EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID AND OTHER CHEMICALS ON THE PROTEIN, PECTIN AND SULFHYDRYL CONTENT OF EPICOTYL SECTIONS OF PEA, AS RELATED TO GROWTH

By

LELAND L. HARDMAN

Bachelor of Science in Education

Nebraska State Teachers College

Chadron, Nebraska

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Thesis Approved:

Walter W. Hansen

Thesis Adviser

Glenn W. Todd

Eddie Basler

J. J. Boggs

Dean of the Graduate School

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CHAPTER I

INTRODUCTION

The early work of Went in 1925-1937, which verified the presence of the postulated growth "substance", presented a problem to plant physiologists which is still not solved today. The problem was to determine the exact site of auxin action in the plant cell and to explain all the observed effects of auxin application.

The investigations have been directed along several routes as researchers have sought the actual primary site, and several hypotheses have evolved which will be discussed in some detail in the next chapter. Much of the evidence presented in support of these hypotheses has merit, but none has yet been presented which accounts for the many observed effects of auxin application. Since there is considerable evidence that the action may be different in each experimental plant and since there are so many physiological effects of auxin, it is entirely possible that there is no single site of action. Several sites may be stimulated and act together to cause the observed response.

This study was designed to investigate the effects of 2,4-dichlorophenoxyacetic acid and certain other chemicals on the cytoplasmic proteins as well as the pectins and sulfhydryls which are found in the cytoplasm and to determine if these effects are related to growth processes.

It is anticipated that the results of these experiments will supply evidence to support the hypothesis which states that the site of auxin

action is in the cytoplasm or one of its constituents.

CHAPTER II

REVIEW OF THE LITERATURE

Reports concerning auxin and its primary mode of action are numerous and varied. Because the literature contains much data supporting several modes of action, it was decided to present a brief, and by no means complete, discussion of the more important hypotheses. This discussion is limited to those hypotheses which are most widely cited in the literature and to those which have much experimental evidence in support of them. They are presented in this thesis for purposes of interpretation and correlation with experimental evidence to be presented in Chapter IV.

A. Auxin Effects on the Cell Wall

Heyn (1940) presented data showing a direct correlation between the growth rate due to auxin and the plastic (irreversible) extensibility of the walls of decapitated Avena coleoptiles. It was Heyn's conclusion that the extension of the wall, or at least changes in its structure, were necessary before growth could occur. In support of Heyn's conclusions, Cleland (1958) presented data showing the importance of wall extensibility to growth. His data showed the effect of indole-3-acetic acid on the plastic and elastic responses of Avena sections and their relationship to growth. Cleland (1960) reported on later work using Ca^{45} which had been incorporated into walls of

Avena and found that IAA had no effect in releasing this labeled element, which would indicate that wall interaction of growth hormone theory was not true because no calcium was released when bonds were broken.

Several experiments determining auxin effects on the enzymes of the cell wall were reported in the literature. The concentration and activity of pectin methyl esterase was reported by Bryan and Newcomb (1954) to be increased with application of indole-3-acetic acid. These same increases in pectin methyl esterase were presented in the data of Neely, et. al. (1950) using 2,4-dichlorophenoxyacetic acid in growth stimulatory concentrations. Albersheim (1963) reported that IAA inhibited the activity of pectin transeliminase on pectin during in vitro experiments. It was postulated that the auxin formed a complex between the enzyme and the product to cause this inhibition. Pectic substances, in general, increased in potato tubers and tobacco pith callus tissue when treated with auxin as shown by Wilson and Skoog (1954). Galston, et. al. (1962, 1963) reported increases in pectic substances with 2,4-D treatment and indicated a possible interaction of these substances with the proteins of the cytoplasm. The pectin increases reported by Galston have not been definitely proven to consist of true pectin (i.e. polymerized methyl galacturonate) and these increases were not correlated with changes in the wall.

These changes in the extensibility of the wall and subsequent changes in the amount of pectins and enzymes associated with the wall do not give conclusive evidence that this is the exact site of the auxin action, but they do certainly show an effect of auxin on the wall structure not explained otherwise. Galston and Purves (1960) stated that in order to cause the wall changes necessary for growth,

auxin would definitely have to influence more than the associated enzymes and pectins; some effect would have to be exerted on lignin and cellulose before a definite connection between cause and effect would exist.

B. Auxin Effects on Water Uptake

Since it is common knowledge that a cell cannot grow without absorbing water and since it has also been observed that cells exhibit increased water uptake in response to auxin treatment, it may be that the action of auxin in this process is important to growth. Attempts have been made to explain these water uptake responses in terms of changes in the osmotic concentration of the cell contents, or in terms of changes in the "active" water uptake processes of the cell. However, no increases in osmotic concentrations have been demonstrated, and these two explanations have been refuted in numerous articles cited by Galston and Purves (1960) and consequently have little support.

Some conclusions concerning the importance of water uptake to growth were reached in an indirect manner by Galston and Hand (1949) and Purves and Hillman (1958). These workers presented data indicating that indole-3-acetic acid stimulated increases in fresh weight at different concentrations than those which stimulated growth in length of pea sections. The concentrations which stimulated growth in length were 0.1 to 1 micrograms per milliliter, while those which stimulated increases in fresh weight were 1 to 10 micrograms per milliliter. These differences in concentration would seem to indicate that separate processes are being affected. Purves and Hillmann (1958) reported that stem sections exposed to high sugar and auxin levels grew almost exclusively in the lateral direction (fresh weight increases), and they concluded

that the auxin action must be one which causes the lateral wall to be weakened, thereby allowing water uptake and consequent increase in diameter.

Since the evidence seems to indicate that separate concentrations are required for growth in length and for lateral growth and since lateral growth is thought to consist of water uptake and swelling of the tissues, it is possible that water uptake is an important aspect of auxin action. It is probable, though, as Thimann (1954) has stated that changes in water uptake are merely secondary effects of auxin action.

C. Auxin Effects on Protoplasmic Viscosity

Northen (1942) reported marked decreases in the viscosity of the cytoplasm of bean stem cells treated with various auxins. Treatments were made using one gram of lanolin paste containing from 1 to 10 milligrams of auxin. Viscosity reductions were noted after treatment with all concentrations within the range of 1 to 10 milligrams auxin. Indole-3-acetic acid, indole-3-propionic acid and a-napthalene acetic acid were equally effective in this action. Northen stated that these experiments indicated that auxins effectively decrease the cytoplasmic viscosity by causing a dissociation of the cellular proteins. The consequent decrease in molecular weights and increased numbers of free or exposed sulfhydryl groups could then account for some of the observed effects of auxin.

Increased streaming rates in the protoplasm, which could be a consequence of auxin reduction of the cytoplasmic viscosity were presented in the data of Sweeny and Thimann (1938, 1942). Using indole-3-acetic acid at concentrations ranging from 0.002 to 0.5 milligrams

per liter these workers showed increased streaming rates in Avena coleoptile cells. The optimum stimulation appeared with concentrations near 0.1 milligram per liter, the effect being observed immediately and continuing for nearly thirty minutes. Supraoptimum concentrations reduced the streaming rate to a level considerably below the controls. Much lower concentrations of IAA were required to promote streaming in Avena root hair cells according to Sweeny (1944). This work seems to be of more importance when one compares that data with the known concentrations of auxins required to stimulate stem or root growth. There is known to be a difference in effective growth stimulation concentrations as large as three orders of magnitude between root and stem. (i.e. 10^{-5}M for stems and 10^{-8}M for roots). It is also well established that a rather narrow range of effective concentrations is a characteristic of auxin responses. Since the changes in viscosity and streaming rates show the auxin concentration effect, we can assume that these changes are indeed due to the auxin treatment and may be important to the growth aspect in some minor way. The conclusion that it is a minor or secondary effect of auxin action comes from data which show that protoplasmic viscosity and streaming rates can be affected by various chemical and temperature treatments, which are probably not beneficial to growth. (Northen, 1942).

D. Auxin Effects on Nucleic Acids and Proteins

Skoog, et. al. (1953, 1957) have stated that auxin exerts its effects in a metabolic interaction with the nucleic acids and their constituents thereby influencing a large variety of processes.

Current evidence indicates that auxin application affects the nucleic acid metabolism in such a way as to cause increases in the RNA and DNA content of the cells. Patau, Das and Skoog (1957) and Silberger and Skoog (1953) showed that tobacco pith callus tissue treated with auxin was higher in DNA and RNA than the controls, while root tissue, which was inhibited in its growth by high concentrations of IAA (10^{-5}M), showed a reduction in DNA and RNA content. West, Key and Hanson (1960) and Key and Hanson (1961) reported large increases in RNA and protein content of soybean seedlings treated with growth inhibitory concentrations of 2,4-D (400-800 ppm). These increases were shown to occur in mature basal tissues just prior to auxin stimulated cell proliferation, which seems to correlate them with growth and synthetic processes. Similar increases were found in isolated stem sections of corn and cucumber which were treated with the same concentrations of auxin. Experiments using growth stimulatory concentrations of auxin (5 ppm) showed a decline in RNA content during the culture period, which did not significantly differ from the decline in RNA content of the control sections undergoing endogenous growth (no auxin).

Roychoudhury and Sen (1964) reported that the DNA and RNA synthesis in isolated coconut milk nuclei was stimulated by appropriate concentrations of auxin. These workers also stated that a-naphthalene acetic acid (C^{14} labeled) was associated with the nuclear material following the stimulation of nucleic acid synthesis. This attachment has been a relatively new discovery in auxin investigations and may indicate that the nucleus is the actual site of auxin attachment and action in this case.

The work presented in relation to the effect of auxin on the nucleic acids of the cell presents some puzzling data which is difficult to associate with the growth aspects of auxin. The first of these is that Roychoudhury and Sen (1964) reported that growth stimulatory concentrations of auxins stimulated the synthesis of DNA and RNA in the nucleus itself but they also reported that only high concentrations of the auxin stimulated their release from the nucleus. Basler (1964) reported that 2,4-D at appropriate concentrations acted to increase the particulate nucleic acids and also to increase the rate of synthesis in the supernatant RNA and particulate nucleic acids. These data along with that of Key and Hanson (1961) and West, Key and Hanson (1960) show that growth substances indeed do have an effect on the nucleic acid metabolism but it remains to be shown exactly how the changes affect the cell as a whole. Roychoudhury and Sen (1964) stated an hypothesis that the effect of auxins on the DNA of the nucleus causes changes in the RNA (messenger) made from it, this in turn influencing any or all the enzyme proteins made from it. Proof of this hypothesis is yet to come, but it is an attractive proposal and could answer many of the questions asked about auxin action.

Sell, et. al. (1949) and Rebstock, et. al. (1952) presented evidence that auxin affects the protein content of red kidney bean plants. Sell, et. al. (1949) showed that stems treated with 0.05 milliliters of a 1000 ppm solution of 2,4-D doubled their protein content in six days. Further analysis of the amino acid content of these proteins indicated that treated proteins were different than control types. Evidence that synthesis was occurring in the treated stems came from carbohydrate analyses which showed a decline of carbohydrate reserves

in those plants showing increased protein content. 30%-50% increases in proteolytic acitivity accompanied the increases in protein content of kidney bean stems treated with 2,4-D by Rebstock, et. al. (1952). A correlation of these changes in protein content with growth is shown in the work of West, Hanson and Key (1960) who reported that the protein content decreased markedly during endogenous growth of cucumber and corn stem sections, and increased in those sections which received high concentrations (400-800 ppm) of 2,4-D. Key and Hanson (1961) state that these changes may be due to some interaction of the auxin in the degradation processes of cell. There probably is no effect on synthesis, other than inhibition but rather an interference with degradation processes in those experiments where high concentrations appear to stimulate production of protein and nucleic acids. In either case there appears to be a definite effect of auxin on the nucleic acids and proteins of the cells in response to treatment,and this effect appears to be stimulation of synthetic processes at the appropriate auxin concentration.

Galston, et. al. (1959, 1962, 1963) have reported an effect of auxin on the proteins of pea stems. They showed that 2,4-D and IAA acted in some way to decrease the amount of heat coagulable protein present in treated sections, without increasing the amount of total (TCA precipitable) protein. Concentrations ranging from 10^{-7}M to 10^{-4}M were effective in this action and changes in the heat coagulation were obtained even with concentrations supraoptimal for growth. Similar changes were reported for roots, but much lower concentrations of the auxin were necessary to initiate changes in these proteins. Associated with the change in the protein were large increases in the pectin

content of the homogenates, the pectin increases occurring at the same concentrations as those which affected the protein.

Several characteristics of the auxin effect in these experiments indicated a metabolic interaction with cellular components; (1) The response was exhibited only in young, apical tissue and no effect was noted in the older more basal tissues. Such results indicate the necessity of active metabolic processes for auxin action. (2) The changes in pectin content and protein heat coagulation response were observed only in homogenates from sections which were treated with the auxin in vivo. This would indicate that certain cellular components are necessary for the action and that it is not merely a physical interaction which causes these effects. (3) The doubling of the pectin content in the treated sections would seem to indicate a stimulation of certain synthetic processes, which could more fully support a hypothesis of metabolic interaction for auxin.

Galston (1963) interpreted these data as indicating an interaction between the pectin and the protein, thereby causing the altered heat coagulability, but analysis of the coagulum indicated that 84% consisted of protein and only 2% was pectin material. These data would seem to show that there was only a slight interaction, if any, between the proteins and the pectins of the cell contents. However, since the effect on the protein is duplicated by addition of commercial citrus pectin to the homogenates of the 2,4-D treated sections, it appears that pectin does influence the protein in some manner.

E. Auxin Effects on the Oxidation-reduction State

Two of the numerous reports concerning the effect of auxins on the oxidation reduction state of the cell contents have indicated that changes in the oxidation state of the sulfhydryl groups (both free and protein bound) may be important in growth processes. (Key and Wold, 1961: Marre and Arrigoni, 1957).

Marre and Arrigoni (1957) stated that the ratio between reduced and oxidized glutathione is closely controlled by growth regulators and that small changes in this ratio have a determining influence on growth and related processes. Application of IAA or 2,4-D in growth stimulatory concentrations caused increases in the reduced glutathione (GSH) content accompanied by decreases in the oxidized glutathione (GSSG) in both Pisum and Avena sections within twenty minutes. On the other hand, ascorbic acid which inhibits growth of the sections at 10^{-3}M and 3 X 10^{-3}M concentrations, favors an increase in the amount of the oxidized glutathione even when applied with IAA or 2,4-D at effective concentrations. The actual importance of the oxidation state of glutathione to growth was further substantiated when these workers found that reduced glutathione at 3 X 10^{-4}M concentration stimulated growth while the same concentration of oxidized glutathione inhibited it. Apparently the reduced type is taken up by the sections, resulting in an excess of GSH, thereby changing the ratio in favor of the more reduced form and stimulating growth processes.

Key and Wold (1961) reported that 2,4-D at growth inhibitory concentrations of 5 X 10^{-4}M caused increases in the ascorbic acid content of treated tissues after 24 hours, with a decline to the control level

after 96 hours. These workers stated that the ascorbic acid-dehydroascorbic acid ratio might be the controlling mechanism of growth processes as well. Indole-3-acetic acid, napthalene acetic acid, and 2,4-5-trichloroacetic acid were equally effective in this action and apparently these chemicals are acting in such a way as to cause a shift towards the more reduced state of ascorbic acid, proteins, soluble sulfhydryls and pyridine nucleotides.

Spragg, et. al. (1959, 1962) presented evidence of the importance of the oxidation state of glutathione to the germination of seeds. It was reported that freshly harvested seeds which had high amounts of the reduced form of glutathione had the highest germination rate, while seeds which had been stored for long period of time had small amounts of the reduced form and a consequent lower germination rate. Analysis of the seeds for total sulfhydryl showed no change in this value, so the difference must have been due to changes in the ratio only. Experiments with germinating seeds indicated that during the first twenty-four hours of germination there was a significant change from the oxidized to the reduced form of glutathione with no change in the total content. These data would seem to indicate that there is a change in the oxidation state of the cell proteins which is associated with growth processes, particularly when it was reported by Spragg, Lievesley and Wilson (1962) that sulfhydryl inhibitors such as N-ethyl maleimide, which significantly decrease the amount of reduced glutathione present in seeds, inhibited germination.

Leopold and Price (1957) presented data which indicated that sulfhydryl inhibitors and certain other chemicals interact with sulfhydryl groups, at least <u>in vitro</u>. Tri-iodobenzoic acid was reported to interact

with reduced glutathione and remove it from the solution at a constant rate. This reaction was not found to be true for all growth regulators, but was observed for the three known sulfhydryl inhibitors maleimides, iodoacetate and p-chloromercuribenzoic acid. The data of Spragg, et al. (1959,1962) and Leopold and Price (1957) would seem to indicate that the reduced form of glutathione is important to growth and give evidence that a controlling mechanism exists in the cell.

Since Price and Taylor (1957) reported that at least 90% of the sulfhydryl of plant tissues is attributed to the proteins of the cells, it is entirely possible that changes in the sulfhydryl-disulfide ratio and its effect on the protein structure could account for the changes in the nature of the proteins of the cytoplasm and serve to explain the changes reported by Northen (1942) and Galston, et al. (1959, 1962, 1963). It is hard to explain, however, how these rather temporary changes in the sulfhydryl content of the cells could exert the many diverse effects reported with auxin treatments, unless we assume that these changes are just a small part of a great series of metabolic changes and interactions which are being affected.

CHAPTER III

MATERIALS AND METHODS

Seeds of garden peas (Pisum sativum, L. var Alaska), obtained from the W. Atlee Burpee Co., seed growers of Clinton, Iowa, were soaked in tap water for five hours before planting in water saturated vermiculite for growth. Aluminum pans (12" X 18" X 2½") were used as containers. The plants were rewatered once during the growth period, usually at midweek to insure adequate water supply for good growth.

A controlled temperature dark room was used as a growth chamber to obtain etiolated seedlings used in most of these experiments. The temperature in this room was maintained at 30°C throughout the growth period and the pans were kept on separate shelves to allow good ventilation.

On the seventh day after planting, the seedlings were in the third internode of stem growth and ready for harvest using methods similar to those of Galston and Kaur (1959). Sections one centimeter long were excised from the third internode directly below the apical crook using a double bladed sectioning tool. This tool consisted of a double edged razor blade attached on either side of a wooden block one centimeter thick. The arrangement of the blades and the attachment of them with small bolts allowed one to easily change the blades, always insuring clean, sharp cuts and assuring uniformity in length of all sections cut.

An experiment consisted of eight separate treatments of eighty sections each. During sectioning the sections were kept in a large petri dish (14 cm. X 2 cm.) containing cool, distilled water to prevent desiccation and to allow randomization of sections from the various plants.

Methods similar to those of Galston and Kaur (1959) were used in the early in vivo experiments. A typical sample was treated in the following manner. Petri plates (9 cm. X 1.5 cm.) were used for the culture dishes, and eighty sections were placed in a dish which contained ten milliliters of solution. This solution consisted of 9 ml. of 2% sucrose buffered with 0.02 M KH_2PO_4 to pH 6.1, and 1 ml. of auxin or other chemical as stated, or in the case of the controls 1 ml. of water was added. The overnight growth period (i.e. 18-20 hr.) was conducted in the same dark, 30° (centigrade) room used for the growth of the seedlings. Later experiments which were conducted used only twenty sections per 10 ml. of solution and utilized four dishes per treatment instead of the original one in order to keep the total number of sections at eighty.

Growth was determined by fresh weight determination methods similar to those used by Galston and Hand (1949). The sections were first blotted on clean paper towels to remove excess moisture and weighed on powdered glassine paper on a Mettler single pan balance (Type H5). These fresh weight determinations were made to the nearest milligram both before and after the overnight growth period. The results were recorded as percentage gain over the original fresh weight of an eighty section group.

After weighing, the sections were rinsed in distilled water, drained and taken to the cold room for the remainder of the processing. Each treatment was ground separately in a pre-chilled mortar and pestle with 14 ml. of cold 0.5 M sucrose solution containing 0.001 M ethylenediaminetetraacetic acid (EDTA). Later experiments used 0.001 M EDTA solution buffered with 0.02 M KH_2PO_4 to a pH of 6.1 for this homogenation. The homogenate was then strained through four layers of clean cheesecloth into polyethylene tubes and centrifuged at 24,000 X G for one hour or 16,700 X G for ½ hour in a Sorvall SS1 centrifuge. The supernatant obtained, after centrifugation, was saved and used in the protein, pectin and sulfhydryl analyses.

Certain experiments were conducted in vitro in which the growth regulator or other chemical was applied to the homogenates of the stem sections. The plants were sectioned and groups of eighty sections were homogenized and centrifuged in the cold room as described previously. Nine ml. of the final supernatant were added to one ml. aliquots of the growth regulator or other chemical in a standard pyrex test tube and allowed to react in the cold room for 15 minutes. Analysis procedures were then completed on these samples after this treatment period.

The heat coagulation response of the supernatant solutions was determined by heating 5 ml. portions in a boiling water bath for ten minute periods. The turbidity resulting from this heating was determined in a Bausch and Lomb monochromatic colorimeter equipped with a #42 filter.

When it was determined that a more quantitative measurement of the protein and amino acid content of the extracts was needed, methods

similar to those of Lowry, et al. (1951) with the Folin-Phenol reagent were employed. A typical protein analysis of the supernatant consisted of placing 1 ml. of the solution in a clean test tube (16 cm. X 2.5 cm.) with 5 ml. of an alkaline copper solution (50 ml. of Na_2CO_3 in 0.10 N NaOH and 1 ml. of 0.5% $CuSO_4 \cdot 5H_2O$ in 1% potassium tartrate). After mixing the solution thoroughly on a Vortex mixer the tube was allowed to stand at room temperature for ten minutes. While the tubes were being stirred rapidly on a Vortex mixer, 0.5 ml. of Folin-Ciocalteu reagent (0.5 N) was added with a blow out pipette. After thirty minutes colorimetric analysis was completed on a Bausch and Lomb colorimeter at 520 mμ or a Klett colorimeter with #54 filter.

Some experiments were conducted to determine the amount of trichloroacetic acid precipitable protein (total) in the supernatant solutions. Five ml. of this supernatant were mixed with an equal volume of 10% TCA in glass centrifuge tubes and allowed to stand in the cold room for 10 to 20 minute periods. After this precipitation period the tubes were centrifuged at 8,000 rpm for ten minutes in a Sorvall SS3. The supernatant was poured off and saved for later protein and amino acid analysis and the remaining precipitate put in solution with 1 ml. of 0.5 N sodium hydroxide. After dilution with 4 ml. of distilled water, one ml. aliquots were removed for protein tests already described. The supernatant was analyzed as described earlier for the original supernatant using 1 ml. aliquots for samples.

The soluble pectin of the extracts was measured using slightly modified methods of McComb and McCready (1952). Two ml. of the supernatant (homogenate centrifuged at 24,000 X G) were added to 10 ml. of

0.05 N sodium hydroxide and allowed to stand at room temperature for 25-30 minutes. At the end of that time the solution was diluted with 10 ml. of distilled water to give an approximate concentration of .002% pectin material. Two ml. of this deesterified material was then added slowly to 12 ml. of cold, concentrated sulfuric acid (32 N). Since this mixture generated intense amounts of heat, it was necessary to keep the tubes in beakers of ice water during the addition. Following a cooling period the tubes were boiled for ten minute periods and cooled to room temperature. To this solution was added 1 ml. of a 0.15% solution of carbazole in ethyl alcohol and colorimetric readings taken after thirty minutes. These readings were taken on a Bausch and Lomb colorimeter at 520 mμ or on a Klett colorimeter with #54 filter.

Sulfhydryl determinations were made on the supernatants (homogenates after centrifugation at 16,700 X G) using two methods. The earlier determinations were made using the nitroprusside methods of Grunert and Phillips (1951) for the determination of glutathione. The procedure for a typical analysis consisted of mixing 0.5 ml. of supernatant with 5 ml. of saturated sodium chloride solution and 1 ml. of 2% sodium nitroprusside, followed by 1.0 ml. of a mixture of 1.5 M sodium carbonate and 0.025 M sodium cyanide. The color intensity was then determined on a Bausch and Lomb colorimeter at 520 mμ. This test proved to be not sensitive enough to detect the presence of glutathione in the samples, so later analyses were made using the colorimetric methods of Klotz and Carver (1961) with greater success. A typical analysis was made in the following manner: 10 ml. of the supernatant was placed in a 25 ml. erlenmeyer flask containing 5 ml.

of the dye-urea solution (2×10^{-4}M pyridine-2-azo-p-dimethylaniline + 4 molar urea in 0.1 M phosphate buffer at pH 5.8). This solution was stirred during the titration by means of a magnetic stirrer in order to assure uniformity of solution. Salyrganic acid (5×10^{-3}M + 1×10^{-2}M sodium chloride in 0.1 M phosphate buffer at pH 5.8) was added by a Micrometric syringe buret and the optical density determinations were made on a Klett colorimeter with #56 filter immediately after each addition.

CHAPTER IV

RESULTS AND DISCUSSION

Growth

Early experiments in which eighty 1 cm. sections were cultured in 10 ml. aliquots of media resulted in very erratic growth patterns among the sections. Accurate determinations of the growth was not possible because the variation was so great. It appeared that in order to obtain as uniform a response as possible to the various concentrations, the number of sections in each dish would have to be reduced.

This problem was overcome by reducing the number of sections in each dish from eighty to twenty. After this change the weight increases were replicable enough to be considered accurate responses to the auxin. The results of the fresh weight determinations for ten separate experiments using 2,4-dichlorophenoxyacetic acid at various concentrations are presented in Table I. These results are expressed as per cent gain in fresh weight during the eighteen hour culture period, and each determination was made on eighty sections (4 treatments of twenty sections each). The average increase in weight for each concentration of 2,4-D used is tabulated at the bottom of the table and presented graphically in Figure 1. This graph shows a typical growth response to auxin concentration in that little effect

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF 2,4-D ON THE FRESH WEIGHTS OF ETIOLATED PEA STEM SECTIONS

	2,4-Dichlorophenoxyacetic acid concentrations						
Control	10^{-7}M	$5X10^{-7}$M	10^{-6}M	$5X10^{-6}$M	10^{-5}M	$5X10^{-5}$M	10^{-4}M
	Per cent increase in fresh weight						
1) 18%	22%	45%	59%	94%	97%	99%	89%
2) 18	26	52	68	96	113	110	107
3) 19	33	47	71	94	86	44	31
4) 14	--	--	64	88	94	80	63
5) 20	--	--	84	113	116	106	115
6) 22	--	--	43	105	125	125	109
7) 17	--	--	99	--	114	--	115
8) 14	--	--	--	86	75	66	--
9) 13	--	--	37	50	63	--	--
10) 17	--	--	30	--	71	86	--
AVE.) 17%	27%	48%	62%	91%	95%	90%	90%

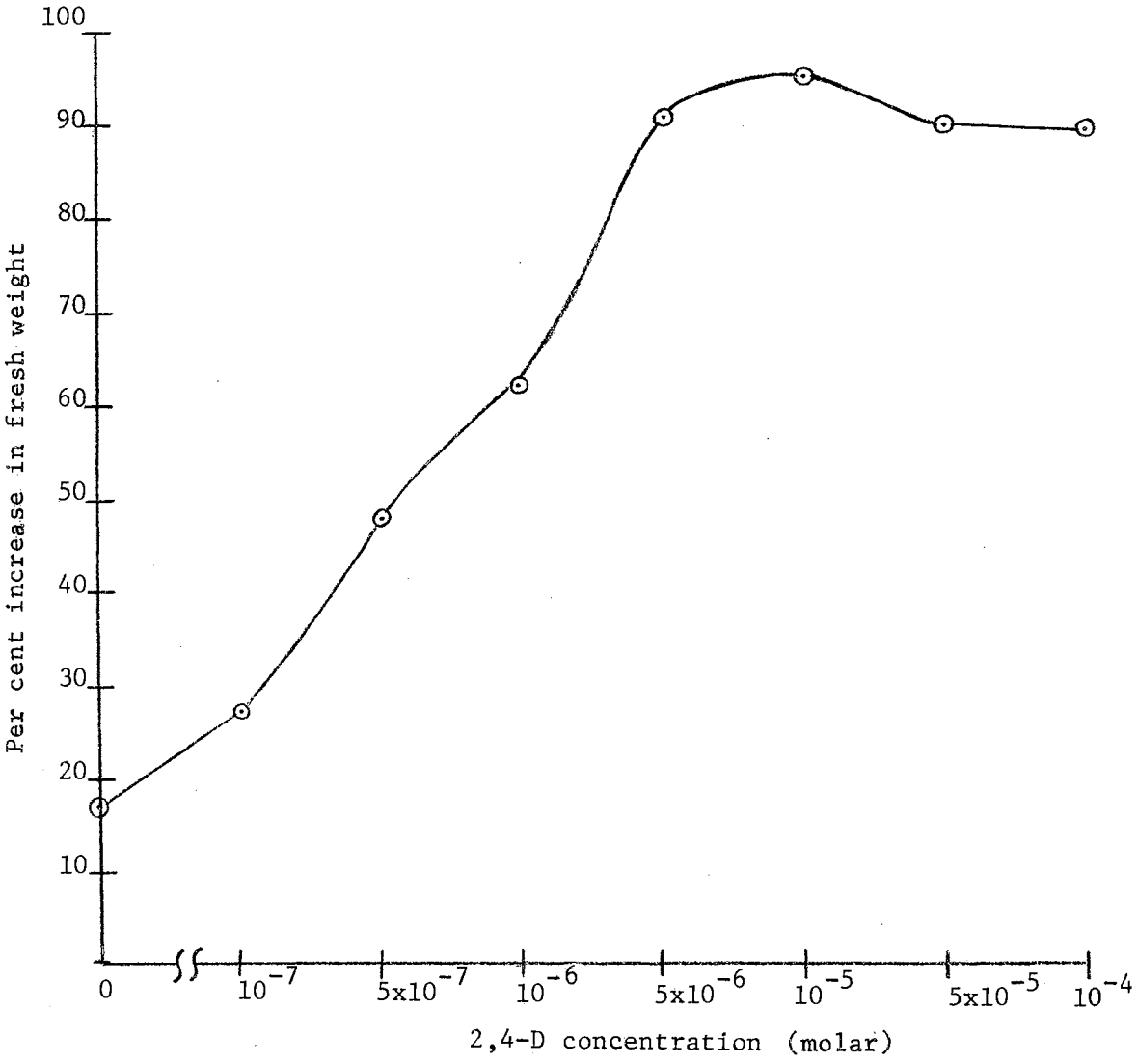

Figure 1. Average percent increase in fresh weight due to various concentrations of 2,4-dichlorophenoxyacetic acid.

is exerted at the lower concentrations with a gradual increase in growth as the concentration increases until the maximum is reached near 10^{-5}M. At concentrations higher than 10^{-5}M the growth effect diminishes and growth retardation occurs.

Table II presents the results of similar fresh weight determinations using reduced glutathione (GSH) and oxidized glutathione (GSSG) instead of 2,4-D as the treatment. These data indicate no effect on the fresh weight by either form of glutathione. Marre and Arrigoni (1957) reported that certain concentrations of glutathione did affect the fresh weight of sections under culture conditions, but only when supplied with IAA at growth stimulatory concentrations.

Heat Coagulation

The experiments undertaken in investigating the effects of certain chemicals on the heat coagulability of the proteins in a particle free homogenate of pea stem sections were conducted to compare the effects of these chemicals with the effects noted by Galston (1959).

Table III shows the results of determinations of the turbidity, due to heat coagulation of the proteins, of the homogenates of sections treated in vivo with various concentrations of 2,4-D. The data is presented as per cent transmittance of the homogenates from eighty sections as determined at 520 mμ on a colorimeter. The data from eight experiments using a wide range of concentrations of 2,4-D are presented. Experiments 1-6 show the effect of the 2,4-D in decreasing the amount of heat coagulum, relative to the controls, at 10^{-7}M through 10^{-4}M concentrations of 2,4-D. These decreases are not necessarily restricted to those concentrations which showed maximum stimulation

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF GLUTATHIONE ON

FRESH WEIGHT OF CULTURED ETIOLATED PEA SECTIONS

Treatment	Experiment Number 1	2	3	4	5	6	7	8	9	10	Ave.
	Per Cent Increase in Fresh Weight										
Control	17%	14%	13%	17%	18%	15%	23%	27%	8%	10%	16%
GSH (10^{-6}M)	19	--	--	--	--	--	--	--	--	--	19%
GSH (10^{-5}M)	18	15	11	17	--	15	--	--	--	--	15%
GSSG "	--	--	--	--	18	--	20	--	--	--	19%
GSH ($5X10^{-5}$)	--	14	12	19	--	--	--	--	--	--	15%
GSSG "	--	--	--	--	21	--	--	--	--	--	21%
GSH (10^{-4}M)	17	14	10	22	--	13	--	27	10	8	15%
GSSG "	--	--	--	--	19	--	21	31	11	14	19%
GSH ($5X10^{-4}$)	--	--	11	24	--	--	--	26	12	12	17%
GSSG "	--	--	--	--	19	--	--	25	13	13	17%
GSH (10^{-3}M)	--	--	--	9	--	--	--	24	10	13	14%
GSSG "	--	--	--	--	21	--	--	25	10	14	17%

TABLE III

THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE HEAT COAGULABILITY* OF PEA STEM PROTEINS

Treatment	1	2	3	4	5	6	7	8
	Per cent Transmittance of Homogenates							
Control	42%	57%	58%	54%	73%	62%	85%	79%
2,4-D (10^{-7}M)	36	51	76	63	--	--	--	--
2,4-D($5X10^{-7}$)	35	47	60	58	--	--	--	--
2,4-D (10^{-6}M)	47	55	75	67	69	78	70	79
2,4-D($5X10^{-6}$)	40	55	72	63	69	80	70	80
2,4-D (10^{-5}M)	43	58	78	66	73	79	72	80
2,4-D($5X10^{-5}$)	35	57	80	64	77	75	75	83
2,4-D (10^{-4}M)	40	68	--	60	76	73	74	77
2,4-D($5X10^{-4}$)	--	--	--	--	--	--	--	--
2,4-D(10^{-3}M)	--	--	--	--	--	--	--	--

* Determination by turbidity method.

of growth by increase in fresh weight. The changes are quite erratic because the method of measuring the turbidity of the solutions does not take into account any solid coagulum present in the tube. The results were quite duplicable when only a slight turbidity resulted from the heat treatment, but when a large amount of coagulum appeared the results were not accurately obtained by this method. Several in vitro experiments were conducted in which the homogenates were exposed to the various concentrations of 2,4-D for fifteen minute periods. These experiments showed no differences due to the treatment and the transmittance values were observed to be quite consistent. No data is presented for these experiments because they were run as preliminary experiments.

Table IV presents the results of five in vitro and one in vivo experiments with urea to observe its effect on the coagulation properties of proteins in the homogenates. These data show no effect when applied to the homogenates in vitro, but do indicate an effect in decreasing the amount of heat coagulable protein in the extracts of sections exposed to urea in vivo for 18 hours. The range of effective concentrations is quite wide (10^{-3}M to 10^{-5}M) and does not appear to be connected with growth of the sections.

Changes in the heat coagulability of the homogenate proteins in response to various concentrations of reduced (GSH) and oxidized (GSSG) glutathione alone and with certain concentrations of 2,4-D are presented in Tables V and VI. Table V shows the results of experiments in which the chemicals were supplied to the sections during the incubation period, and the homogenates were extracted and boiled to coagulate the heat sensitive proteins. The values are again quite erratic due to variation

TABLE IV

EFFECT OF UREA ON THE HEAT COAGULABILITY*

OF PEA STEM PROTEINS

Treatment	1	2	3	4	5	6
		in vitro experiments				in vivo exp.
	Per cent transmittance of Homogenates					
Control	81%	77%	79%	60%	81%	72%
Urea (10^{-2}M)	82	79	--	65	--	--
Urea ($5X10^{-3}$M)	84	81	--	--	--	--
Urea (10^{-3}M)	83	76	--	59	--	89
Urea ($5X10^{-4}$M)	82	77	--	--	--	93
Urea (10^{-4}M)	82	79	81	--	81	88
Urea ($5X10^{-5}$M)	82	--	81	--	82	--
Urea (10^{-5}M)	82	--	82	--	82	93
Urea ($5X10^{-6}$M)	--	--	--	--	--	77
Urea (10^{-6}M)	--	--	--	--	--	77

*Determination by turbidity method.

TABLE V

EFFECT OF OXIDIZED AND REDUCED GLUTATHIONE ON HEAT COAGULABILITY*OF PEA STEM PROTEINS

Treatment	Exp. 1	Exp. 2
	Per cent transmittance of Homogenates at 520 mμ	
Control	67%	55%
2,4-D (10^{-5}M)	59%	55%
GSSG (10^{-4}M)	65%	72%
GSH (10^{-4}M)	61%	80%
GSSG ($5X10^{-4}$M)	64%	81%
GSH ($5X10^{-4}$M)	64%	77%
GSSG (10^{-3}M)	60%	59%
GSH (10^{-3}M)	62%	88%

*Determination by turbidity method.

TABLE VI

EFFECT OF VARIOUS COMBINATIONS OF GLUTATHIONE AND 2,4-D ON HEAT COAGULABILITY*OF PEA STEM PROTEIN

Treatment	in vivo	in vitro
	Per cent transmittance of homogenates at 520 mμ	
Control	78%	
2,4-D (10^{-5}M)	72%	
GSSG (10^{-4}M)	63%	
" + 2,4-D	68%	
GSSG ($5X10^{-4}$M)	78%	
" + 2,4-D	76%	
GSH ($5X10^{-4}$M)	77%	
" + 2,4-D	71%	
Control	67%	60%
2,4-D (10^{-6}M)	60%	60%
GSSG ($5X10^{-5}$M)	48%	74%
" + 2,4-D	53%	62%
GSSG (10^{-4}M)	57%	61%
" + 2,4-D	53%	62%
GSSG ($5X10^{-4}$M)	56%	61%
" + 2,4-D	60%	65%
Control	52%	52%
2,4-D ($5X10^{-5}$M)	48%	42%
GSSG ($5X10^{-4}$M)	61%	57%
" + 2,4-D	60%	59%
GSSG (10^{-3}M)	--	49%
" + 2,4-D	--	58%
GSH (10^{-3}M)	57%	31%
" + 2,4-D	60%	59%

*Determination by turbidity method.

in amount of coagulum present in the tubes and in only one case is there any indication of decreased heat coagulability (Exp. 2). Since these changes were not evident in other experiments their importance is not known. Table VI shows the results of two experiments in which the reaction of the proteins of the sections treated in vivo are compared with homogenates which were treated in vitro with various concentrations and combinations of these chemicals.

None of the experiments using the turbidity method showed consistent additive effects when either form of glutathione was added in conjunction with 2,4-D, nor were there large consistent effects of the 2,4-D on the heat coagulation.

Folin Phenol Protein Analysis

These determinations were begun after it became apparent that the measurement of the turbidity of the homogenates for purposes of determination of changes in the heat coagulation was not accurate enough because of variation in the amount of coagulum. Preliminary experiments with the methods of Lowry, et al. (1951) indicated that differences in amounts of proteins and amino acids in the homogenates were due to different concentrations of 2,4-D applied to the stem sections in vivo. Some disadvantages of the methods were reported by Lowry, et al. (1951): (1) Both proteins and free tyrosine are measured, (2) different proteins give differing degrees of color, depending upon the tyrosine content, and (3) the color intensity is not directly proportional to the concentration of tyrosine present in the solutions, particularly at high concentrations. It was anticipated, however, that these analyses would detect differences large enough to overshadow

the disadvantages in technique.

Table VII shows the results of protein analyses on the total homogenate after centrifugation from sections treated with various concentrations of 2,4-D in vivo. The analyses were run on samples before and after coagulation and removal of the heat sensitive proteins in order to obtain data on the differences in amounts of proteins and amino acids present in the solution, as well as to determine how much was being removed by the heating.

The total reactable material (milligrams/ml. of protein as compared with a standard curve prepared with egg albumin) is shown by the upper curve in Figure 2. This curve indicates the average amount of material present in the sections before removal of the heat coagulable protein. The middle curve of the figure shows the amount, in milligrams, of heat coagulable protein removed from sections treated at various concentrations of 2,4-D. The lower curve shows the increase in fresh weight initiated by the various concentrations of 2,4-D.

It appears from the graph that the total reactable material in the solutions before heating is increased slightly at lower concentrations of 2,4-D, while the higher concentrations cause a reduction in amount of reactable material. One can readily see that increases in reactable material are not associated with the growth response, in fact they occur at low concentrations not showing a great increase in fresh weight. These results resemble those of Fang and Yu (1965) who found that concentrations of IAA and 2,4-D causing increases in protein synthesis had no effect on fresh weight. The graph also shows that there is little change from the control in the amount of heat coagulable protein present in the sections treated with various concentrations of 2,4-D.

TABLE VII

EFFECT OF 2,4-D ON THE PROTEIN CONTENT

OF ETIOLATED PEA STEM SECTIONS

Treatment	B*	A**	Protein (mg/ml) B	A	B	A	Ave. B-A
Control	1.025	.625	.745	.470	.757	.420	.304
2,4-D (10^{-7}M)	1.035	.865	.865	.500	.855	.490	.300
" ($5X10^{-7}$M)	1.040	.815	.885	.525	.900	.485	.323
" (10^{-6}M)	1.033	.900	.805	.520	.945	.505	.286
" ($5X10^{-6}$M)	1.036	.840	.825	.508	.905	.637	.261
" (10^{-5}M)	1.030	.770	.770	.545	.710	.498	.232
" ($5X10^{-5}$M)	.965	.547	.905	.555	.840	.475	.378
" (10^{-4}M)	.827	.480	.840	.480	.705	.495	.305

* B equal total reactable material.

** A equal reactable material after heating and removal of coagulum.

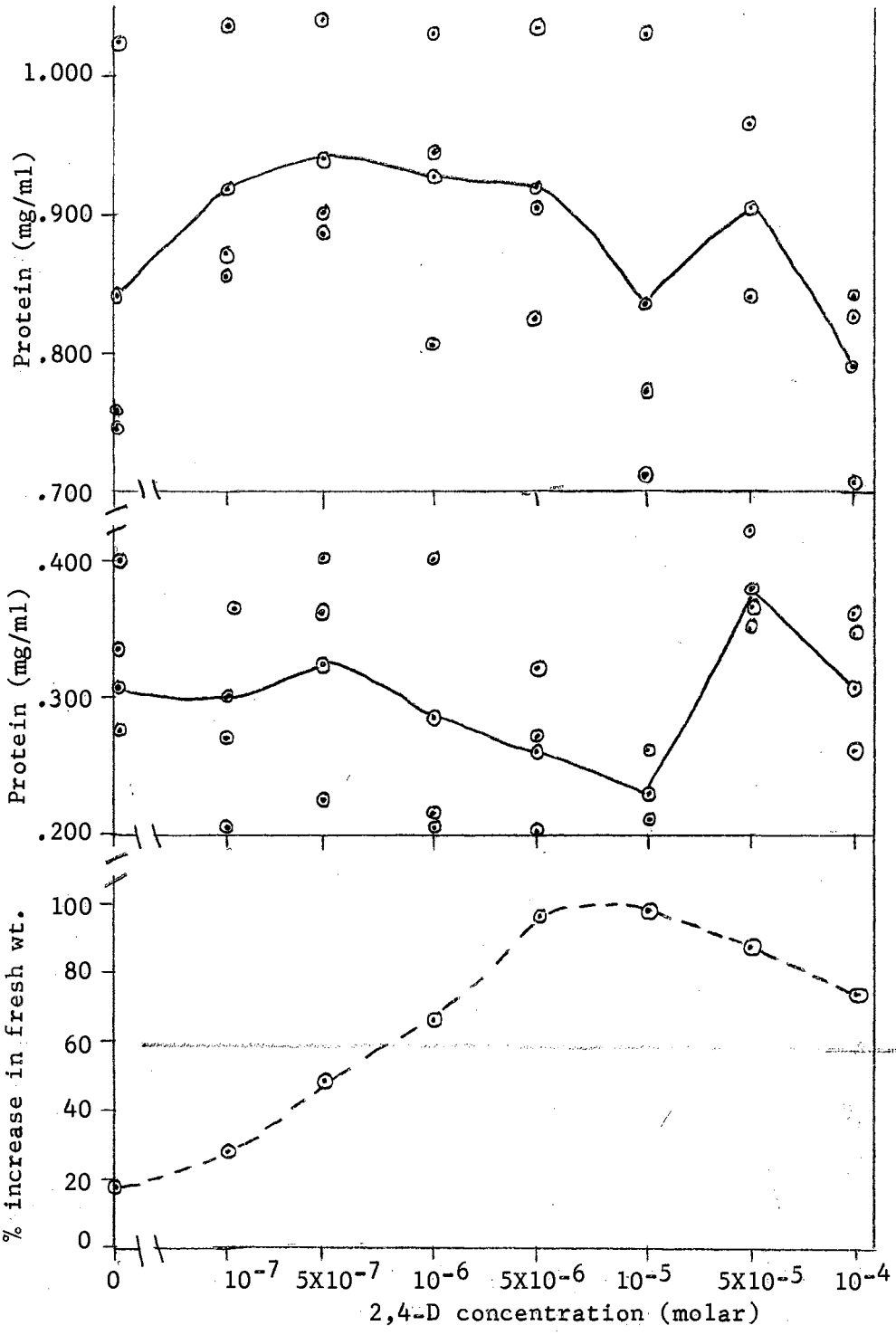

Figure 2. Effect of 2,4-D concentration on the protein and amino acids in the homogenates, as determined by Folin Ciocalteu method. The upper curve denotes total reactable material, the middle curve heat coagulable protein and the lower curve indicates per cent increase in growth at the respective concentrations of 2,4-D.

The results of the Folin analysis on the homogenates of stem sections treated in vivo with various concentrations of oxidized (GSSG) and reduced (GSH) glutathione are presented in Table VIII. Determinations were made on the solutions as in the 2,4-D experiments and the protein content in milligrams/ml for each treatment was obtained by comparing with a standard curve of egg albumin. It appears from these data that no significant increase in the amount of reactable material is mediated by either of these types of glutathione at any of the concentrations tested. Examination of the average heat sensitive protein content of the sections for each treatment (B-A value) shows that all except one have less, regardless of the oxidation state of glutathione applied. The one exception was oxidized glutathione when applied at 5×10^{-4}M concentration which showed a slight increase. Table IX shows the results of two experiments in which various combinations of GSH and GSSG and 2,4-D were used to determine if any additive effects were exerted by glutathione on the response already exhibited by 2,4-D. No additive effects on growth or protein content were noted in these experiments at any of the concentrations and combinations tested.

Table X presents the results of experiments conducted to determine the amount of reactable material present in the supernatant solutions before and after precipitation with 10% TCA, as well as the quantity being precipitated by the TCA. The total reactable material present in the solutions follows the trend observed in the earlier experiments, with higher amounts being present in those samples treated with 10^{-6}M and 10^{-5}M 2,4-D concentrations with little difference noted between the 10^{-4}M treatment and the control. This same trend is indicated in the analysis of the solutions for reactable materials after removal of the

TABLE VIII

EFFECT OF OXIDIZED AND REDUCED GLUTATHIONE

ON THE PROTEIN CONTENT OF PEA STEM SECTIONS

Treatment	Protein (mg/ml)						Ave.
	B*	A**	B	A	B	A	B-A
Control	.980	.370	.900	.535	.830	.520	.428
GSH (10^{-5}M)	---	---	---	---	.790	.452	.338+
GSH ($5X10^{-5}$M)	---	---	---	---	.810	.430	.386+
GSH (10^{-4}M)	.757	.383	.972	.565	.840	.477	.381
GSH ($5X10^{-4}$M)	.705	.400	.915	.488	.816	.465	.361
GSH (10^{-3}M)	.805	.405	.915	.550	.687	.405	.349
GSSG (10^{-5}M)	---	---	---	---	.687	.412	.275+
GSSG ($5X10^{-5}$M)	---	---	---	---	.695	.405	.290+
GSSG (10^{-4}M)	.660	.345	.972	.625	.760	.405	.339
GSSG ($5X10^{-4}$M)	.830	.373	1.025	.525	.810	.461	.435
GSSG (10^{-3}M)	.672	.357	.947	.547	.782	.461	.345

* B equal total reactable material

** A equal reactable material after heating and removal of coagulum.

+ Values of only a single determination.

TABLE IX

EFFECT OF VARIOUS COMBINATIONS OF GLUTATHIONE AND 2,4-D ON THE PROTEIN CONTENT OF PEA STEM SECTIONS

Treatment	Protein Content (mg/ml) Before	After	B-A
Control	.668	.447	.221
2,4-D 10^{-6}M	.800	.527	.273
" 10^{-5}M	.770	.507	.263
GSH 10^{-5}M	.812	.546	.266
" 10^{-4}M	.775	.452	.323
2,4-D 10^{-6}M + GSH 10^{-5}M	.725	.447	.278
2,4-D 10^{-6}M + GSH 10^{-4}M	.775	.447	.278
2,4-D 10^{-5}M + GSH 10^{-5}M	.775	.502	.273
Control	.705	.545	.160
2,4-D 10^{-6}M	.770	.610	.160
" 10^{-5}M	.770	.590	.180
GSSG 10^{-5}M	.770	.520	.250
" 10^{-4}M	.705	.520	.185
2,4-D 10^{-6}M + GSSG 10^{-5}M	.743	.560	.183
2,4-D 10^{-6}M + GSSG 10^{-4}M	.673	.535	.138
2,4-D 10^{-5}M + GSSG 10^{-4}M	.775	.547	.228

TABLE X

EFFECT OF 2,4-D ON PROTEIN CONTENT OF STEM HOMOGENATE SUPERNATANTS (16,700 X G)*

Treatment	Protein (mg/ml) Supernatant Before TCA ppt.	TCA ppt.	Supernatant After TCA ppt.
Control	.780	.382	.450
2,4-D (10^{-6}M)	.870	.379	.476
2,4-D (10^{-5}M)	.845	.382	.457
2,4-D (10^{-4}M)	.793	.381	.440

*Average of four experiments.

TCA precipitated proteins except that the amounts are considerably smaller. Analysis of the amount of protein precipitated by 10% TCA showed no differences in amounts of protein present between the different treatments. Since the TCA precipitation removes only protein and no amino acids it is possible that the differences being detected in these experiments upon treatment with certain levels of 2,4-D are in free tyrosine content or in some other reactable substance in the cytoplasm, rather than the protein as first thought.

Pectin Analysis

The results of the analysis of the homogenates for pectin content after in vivo treatment with various concentrations of 2,4-D are shown in Table XI and in Figure 3. Data are presented in this table for determinations made before and after removal of the heat coagulable protein of three separate experiments which were close replicates. The optical density values for the 3 experiments are shown plotted as averages in Figure 3 and increases are indicated in the amount of pectin present in the homogenates of sections exposed to concentrations of 2,4-D ranging from 10^{-7}M to 5 X 10^{-6}M. The maximum increase seems to be exhibited by those homogenates which were obtained from sections treated with 5 X 10^{-6}M 2,4-D with a sharp decline in amounts at concentrations higher than this. The values for the analyses after removal of the heat coagulum follow the same trend as the initial determinations with a maximum amount of pectin present at 5 X 10^{-6}M treatment. The increase in pectin content appears to occur at the same concentrations of 2,4-D as those which caused the increases in protein and, as before, they do not necessarily correlate with the growth of the sections.

TABLE XI

EFFECT OF 2,4-D ON THE SOLUBLE PECTIN CONTENT

OF ETIOLATED PEA STEM SECTIONS

Treatment	Optical density at 540 mu						Ave.		
	B*	A**	B	A	B	A	B	A	B-A
Control	.426	.410	.336	.338	.332	.330	.364	.360	.005
2,4-D (10^{-7}M)	.490	.446	.376	.356	.364	.368	.410	.390	.020
2,4-D ($5X10^{-7}$M)	.584	.494	.368	.356	.400	.392	.450	.414	.042
2,4-D (10^{-6}M)	.490	.466	.400	.394	.406	.434	.432	.432	.001
2,4-D ($5X10^{-6}$M)	.502	.480	.400	.380	.416	.440	.440	.434	.006
2,4-D (10^{-5}M)	.448	.434	.384	.380	.416	.410	.416	.408	.008
2,4-D ($5X10^{-5}$M)	.398	.344	.374	.394	.356	.384	.376	.374	.001
2,4-D (10^{-4}M)	.322	.294	.348	.348	.336	.342	.336	.328	.007

* B equal total protein plus reactable free amino acids.

** A equal protein remaining after heat coagulation.

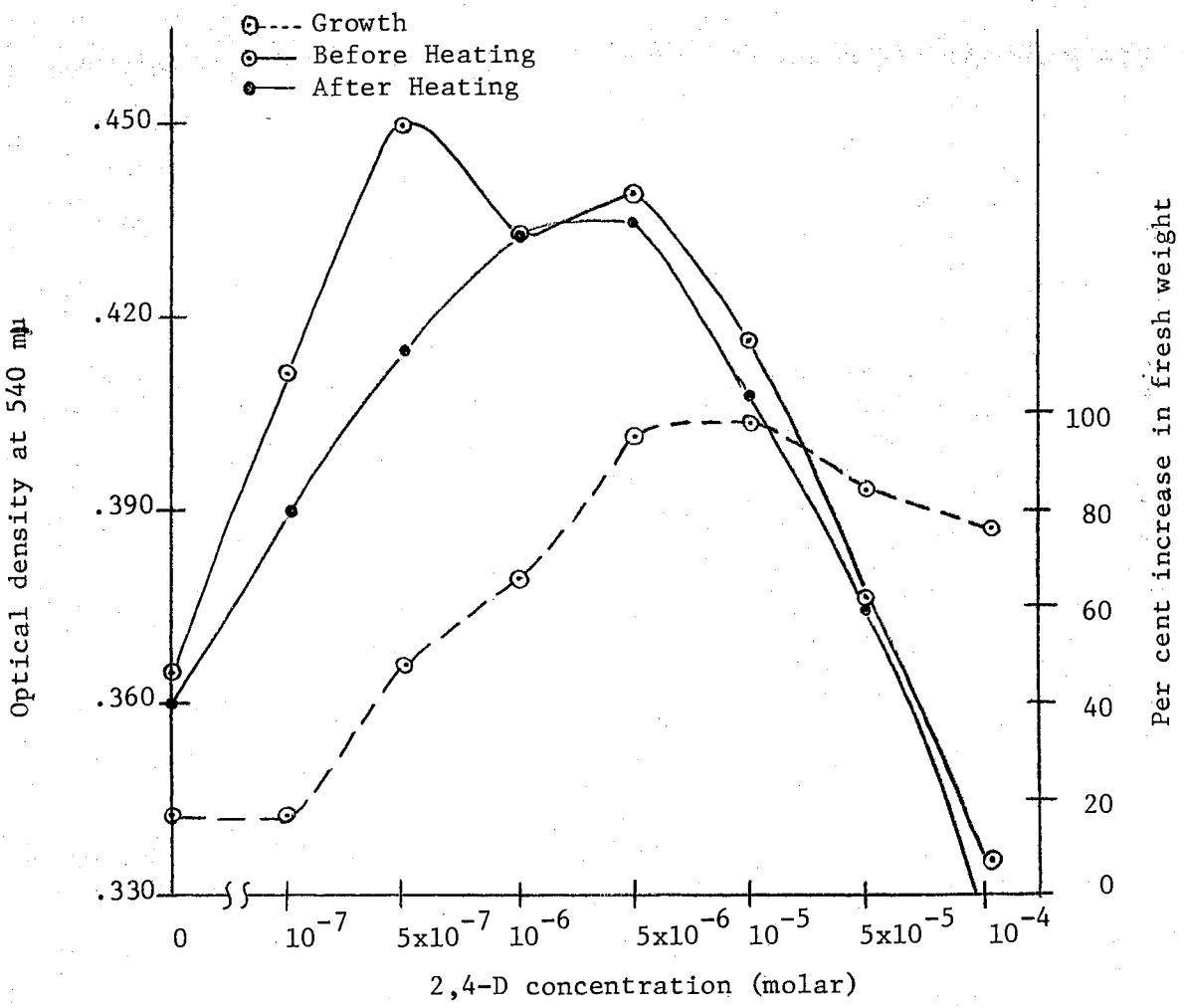

Figure 3. Effect of 2,4-D concentrations on the pectin content of the homogenates before and after heat coagulation.

Tables XII and XIII show the results of similar determinations of pectin content in sections exposed in vivo to various concentrations of oxidized and reduced glutathione as well as certain combinations of 2,4-D and glutathione. These experiments seem to indicate no effect of these chemicals or their combinations on the pectin content of the homogenates, since no consistent changes could be detected there.

Sulfhydryl Determinations

Preliminary experiments in which the analysis for sulfhydryl groups in the homogenates was accomplished using the methods of Grunert and Phillips (1951) met with failure in all trials. Analysis of several samples failed to indicate any sulfhydryl material in any of the extracts and comparison of several of these sample analyses with analyses of solutions known to contain adequate amounts of sulfhydryl in the form of reduced glutathione showed that none was being detected in the homogenates.

Later experiments using the colorimetric analysis methods of Klotz and Carver (1961) resulted in somewhat better determinations of the sulfhydryl content of the homogenates. Preliminary experiments conducted to determine the best amount of homogenate to use in each titration to obtain good end points indicated that as little as 10 ml. was sufficient. Some preliminary experiments using 25 ml. aliquots gave results similar to those using only 10 ml. amounts so it was decided to use the smaller aliquots because of the limited availability (14 ml.) of homogenates from each treatment.

Titration data from five separate experiments which were conducted to determine if any variation in sulfhydryl content could be detected

TABLE XII

EFFECT OF OXIDIZED AND REDUCED GLUTATHIONE

ON THE PECTIN CONTENT OF PEA STEM SECTIONS

Treatment	Optical density at 540 mu							
	B*	A**	B	A	B	A	B	A
Control	.206	.264	.342	.334	.404	---	.317	.299
GSH (10^{-5}M)	---	---	---	---	.404	---		
GSH ($5X10^{-5}$M)	---	---	---	---	.402	---		
GSH (10^{-4}M)	---	.254	.354	.360	.412	---	.383	.307
GSH ($5X10^{-4}$M)	.306	.276	.330	.320	.386	---	.341	.298
GSH (10^{-3}M)	.332	.384	.374	.342	.340	---	.349	.313
Control	---	---	---	---	.288	---		
GSSG (10^{-5}M)	---	---	---	---	.248	---		
GSSG ($5X10^{-5}$M)	---	---	---	---	.236	---		
GSSG (10^{-4}M)	.286	.262	.354	.360	.250	---	.297	.311
GSSG ($5X10^{-4}$M)	.294	.274	.326	.388	.254	---	.291	.331
GSSG (10^{-3}M)	.278	.248	.374	.344	.258	---	.303	.296

* B equal total protein plus reactable free amino acids.

** A equal protein remaining after heat coagulation.

TABLE XIII

EFFECT OF VARIOUS COMBINATIONS OF GLUTATHIONE AND 2,4-D ON THE PECTIN CONTENT OF PEA STEM SECTIONS

Treatment	Before	After
Control	.352	.380
2,4-D 10^{-6}M	.400	.394
" 10^{-5}M	.394	.400
GSH 10^{-5}M	.408	.400
" 10^{-4}M	.346	.368
2,4-D 10^{-6}M + GSH 10^{-5}M	.336	.358
2,4-D 10^{-6}M + GSH 10^{-4}M	.316	.350
2,4-D 10^{-5}M + GSH 10^{-5}M	.366	.372
Control	.408	.420
2,4-D 10^{-6}M	.472	.480
" 10^{-5}M	.476	.456
GSSG 10^{-5}M	.268	.422
" 10^{-4}M	.404	.404
2,4-D 10^{-6}M + GSSG 10^{-5}M	.466	.434
2,4-D 10^{-6}M + GSSG 10^{-4}M	.402	.386
2,4-D 10^{-5}M + GSSG 10^{-4}M	.426	.420

due to treatment with various chemicals are presented in Figures 4-8. The figures illustrate the variation which occurs in the separate experiments and are interpreted on the basis of microliters of salyrganic acid required to titrate all the free sulfhydryl groups in the centrifuged supernatant. This value is obtained by following the base line obtained in each titration over to the point at which the optical density values become linear with each addition of salyrganic acid. This linear increase indicates that one is now titrating directly the dye used as the end point indicator. The end point then is read directly in microliters of salyrganic acid per sample and comparison of the amounts of sulfhydryl material can be made using these values.

Figure 4 shows the titrations of samples treated with three concentrations of 2,4-D compared with a control which received no 2,4-D. It is apparent from these data that the 2,4-D treated sections contained a greater amount of sulfhydryl in each case than did the control. 10^{-4} and 10^{-6}M treatment appeared to have the greatest effects in increasing the SH content.

Figure 5 shows that the control has the greatest amount of sulfhydryl material when compared with the other treatments. The trend shown by 10^{-5}M 2,4-D in Figure 4 is reversed in this experiment, and the addition of 1 ml. of GSH (10^{-4}M) *in vitro* to the homogenates which would be expected to increase the amount of sulfhydryl present actually decreased it.

Figure 6 shows the results of application of TIBA to the sections, *in vivo* for varying lengths of time. It would appear that TIBA is effective in reducing the amount of sulfhydryl present in the extracts, particularly when supplied to the sections for short periods of time.

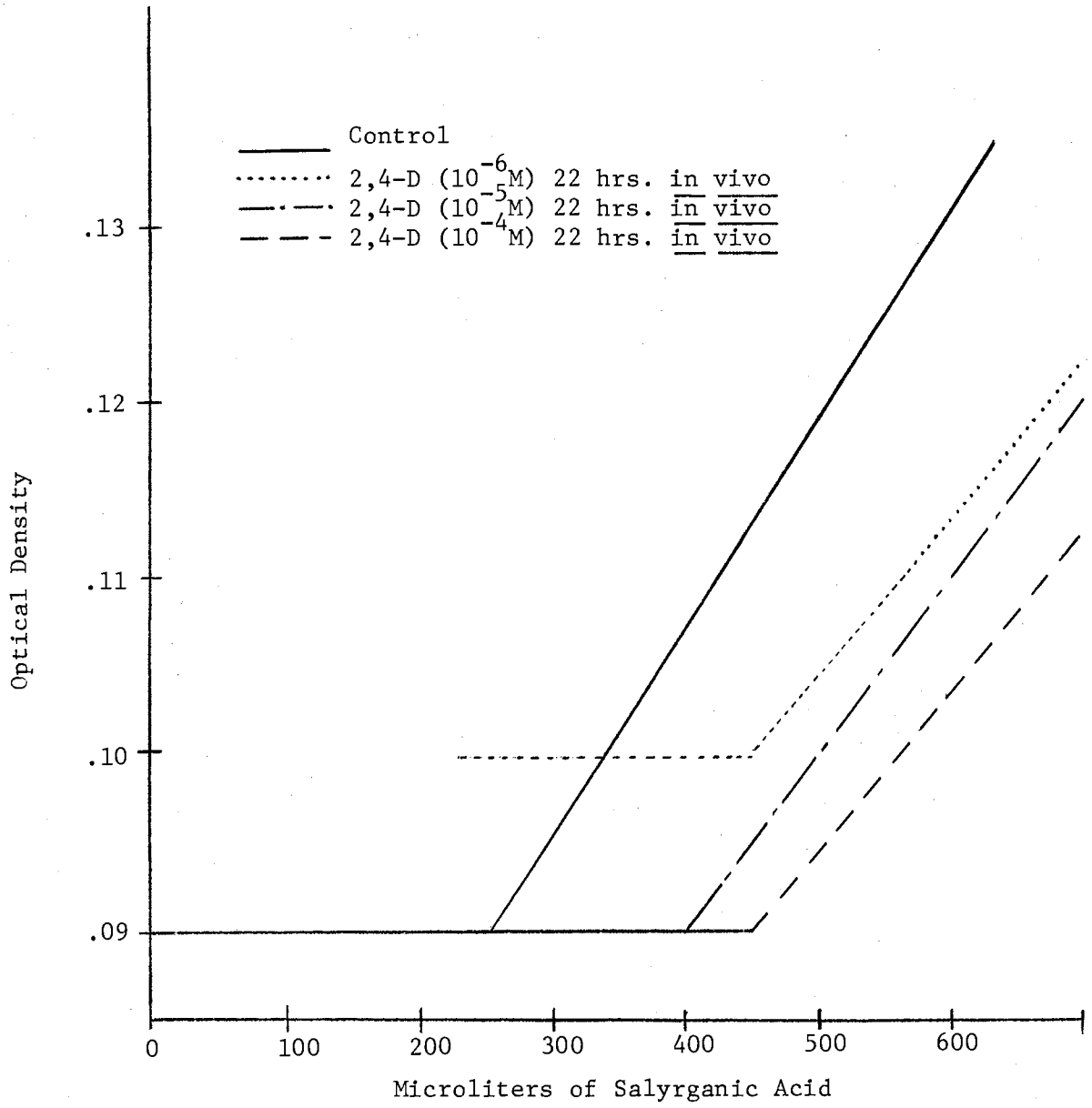

Figure 4. SH content of homogenates after treatment with various concentrations of 2,4-D.

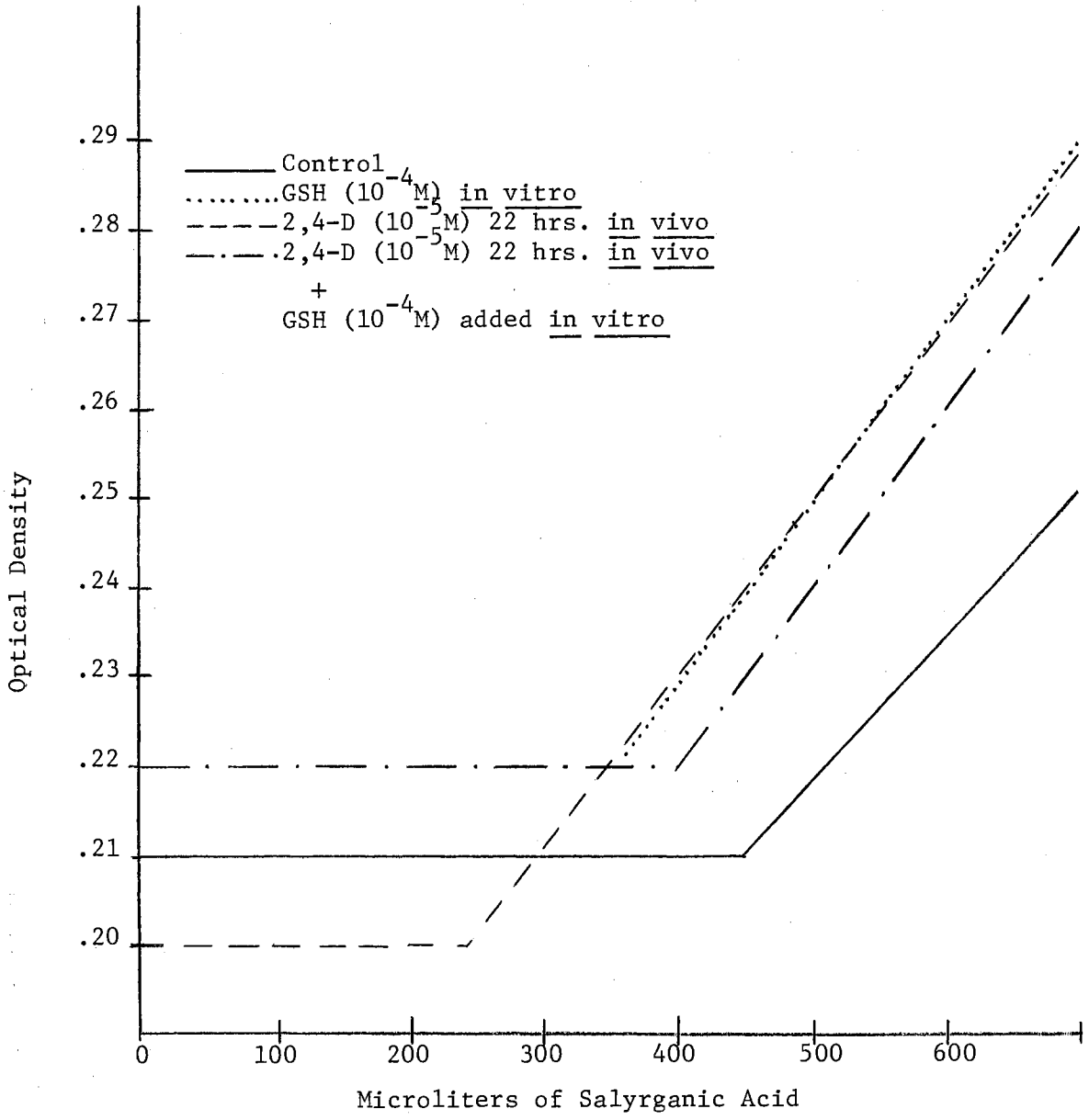

Figure 5. SH content of homogenates after treatment with 2,4-D and reduced glutathione.

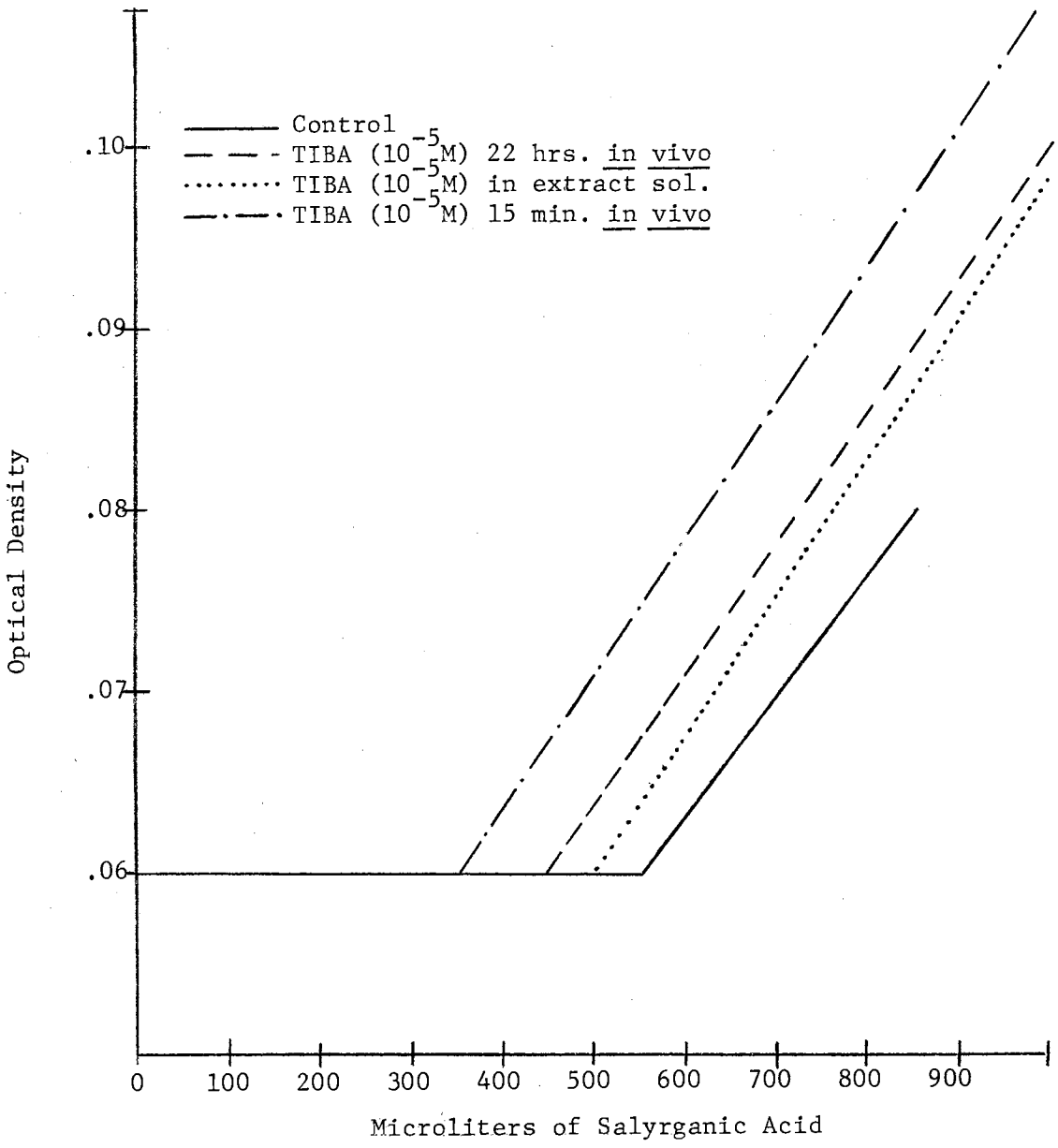

Figure 6. SH content of homogenates after treatment with various concentrations of TIBA.

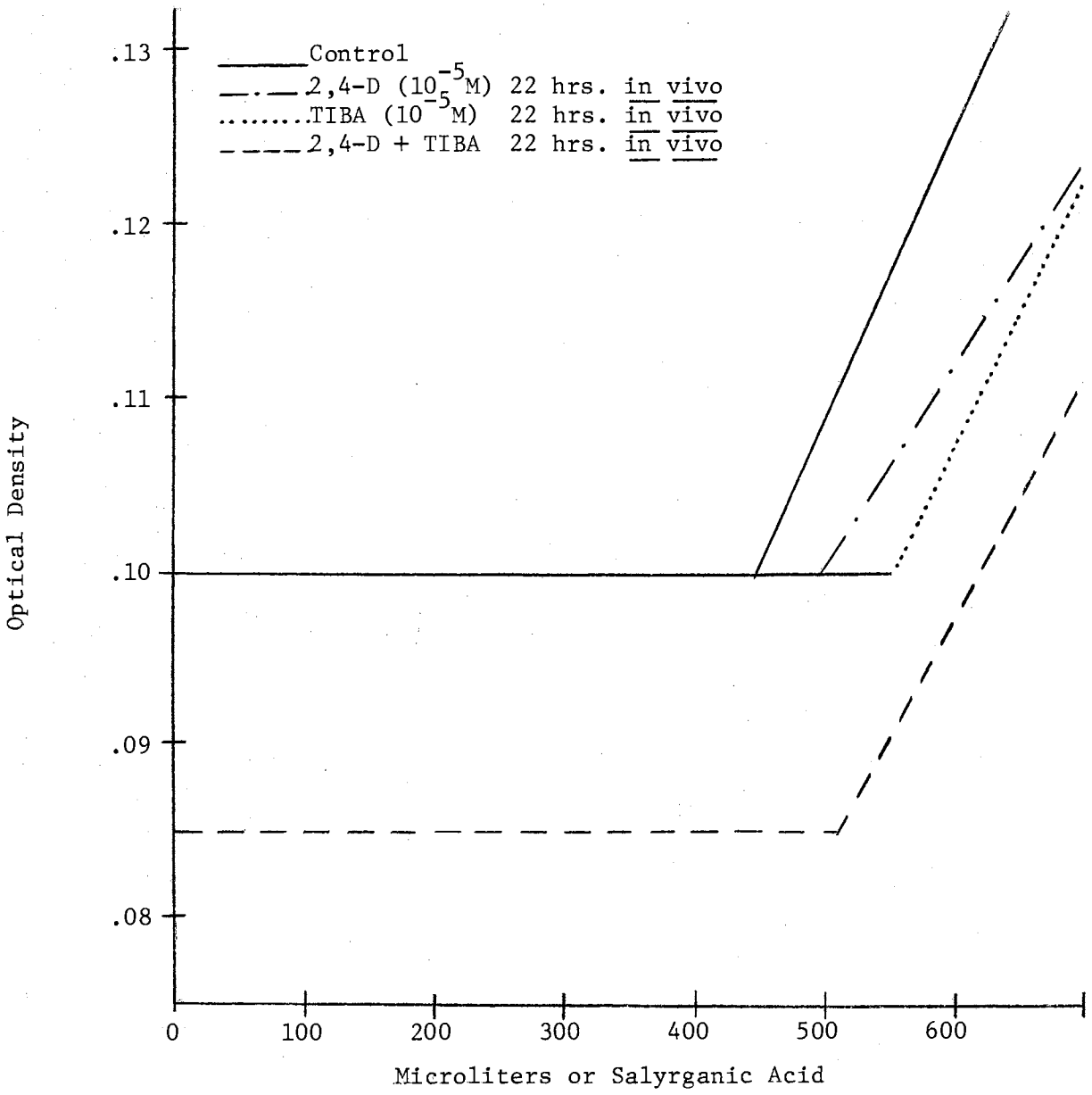

Figure 7. SH content of homogenates after treatment with 2,4-D and TIBA.

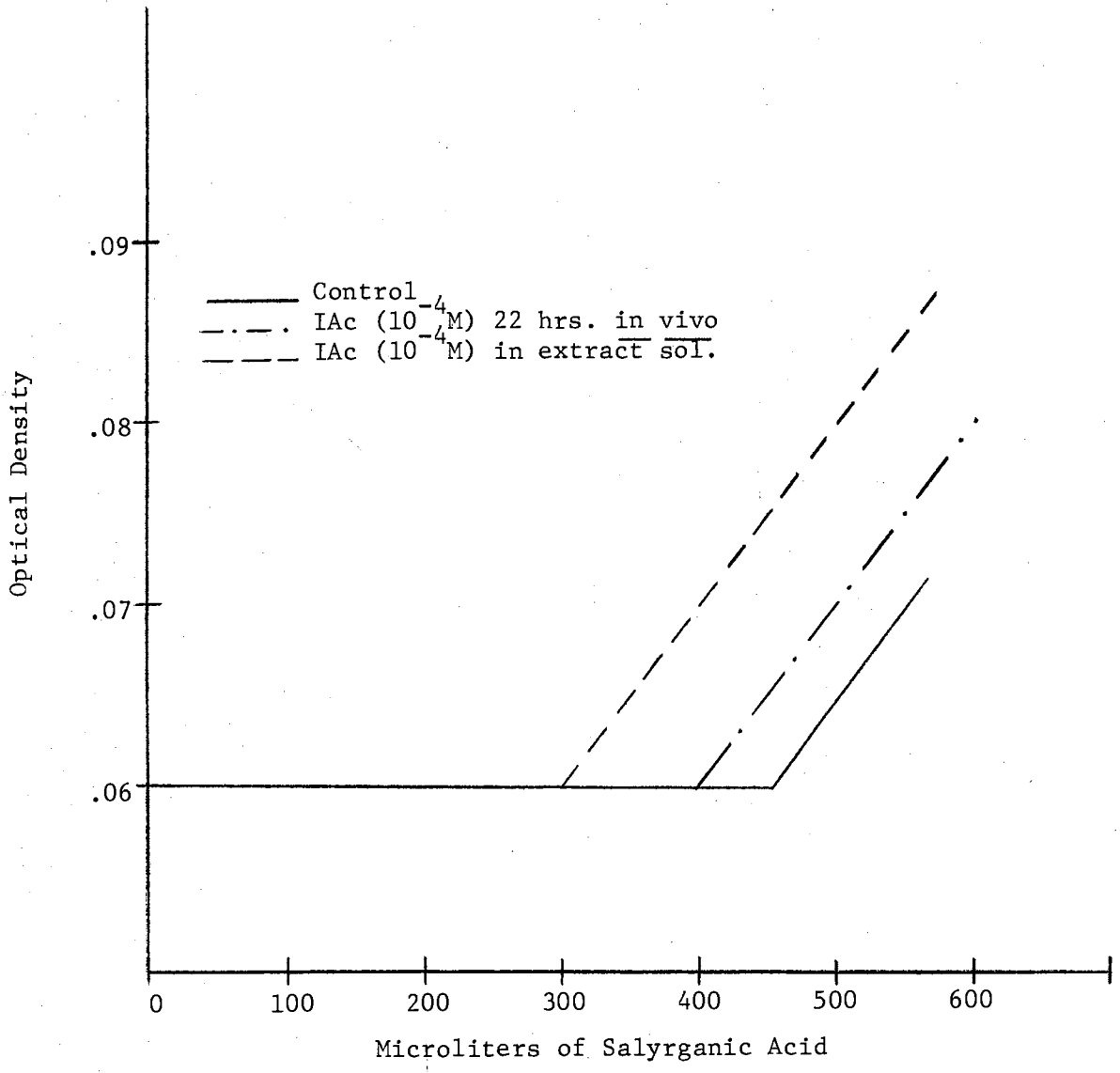

Figure 8. SH content of homogenates after treatment with iodoacetate, *in vivo* and *in vitro*.

Figure 7 shows the results which reverse the trends evident in the TIBA treatments of Figure 6, in that all applications of it in this case show increased amounts of sulfhydryl material in the homogenates. 2,4-D (10^{-5}M) is again effective in increasing the amount of sulfhydryl material relative to the control.

Finally in Figure 8 are presented the results of the experiments in which a known sulfhydryl blocking agent, iodoacetate, was applied to the sections and their homogenates. From these data it is evident that iodoacetate does remove or tie up the sulfhydryl material; whether it is applied in vivo or in vitro does not seem to matter, but the presence of this chemical in the extract solution reduces the sulfhydryl content below the other treatments.

Other experiments revealed no consistent trends which would relate an effect of any chemical employed, except 2,4-D and iodoacetate, to sulfhydryl content. On the basis of these data no definite conclusions can be made about the effectiveness of 2,4-D, TIBA, GSH or iodoacetate in enhancing or reducing the sulfhydryl content of sections or homogenates to which they have been applied.

CHAPTER V

SUMMARY

Investigations were conducted to determine the effects of 2,4-dichlorophenoxyacetic acid, oxidized and reduced glutathione, urea, triiodobenzoic acid and iodoacetate on some of the cytoplasmic constituents of etiolated pea epicotyl sections. In some cases correlation with growth effects of the chemical were made.

2,4-D was found to stimulate increases in fresh weight of sections under culture conditions and a typical auxin growth curve was obtained with a maximum response at 10^{-5}M concentration. Determinations of changes in the heat coagulation response by turbidity measurements of the supernatant (homogenate after 24,000 X G) showed a general decrease in the amount of coagulum present in samples treated with concentrations between 10^{-7}M and 10^{-4}M. These decreases were not exhibited in extracts treated with 2,4-D *in vitro*. Protein determinations on the 16,700 X G supernatant with the Folin phenol method showed that lower concentrations of 2,4-D were generally effective in increasing the reactable material present in the sections, while the higher concentrations caused a decline in amount of reactable material below the control level. Total protein (TCA precipitable) determinations on the samples indicated no differences. It appears then that the changes being reported are in the amount of free tyrosine or other reactants and not total protein. Soluble pectin determinations indicated some changes in response to 2,4-D

concentrations below 5 X 10^{-6}M, with a sharp decline in amounts at higher treatment concentrations. These appeared at nearly the same concentrations as those which stimulated amino acid increases discussed above. Only slight changes in the sulfhydryl content were measured in response to 2,4-D treatment and indicated no consistent trends.

Treatment with glutathione showed no effect on fresh weight increases in either its oxidized or reduced form, and no consistent effect was noted in decreasing heat coagulation, as measured by turbidity methods. Folin phenol determination of the reactable material in these sections indicated no consistent changes in content due to glutathione alone or in combination with 2,4-D. No effect was observed on the pectin content or the sulfhydryl content due to glutathione treatment.

Urea showed no effect on heat coagulation of proteins as measured by turbidity methods, when administered in vitro, but a slight effect of decreasing the heat coagulable protein in vivo was noted at concentrations between 10^{-3}M and 10^{-5}M.

Determinations of sulfhydryl content of the homogenates were made after treatment with TIBA, IAc, 2,4-D and GSH with little significant result. No consistent changes were measured in the sulfhydryl content due to these chemicals, except that the sulfhydryl blocking chemicals IAc and TIBA may act to reduce the amount of SH in the extracts treated in vitro.

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VITA

Leland L. Hardman

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID AND OTHER CHEMICALS ON THE PROTEIN, PECTIN AND SULFHYDRYL CONTENT OF EPICOTYL SECTIONS OF PEA, AS RELATED TO GROWTH.

Major Field: Botany and Plant Pathology

Biographical:

Personal Data: Born in Hot Springs, South Dakota, October 21, 1939, the son of Charles R. and Mildred L. Hardman.

Education: Attended grade school in Igloo, South Dakota; graduated from Provo High School, Igloo, South Dakota in May, 1957; received the Bachelor of Science in Education degree from the Nebraska State Teachers College, Chadron, Nebraska, with majors in biology and chemistry in May, 1961; completed requirements for the Master of Science degree in August, 1965.

Professional Experience: Undergraduate laboratory teaching assistant in Biology at Nebraska State Teachers College, Chadron, Nebraska, for the academic year 1960-1961; full time laboratory instructor at Nebraska State Teachers College from May 1961 - June 1962; graduate teaching and research assistant in Department of Botany and Plant Pathology, Oklahoma State University, Sept. 1962 - May 1965.

Professional Organizations: Associate member of the Society of the Sigma Xi and Oklahoma Academy of Science.