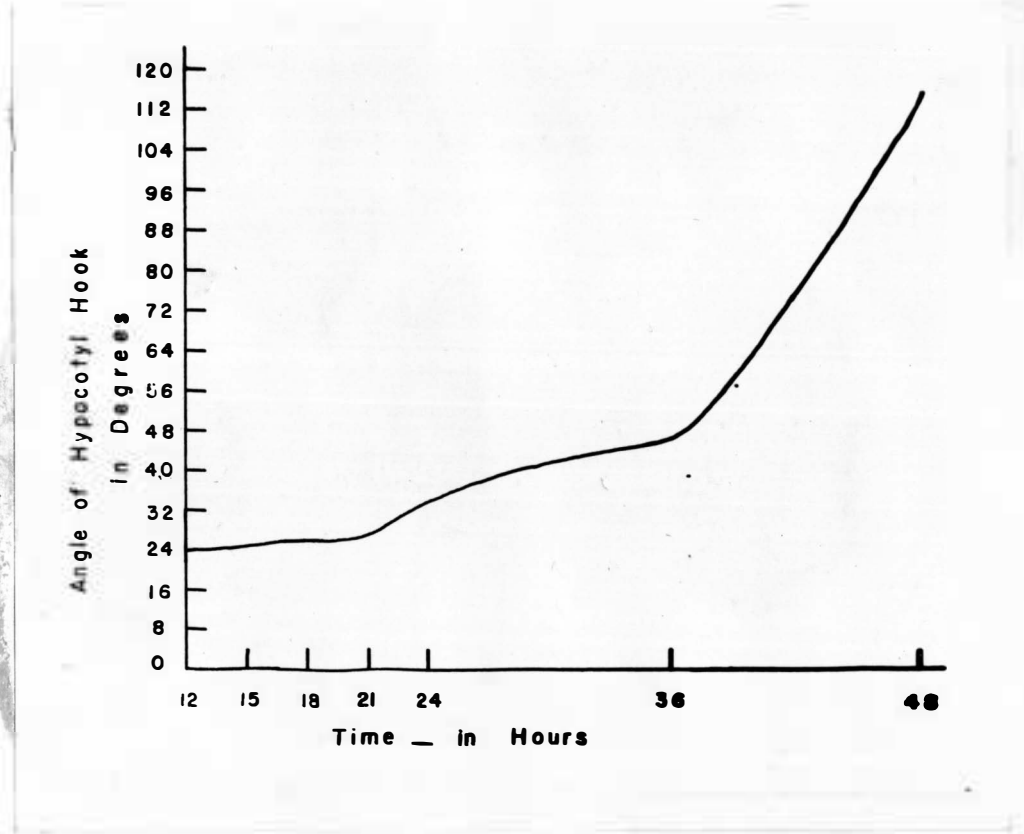

Figure 6. Curve showing reaction of cucumber hypocotyl hook to IBA at 1000 ppm over a period of 48 hours

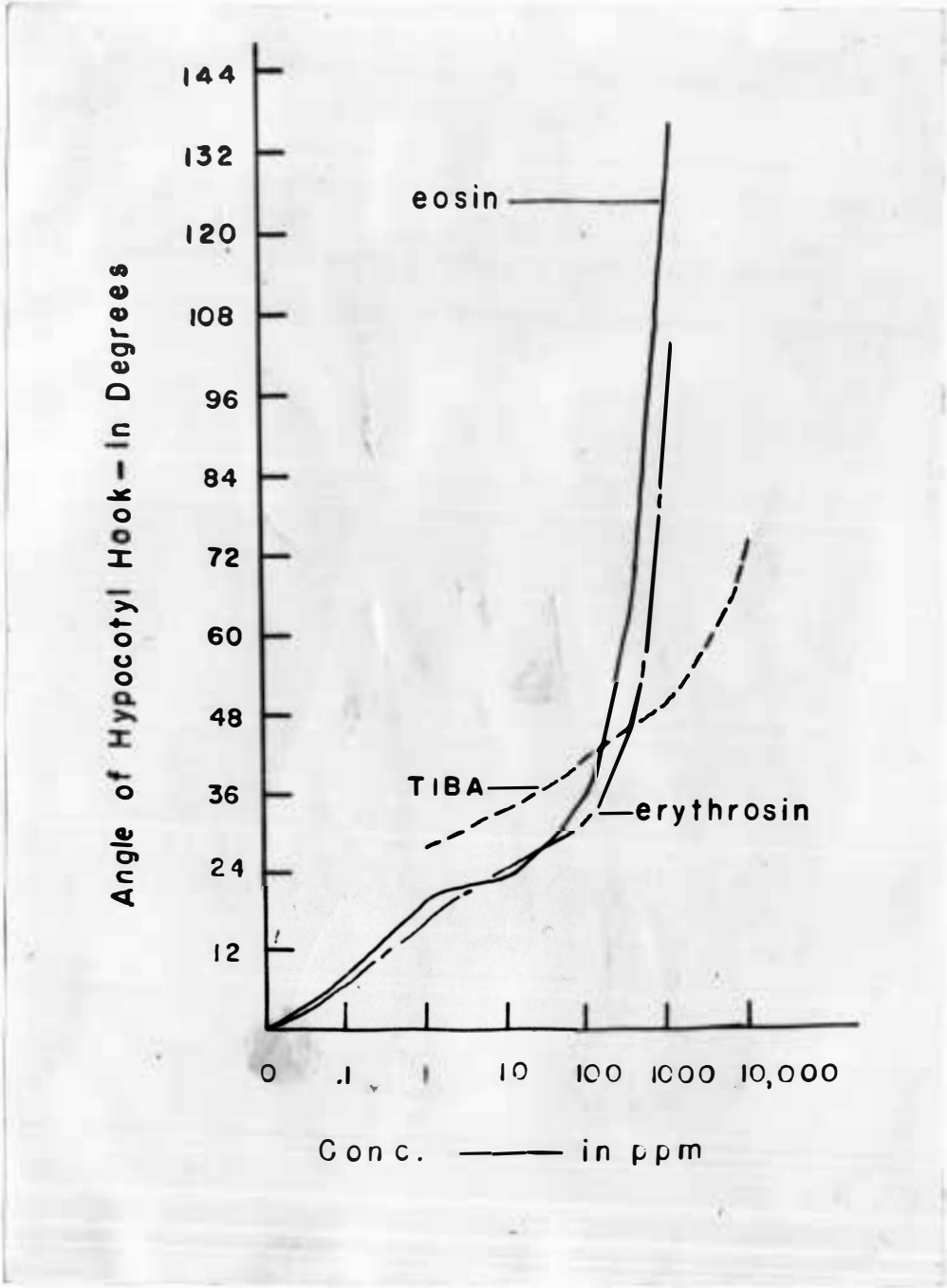

Figure 7. Degree of inhibition of hypocotyl hook angle when eosin, erythrosin, and TIBA are applied to the top of the hook at various concentrations

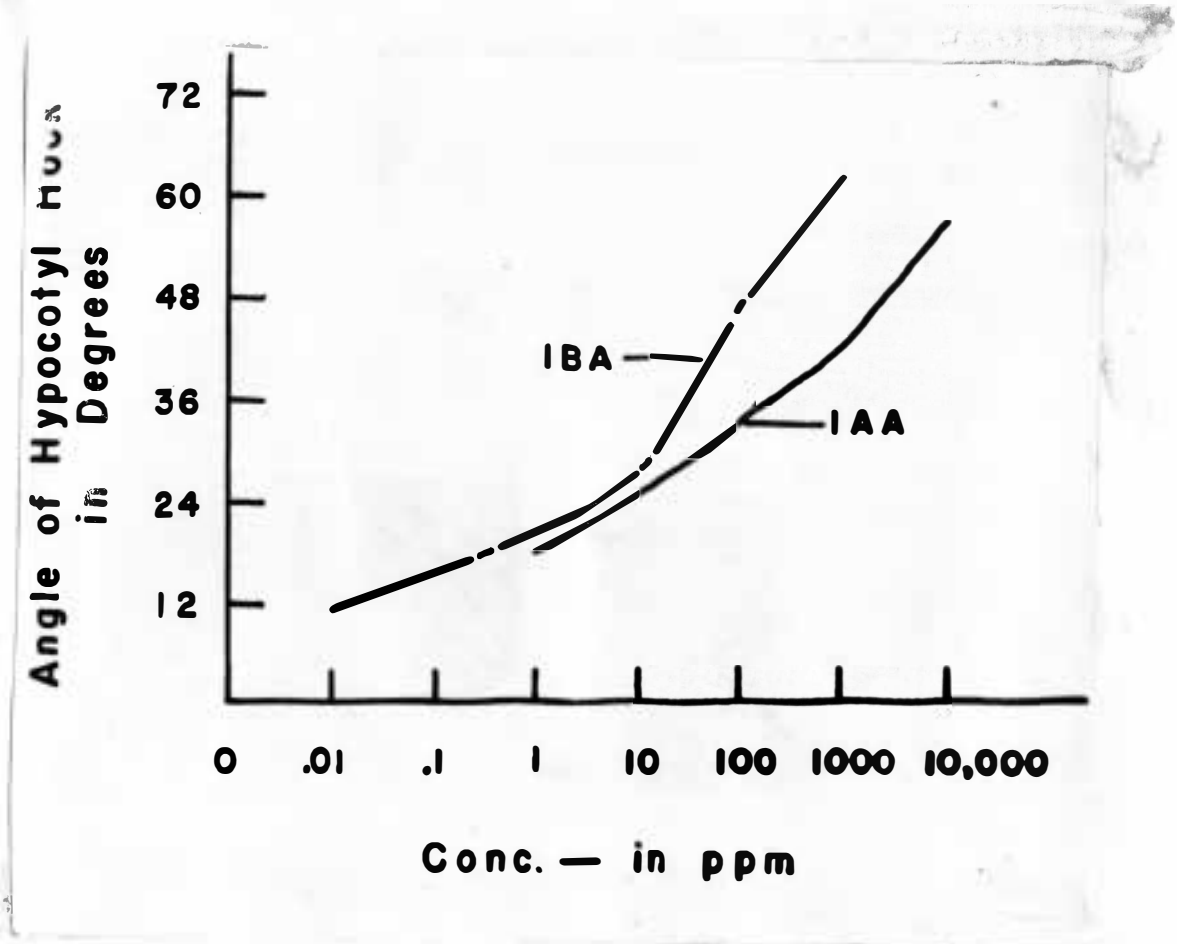

Figure 8. Degree of stimulation of hypocotyl hook angle when IAA, and IBA are applied to the top of the hook at various concentrations

Various substances were applied by themselves on the top of the hook, NAA showing the greatest negative curvature, and eosin the greatest positive curvature (fig. 9). Some of these substances were then mixed where NAA produced a 0° curvature, 2,4,6-T a 30° curvature, TIBA a 115° curvature, erythrosin a 160° curvature, and eosin a 196° curvature, with all the others intermediate between 2,4,6-T and TIBA (fig. 10). Here too results indicated reinforcement of the NPA action by translocated stimulators, and non-translocated inhibitors, while the action of NPA was inhibited by non-translocated stimulators, and translocated inhibitors.

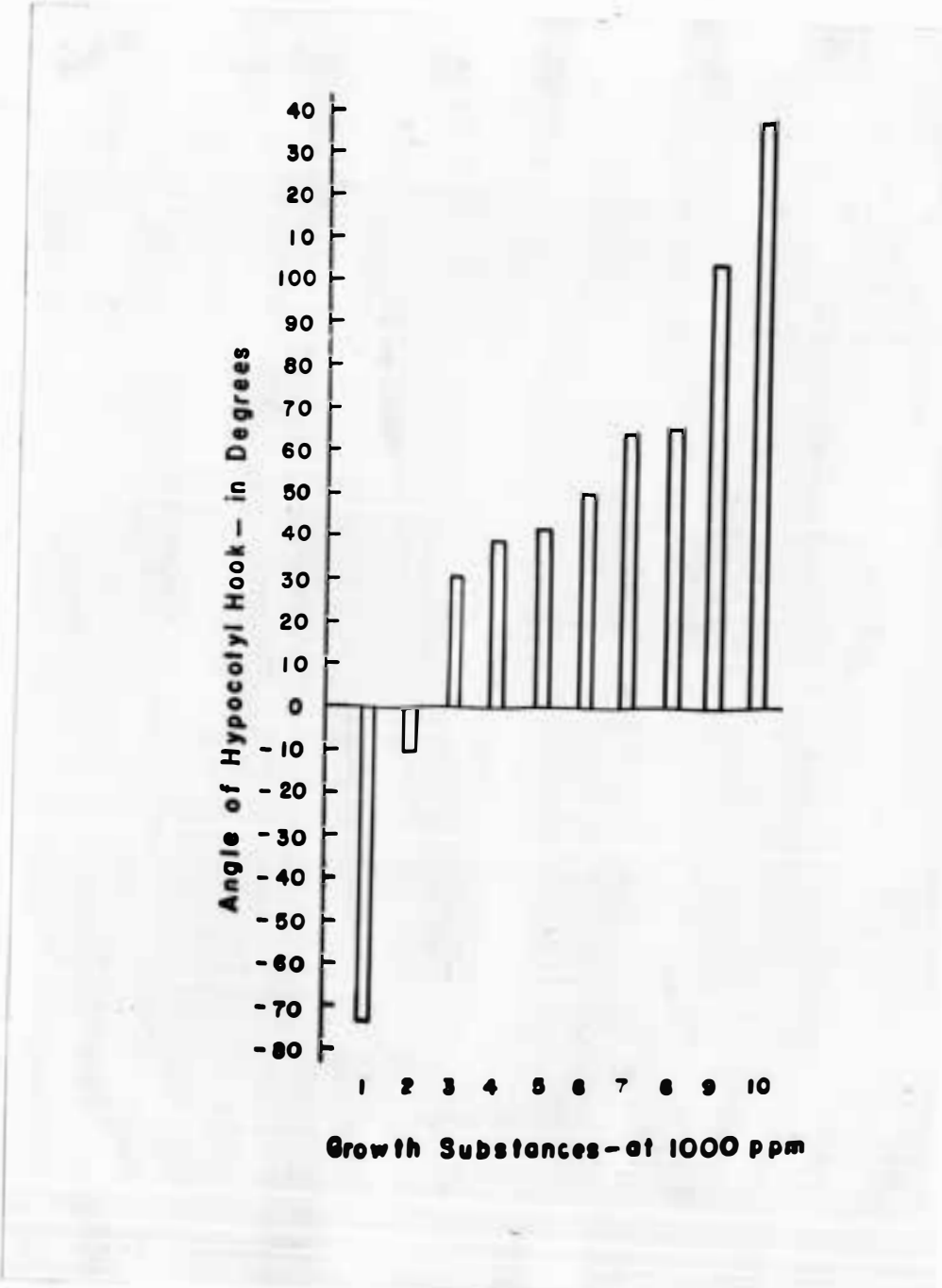

Figure 9. Degree of opening of the hypocotyl hook when treated on the top of the hook with growth substances at 1000 ppm. 1-NAA; 2-2,4,6-T; 3-coumerin; 4-2,4,5-T; 5-IAA; 6-TIBA; 7-NPA; 8-IBA; 9-erythrosin; 10-eosin

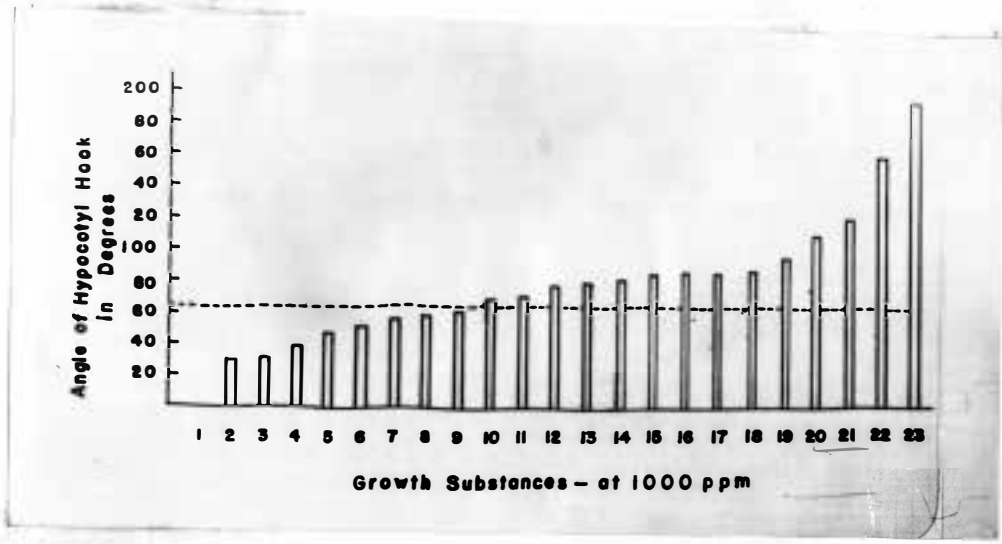

Figure 10. Degree of straightening of the hypocotyl hook, when growth substances were mixed with NPA, and applied to the top of the hook for 24 hours. All concentrations are at 1000 ppm. NPA alone produced a 63° opening (dashed line). 1 - NAA; 2 - 2,4,6-T; 3 - 2,4-D; 4 - phenyl lactic acid; 5 - gibberellic acid; 6 - 4ClN; 7 - malonic acid; 8 - CCA; 9 - fumaric acid; 10 - PCA; 11 - maleic hydrozide; 12 - pyruvic acid; 13 - nicotinic acid; 14 - succinic acid; 15 - 2,4,5-T; 16 - coumerin; 17 - CCA; 18 - TCA; 19 - IAA; 20 - IBA; 21 - TIBA; 22 - erythrosin; 23 - eosin

DISCUSSION

The solution method used involved considerable care and time in preparation. The results were not entirely satisfactory and a fairly long period of time was needed to allow the seeds to react to the growth substance. These facts persuaded this investigator to drop this procedure.

The agar method was also eliminated due to the fact that the hypocotyl sections placed on the agar dried up long before any reactions to the growth substance included in the agar could be measured.

The lanolin paste method proved to be the most effective of the methods tried, and also seemed to hold the most promise of positive results. No difficulty was experienced in preparing the various concentrations in this paste form, nor in diluting some of them to various lower concentrations. The main problem was that of application of the paste to the hypocotyl hook. When applying the paste to the top of the hook, care had to be taken to insure that the application would not run down the side of the hook and influence the results. Application with a #1 artist's brush was attempted, but proved to be unsuitable, in that in order to get a drop from the brush, the lanolin had to be maintained at a very exact temperature - if too high, the lanolin would run down the sides of the hook - if too low, the lanolin would not form the drop. The method employed was that of using the flat end of toothpicks, which allowed easy application of the lanolin at room temperature. This procedure was used for application

to both top and bottom of the hooks.

The dissolving of the growth substances in proper solvents before attempting to mix them with the lanolin was necessary to insure fast, complete diffusion throughout the lanolin. It was found, by Leopold (6), that if crystals are dissolved directly into the lanolin, a solution is obtained which is 10 times less effective than if the crystals are first dissolved in a small amount of alcohol or ether. This was also borne out in preliminary experiments conducted by this investigator, whose results showed little activity of some growth regulators that were placed directly into lanolin, as compared to fairly active reactions to the growth substances which had first been dissolved in an appropriate solvent.

There were many factors influencing the reaction of the growth substances and the hypocotyl hooks. One of these was light. It was found that though light did cause the hook to straighten out, it took a considerable amount of it to do so, and the seedlings could be treated in subdued light for up to 5 minutes, without causing them to open the hook angle naturally. The seedlings were kept in a light-proof incubator the remainder of the time, and light was therefore eliminated as a variable. Temperature was also a factor to consider. The incubator was maintained at a temperature of 24° C, which showed satisfactory results. This is also in the range of optimum sensitivity for the oat coleoptile test (6). Age of the plant was taken into consideration as a factor, and a range of optimum sensitivity was used for

the treatments. These various factors are also important in other bio-assay tests, and in the case of the oat coleoptile test, have been dealt with very well in attempting to control growing conditions (6).

The waxy surface, or cuticle of the hypocotyl posed a problem at first, in that the application of the growth substances did not have a chance to penetrate to the interior before the hook grew past the point of application. By adding a wetting agent, TWEEN 20, the growth substances were able to reach the interior of the hook and cause an effect of opening or closing of the hypocotyl angle. Mitchell (14) feels the effectiveness of the wetting agent, or surface-active substance may be due, in part, to their ability to spread out the chemical and make it adhere closely to the plane surface. Staniforth and Loomis (14) doubt that the surface tension is actually reduced appreciably by these agents.

Variations of results from day to day are probably due to the variation of sensitivity of the different plants, and also variation of sensitivity of each plant from day to day. This has been mentioned by Went (19) as a point of variability of the Avena coleoptile test, too.

Application to the hook of the cucumber seedling was decided on because a reduction or increase of growth at the point of application would cause the hook to "spin" in some characteristic manner. In the case of IAA, the permeability of plant cells is changed, and the cells tend to take up more water, and grow longer (4), thereby indicating a growth stimulating effect. Those substances that are most active in this sense are the substituted phenoxyacetic acids. They also must have

the proper structure (2).

Variations with growth substances also occur when different parts of the same plant are treated (3, 12), roots usually needing lower concentrations to show responses, and stems needing quite high concentrations. Most growth substances will have both promoting, or stimulating, and inhibiting effects (8), depending on place of application, concentrations used, and other conditions. Coumarin, usually thought to be an inhibitor of growth, shows some auxin activity with Helianthus at 250 ppm (10).

Comparing the curvature obtained with eosin alone, placed on the top of the hook, with the standard scale set up by Went for the oat coleoptile test (15), eosin produced a -1.167 AE, where 1 AE = the amount of material needed to affect a 10° curvature in 2 hours. The negative sign is indicative of the fact that eosin is a growth inhibitor, and Went's scale is set up for growth stimulators.

Some results in this research were rather unexpected. NPA, IAA, and IBA, when applied to the top of the hooks, caused an opening of the hook angle. As these substances are usually considered growth stimulators, the results proved to be opposite to what might be expected. On the other hand, NAA also considered a growth stimulator, yielded a strong negative curvature, or closing of the hook angle when applied to the top of the hook.

Treatment was then applied to the underside of the hook, in an attempt to correlate any results obtained this way with the results

previously obtained. Some definite relationships were established when the two sets of data were compared. It was seen that IAA, IBA, NPA, 2,4,5-T, 2,4,6-T, all gave comparable results when used on the underside, as compared to results when they were used on the topside. An explanation for this is when the substances were applied to the top of the hook, some of them were able to be translocated rather easily, and their effect on growth actually took place at a site on the underside of the hook.

The topic of translocation of growth substances is a complex one (1). Rate of penetration (which in this case, was dealt with with a wetting agent, to some degree) rate of transport or translocation, and rate of inactivation or stimulation of tissues all greatly affect the ultimate response (1). Discrepancy between results obtained from treatment on top and treatment on the bottom of the hooks, can in part be a reflection of slow penetration or translocation (1). The substances yielding opposite results when treatment is at the two different sites, indicate a nearly complete lack of mobility. NAA, eosin, erythrosin, and TIBA showed strong tendencies to remain at the point of application. Eosin produced the greatest angle opening when applied to the top, and the greatest angle closing when applied to the bottom. Erythrosin was next in this respect, TIBA next, NAA showed strong stimulatory effects, while not being translocated. The fact that NAA is not easily translocated is substantiated by Audus (1). Comparison of IAA, IBA and NAA showed them to be approximately the same in activity,

which is suggested by Went and Thimann (19).

Competition takes place at particular sites in some instances when two substances are applied simultaneously. The substances having the greater affinity for the site, will occupy it and greatly reduce the effect of the competitor (17). Form, physical nature, electronic configuration, size, and space filling capacity are the important properties to be considered here. When applications of mixtures of substances were placed on the tops of the hooks, results showed differential translocation taking place. Sometimes competition would take place, as with NPA and 2,4,6-T, where both would be translocated to the bottom of the hook, one being a stimulator, and the other an inhibitor, with a resultant 0° curvature. Most of the time, the inhibitors remained on top, and the stimulators were translocated to the bottom and the action of the two reinforced each other. This was true with the inhibitors, eosin, erythrosin, and TIBA, and the stimulators IAA, IBA, and NPA. The reactions show that some of the substances will enhance each other's activity (13), while others will tend to inhibit each other's action (5, 7).

Due to the complexity of the reactions taking place in treating the cucumber hypocotyl hooks, especially that of the translocation process, it is felt that at this time the cucumber hypocotyl has limited use as a bio-assay test for growth substances. The main point brought out by this investigation was that of the ability of these treatments to distinguish between stimulators, and inhibitors, and substances capable of being translocated, and not translocated. In this sense,

then, the procedures described here are valuable for these distinctions. When more is learned of the process of translocation, and the rates at which it takes place, the cucumber hypocotyl could then be used as a bio-assay test, due to its relative sensitivity to growth substances.

SUMMARY

Three approaches were tried in an attempt to establish a simplified growth regulator test using cucumber seedlings. The first two proved to be unsuitable.

A third method, that of using lanolin, with the growth regulators dissolved in it, proved to be very effective. This paste could be applied to the hypocotyl hook, while the seedling was still growing, and measurements taken on the effect the substance had on the degree of straightening of the hook. Various substances could be compared then to determine their relative activity as growth regulators. It was found that eosin and erythrosin were both highly active inhibitors, in that they tended to straighten out the hook to a great degree. Other substances were also used, and some combinations of substances were applied. It was found that some substances usually thought of as stimulators, acted like inhibitors when applied to the top of the hook. When application was then made to the bottom of the hook, comparable results were obtained. This indicated a translocation process taking place. Those substances, such as IAA, IBA, and NPA which are thought of as stimulators were found to be translocated to the bottom of the hook, where they stimulated growth, resulting in an opening of the hypocotyl hook. Other substances, such as eosin, and erythrosin remained on the top of the hook, causing inhibition, and again opened the hypocotyl hook. NAA, a stimulator, was not translocated, and closed the hook, when applied to the top, while opening it when applied to the bottom.

The fact that some substances are translocated and others are not, complicates the picture of using the hypocotyl hook as a bio-assay for growth substances. It is felt, therefore that the use of the hypocotyl in this sense is limited, even though the hypocotyl is definitely sensitive to these substances. Though it was not evident for quite a while, it became apparent after repeated experimentation that through the use of applications on top, and then on the bottom of the hook, growth substances could be categorized as to stimulators, or inhibitors, and whether or not they were capable of being translocated. When the process of translocation is fully understood, the cucumber hypocotyl hook may well prove to be a valuable tool as a bio-assay test for growth regulators, due to its relative sensitivity to the growth regulators.

LITERATURE CITED

1. Audus, L. J. 1953. Plant growth substances. Interscience Publishers, Inc., New York.

2. Crosby, Donald G., and A. J. Vlitos. Aug. 29, 1958. n-butyl-5-chloro-2-pyrimidoxyacetate - a plant growth analog. Science 128:480.

3. Eyster, H. C. Sept. 19, 1947. Promotion and inhibition of growth. Science 106:265.

4. _____. Nov. 30, 1957. Find hormones related. Science Newsletter 72:340.

5. Laloraya, M. M., and S. A. Naqvi. April 28, 1961. Auxin gibberellic acid interaction in controlling the hypocotyl growth in seedlings. Science 133:1357-1359.

6. Leopold, A. Carl. 1955. Auxins and plant growth. University of California Press, Berkeley.

7. Leopold, A. C., and W. H. Klein. July 6, 1951. Maleic hydrazide as an antiauxin in plants. Science 114:9.

8. Linser, H., and K. Kaindl. July 20, 1951. The mode of action of growth substances and growth inhibitors. Science 114: 69-70.

9. Machlis, Leonard, and John G. Torrey. 1959. Plants in action. W. H. Freeman and Co., San Francisco.

10. Neumann, J. June 19, 1959. An auxin-like action of coumarin. Science 129:1676.

11. _____. June, 1955. Plant regulators, CBCC positive data series. United States Department of Agriculture.

12. Schocken, Victor. May, 1949. Plant hormones. Scientific American 180:40-43.

13. Schroeder, C. A., and C. Spector. Oct. 11, 1957. Effect of gibberellic acid and indole-acetic acid on growth of excised fruit tissue. Science 126:701.

14. Skoog, Folke (editor). 1951. Plant growth substances. University of Wisconsin Press; William Byrd Press, Inc., Richmond, Virginia.

15. Tukey, H. B. (editor). 1954. Plant regulators in agriculture. John Wiley and Sons, Inc., New York.

16. Verdoorn, Frans (editor). 1948-1949. Chronica Botanica Vol. 12. Chronica Botanica Co., Waltham, Mass.

17. Wain, R. L., and Wightman (editors). 1956. The chemistry and mode of action of plant growth substances. Academic Press, Inc., New York.

18. Went, Frits W. 1957. The experimental control of plant growth. Chronica Botanica Co., Waltham, Mass.

19. Went, F. W., and Kenneth V. Thimann. 1937. Phytohormones. The MacMillan Co., New York.