THE EFFECT OF GROWTH REGULATING COMPOUNDS ON ISOLATED STEM SECTIONS

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INTRODUCTION

Recently, plant physiologists have witnessed what in all probability will be a milestone in understanding plant growth. This is due to observations that growth is regulated by hormone-like chemical compounds, referred to as plant growth substances. It is with several of these growth regulating substances that the present investigation was concerned.

The first growth substance discovered was "auxin". Fritz Went (1926), using the coleoptile of an oat seedling, discovered the presence of a certain growth promoting factor . After the removal of the tip of the coleoptile, Went placed the excised tip on a block of agar. He then discarded the tip, removed the tip of a second coleoptile, and placed the block of agar to one side of the stump of this plant. The fact that the seedling bent away from the agar block side indicated growth had occured more rapidly under the block. This led Went to conclude that the coleoptile tip produced a hormone that influenced plant growth.

The discovery of Went was a turning point in the entire outlook on plant growth. Before the discovery, many botanists believed that hormones occured only in animals and did not exist in plants. Since the 1920's, plant physiologists have devoted much time to the study of plant

growth substances and have published numerous papers.

The exact cellular mechanism of action of these substances is under intense investigation, and, in all probability, varies with the different kinds of growth chemicals. Although relatively few compounds have been isolated in pure form, there may be hundreds of different chemicals in this group. There is accumulating evidence that different substances may act by affecting respiration, enzyme activation, plasticity of the cell wall, cell division, cell enlargement, and other physiological activities in the plant. (Hill, et al, 1962, p. 189)

Botanists are finding these substances invaluable in studying plant growth, as well as valuable clues into the processes that cause seeds to germinate and plants to flower and bear fruit.

MATERIALS AND METHODS

A bio-assay was performed testing the effects of six chemical compounds on isolated stem sections from the uppermost modes of three plants: (1) Nicotiana tabacum; (2) Helianthus annuus; and (3) Zinnia elegans. The six chemical compounds tested were the following: (1) biotin; (2) colchicine; (3) gibberellic acid; (4) indole-3-acetic acid; (5) kinetin; and (6) 2,4-dichlorophenoxyacetic acid.

In preparation of stem material, sections of the stem were treated in the following manner:

(1) cross-sections of uniform size (5 millimeters) and weight (70 milligrams) were obtained from the upper nodes of the plant stems;

(2) the stem sections were surface-sterilized with
   (a) a 50% ethyl alcohol solution for one minute,
   (b) a 1:5 aqueous solution of sodium hypochlorite for two to three minutes,
   (c) sterile distilled water for two to three washings;

(3) the epidermis of Nicotiana was removed from all sections to eliminate buds; the epidermis was intact on Helianthus and Zinnia;

(4) transfer was made to various concentrations of media.

In preparation of the media containing the six chemical compounds, the following procedure was followed: (Morrison, Personal Communication)

(1) a stock solution for each compound was made up to 10^{-3} Molar;

(2) the stock was diluted to the concentrations 10^{-5}M, 10^{-7}M, 10^{-9}M, 10^{-11}M, and 10^{-13}M;

(3) a control was prepared for each series;

(4) after reaching the desired dilution, specific amounts of chemical compounds (White's basal mineral solution) were added to complete the medium; the chemicals included in the medium were

Mineral Salts	Grams/Liter
$Ca(NO_3)_2.4H_2O$	28
KNO_3	16
$MgSO_4$	14
KCl	13
KH_2PO_4	5
NH_4NO_3	5
$FeSO_4.7H_2O$	5
	Milligrams/Liter
KI	150
$ZnSO_4.7H_2O$	534
H_3BO_3	320
$MnSO_4.H_2O$	985

Amino Acid	Milligrams/Liter
Glycine.	400
Vitamins	
Thiamine HCl	20
Nicotinic Acid	100
Pyridoxine HCl	20
Carbon Source	
Sucrose	20 Grams/Liter

(5) PH was adjusted to 6.5 for each solution;

(6) 1% agar was used to solidify the medium;

(7) the medium was dispensed in 25 milliliter aliquots into test tubes and sterilized.

Four sections were used in each test, at each concentration, and for each chemical tested. This was a minimum number necessary to arrive at a statistically significant result.

The cultures were maintained at 21 C under a light intensity of 80 F.C. for a period of twenty-eight days.

At the end of twenty-eight days, each section was removed and weighed. The criteria for determining the effects of the chemical compounds were the following: (1) the reaction range of the compounds at the various concentrations; (2) an overall increase in size and weight; (3) formation of adventitious organs; and, (4) cellular induction.

RESULTS AND DISCUSSION

THE EFFECT OF BIOTIN ON PLANT STEM SECTIONS (TABLE I)

The effect of biotin on the three sections in this experiment was varied. The control represented the normal growth of plant sections growing on a nutrient solution. The deviations from this normal growth indicated the effects of biotin after standard deviation was determined.

Nicotiana gained most weight in the lower concentrations of biotin. In the concentration 10^{-11}M, the plant sections increased in weight 101% above the control; and, in the lowest concentration, 10^{-13}M, there was a 98% increase in weight above the control.

Helianthus responded with the greatest increase in weight to the lower concentrations of biotin. The greatest increase was found in the lowest concentration, 10^{-13}M.

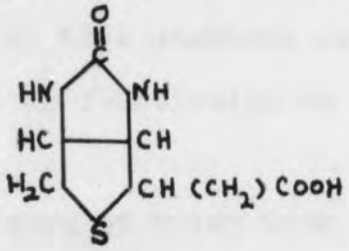
Zinnia gained most weight in the highest concentration of biotin, 10^{-3}M. In this concentration, there was a 106% increase in weight above the control.

Microscopic examination of all the stem sections revealed extreme enlargement of parenchyma cells. The parenchyma cells of the in vitro stem were three times the size of the in vivo stem parenchyma cells. The cells of the parenchyma tissue were the only portion of the stems affected by biotin.

Adventitious roots were formed on Helianthus sections only. These developed in the lower concentrations of biotin, 10^{-7}M, 10^{-9}M, 10^{-11}M, and 10^{-13}M.

These results indicated that biotin was an effective growth regulator depending on the plant section tested and the concentration used.

Biotin (Merck Index, 1960, p.150) is a member of the family of B vitamins, having the structure



Biotin is present in minute amounts in all living cells. It participates in many different biochemical reactions and transformations: it helps convert carbon dioxide into carbohydrates; it acts in removing amino groups from certain amino acids and the carboxyl group from certain other organic acids that are key intermediates in the breakdown of carbohydrates; it plays an essential role in the synthesis of aspartic acid and of fatty acids; and there is evidence that it is involved in glucose oxidation and metabolism of pyruvic acid. This list indicates that biotin participates in the metabolism of the three principle constituents of living organisms: carbohydrates, fats, and proteins. (Woodward, 1961, p.146)

The experimental work of Lones, Rainbow, and Woodward (Woodward, 1961, p.146) demonstrates that perhaps this vitamin acts to synthesize specific enzymes rather than to assist in chemical function. Other recent work indicates that biotin can also function as a coenzyme. Wakil (Woodward, 1961,p. 146) has obtained an enzyme involved in the synthesis of fatty acids that requires the vitamin.

From the literature on biotin, it is probable that growth is regulated by this chemical compound by either synthesizing enzymes or functioning as a coenzyme.

THE EFFECT OF COLCHICINE ON PLANT STEM SECTIONS (TABLE II)

The control represented the normal growth of plant sections growing on a nutrient solution. The deviations from this normal growth indicated the effects of colchicine after standard deviation was determined.

In all concentrations of colchicine, the growth of Nicotiana was inhibited by colchicine. This was shown by the negative percentages, indicating no growth in the experimentals.

In the higher concentrations of colchicine, Helianthus was inhibited , as was shown by the negative percentages. In the lower concentrations, there was an increase of 13% in 10^{-7}M and 14% in 10^{-9}M above the weight of the control.

The response of Helianthus and Zinnia to colchicine was similar. Zinnia was inhibited in the higher concentrations, but increased in growth in the lower concentrations. Zinnia responded slightly more to colchicine than did Helianthus. Helianthus and Zinnia are both members of the Compositae family and this similar reaction to colchicine suggests some metabolic similarity.

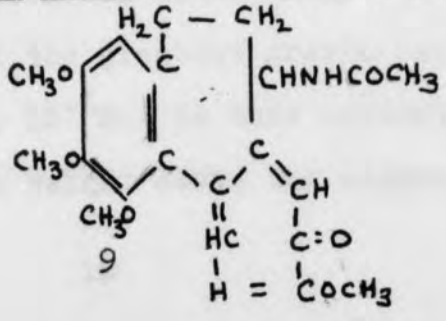
Microscopic examination revealed greatly enlarged parenchyma cells in all three plant sections. The in vitro stem parenchyma cells were from two to three times the size of the in vivo stem parenchyma cells.

Adventitious roots were formed only on Helianthus sections. These organs developed in the lower concentrations of colchicine, 10^{-7}M and 10^{-9}M.

These results indicated that colchicine was an effective growth regulator depending on the plant section tested and the concentration used.

Colchicine is a vegetable alkaloid obtained from the corms or seeds of the autumn crocus, Colchicum autumnale, a perennial plant of the family Liliaceae. (Avery and Johnson, 1947, p. 283)

Colchicine (Merck Index, 1960, p.238) has the structure



Colchicine is very effective in doubling the chromosome number of cells exposed to it. It is known to act by preventing cell division by interfering with spindle formation during mitosis. (Swanson, 1957, p.382) The result of this inhibition of normal mitosis is the polyploid cell. This increase in the number of chromosomes in a cell results in an enlarged cell. (Avery and Johnson, 1947, p.283)

Loo and Tang (Newcomer, 1945, p.677) reported that colchicine in low concentrations accelerated germination of seeds and growth of the mungo bean, cabbage, rice and wheat.

Because of its relative specificity of cytological effect on mitosis, colchicine could be used to provide valuable information regarding the mechanism of mitosis.

THE EFFECT OF GIBBERELLIC ACID ON PLANT STEM SECTIONS (TABLE III)

The control represented the normal growth of plant section on a nutrient solution. The deviations from this normal growth indicated the effects of gibberellic acid after standard deviation was determined.

Nicotiana showed the greatest growth response in the higher concentration, 10^{-5}M. In this concentration, there was a 42% increase in weight above the control. In the

lower concentrations, 10^{-7}M and 10^{-9}M, there was a decrease in weight below the control, indicating an inhibitory effect.

Helianthus showed the greatest growth response in the concentration 10^{-7}M. In this concentration, there was a 63% increase in weight above the control. There was no inhibition here.

The response of Zinnia to gibberellic acid was similar to the response of Nicotiana. At 10^{-5}M, there was a 10% increase above the control. This was the greatest growth response of Zinnia. In the lower concentrations, 10^{-7}M and 10^{-9}M, there was a decrease in weight below the control indicating an inhibitory effect.

Microscopic examination of the sections revealed an increase in both cell division and cell enlargement of the parenchyma cells. The enlarged in vitro parenchyma cells were three times the size of the in vivo parenchyma cells.

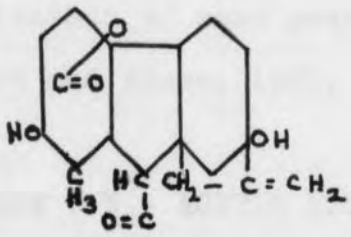
There was no formation of any adventitious organs on any section in gibberellic acid.

These results indicated that colchicine was an effective growth regulator depending on the plant section tested and the concentration used.

Gibberellic acid is a plant growth metabolite isolated first from the ascomycetous fungus, Gibberella fujikuroi.

This fungus caused the rice disease, bakana byu or "foolish seedling". Plants having this fungus grew markedly taller than the healthy ones. Interest in this disease led to the discovery of the gibberellins by the Japanese. Although several kinds of gibberellins have been found, gibberellic acid is perhaps the most important. (Lang, 1959, p.148)

Gibberellic acid (Merck Index, 1960, p.269) has the structure



The effect of gibberellic acid is attributed basically to cell elongation, but recent experiments on the effect of gibberellic acid on the growth of excised fruit tissue have shown that gibberellic acid also produces cell division. (Lang, 1959, p.148)

The metabolic effects have been sought for a long time. No clear-cut linkage with any metabolic pathway has yet been established, but analyses so far have shown the greatest changes among carbohydrate constituents. Besides this effect, gibberellic acid is known to promote respiration of growing parts of treated plants and seeds, to cause variations in the level of certain enzyme activities,

and to reduce the effects of certain growth inhibitors such as maleic hydrazide and coumarin. (Stowe and Yamaki, 1959, p.812)

Plants treated with gibberellic acid generally grow at least two to three times as tall as untreated ones. Gibberellic acid causes certain dwarf varieties of plants to grow as high as tall varieties, allows certain species to grow over a broader temperature range, promotes flowering before the proper time, and eliminates the light requirement for the germination of some seeds, such as those of lettuce. (Greulach and Adams, 1962, p.329)

THE EFFECT OF INDOLE - 3 - ACETIC ACID ON PLANT STEM SECTIONS (TABLE IV)

The control represented the normal growth of plant sections growing in a nutrient solution. The deviations from this normal growth pattern indicated the effects of indole-3-acetic acid (I.A.A.) after standard deviation was determined.

Indole-3-acetic acid promoted growth of Nicotiana at only one concentration, 10^{-5}M. At this concentration, there was an 18% increase in weight above the control. At all other concentrations, there was a decrease in weight below the control. The explanation for this decrease may be either that I.A.A. is inhibitory to Nicotiana at these

concentrations, or that Nicotiana in light is insensitive to this compound. Since light is known to affect auxin distribution in the plant, the second explanation is probably correct. Because of lack of space and facilities, this experiment with I.A.A. was conducted only in light. The effect of light may be a factor in the variation of the values obtained in the experiment.

Helianthus showed the greatest growth response in the higher concentration, 10^{-5}M. At this concentration, there was a 35% increase in weight above the control.

The response of Zinnia was somewhat similar to that of Helianthus. The greatest increase in weight of Zinnia was in response to the higher concentration, 10^{-5}M. At this concentration, there was a 59% increase in weight above the control. Zinnia showed the greatest response of all three sections to I.A.A.

Microscopic examination of all three sections revealed an increase in cell enlargement of parenchyma cells. The in vitro stem parenchyma cells were three to four times the size of the in vivo stem parenchyma cells. In addition, there was an increase in cell division of parenchyma cells of Nicotiana.

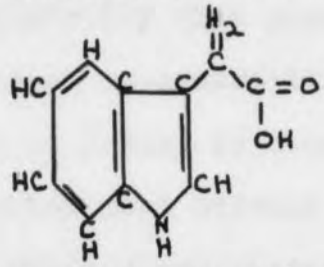
There was no formation of any adventitious organs on any sections in this compound.

These results indicated that I.A.A. was an effective

growth regulator depending on the plant section tested and the concentration used.

It has been conclusively demonstrated that "auxin" is indole-3-acetic acid. (Bonner and Galston, 1952, p.356) Certain plant tissues possess an enzyme system which catalyzes the transformation of the amino acid, tryptophane, to I.A.A. (Bonner and Galston, 1952, p.358)

I.A.A. (Merck Index, 1960, p.554) has the structure



A basic question concerning the auxin is that of the mechanism by which it exerts its manifold effects on plant growth. It is known that through the influence of auxin on the plasticity of cell walls, cell elongation occurs. Cell elongation is, however, only one of the responses elicited by auxin. Most investigators now hold that this effect on cell wall plasticity is one of the possible results of a more general and basic effect of auxin on metabolism. It seems reasonable that I.A.A. is involved in a key reaction, the end result of which may depend on the nature of the tissue employed. That the key reaction controlled by I.A.A. is somehow related to respiration is indicated by the fact that in many different

tissues, the rate of respiration is immediately increased by the application of auxin in concentrations suitable to stimulate growth. However, detailed knowledge of the way in which respiration and I.A.A. induced respiration are linked to varied growth response is not known. It is probable that I.A.A. may function as a prosthetic group of an enzyme. (Bonner and Galston, 1952, p.384)

From various experiments by Jablonski and Skoog (1954), it was shown generally that plant tissues respond more favorably to lower concentrations of I.A.A. Although the primary action of I.A.A. is elongation of the cell, recent evidence indicates that certain concentrations of this compound on certain plant tissue, especially tobacco tissue, induces and increases cell division.

Indole-3-acetic acid is involved in many activitie s that include inhibition of the lateral bud growth in the presence of a terminal bud, inhibition of abscission, stimulation of growth of cambium and other meristematic activity, and promotion of water uptake. (Bonner and Galston, 1952, p.370)

THE EFFECT OF KINETIN ON PLANT STEM SECTIONS (TABLE V)

The control represented normal plant growth ina a nutrient solution. The deviations from the control indicated the effect of kinetin on the plant sections after

standard deviation was determined.

Nicotiana showed the greatest growth response in the concentration, 10^{-7}M. At this concentration, there was a 104% increase in weight above the control. There was a decrease in weight below the control at the highest concentration, 10^{-3}M. Nicotiana showed the greatest response to kinetin.

Helianthus showed the greatest growth response in the concentration, 10^{-7} M. At this concentration, there was a 22% increase in weight above the control. The concentrations of 10^{-3}M and 10^{-9}M brought a decrease in weight below the control.

The results of the effect of kinetin on Zinnia indicated that there was an increase in weight above the control at only one concentration, 10^{-5}M, and that at all other concenterations, there was a decrease. This 4% increase may not be a significant figure, but may be the result of an error in technique or of tissue variation.

Microscopic examination of the three sections revealed an increase in cell division of the parenchyma cells.

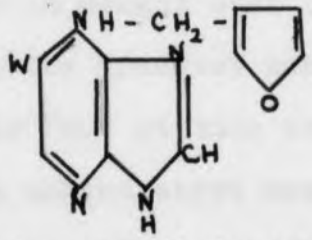
Adventitious roots were formed on Helianthus sections. These organs developed at the lower concentration of 10^{-11}M and 10^{-13}M.

Indole-3-acetic acid was added to all the kinetin media as kinetin must have a small amount of this compound present if it is to act.

These results indicated that kinetin in the presence of indole-3-acetic acid is an effective growth regulator depending on the plant section tested and the concentration used.

Kinetin is derived from adenine, a component of the nucleic acid, desoxyribonucleic acid (DNA). It was discovered by Miller and Skoog (1955), when they used a four year old bottle of DNA on some work with yeast extract. This chemical can be extracted from the sperm of herring and other organisms, and is found in some plant organs and micro-organisms. (Miller and Skoog, 1955, p.1392)

Kinetin (Merck Index, 1960, p.590) has the structure



Kinetin promotes growth by promoting cell division in plant tissue. However, it must have a small amount of indole-3-acetic acid present in order to do so. (Miller and Skoog, 1955, p.1392) A Wisconsin group of plant physiologists have grown an entirely new plant from pieces of tissue taken from the pith of the stem of tobacco plant and placed in a kinetin medium containing indole-3-acetic acid. (Lang, 1959, p.144) It has been found that for most plant tissues, kinetin is most effective in promoting

growth at lower concentrations. (Kefford, 1963, p.1497)

THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON PLANT STEM SECTIONS (TABLE VI)

The control represented the normal growth of plant sections on a nutrient solution. The deviations from this control represented the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) after standard deviation was determined. Nicotiana stem sections were not available for this experiment.

Helianthus showed the greatest response to 2,4-D at the concentration of 10^{-7}M. At this concentration, there was a 63% increase in weight over the control.

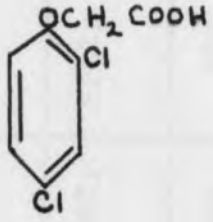
Zinnia showed the greatest response to 2,4-D at the concentration of 10^{-9}M. At this concentration, there was a 104% increase in weight above the control.

Microscopic examination revealed an increase in both cell division and cell enlargement of parenchyma cells. The in vitro stem parenchyma cells were three times the size of the in vivo stem parenchyma cell.

Adventitious roots were formed on Helianthus. These organs developed at the low concentration of 10^{-11}M.

These results indicated that 2,4-D was an effective growth regulator depending on plant section tested and concentration used.

2,4-D is a synthetic compound used widely as a selective herbicide. It has the structure (Merck Index, 1960, p.346)



2,4-D has many auxin-like effects when used in extremely low concentrations. According to Greulach and Adams (1962), 2,4-D in higher concentrations will inhibit growth. The results obtained from this experiment did not support this in regard to Helianthus and Zinnia. However, the use of in vitro stem sections on a medium with a carbon source may cancel out the herbicidal characteristics of 2,4-D. Whereas, in vivo plants supply their own carbohydrate through the mechanism of photosynthesis. Addition of 2,4-D to the latter disrupts the photosynthetic-respiratory balance by speeding up respiration. This in turn utilizes the cellular carbohydrate reserve, and finally results in the death of the cell and the organism.

2,4-D is effective in the regulation of cell elongation and other auxin-like activities. In an experiment with the hypocotyl of the soybean, after the application of concentrations of 2,4-D, the pith cells started to actively divide. (Sun, 1955, p.641) Thus, 2,4-D may regulate growth by stimulating both cell elongation and cell division.

TABLE I. THE EFFECT OF BIOTIN ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS						
		CONTROL	10^{-3} M	10^{-5} M	10^{-7} M	10^{-9} M	10^{-11} M	10^{-13} M
Nicotiana	Weight in Milligrams Standard Deviation	139	250±1.7	230±1	250±1.58	240±1.4	280±1	276±0
	% Deviation		79%	65%	79%	73%	101%	98%
Helianthus	Weight in Milligrams Standard Deviation	380	460±2	505±1.4	487±1	487±1	522±2	575±1.7
	% Deviation		21%	33%	28%	28%	37%	51%
Zinnia	Weight in Milligrams Standard Deviation	124	256±2	223±1.8	200±0	200±0	223±1.58	200±1
	% Deviation		106%	80%	61%	61%	80%	61%

CORRECTION

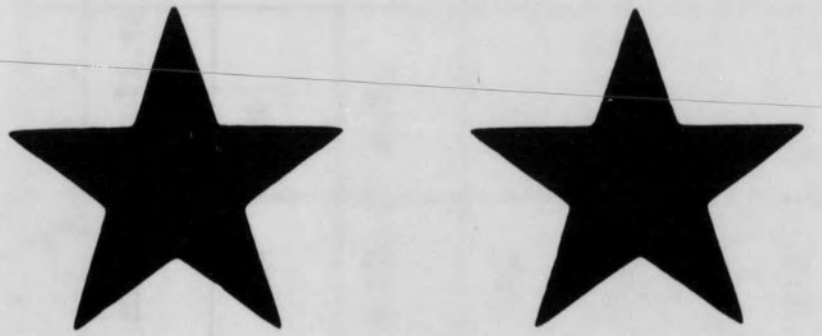


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TABLE I. THE EFFECT OF BIOTIN ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS						
		CONTROL	10^{-3} M	10^{-5} M	10^{-7} M	10^{-9} M	10^{-11} M	10^{-13} M
Nicotiana	Weight in Milligrams Standard Deviation	139	250±1.7	230±1	250±1.58	240±1.4	280±1	276±0
	% Deviation		79%	65%	79%	73%	101%	98%
Helianthus	Weight in Milligrams Standard Deviation	380	460±2	505±1.4	487±1	487±1	522±2	575±1.7
	% Deviation		21%	33%	28%	28%	37%	51%
Zinnia	Weight in Milligrams Standard Deviation	124	256±2	223±1.8	200±0	200±0	223±1.58	200±1
	% Deviation		106%	80%	61%	61%	80%	61%

TABLE II. THE EFFECT OF COLCHICINE ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS				
		CONTROL	10^{-3}M	10^{-5} M	10^{-7}M	10^{-9}M
Nicotiana	Weight in Milligrams Standard Deviation	140	70±0	70±1.7	80±2	87±1.4
	% Deviation		-50%	-49%	-41%	-38%
Helianthus	Weight in Milligrams Standard Deviation	280	90±2	176±1.7	317±1.58	320±1
	% Deviation		-63%	-37%	13%	14%
Zinnia	Weight in Milligrams Standard Deviation	132	70±1	130±1	160±1.4	150±1.7
	% Deviation		-47%	-1.5%	21%	14%

TABLE III. THE EFFECT OF GIBBERELLIC ACID ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS				
		CONTROL	10^{-3}M	10^{-5}M	10^{-7}M	10^{-9}M
Nicotiana	Weight in Milligrams Standard Deviation	127	140±0	180±1	120±1.7	90±0
	% Deviation		10%	42%	-5.5%	-29%
Helianthus	Weight in Milligrams Standard Deviation	212	242±1	312±2.2	345±2	297±1.4
	% Deviation		14%	47%	63%	40%
Zinnia	Weight in Milligrams Standard Deviation	109	115±1.4	120±0	90±2	85±2
	% Deviation		5.5%	10%	-17%	-22%

TABLE IV. THE EFFECT OF INDOLE-3-ACETIC ACID ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS						
		CONTROL	10^{-3}M	10^{-5}M	10^{-7}M	10^{-9}M	10^{-11}M	10^{-13}M
Nicotiana	Weight in Milligrams Standard Deviation	103	80±1.58	122±1.6	100±1.8	75±2	77±1.7	87±1
	% Deviation		-22%	18%	-3%	-27%	-25%	-16%
Helianthus	Weight in Milligrams Standard Deviation	410	455±1.7	555±1	462±1.4	425±2	487±0	442±1
	% Deviation		11%	35%	13%	4%	19%	8%
Zinnia	Weight in Milligrams Standard Deviation	123	166±1.4	196±1.5	155±1.7	126±1	133±1.8	126±0
	% Deviation		35%	59%	26%	2%	8.1%	2%

TABLE V. THE EFFECT OF KINETIN ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS						
		CONTROL	10^{-3}M	10^{-5}M	10^{-7}M	10^{-9}M	10^{-11}M	10^{-13}M
Nicotiana	Weight in Milligrams Standard Deviation	139	95±1	260±1.4	283±2	270±0	256±1	196±1
	% Deviation		-32%	87%	104%	87%	84%	41%
Helianthus	Weight in Milligrams Standard Deviation	470	467±1	535±1.8	572±0	440±2	527±1.4	570±1
	% Deviation		-3%	14%	22%	-6.9%	12%	21%
Zinnia	Weight in Milligrams Standard Deviation	125	85±0	130±1	113±2	123±1.5	96±1.7	90±1
	% Deviation		-33%	4%	-10%	-2%	-23%	-28%

TABLE VI. THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON PLANT STEM SECTIONS

PLANT SECTIONS		DILUTIONS						
		CONTROL	10^{-3}M	10^{-5}M	10^{-7}M	10^{-9}M	10^{-11}M	10^{-13}M
Helianthus	Weight in Milligrams Standard Deviation	460	630±1	690±2.2	750±1.7	720±2	710±1.5	700±0
	% Deviation		37%	50%	63%	57%	54%	52%
Zinnia	Weight in Milligrams Standard Deviation	137	235±1.7	247±1.5	275±1	280±0	258±2	260±2
	% Deviation		72%	80%	100%	104%	88%	90%

Conclusions

The results of this experiment indicate that plant stem sections differ in their response to growth substances in various concentrations. The following conclusions may be made from this data on the basis of (1) range of reaction of the six compounds, (2) mechanisms of growth, (3) cellular induction, and (4) formation of adventitious organs.

(1) Range of reaction of the six compounds:

a) Nicotiana: Increase in growth in lower concentrations of biotin, kinetin; Increase in growth at higher concentrations of gibberellic acid, I.A.A.; Decrease in growth at all concentrations of colchicine, lower concentrations of gibberellic acid, all concentrations of I.A.A., except 10^{-5}M, the highest concentration of kinetin.

b) Helianthus: Increase of growth in lower concentrations of biotin, colchicine, gibberellic acid, kinetin, and 2,4-D; Increase of growth in higher concentrations of I.A.A.; Decrease in growth in higher concentrations of

colchicine , and at the concentrations 10^{-3}M and 10^{-9}M of kinetin.

c) Zinnia: Increase of growth in lower concentrations of colchicine, 2,4-D; Increase of growth at higher concentrations of biotin, gibberellic acid, I.A.A., kinetin; Decrease in growth in higher concentrations of colchicine, lower concentrations of gibberellic acid, all concentrations of kinetin, except 10^{-5}M.

(2) Mechanisms of Growth:

In all three sections growth, or increase in size and weight, resulted from cell division, cell enlargement, or both.

(3) Cellular Induction:

In all three sections, the cells directly affected were the parenchyma cells.

(4) Formation of Adventitious Organs:

Adventitious roots were developed on Helianthus sections in low concentrations of biotin, colchicine, kinetin, and 2,4-D.

From this experiment, it can be concluded that biotin, colchicine, gibberellic acid, indole-3-acetic acid, kinetin, and 2,4-dichlorophenoxyacetic acid are growth regulators

in the plant stem sections of Nicotiana, Helianthus, and Zinnia. The response of these three stem sections differs according to the growth substance used in terms of the four criteria already mentioned.

The results of this experiment may be of significance in both a practical and a theoretical sense. The may perhaps have a bearing on the food industry, as well as shed further light on the mechanism of growth in plants.

BIBLIOGRAPHY

Avery, George S. and Elizateth R. Johnson. 1947. Hormones and Horticulture. McGraw-Hill Book Company, Inc., New York.

Bonner, James and Arthur W. Galston. 1952. Principles of Plant Physiology. W. H. Freeman and Company, San Francisco.

Greulach, Victor A. and J. Edison Adams. 1962. Plants. An Introduction to Modern Botany. John Wiley and Sons, Inc., New York.

Hill, J. Ben, et al. 1960. Botany. McGraw-Hill Book Company, Inc., New York.

Jablonski, John R. and Folke Skoog. 1954. Cell Enlargement and Cell Division in Excised Tobacco Pith. Biological Abstracts 28: 19257.

Kefford, N.P. 1963. Natural Plant Growth Regulators. Science 142: 1495-1505.

Lang, Anton. 1959. Chemistry of growth in Plants. Natural History 68: 142-150.

Miller, C.O. and Folke Skoog. 1955. Kinetin; a Cell Division Factor from DNA. Journal of the American Chemistry Society 77: 149.

Newcomer, Earl H. 1945. Colchicine as a Growth Stimulator. Science 101: 677.

Stecher, Paul G., Editor. 1960. The Merck Index of Chemicals and Drugs. Merck and Company, Inc., Rahway, New Jersey.

Stowe, B.B. and T. Yamaki. 1959. Gibberellins: Stimulators of Plant Growth. Science 129: 807-816.

Sun, C.N. 1955. Anomolous Structure in Hypocotyls of Soybean Following Treatment in 2,4-D. Science 121: 641.

Swanson, Carl P. 1957. Cytology and Cytogenetics. Prentice-Hall, Englewood Cliffs, New Jersey.

Went, F.W. 1926. On Growth Substances in the Coleoptile of Avena Sativa. Great Experiments in Biology. 148-152 Prentice-Hall, Englewood Cliffs, New Jersey, 1955.

Woodward, John D. 1961. Biotin. Scientific American 204: 139-146.