SOME METABOLIC EFFECTS OF D-CAMPHORSULFONIC ACID IN HIGHER-PLANT TISSUES

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INTRODUCTORY REMARKS

Research in the field of plant growth regulators has, during the past twenty-five years, yielded much information concerning the properties of compounds which possess physiological activity. The behavior of a wide variety of plants and plant tissues under the influence of regulators has been studied in some detail, but generally from the point of view of growth. In recent years, the development of synthetic compounds having a high degree of activity for use in controlling plant growth has been possible through application of fundamental knowledge gained by systematic research in growth-regulator pharmacology. The use of growth-active substances as agricultural chemicals is a comparatively recent development. Much fundamental work remains to be done in elucidating the metabolic processes of plant growth and their relation to growth-active substances. Research on the action of synthetic growth substances has shed light on many influences which the compounds can exert on growth rate, respiration, water-balance, cell division, uptake of mineral nutrients, membrane permeability and other phenomena associated with living plants.

Intermediary metabolism in plants has received intensive study only in recent years. Along with this

development has arisen a new interest in the mechanism of the action of substances which influence plant growth. Results to date in demonstrating this mechanism of action of any type of natural or synthetic growth substance has been fragmentary and singularly unrewarding considering the amount of effort expended in this work.

Establishment of a sound theoretical basis for controlling plant growth can come only after a thorough investigation of metabolic interactions underlying growth regulator action. Most synthetic growth substances have been recognized through their similarity to auxins of natural origin. Activity of herbicides such as 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chloro-phenoxyacetic acid have been discovered through routine screening of large numbers of organic chemicals for their effects on plant growth.

In the course of an Oregon State Agricultural Experiment Station project concerned with control of Elodea densa, nearly 1000 organic chemicals were tested for toxic or inhibitory action in attempts to find an effective control agent for this plant (17; 18). It was hoped that a compound could be found which would control growth of Elodea without injuring fish or other organisms in lakes. A control chemical with specific auxin - like

activity would be most desirable.

Early screening operations indicated that d-10-camphorsulfonic acid (hereinafter CSA) had some regulatory activity in E. densa.[1] In high concentrations, growth was little affected or slightly retarded, but at lower concentrations (ca. 25 parts per million) rapid budding and growth were stimulated. Other reports indicated that camphorsulfonic acid (CSA) might exert a regulatory action in bean seedlings.[2]

The writer's early participation in the weed control project involved a study of ecological factors favoring the growth of E.densa in Siltcoos Lake, Oregon. At the conclusion of this phase of the writer's investigations it was suggested to him that a more detailed study of the properties of CSA might bring to light useful information concerning its chemical activity as a control. The preliminary studies were begun which led to the investigations outlined in this thesis.

This study of CSA began with screening tests for its activity by means of conventional assay procedures using the responses of oat (Avena sativa) coleoptile sections. Growth studies were also made with Elodea densa. Known growth regulators were used in these studies and the

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[1]Bond, Carl E. Personal communication.

[2]Kraus, E. J. Personal communication.

action of CSA compared with them. When it was discovered that CSA had activity similar but not identical with that of conventional growth regulators an investigation of the metabolic effects of CSA was undertaken to discover whether or not these were similar to the metabolic effects of natural growth substances (auxins). The original studies were made with Elodea, but for greater precision of measurement later studies were performed with more uniform biological material. Because the preliminary studies indicated an auxin-like activity by CSA subsequent work was designed to elucidate its effects on respiration and phosphate uptake. Respiration studies were made in an attempt to study the effects of CSA on respiration of potato tuber tissue. Further work was done with radioactive phosphorus in a study of the effects of CSA on phosphate uptake and incorporation in potato tuber tissue and in Elodea.

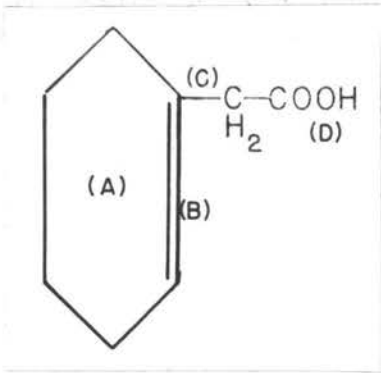
LITERATURE REVIEW

A plant growth regulator, or auxin, has been defined as "an organic substance which promotes growth (i.e., irreversible increase in volume) along the longitudinal axis, when applied in low concentrations to shoots of plants freed as far as is practical from their own inherent growth-promoting substance. Auxins may, and generally do, have other properties, but this one is critical." (36, p.4). This definition covers the large group of compounds, both natural and synthetic, which exhibit growth-stimulatory properties in plants. Substances produced naturally in plants which control growth or other physiological functions have been termed "phytohormones" by Went and Thimann (46). These substances in common with their auxinal analogs, exert their effects at a site removed from their places of production within the plant and are active in minute amounts.

Koepfli, Thimann and Went (20, p. 179) published in 1937 the minimum structural requirements for a growth-active substance. These, briefly enumerated, were:

1. A ring system in the nucleus of the molecule (A).
2. At least one double-bond in the ring (B).
3. A side-chain in the ring (C).
4. A carboxyl group (or an equivalent precursor) at least one carbon atom removed from the ring on the side chain (D).

5. There must be a definite space-relationship between the ring and the side chain. The cis forms of tested compounds were the active ones.



I.

Example (I) above illustrates these basic properties. While these five characteristics seemed to describe the naturally occurring auxins, other compounds were found which did not exhibit growth-activity although they met the prescribed structural requirements. Still others were found which did not meet all of the requirements but showed some degree of auxinal activity.

Veldstra (40, p.145) condensed the five criteria into two broader ones:

1. There must be a basal ring system having high surface activity.
2. There must be a carboxyl group (or its dipole) in a definite spatial relationship with the ring, i.e., out of its plane.

Further work with compounds falling outside either of the above classifications led Veldstra and Booij (42, p.309) to redefine the criteria more specifically:

1. "A basal ring system with a high interface activity."

2. "A carboxyl group (polar part), in general a group of acidic character, in such a spatial position with respect to the ring system, that on adsorption of the active molecule to a boundary (the nonpolar part playing the most important role), this functional group will be situated as peripherally as possible."

Skoog et al. (35, p.573) considered auxin activity to depend upon a specific structural configuration in the molecule to enable it to occupy a definite position in the large enzyme aggregate with which it was thought to combine. A further requirement for auxins was postulated by them to be the possession of a specific, chemically reactive group, or polar part. They suggested that auxin acted in a manner similar to that of a coenzyme. (35, p. 573). No definite knowledge is available at present to indicate that auxins act as coenzymes or coenzyme precursors (33, p.383; 42, p.308).

It is generally conceded that an auxin must consist of a polar side-chain and a ring-system. The shape and area of the ring has been found to govern the degree of growth activity exhibited by auxins. Veldstra states that this is explained by changes in the hydrophil/lipophil balance between the polar and non-polar parts of the molecule (41, p.161).

The effects of auxins on plant cells are numerous. This has caused confusion as to the exact mechanism of auxin action. Such diverse processes as cell elongation, cell division, and fruit set are all affected by auxins.

(36, p.51). This has led to the adoption by many workers of the view that auxin exerts its primary effects through a "master reaction" whose pathway is determined by the particular biochemical environment prevailing at the time of action (15, p.227).

The mechanism of auxin action has received much attention in investigations of the relationship between respiration and auxin-induced growth. That auxin-induced growth is intimately linked with aerobic respiration was recognized early by Bonner (5, p.71; 6, p.10). An increase in growth of plant tissues caused by auxin is generally accompanied by an increase in respiration rate. This is not a universal observation, however. Under suitable conditions, auxin-induced growth response need not be accompanied by a respiratory increase (8, p.435).

Mitochondria isolated from young bean seedlings and treated with indole-3-acetic acid (IAA) show no increase in oxygen uptake during the oxidation of pyruvate to CO_2 and water (9, p.80). Pre-treatment of the bean seedlings with IAA does not cause increased respiration in mitochondrial suspensions prepared from them. (loc.cit.) This indicates that an intact cell-system is necessary for auxin to elicit respiratory responses. The implication of these findings is that auxin does not act upon a respiratory enzyme or a system of respiratory enzymes directly, but that other cell components mediate the

steps between auxin and the ultimate effects of its action. That phosphate transfer may be involved in the action of auxin has been considered possible. (9, p.82). Phosphate transfers are important links in oxidative metabolism, in so-called "high energy" phosphate bonds which involve transfer of energy via adenosinetriphosphate (ATP). The synthesis of this latter substance is normally coupled to respiratory oxidation through phosphorylations which occur at numerous places along metabolic pathways within a cell.

Additional evidence for an auxinal role in aerobic metabolism is found in experiments with "active" water uptake in plant tissues. It has been reported that potato tuber slices immersed in water containing small amounts of auxin take up and retain water in amounts greater than those of potato slices immersed in water alone (11, p.684; 23, p.274; 28, p.825). If oxygen is excluded from these tissues, depressing aerobic respiration, water-uptake rates are diminished markedly. Active uptake is the accumulation of water in amounts greater than that caused by normal osmosis. This supra-osmotic accumulation of water or salts requires the expenditure of metabolic work, and the relation of auxin to the performance of this work must logically be through control of reactions which utilize metabolic energy.

The use of respiratory inhibitors has been instru-

mental in shedding light on the relationships between growth and respiration. Bonner found that 2,4-dinitrophenol (DNP) could inhibit growth in the Avena coleoptile without exerting any appreciable effect on respiration rate (7, p.331). In appropriate concentrations, DNP was found by him to remove the ability of the tissues to grow or respire more rapidly in response to auxin. Dinitrophenol prevents the formation of organic phosphate compounds in living cells, and its mechanism is thought to consist in uncoupling oxidation from phosphorylation (25, 809). In proper concentrations, the compound will inhibit the synthesis of ATP. Reactions which require ATP-energy and which therefore depend upon respiration as a source of ATP are inhibited by DNP. When DNP is applied to tissues in very low concentrations (ca. 10^{-5} Molar) the respiration rate often rises in response to the uncoupling of respiration from the synthesis of ATP (14, p.664).

Robertson et al. (30, p.260) reported that salt-uptake in carrot slices could be inhibited by DNP in concentrations which also raised the endogenous respiration rate. Hackett and Thimann found that DNP inhibits active water uptake by potato slices (16, p.556). The significance of these observations lies in the fact that ATP is required as a driving-agent for active uptake of both salts and water. The fact that auxins fail to stimulate respiration in DNP-inhibited tissues suggests that auxins

are active through that part of the system which utilizes the ATP formed during oxidative respiration. The dependence of auxin activity on an intact phosphorylative system indicates that auxins may act in coupling respiration (or more particularly the high-energy phosphates produced by respiration) to active uptake and growth.

The term "auxin" used in the foregoing discussion has been within the framework for the definitions of active substances laid down by principal workers in the field and outlined in the opening paragraphs of this review.

No substances with auxin activity have been found which lack a double bond in the ring nucleus (41, p.161). Camphorsulfonic acid and compounds structurally related to it have received virtually no attention in respect to their possible possession of growth activity. The only report, made in 1946 by Thompson et al. (37, p.491), listed camphoroxime as having a definite inhibitory action on the elongation of internodes of young kidney bean plants when applied to the stems in oil emulsion. The same compound was also found to have a slight stimulatory effect on elongation of the primary roots of germinating corn seedlings. These tests were made as a part of a large screening program involving more than 1000 organic compounds conducted by the Chemical Warfare Service.

Changing the carboxylic (-COOH) group on the side-chains of active compounds to the sulfonic ($-SO_3H$) group

often does not remove activity, but may change the degree of activity (41, loc cit.). Sulfonic acid analogs of many growth substances have been prepared and assayed for activity. Indole-3-methanesulfonic acid (41, p.162) is an example of an active sulfonic acid analog. On the other hand, naphthalene-1-methanesulfonic acid is inactive in some assay methods for auxin activity. Bonner and Thimann (10, p.70) reported that 1-amino-2-naphthol-4-sulfonic acid stimulated growth of Avena coleoptiles.

The physiological activity of any compound is predicated upon its ability to gain access to susceptible sites of action within the cell. Selective permeability of the plasma membrane regulates the entry of various ionic and molecular species, and this factor must be taken into account when considering the properties of an active molecule. Naturally-occurring auxins and certain synthetic growth-substances are predominantly acidic in nature, as evidenced by their pKa values, which range from 3 to 5 (41, p.192). At physiological pH values, depending upon the pKa value of a given growth substance, the active compound may be present in solution mostly as an anion (32, p.188). Since the permeability of plasma membranes is generally lower to ionic radicals than to undissociated molecules, the state of dissociation of a growth substance may control the amount entering the cell.

The acidity of sulfonic derivatives of auxins is generally higher than that of carboxyl forms, rendering them more fully dissociated at physiological pH values (41, p.162). This is held by some to be strong evidence that activity of the sulfonic derivatives resides entirely within the anionic forms (op cit, p.192).

In order to produce a compound which does not ionize freely, an acid may be converted to its corresponding ester, amide or nitrile. This has been done with some growth substances in attempts to minimize problems associated with penetration of cell membranes by anionic forms. The activity of esters and amides of growth substances has been investigated by Kögl and Kostermans (22, p.211) who found that the ethyl and methyl esters of IAA were active. They attributed this to partial hydrolysis inside the cell. In a series of homologous esters, activity decreased with increasing length of the alcohol chains.

Estimation of the activity of auxins has been the subject of intensive study for many years. A biological assay method is mandatory for auxins because of the unique nature of their activity. Went (43; 44) developed a method utilizing the curvature of young Avena coleoptiles resulting from application of the test substances in a small agar block assymetrically around the end of a decapitated coleoptile. The curvature of the coleoptile is

directly related to concentration of auxin in the applied block. This method requires careful control of all procedures, from the selection of a pure line of oat seed, continuing through the germination and preparation of the seedlings, to the actual test, which must be done under carefully regulated conditions of humidity and temperature.

Another method for testing auxin activity is measurement of the elongation of sections of decapitated oat coleoptiles (31; 36). In this method, elongation is approximately proportional to the logarithm of auxin concentration in the solution in which the coleoptile sections are placed. Oat seedlings are prepared in much the same manner as that for the curvature test by decapitation to remove the tips in which auxin is produced. The central primary leaf is removed from the coleoptiles and short (2 to 5 mm.) sections of the outer cylinder are cut and placed on the surface of solutions containing test compounds. Several sections (from 5 to 10 or more) are placed on the teeth of fine combs or threaded on fine glass needles and the group of sections floated on top of the test solutions so that some part of the sections breaks the surface of the solutions, to allow access to atmospheric oxygen. After suitable lengths of time, usually 70 to 90 hours, the increase in length of the sections is measured. It has been discovered (31, p.268) that a low concentration of sucrose in the test solutions allows more

elongation than is possible in water. For this reason, sucrose is usually added in 1 to 2% dilution to test solutions for straight-growth assays.

The curvature of slit plant organs has been used for assaying auxins, and this method permits less rigorously controlled experimental conditions (45, p.547-555). The internodes of young, etiolated pea stems if slit lengthwise and immersed in auxin solutions, were found by Went (op. cit.) to curve inward toward each other. This curvature was found to be more nearly proportional to the logarithm of auxin concentration than to the concentration itself. Other plant organs that have been used in a similar manner are slit dandelion flowerstalks (19), Helianthus hypocotyls (12) and slit coleoptiles of oats and corn. When such tissues are placed in water alone, the slit halves curve outward due to tissue tension. When the water contains auxin, the ends of the slit halves curve inward toward each other. The curvature may amount to a tight inrolling under unusually favorable conditions of auxin concentration, pH, osmotic pressure and temperature. Any single growth-active compound will not always elicit similar degrees of response from different tissues or in different techniques for auxin assay. For this reason, more than a single type of assay is usually made when testing a compound for growth-activity.

With reference to the objective of this thesis, it is interesting to note the results of some experiments conducted by King (20, p.127-151) on the effects of growth-regulating substances in Elodea densa. Additions of such compounds as IAA, indole-3-propionic acid (IPA), indole-3-butyric acid (IBA), alphanaphthaleneacetic acid (NAA), thiamine, l-histidine, l-tryptophane and ascorbic acid to cultures of this plant growing in nutrient solutions caused varying degrees of shoot and root response.

Root stimulation was shown by IPA at 1 ppm. and by others of the indole series in higher concentrations. Elongation of budding stem segments was stimulated by concentrations of NAA to 5 ppm. and by indolyl compounds in higher concentrations. Compounds of the indole type and NAA caused the formation of root hairs. Normally, Elodea does not form root hairs unless it is anchored in a substratum (27).

King (20, p.147) found that thiamine, histidine and tryptophane inhibited shoot elongation in illuminated cultures. Calcium pantothenate was also inhibitory to shoot elongation in King's study.

The use of Elodea as a test plant in auxin studies is convenient and may be somewhat comparable to methods utilizing the elongation of oat coleoptiles or pea stem internode curvature. King suggested that the root-hair response of Elodea would appear to have value as a diagnostic

method for classifying and characterizing certain of the growth-regulating substances (20, p.150).

MATERIALS AND METHODS

EXPERIMENTAL

Several experimental approaches were used to obtain a generalized picture of some effects of CSA in Elodea densa and in potato tuber tissue.

Investigation into the nature of CSA activity included straight growth measurements of Avena coleoptile and pea stem curvature tests. Phosphate uptake experiments employing radioactive phosphorus were performed to determine the effects of CSA on the uptake of phosphate and its incorporation into organic metabolites. Paper chromatographic techniques were used to identify organic phosphate esters.

Further investigation involved a study of the effects of CSA on respiration of potato tuber tissue. In conjunction with this, 2,4-dinitrophenol (DNP) was used to determine the interaction between the inhibition produced by this compound and the respiratory effects of CSA. Auxin-stimulation of respiration and its interaction with CSA were also investigated.

Some experimental work was also done utilizing the ethyl ester of CSA.

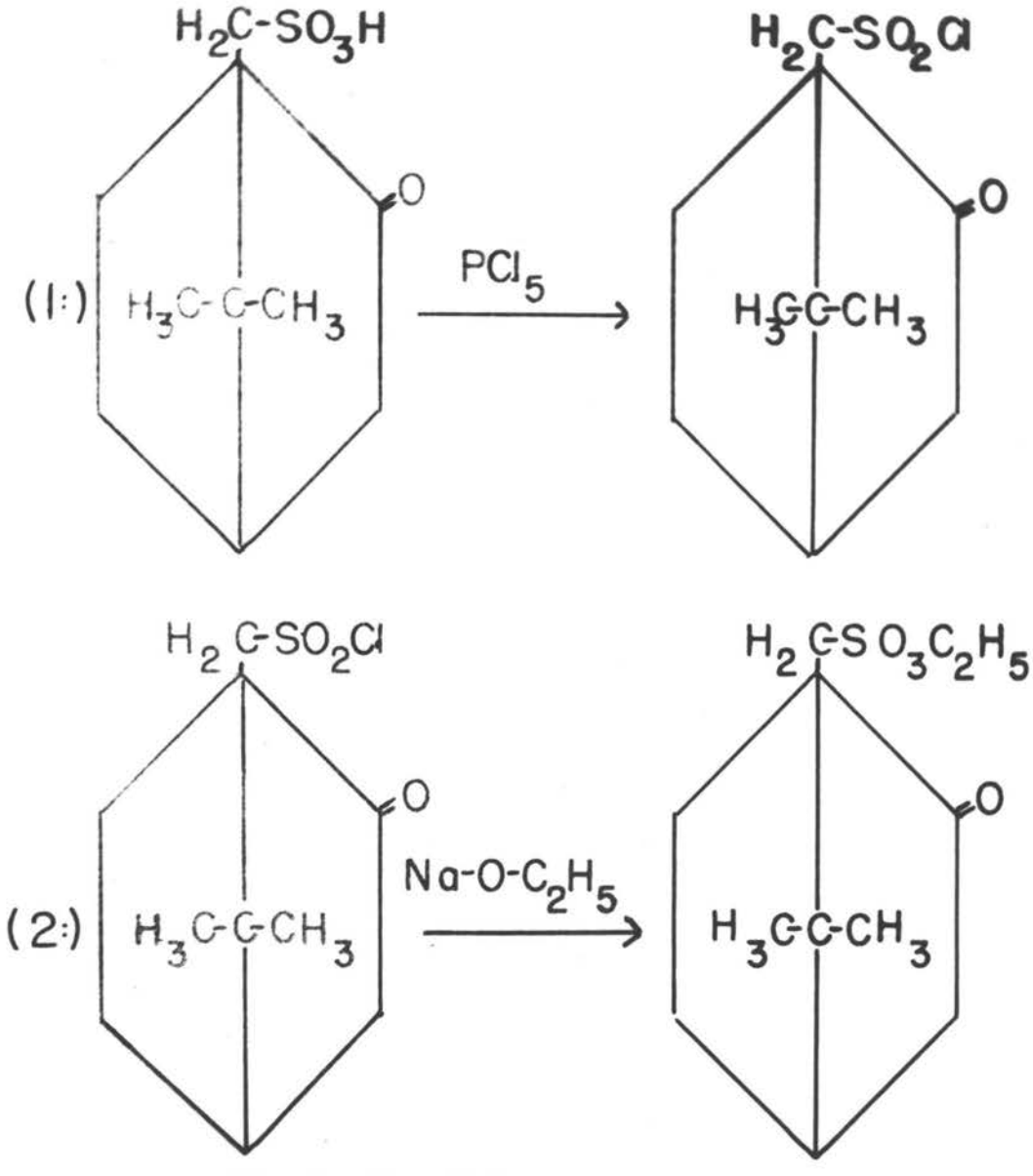
METHODS

1. Synthesis of ethyl camphorsulfonate.

The ethyl ester of d-camphorsulfonic acid (Et-CSA) was prepared from the free acid in two steps. The acid chloride was made from camphorsulfonic acid according to the method of Reychler (29, p.124). This involves conversion of CSA to the acid-chloride with PCl_5. The ethyl ester was then prepared by reacting the acid chloride with sodium ethoxide by a procedure reported by Edminson and Hilditch (13, p. 228). The two steps of the synthesis of Et-CSA are diagrammed in Plate I.

To prepare the acid chloride, equimolar quantities of d-10-camphorsulfonic acid[3] and PCl_5 are weighed out and transferred to a large mortar. Upon trituration, the two solid substances change rapidly to a creamy paste. This is accompanied by the evolution of HCl fumes and heat. A well-ventilated hood is necessary for this operation. After grinding the mixture to a uniform consistency, the mortar is covered with a glass plate and the mixture allowed to stand for one to two hours. The mass is then poured into a large volume of ice water, whereupon the acid chloride separates out as a solid, waxy mass. This is filtered off

[3]Eastman white label #63.



SYNTHESIS OF ETHYL CAMPHORSULFONATE

Plate I

and dissolved in C.P. ethyl ether. The resulting solution is shaken with anhydrous $CaCl_2$ to remove water. After allowing the solution to stand over the $CaCl_2$ for an hour, the solids are removed by filtration and the ethereal solution evaporated to dryness under vacuum at room temperature.

The acid chloride of CSA thus obtained is a waxy solid, melting without decomposition at 67-68°C. It is insoluble in water, slightly soluble in petroleum ether and readily soluble in ethyl ether. The compound decomposes slowly when left in contact with air at room temperature, so it is prepared in quantities which will be used within a short time.

To prepare the CSA ethyl ester, equimolar quantities of sodium ethoxide (freshly prepared by the action of metallic sodium on anhydrous ethanol) and camphorsulfonyl chloride are placed in an 8" Pyrex test tube and the mixture is heated gently in a steam or hot-water bath. The esterification goes rapidly to completion, evidenced by the thickening of the mixture in the test tube. The reaction mixture is poured into ice water. The ester solidifies and is filtered out, collected, and recrystallized from 80% aqueous methanol. After recrystallization, the ester is dried in a vacuum dessicator over $CaCl_2$. The Et-CSA thus prepared has a molecular weight of 260.29. At a concentration of 5% in chloroform it has an optical

rotation of +43.9 degrees at 23°C. It is insoluble in water and must be applied in an emulsion. "Triton X-100"[4] in 0.05% concentration was found satisfactory for use in emulsifying Et-CSA.

2. Avena coleoptile elongation assays.

Oats of the "Gray" variety were hulled and placed in distilled water to soak for two hours. The seeds were then transferred to moist filter-paper pads in Petri-plate halves in lots of 50 to 65 seeds per dish. The plates were then placed in a large polystyrene dish with a tight-fitting lid. In the dish was also placed an open container holding a saturated solution of $Na_2CO_3 \cdot 10H_2O$ to maintain a constant humidity of about 89% within the dish. The cover was placed on the large plastic dish which was then placed in an incubator maintained at 25°C. No illumination was provided in the incubator. The condition of the germinating seeds was checked daily and water added if necessary to maintain moistness of the filter paper pads. After the coleoptiles reached an average length of 3 cm., requiring about 72 hours, the plastic dish was removed from the incubator in subdued illumination and the seedlings subsequently handled in weak red light from a photographic safelight.

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[4]This compound, an alkylaryl polyether alcohol, is marketed by the Rohm and Haas Company.

The coleoptiles were then decapitated at a distance of 1.0 mm. from the apex and allowed to remain in weak red light for three hours. The coleoptiles were then redecapitated to remove the physiologically-regenerated tip end. After 30 minutes the coleoptiles were cut carefully with stainless-steel razor blades spaced 5 mm. apart with washers. An average of three sections was cut from each seedling. The sections were threaded on fine glass needles, the central primary leaf tissue being pushed out of the outer cylinder at this time. Ten coleoptile sections were placed upon a single glass needle and the assembly floated on the surface of test solutions in Petri plates containing 10.0 ml. of solution per plate.

Test solutions were made up containing CSA at .0001 M. (23.2 ppm.) and .001 M. (232 ppm.), Et-CSA at .0001 M. (26 ppm.) and .001 M. (260 ppm.), and IAA 1 ppm. (.000057 M). Solutions containing both CSA and IAA were prepared as follows: IAA 1 ppm. and CSA .0001 M., IAA 1 ppm. and CSA .001 M., IAA 1 ppm. and Et-CSA .0001 M., IAA 1 ppm. and Et-CSA .001 M. All solutions were made up in 1% sucrose solution and brought to pH 6.0 before use to prevent acidity from releasing bound auxin in the tissues.

3. Pea tests.

Assays using split stem segments of etiolated pea seedlings were carried out according to the general

instructions of Went (45). Pea seeds of the "Tall Telephone" variety were soaked in shallow water for several hours before transferring to glass dishes containing moist "Vermiculite". The dishes were placed in a dark cupboard and the seeds allowed to germinate and grow to a length ranging from 5 to 20 cm. Seedlings of uniform height and diameter were selected and cut off 5 mm. below their terminal buds. Five-centimeter lengths were then cut off from the apical end of the stem and a median slit was made with a stainless-steel razor blade for approximately 3 cm. from the distal end. The slit sections were transferred to 10 ml. of test solutions in Petri plates. The test solutions used for pea assays were the same as those used for Avena elongation tests. The solutions were adjusted to pH 6.0 at the beginning of the test to minimize artifacts resulting from acid-curvature (39, p. 36).

Curvature of slit internodes was obtained within 12 hours after the introduction of the pea stem sections into the active solution. At this time the sections were removed from the solutions, blotted and photographed to record comparisons between the various treatments. Plate II depicts results obtained from such a test.

4. Elodea growth studies.

To determine the gross effects of CSA and Et-CSA on the growth of Elodea densa, liquid culture assays were run

with different compounds to determine the interaction of these compounds with CSA in stimulating or inhibiting growth. Compounds chosen for testing, in addition to CSA and Et-CSA, were among those used by King (24) in his study of the effects of growth-regulators on the growth of E. densa. A satisfactory liquid nutrient medium for growing plants in sand, Vermiculite, or liquid cultures is the modification of Shive's solution known as R5S2 (31, p.243). This is basically a three-salt solution with small amounts of trace elements and iron added. For growing submersed aquatic plants the solution has been used at ½-strength with good success. For a 20-liter volume of solution, the composition of the basic medium is as follows: KH_2PO_4, 12 gm., $Ca(NO_3)_2$, 7 gm., $MgSO_4 \cdot 7H_2O$, 18 gm. To this is added about 0.5 gm. of a chelated iron compound, sodium ferric ethylenediaminetetraacetate, made by the Alrose Chemical corporation. Iron in this form is stable and maintained in the solution several times as long as when added as citrate or tartrate. Trace elements are added to the solution as a mixture of the following salts, of which 20 ml. are added to 20 liters of solution:

H_3BO_3: 0.6 gm./l.
$MnSO_4$: 0.4 gm./l.
$ZnSO_4$: 0.05 gm./l.
$CuSO_4$: .05 gm./l.
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: .024 gm/l.

Besides the CSA and Et-CSA the following compounds were added to the basic mineral nutrient solution as growth-regulators: l-histidine, IAA, biotin, yeast extract tryptophane, thiamine, and adenine sulfate. King (20) found that shoot elongation of Elodea was inhibited slightly in the light by l-tryptophane and l-histidine. Indoleacetic acid and naphthaleneacetic acid stimulated shoot growth (20, pp.146-147). Thiamine, ascorbic acid and l-histidine showed similar mild degrees of inhibition of shoot elongation. These compounds, supplied alone and together with CSA and Et-CSA, were used primarily to show that CSA did not inhibit synthesis of some compounds or enzymes.

Elodea growth assays were made in two different ways. In some experiments, 500 ml. Erlenmeyer flasks containing 200 ml. of nutrient solution were employed. Test compounds were made up in concentrated stock solutions and added in small amounts, so that from 0.1 to 2.0 ml. of solution were used to supply the required concentrations in the culture flasks. Nutrient and test-compound solutions were changed every two days in these experiments. It was found that the yeast-extract and tryptophane-containing cultures became contaminated rapidly with microorganisms, even with a change of nutrient every two days.

A later series of experiments was run using 250 ml. beakers to contain the solutions and plant material. The solutions were presented to the plants in alternation:

nutrient solutions containing the test compounds were supplied to the plants for 10 hours, then the solutions were replaced with mineral nutrient solution alone for 24 hours. This cycle of solution changes was maintained for the duration of the experiments.

Aseptic techniques were not found practicable due to the difficulty of surface-sterilizing Elodea without damaging it. Surface-sterilization with 8-hydroxyquinoline benzoate (and -sulfate) was attempted but these compounds were somewhat toxic to Elodea in bacteriocidal concentrations.

Elodea was obtained for the assays under conditions planned to guard against undue variability in plant material. Plants were harvested at different times from one sampling area in Siltcoos Lake, Oregon, then transported to a large, metal holding tank maintained at the stream-pollution laboratory of the Oregon State College Fish and Game Management Department. As they were needed, plants were removed from the holding tanks and taken to the laboratory where they were placed in R5S2 solution. Under a 14-hour photo-period, rapid budding ensued from bud nodes on the stems. When new shoots were from 5 to 10 centimeters long they were removed, washed in distilled water, and used for growth experiments. A supply of material of uniform age, size, internodal distance and previous physiological history was obtained in this way.

Each culture flask contained four or five apical sections of Elodea. Lengths were recorded at the beginning of each experiment by placing all of the sections from one flask end-to-end along the edge of a meter stick or centimeter ruler. The stems were placed with the proximal end of one section just touching the inner apex of the terminal leaf whorl of the preceding section. This was adopted for all experiments in order to minimize variations in technique. Measurements were made again at the end of nine days.

All growth studies with Elodea were run at room temperature under "cool-white" fluorescent tubes. Intensity of illumination at the surface of culture solutions was 430 footcandles, measured with a Weston footcandle meter. King (20, p.149) found that reasonably good growth was obtained with illumination of approximately this intensity.

Another experiment was performed in which Elodea was placed in a 0.067 M. phosphate buffer of pH 6.7 to which were added under the following conditions:

Treatment:

1. Control. Phosphate buffer alone.
2. Dinitrophenol 0.00001 M.
3. Dinitrophenol 0.00001 M. and CSA .001 M.
4. Camphorsulfonic acid alone, .001 M.

The buffer used in these experiments was made up with boiled tap water. Five-hundred ml. culture volumes were

used with two 10-cm. actively-growing Elodea stem tips per flask. The flasks were placed under fluorescent illumination of 430 fc. intensity for ten days. Eighteen-hour periods of illumination and 6-hour dark periods were used for this experiment. At the end of 10 days the plants were removed from the flasks and photographed in color. The results of this experiment are illustrated in Plate III.

5. Respiration experiments.

In preliminary trials of Elodea in respiratory experiments, difficulty was encountered in obtaining uniform tissues and in getting enough plant material into each flask to yield a useable respiration rate. Conventional Warburg flasks are too small to permit the use of sufficiently large amounts of Elodea tissue. Potato tuber tissue was selected for these experiments because it is uniform and easy to handle. Also it has been much used in the experiments of other workers and its responses to auxins have been studied by previous investigators (2; 16; 24; 28, p.825).

The "direct method" of Warburg was employed in these investigations (38, p.1-16). Equipment available for this work included a constant-temperature bath with an adjustable shaker mechanism which has a capacity of twenty Warburg manometer and flask assemblies. All respiratory experiments were made at 30°C. with a shaking-rate of

approximately 100 oscillations per minute. Double-sidearm Warburg vessels having an average volume of 23 ml. were used. Each flask contained 2.0 ml. of buffer in the main compartment and 0.2 ml. of 5% KOH solution in the center-well.

In some experiments, barbiturate acid NaOH buffers were used, and in others phosphate buffers were employed. Most reported studies involving respiration of auxin-stimulated or DNP-inhibited tissues have involved the use of phosphate buffering systems, but for the purpose of this investigation it was found desirable to compare the respiration of potato slices in phosphate buffer and an inert buffer. Barbiturate buffers of 0.01 M. strength and pH of 5.0 to 5.3 held the pH of the solutions in the vessels within 0.2 pH unit of the specified value during an experimental run, as did phosphate buffers of 0.03 M. strength and pH values ranging from 5.0 to 6.0. Solutions of CSA and other test compounds were made up in buffer and the pH of these solutions adjusted to pH 5.0 before use in the respiratory experiments.

For each experiment, slices were prepared from a single potato tuber by removing plugs of tissue with a sharp cork borer of 8 mm. inside diameter. Immediately after cutting, the plugs were rinsed in distilled water to remove excess starch. Slices 0.5 mm. in thickness were cut from the plugs on a sliding microtome. The slices

were rinsed in distilled water and blotted on filter paper immediately before being placed in the flasks. Ten slices were used in each Warburg flask, giving an average fresh-weight of tissue per flask of 256 mg. Slices were transferred to the flasks immediately instead of being soaked in running water for several hours before use. Barron et al. (2, p.379) suggest that preliminary soaking of potato slices, while causing an increased respiration rate, also brings about undesirable changes in cell membranes and causes the loss of water-soluble coenzymes.

After transferring potato slices to the flasks, which contained the required amount of buffer, the edges of the flask center-cups were greased and 0.2 ml. of 5% KOH solution were added to all cups except that of the thermobarometer. One-inch squares of filter-paper were pleated and inserted into the center cups to provide a large surface for the absorption of CO_2. Each experimental run usually consisted of one thermobarometer and nine test conditions, twice replicated.

The flasks, containing sample, buffer, and KOH solution, were attached to the manometers and the assemblies set in place on the shaker-bath. From 45 to 60 minutes were allowed for equilibration before the manometer stopcocks were closed and readings begun. This permitted equilibration of the Warburg assembly and at least partial equilibrium between the sample and the chemical substances.

6. Tracer phosphate studies.

A. Treatment of plant materials.

Incorporation of radioactive phosphate into potato tuber slices and Elodea apical stem segments was studied by supplying $H_3P^{32}O_4$ to the tissues in 0.03 M. barbiturate buffer of pH 5.1. In all experiments, 10 ppm. of carrier phosphate were added as KH_2PO_4 to furnish a stable source of inorganic phosphate to the tissues. Supplements of CSA or DNP were added to the buffer solutions under three conditions: .0002 M. CSA alone; .0002 M. DNP alone; .0002 M. CSA together with .0002 M. DNP. Control samples were run with all experiments.

The amount of tracer phosphate added to samples was governed by the amount of isotope available at the time the experiment was run. Solutions were made up to a standard volume in buffer, usually 20 ml., and placed in small conical flasks. The tissue samples (potato tuber slices or Elodea stem tips) were then added. In the potato experiments, from 50 to 80 slices were used per flask. When Elodea was used, stem tip segments were blotted and weighed and their proximal ends trimmed until only 1.0 gram of tip tissue remained. After introduction of the tissues the isotope was added, in amounts ranging from 7 to 30 microcuries per flask. In each experiment, all flasks received an equal amount of isotope. The flasks, containing buffer, tissues, isotope, and compounds to be

tested, were then plugged lightly with cotton and placed in a shaker for 9 to 13 hours at room temperature.

B. Extraction of the tissues.

After shaking with the test solutions for a given length of time the tissues were removed from the flasks and washed three times in distilled water to remove external inorganic phosphate. Tissues from each flask were extracted with boiling 80% ethanol and by grinding in a mortar with fine carborundum sand. Grinding and extraction were continued until practically no radioactivity could be detected in the residue. The washings from each sample were made up to 20 ml. with ethanol and centrifuged at 2000 rpm. for 5 minutes to remove suspended matter. The supernatant extracts were then decanted and concentrated to 10.0 ml. in a vacuum dessicator.

To determine the total radio-activity of the samples two 1.0 ml. aliquots of each extract were pipetted into stainless steel cup planchets and dried for counting. The counts obtained from these aliquots were multiplied by 20 to give the total activity in the tissue. The remaining extract was used for chromatography.

C. Chromatographic separation of phosphate esters.

Separation of organic phosphate compounds was accomplished by means of ascending filter paper chromatography with an ammonia-methanol-water solvent described by Bandurski and Axelrod (1, p.406). The composition of the

solvent is: C.P. methanol, 6 parts; 28% ammonia water, 1 part; distilled water, 3 parts. Whatman #1 filter paper sheets were cut into strips 1" wide and 18-3/4" long for use in chromatographic separation of the extracts.

The extracts obtained as outlined above were applied to the filter paper strips in narrow bands approximately 3" from one end, with fine glass pipettes. Sufficient extract was applied to each strip to give a count of 500 to 1000 counts per minute (cpm.).

The chromatogram strips were developed in Pyrex chromatography jars equipped with stainless-steel racks. The strips were placed on the racks so that the extract bands were spaced at a sufficient distance from the solvent troughs to require about 1 hour for the solvent to reach them. In this way, the solvent vapors were able to saturate the atmosphere in the jars before development of the chromatograms began. The jars were covered with glass covers sealed with petrolatum. Development was done in a cold room at 3°C. The chromatography jars had been previously cooled to this temperature before use. About 16 hours were required to obtain a solvent-front migration of 30 cm., the maximum length permitted by the size of the apparatus.

After development, the distance that the solvent had travelled was marked on each strip, after which the strips were removed from the jars. After drying in air at room

temperature the positions of the radioactive fractions in the extracts were determined by counting one-centimeter intervals along the solvent path, starting at the center of the original extract application. Duplicate strips were prepared for all extracts from a single experiment. One of the strips was counted throughout its entire length and the second strip was compared at points showing activity on the original strip. Close agreement was obtained between duplicate strips in all experiments.

By attaching an intact chromatogram strip to a calibrated strip of pressed fiberboard which slides freely in the grooves under the counting window of a Tracerlab lead counting-chamber it is possible to position consecutive one-centimeter sections of the paper strips under the counter-tube. A brass mask with a rectangular window 2.5 x 1.0 cm. is placed over the mica window of the counter tube to delimit the area from which beta-particles reach the tube from the paper strip. Counting was facilitated by a Tracerlab Utility Scaler equipped with a Tracerlab TGC-2 Geiger-Müller tube and a Berkeley timer.

Values of Rf, an expression of the ratio

$$Rf = \frac{\text{distance travelled by a given spot on chromatogram}}{\text{distance travelled by solvent front,}}$$

have been published by Bandurski and Axelrod (1, p.408) for the commonly-occurring biological organic phosphates

when developed on a paper chromatogram with an ammonia-methanol-water solvent system.

As a check on the reproducibility of the published method, known (non-radioactive) phosphate compounds were applied to strips and separated chromatographically in a manner identical to that used for the plant extracts. The strips were treated with a perchloric acid-HCl mixture (3, p.88), heated in an oven to hydrolyze the organic phosphate linkages, and then sprayed with a molybdic acid solution. The strips were air-dried and hung in an atmosphere of H_2S to develop color spots corresponding to the positions of the migrated compounds on the paper strips. The two compounds thus checked, Glucose-1-phosphate and 3-phosphoglyceric acid, showed Rf values close to those published for these compounds, namely, 0.63 and 0.35, respectively (1, p.408). Thus it is likely that the identity of most of the peaks on the plant-extract chromatograms may be determined by referring to the published values.

RESULTS

1. *Avena* coleoptile elongation.

The activity of CSA and Et-CSA in producing auxin-like responses in *Avena* coleoptile sections is summarized in tables I, II, and III. Elongation of the control groups of coleoptile segments receiving no test substances in solution are in close agreement. In the three series, the percentages of elongation are 13.6%, 13.5% and 13.6%. These values represent elongations of groups of ten sections reported as percent increase of the original lengths. There is general agreement among the other treatments in the three series. Maximum elongation of the sections was obtained with simultaneous application of indole-3-acetic acid together with .001 M. CSA or .0001 M. Et-CSA. Ethyl camphorsulfonate applied together with IAA was most effective in stimulating elongation when applied at .0001 M.

When Et-CSA was applied at .001 M., either alone or in combination with IAA, marked inhibition occurred. Ethyl camphorsulfonate at .0001 M. inhibited elongation, but less so than at .001 M.

Camphorsulfonic acid applied alone stimulated elongation in all three series at .0001 M., but at .001 M. it caused slight stimulation in the first two series and strong inhibition in the third.

Indoleacetic acid was able to overcome the inhibition of .0001 M. Et-CSA but not of .001 M. Et-CSA. It increased elongation with .001 M. CSA but with .0001 M. CSA it caused strong inhibition in all series.

Avena coleoptile elongation data show that CSA stimulates elongation at .0001 M. Both forms of CSA are inhibitory at .001 M., but the ester is more inhibitory than the acid at both .0001 M. and .001 M.

Differences in permeability to anionic camphorsulfonate and to the ethyl ester may help explain the differences in activity of the two forms of CSA. Apparently, the ester enters the plant cell more readily than does the ionic form. This is reflected in higher inhibitions resulting from Et-CSA than from the free acid. That CSA is active as an anion is suggested by higher activities resulting from .0001 M. applications which permitted a lower concentration of CSA anions within the tissues. These results are in agreement with the report of Bond concerning the effects of CSA on Elodea densa at high and low concentrations.

Table I

Avena coleoptile section elongation: 84 hours.

Conditions of test.	Initial length.	Final length.	d	% Elongation
Controls	44 mm.	51 mm.	7	13.6
Indoleacetic acid, 1 ppm.	40	47	7	17.5
Camphorsulfonic acid, .0001 M.	35	44	9	25.7
Camphorsulfonic acid, .001 M.	35	40	5	14.3
Ethyl camphorsulfonate, .0001 M.	36	40	4	11.1
Ethyl camphorsulfonate, .001 M.	40	40	0	0
Indoleacetic acid, 1 ppm. plus Camphorsulfonic acid, .0001 M.	40	42	2	5.0
Indoleacetic acid, 1 ppm. plus Camphorsulfonic acid, .001 M.	39	50	11	28.2
Indoleacetic acid, 1 ppm. plus Ethyl camphorsulfonate, .0001 M.	45	60	15	33.3
Indoleacetic acid, 1 ppm. plus Ethyl camphorsulfonate, .001 M.	39	40	1	0.25

Table II

Avena coleoptile section elongation: 90 hours.

Conditions of test.	Initial length.	Final length	d	% Elongation
Controls	44 mm.	50 mm.	6	13.5
Indoleacetic acid, 1 ppm.	42	49	7	16.7
Camphorsulfonic acid, .0001 M.	45	54	9	20.0
Camphorsulfonic acid, .001 M.	43	50	7	16.3
Ethyl camphorsulfonate, .0001 M.	36	38	2	5.5
Ethyl camphorsulfonate, .001 M.	40	41	1	2.5
Indoleacetic acid, 1 ppm. plus Camphorsulfonic acid, .0001 M.	41	44	3	7.3
Indoleacetic acid, 1 ppm. plus Camphorsulfonic acid, .001 M.	40	52	12	30.0
Indoleacetic acid, 1 ppm. plus Ethyl camphorsulfonate, .0001 M.	40	50	10	25.0

Table III

Avena coleoptile section elongation: 84 hours

Conditions of test.	Initial length.	Final length.	d	% Elongation
Controls	44 mm.	50 mm.	6	13.6 mm.
Indoleacetic acid, 1 ppm.	42	50	8	19.0
Camphorsulfonic acid, .0001 M.	45	54	9	20.0
Camphorsulfonic acid, .001 M.	44	47	3	6.8
Ethyl camphorsulfonate, .0001 M.	45	50	5	11.1
Ethyl camphorsulfonate, .001 M.	44	45	1	2.2
Indoleacetic acid, 1 ppm. plus Camphorsulfonic acid, .0001 M.	41	42	1	2.5
Indoleacetic acid, 1 ppm. plus Camphorsulfonic acid, .001 M.	45	58	13	28.9
Indoleacetic acid, 1 ppm. plus Ethyl camphorsulfonate, .0001 M.	44	55	11	25
Indoleacetic acid, 1 ppm. plus Ethyl camphorsulfonate, .001 M.	45	47	2	4.4

2. Curvature of slit pea stem internodes.

Plate II illustrates the responses obtained from slit pea seedlings upon treatment with IAA, CSA and Et-CSA. Stem sections were in contact with the test solutions for 12 hours before being photographed. Five sections were used per test condition, but the two most typical of these were selected for photographing. This was done in the interest of space economy, for the use of five sections from each of the tests would have presented photographic difficulties.

Definite inward curvature of the slit halves of the internodes was caused by IAA alone, as shown by the photograph. Ethyl camphorsulfonate at .0001 M. and .001 M. caused marked straightening of the slit halves, an auxin-like action. When 1 or 2 ppm. of IAA was applied together with Et-CSA the straightening was also evident. Camphorsulfonic acid applied alone at .0001 M. caused a slight recurvature of the slit halves, but no curvature was caused at .001 M.

Compared with the Avena elongation tests, the pea tests are not as instructive in pointing to a relationship between IAA and camphorsulfonate. Ethyl camphorsulfonate seems to have definite activity in the pea test, a property not shown by the free acid. Responses to CSA alone show that .001 M. CSA causes no recurvature nor is inhibitory. At .0001 M. CSA there is a slight

indication of response. Indoleacetic acid applied together with CSA at .0001 M. and .001 M. causes a slight response, but this is not markedly different from the response obtained from CSA alone.

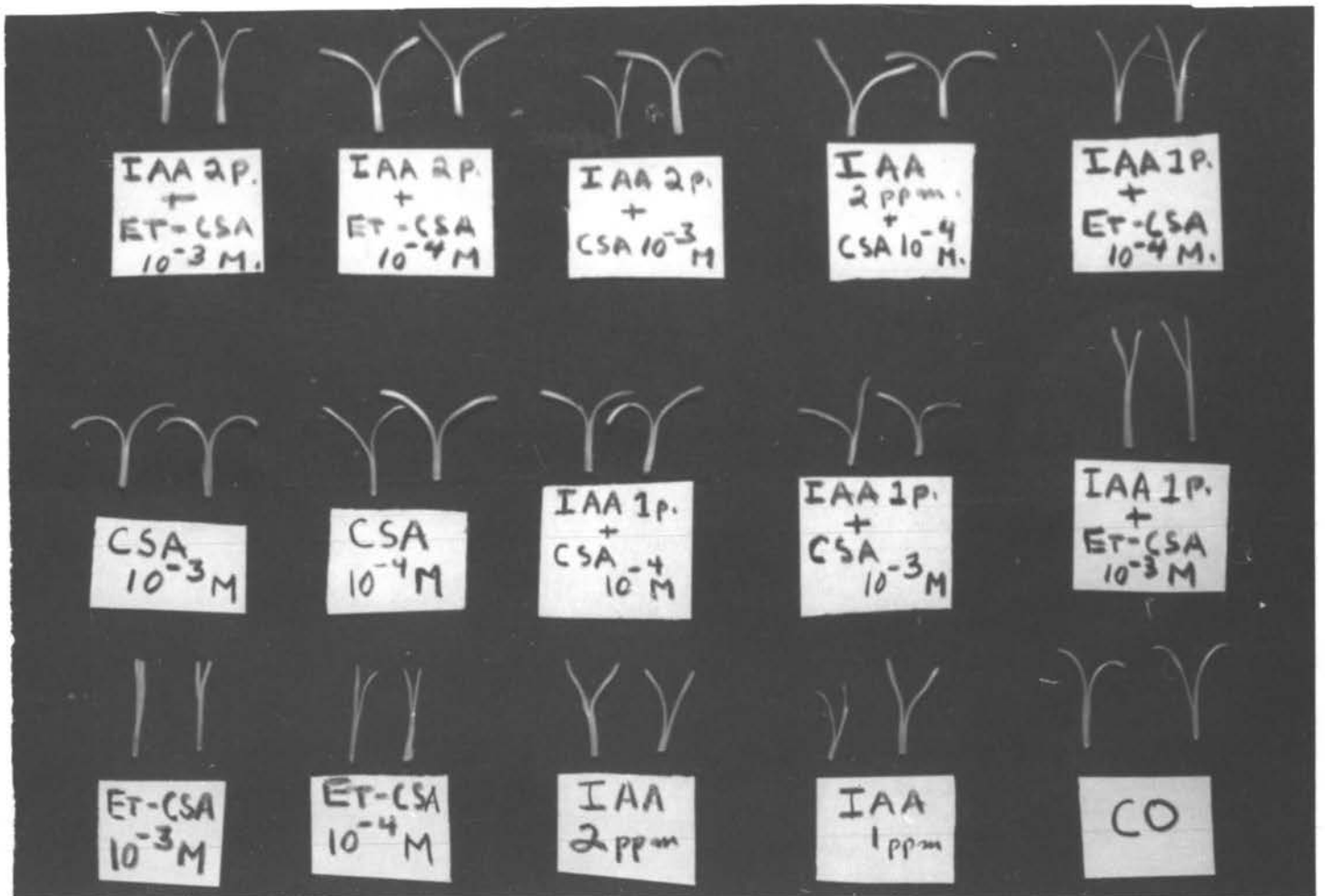


Plate II
Responses of slit pea internodes to IAA, CSA and Et-CSA

3. *Elodea* shoot growth.

Tables IV and V list results of two series of tests intended to determine the responses of *Elodea densa* shoots to .0001 M. CSA and Et-CSA, as well as to a number of other compounds. These compounds were chosen from those reported by King (20) to have activity in stimulating or inhibiting growth of *Elodea* shoots. Results from tests of *Elodea* responses are not as consistent as those from *Avena* elongation tests. The series of Table IV was done with Et-CSA only and the series of Table V was done with CSA and Et-CSA. Both the free acid and its ethyl ester stimulated growth of *Elodea* stem apices, and in combination with indolebutyric acid this activity is heightened. A curious effect was noted in the samples receiving both IBA and camphorsulfonate (Table V) in that after growth had been stimulated there was bleaching of terminal leaf whorls on the test stem sections. In samples receiving IBA alone this effect was noted, and its degree was increased when CSA and Et-CSA were applied with IBA. Ethyl camphorsulfonate aggravated the damage more than the free acid did. As in *Avena*, IAA and .0001 M. Et-CSA in combination stimulated rapid elongation.

With histidine, which itself inhibits growth of *Elodea*, Et-CSA elicits additional signs of damage as evidenced by death of the proximal ends of stem segments and slight chlorosis. Whether this is primarily an effect of

histidine or Et-CSA is difficult to determine from these data. Either compound alone did not exhibit particularly harmful effects.

Biotin caused an apparent inhibition of growth, and Et-CSA caused additional damage symptoms when presented with it.

The apparent inhibition caused by yeast extract in the first series of tests (Table IV) may possibly be attributed to anaerobiosis and ammonia liberation caused by microorganisms which rapidly contaminated the samples containing yeast extract. In the later series of tests (Table V) solutions were changed more often and damage is not so apparent as in the earlier series. The importance of frequent solution changes in growth studies of this kind is apparent when certain compounds are added to the cultures which favor the growth of microorganisms. King (20, p.129, 131, 132, 141) did not change his culture solutions except at varying intervals of 4 to 17 days -- in some instances not at all -- and it is doubtful whether many of his test compounds remained stable in solution for more than two or three days under continued illumination and in the presence of microorganisms.

Tryptophane alone did not stimulate elongation in either of the two series. Tryptophane applied with Et-CSA appeared to inhibit growth in the first series and to stimulate it slightly in the second series.

Adenine sulfate inhibited growth strongly in the second series, and this was partly reversed by CSA and Et-CSA.

The results of this study point to stimulatory activity of CSA and Et-CSA in growth of Elodea stem sections. This activity appears to augment that of IAA and IBA, but to be inhibited by adenine sulfate and histidine. Thiamine and biotin were also inhibitory, but slight reversal of thiamine inhibition was caused by Et-CSA.

Table IV

Elodea shoot growth.
(Solutions changed every two days)

Treatment	Initial length.	9-day length.	Change.
Controls	34.4 cm.	39.8 cm.	5.4 cm.
Histidine, 4 ppm.	22.9	22.7	-0.2
Histidine, 4 ppm. plus Ethyl camphorsulfonate, .0001 M.	23.8	20.5	-3.3
Indoleacetic acid, 4 ppm.	23.9	28.8	4.9
Indoleacetic acid, 4 ppm. plus Ethyl camphorsulfonate, .0001 M.	8.7	23.3	14.6
Biotin, 4 ppm.	22.7	26.5	3.8
Biotin, 4 ppm. plus Ethyl camphorsulfonate, .0001 M.	14.1	11.5	-2.4
Yeast extract, 12 ppm.	22.0	19.6	-2.4
Yeast extract, 12 ppm. plus Ethyl camphorsulfonate, .0001 M.	13.0	18.7	5.7
Tryptophane, 4 ppm.	13.1	14.3	1.2
Tryptophane, 4 ppm. plus Ethyl camphorsulfonate, .0001 M.	16.4	14.0	-2.4
Ethyl camphorsulfonate .0001 M.	17.1	19.3	2.2

Table V

Elodea shoot growth.

Alternating 10-hour periods of test solutions and 24-hour periods of mineral nutrient alone.

Treatment.	Initial length.	9-day length.	Change.
Controls	16.0 cm.	16.5 cm.	0.5 cm.
Camphorsulfonic acid, .0001 M.	15.5	16.5	1.0
Ethyl camphorsulfonate, .0001 M.	15.0	16.0	1.0
Indolebutyric acid, 5 ppm.	19.2	21.5	2.3
Indolebutyric acid, 5 ppm. plus Camphorsulfonic acid, .0001 M.	15.0	18.0	3.0
Indolebutyric acid, 5 ppm. plus Ethyl camphorsulfonate, .0001 M.	19.0	20.5	1.5
Yeast extract, 10 ppm.	18.5	19.0	0.5
Yeast extract, 10 ppm. plus Camphorsulfonic acid, .0001 M.	16.0	17.0	1.0
Yeast extract, 10 ppm. plus Ethyl camphorsulfonate, .0001 M.	18.5	19.5	1.0
Thiamine, 5 ppm.	17.0	17.5	0.5
Thiamine, 5 ppm. plus Camphorsulfonic acid, .0001 M.	15.0	15.5	0.5
Thiamine, 5 ppm. plus Ethyl camphorsulfonate, .0001 M.	18.0	19.0	1.0

Table V, continued

Treatment.	Initial length.	9-day length.	Change
Tryptophane, 5 ppm.	20.0 cm.	20.5 cm.	0.5 cm.
Tryptophane, 5 ppm. plus Camphorsulfohic acid .0001 M.	17.3	18.3	1.0
Tryptophane, 5 ppm. plus Ethyl camphorsulfonate, .0001 M.	17.0	18.0	1.0
Adenine sulfate, 5 ppm.	11.0	11.0	0.0
Adenine sulfate, 5 ppm. plus Camphorsulfonic acid, .0001 M.	12.0	12.3	0.3
Adenine sulfate, 5 ppm. plus Ethyl camphorsulfonate, .001 M.	12.0	12.3	0.3

Plate III illustrates the responses of Elodea stem sections to dinitrophenol and CSA. After ten days in phosphate buffer at pH 6.7, the plants receiving .0001 M. DNP were damaged severely, as evidenced by almost complete disintegration of the tissues. When .001 M. CSA was supplied the stem sections, moderate yellowing of the leaves occurred. Dinitrophenol and CSA supplied together caused little or no changes in the appearance of the stems or leaves. This is an interesting result in the light of respiratory findings to be discussed in a later section.

1

2

3

4

Elodea densa from cultures grown in .067 M. phosphate buffer (pH 6.7) for 10 days under fluorescent illumination.

1. Controls.
2. Dinitrophenol, .00001 M.
3. Dinitrophenol, .00001 M. and camphorsulfonic acid, .001M.
4. Camphorsulfonic acid, .001 M.

Plate III

4. Respiration studies.

Figures 1 through 5 portray graphically the results of experiments involving the effects of CSA on respiration rates of potato tuber slices. The results depicted in the graphs are from single experiments chosen as typical of a series of experiments. A summary of the effects of CSA which were observed is given in the following paragraphs. References to the figures which apply to each statement are given at the end of each item.

1. Camphorsulfonate depresses endogenous respiration rates of potato tuber tissue in an inert (barbiturate) buffer (2-D,E).
2. Camphorsulfonate stimulates endogenous respiration rates of potato tuber tissue in a phosphate buffer or in a barbiturate buffer containing phosphate (1-D,E; 3-B; 5-B).
3. Indoleacetic acid stimulates endogenous respiration rates of potato tuber tissue in a phosphate buffer (1-B)
4. Indoleacetic acid depresses endogenous respiration rates of potato tuber tissue in an inert buffer (2-B).
5. Camphorsulfonate augments IAA in stimulating endogenous respiration rates in a phosphate system (1-C,F).
6. Camphorsulfonate augments the depression of respiration rates by IAA in an inert buffer (2-C,F).
7. When dinitrophenol (DNP) is present in an inert buffer it depresses endogenous respiration rate at .0002 M. and .001 M. (4-B,C).

8. When DNP is inhibiting respiration rate in an inert buffer at .0002 M., CSA reduces the degree of inhibition at .001 M., but less so at .0001 M. (4-E,D).
9. When DNP is inhibiting at .001 M., CSA is relatively ineffective in reversing the inhibition at .001 M. or .0001 M. (3-E; 4-F; 5-E).
10. In a phosphate-containing buffer (pH 5.9), DNP shows marked inhibition of respiration rate at .0002 M. (5-C; 3-C). The degree of respiratory inhibition by DNP at .001 M. does not appear to be as marked in phosphate systems as in inert systems (5-D; 3-D).
11. Camphorsulfonate appears to augment DNP-inhibition in phosphate systems (3-E,F; 5-E,F).

These observations were made from experiments using potato tuber slices only. Some attempts were made to use Elodea in respiratory experiments but the volume of tissue required was so large that utilization of standard-sized Warburg vessels was impractical.

The effects of IAA and CSA on respiration rate of potato tuber slices in phosphate buffer.

Stimulation of the respiration rate of potato tuber slices in phosphate buffer by IAA and CSA is shown in Figure 1. The rate of respiration in control flasks is shown by curve A. Indoleacetic acid alone accelerated respiration rate about 90%, as shown by curve B. Camphorsulfonic acid also stimulated respiration rate, as shown by curves

D and E. At .0001 M., CSA stimulated respiration rate almost as much as IAA did at a similar concentration. Increasing the concentration of CSA to .001 M. gave a further increase in respiration rate. Camphorsulfonate and IAA together gave an even greater respiration rate than CSA or IAA alone. It is significant that CSA acted in a manner similar to that of IAA in this experiment.

Because this experiment was done with phosphate buffer the increases in respiration rate may be attributed tentatively to an increase in "salt respiration" concomitant with increased active uptake. The observations of Hackett and Thimann (16, p.554) that IAA and naphthaleneacetic acid stimulate the active uptake of water by potato discs suggest that the stimulations observed in this experiment are attributable to an increased active uptake mediated by auxin. The similarity of respiratory responses elicited by CSA to those of IAA indicate that the effect of this compound may be similar to that of auxin.

The effects of IAA and CSA on respiration rate of potato tuber slices in barbiturate buffer.

The set of curves illustrated in Figure 2 represents the results of an experiment performed under conditions similar to those of the previous experiment except that a barbiturate buffer was substituted for phosphate buffer. Curve A represents the respiration rate of the control samples. It will be noted that the respiration rate is

higher in these samples than in those of the previous group. This difference was consistent in replications of the samples and might be explained by differences in tubers from experiment to experiment. Slices from a single tuber were used in each experiment for the purpose of holding tissue variations down to as small a value as possible. In contrast to the effects found in the previous experiment, respiration rates were inhibited by IAA at .0001 M. Camphorsulfonate also depressed respiration rates at .0001 M. and .001 M. Curves B, D and E illustrate these effects. The degree of inhibition by CSA was related to its concentration in the same way stimulation was related to concentration in the previous experiment. The higher concentration of CSA .001 M. depressed respiration rate more than the .0001 M. level. Application of IAA together with CSA (Curves C and F) depressed respiration rate more than either compound alone when .001 M. CSA was added to .0001 M. IAA. When CSA was applied at .0001 M. with IAA at .0001 M., the inhibition was not as great as IAA applied alone at .0001 M. (Compare curves B and C).

French and Beevers (14, p.661) found that .0001 M. IAA stimulated respiration of corn coleoptile sections in phosphate buffer. The findings of the previous experiment confirm the effectiveness of .0001 M. IAA in stimulating respiration of potato discs in a phosphate system. In an inert system, the same concentration acted to depress

respiration in a degree similar to its stimulation of respiration in phosphate buffer. The mechanism by which IAA acts in causing the respiratory responses is not known, but it might be assumed from the findings of the experiments illustrated in Figures 1 and 2 that the action of IAA in phosphate buffers and in inert buffers are complementary, i.e., the mechanism which is stimulated in the presence of phosphate is inhibited in its absence. This could be an effect of auxin or of substrate (phosphate) but since this thesis concerns the effects of CSA rather than the theory of auxin action this was not investigated further.

The effects of CSA and DNP on respiration rate of potato tuber slices in phosphate buffer.

In the series of curves illustrated in Figure 3, the effects of DNP on respiration rates of potato slices in phosphate buffer are illustrated. Curve A represents the respiration rates of control samples. Curves C and D show the depression in respiration rate produced by moderately high concentrations of DNP, .0002 M. and .001 M. When CSA was supplied alone, respiration rates increased (curve B) as in the experiment of Figure 1. Adding DNP and CSA together, however, caused a marked drop in respiration rate, as evidenced by curves E and F. There was a temporary rise in respiration rate in these samples for about 45 minutes preceding a drop in oxygen uptake. This

was probably a reflection of a changing equilibrium between the tissue slices and the compounds in solution surrounding the slices. The combined action of DNP and CSA was so strongly inhibitory that an apparent "negative respiration" took place. One concentration of CSA is shown, .0002 M., and this level was effective in causing a nearly identical inhibition with two different concentrations of DNP: .0002 M. and .001 M.

The effects of CSA and DNP on respiration rate of potato tuber slices in barbiturate buffer.

The action of CSA and DNP in affecting respiration rates of potato slices in barbituric acid buffer is illustrated in Figure 4. As shown by curves B and C, dinitrophenol at .0002 M. and .001 M. was inhibitory, but CSA supplied at .001 M. together with .0002 M. DNP was able to raise the respiratory rate from the level shown in curve B to that of curve E, from 54% to 86% of controls. At a lower concentration, .0001 M., the effects of CSA in reversing DNP inhibition were not significant. This is depicted in curves B and D. When DNP was present in a relatively high concentration, .001 M., then CSA at .0001 M. or .001 M. was unable to affect significantly the inhibited respiratory rate, as shown in curve F.

When external phosphate is absent the effect of CSA on DNP-inhibited tissues is that of partially reversing the inhibition of DNP. This is in contrast to the res-

ults obtained in the previous experiment, where CSA augmented the inhibition produced by DNP. These findings point to an interaction between CSA and DNP, either directly at a site of action with the organized cell or separately, at different points within the metabolic pathways of the cell. The possibility that CSA and DNP could interact outside of the tissues and form a salt or ester is contraindicated by the acidic nature of both of the compounds. The ultimate effect of each of the compounds on the metabolism of the tissues must lie in a real effect upon a key site, or sites, within the cell.

The effects of CSA and DNP on respiration rate of potato tuber slices in barbiturate buffer containing phosphate.

The results of this experiment, employing a barbituric acid buffer to which .01 M. KH_2PO_4 has been added, confirm the importance of an external inorganic phosphate supply to the nature of CSA and DNP interaction. As in the experiment of Figure 3, .0002 M. CSA stimulated respiration rates about 25% over these of control samples (curve B). A similar action was observed in the experiment of Figure 1, but the degree of stimulation by .0001 M. CSA in that experiment was greater than the stimulation produced in this experiment. A higher concentration of inorganic phosphate in the first experiment may account for this. The results of the experiment of Figure 3 are in agreement with those of this experiment (Figure 5), in

which camphorsulfonate was also unable to reverse inhibition produced by DNP. This is shown by curves E and F. The presence of CSA and DNP together with phosphate is quite inhibitory, and this effect is apparently independent of the presence of barbiturate.

The action of CSA and DNP together in the experiments of Figures 3 and 5 is interesting in the light of an apparent mutual antagonism between DNP and CSA shown in the Elodea growth experiment the results of which are illustrated in Plate III (p.51). Dinitrophenol alone and CSA alone caused extensive damage to Elodea in phosphate buffer. Supplied together, the compounds were not as harmful as they were when applied singly. Further experimentation with Elodea is indicated in studying this interaction.

Summarized, the effect of CSA on respiration rate of potato tuber tissue is an auxin-like stimulation of respiration rate in the presence of external inorganic phosphate and a depression of respiration rate in an inert buffer. These effects are apparently reversed by DNP, which causes CSA to depress respiration rate in phosphate-containing systems and to stimulate DNP inhibited tissues in inert buffers.

FIGURE 1

Effects of camphorsulfonic acid (CSA) and indoleacetic acid (IAA) on respiration rate of potato tuber slices in 0.03 M. phosphate buffer, pH 6.0. Temperature: 30°C.

A: Controls.
B: IAA, .0001 M.
C: IAA, .0001 M. and CSA, .0001 M.
D: CSA, .001 M.
E: CSA, .0001 M.
F: IAA, .0001 M. and CSA, .01 M.

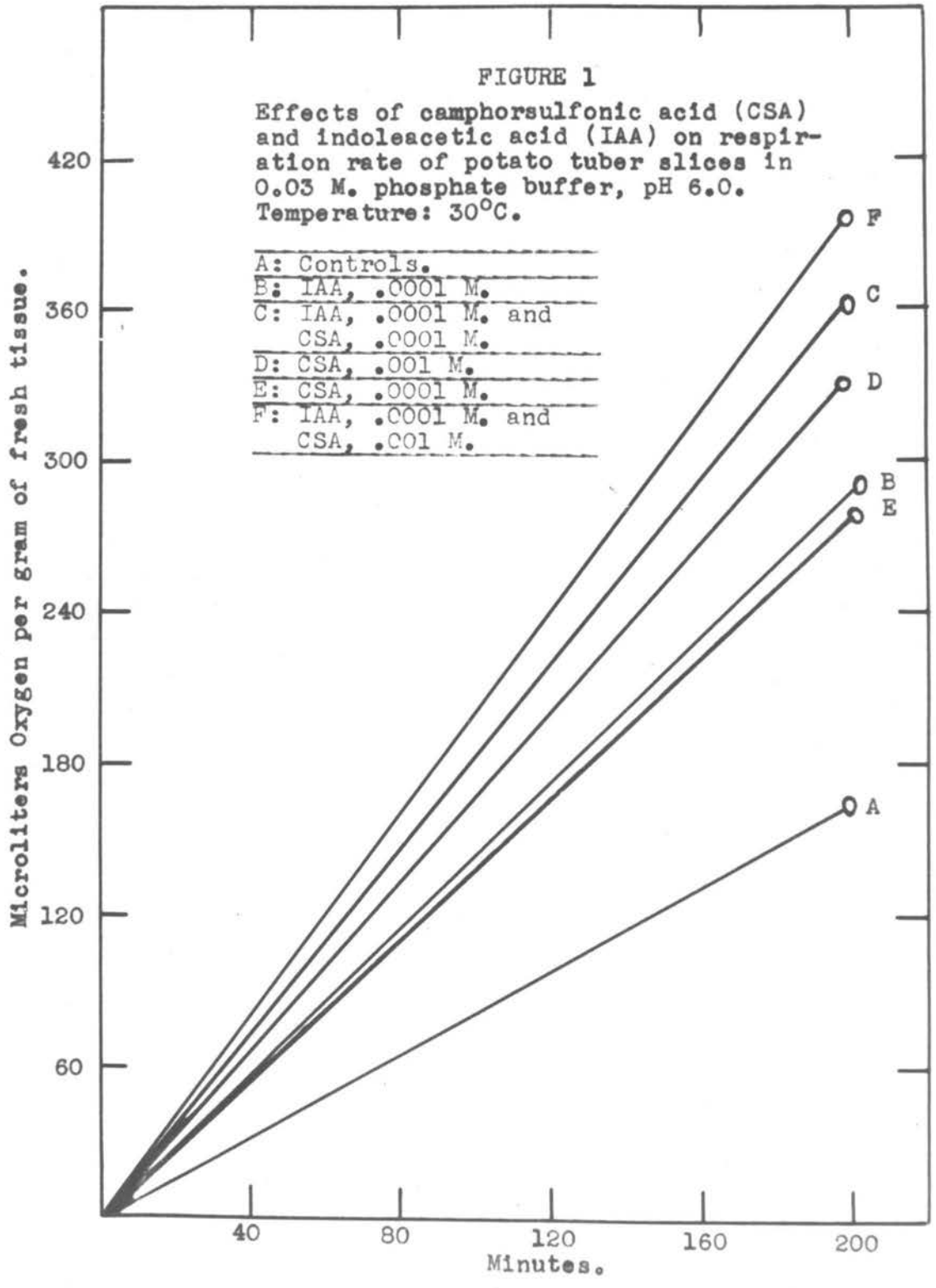
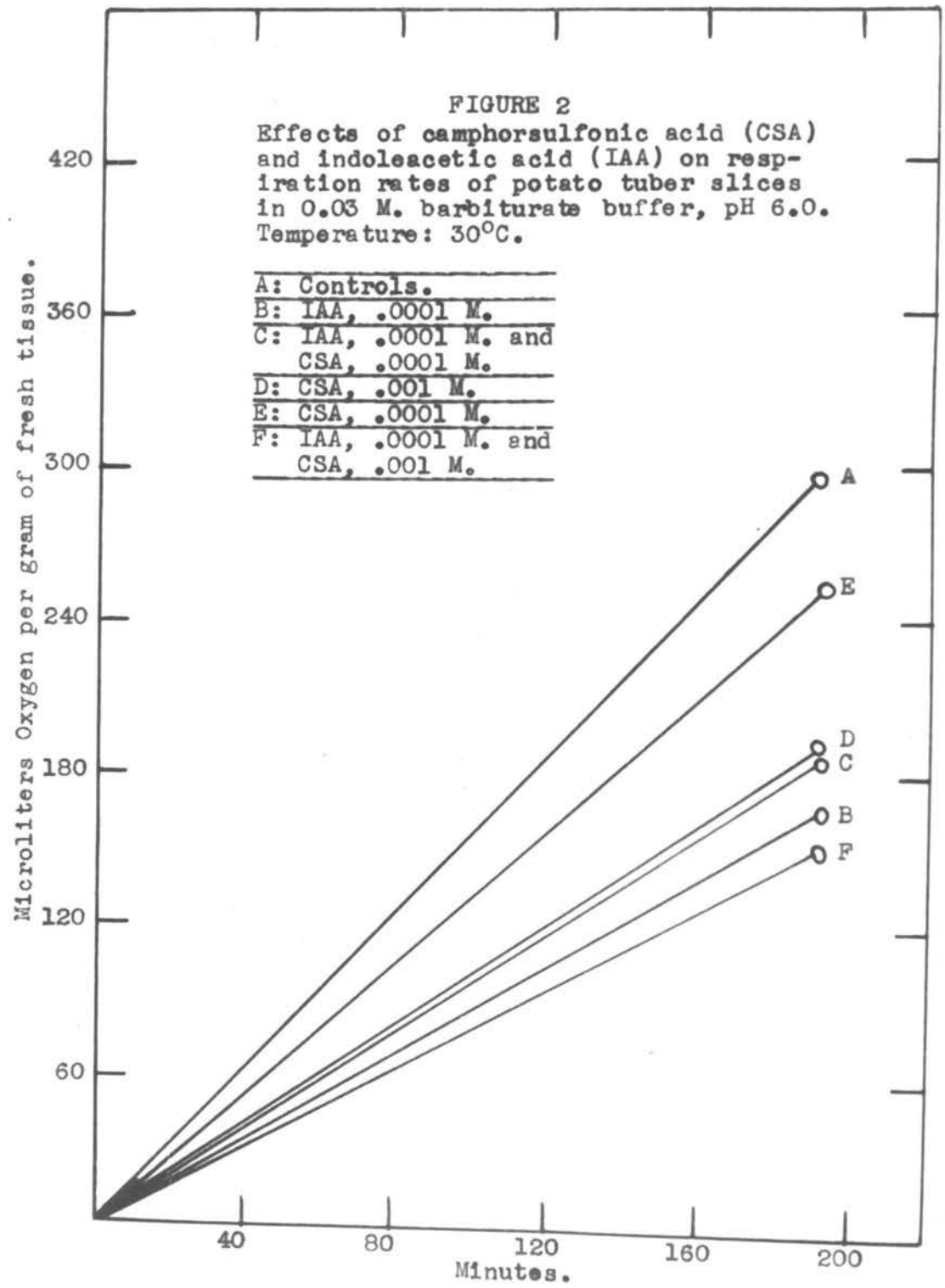


FIGURE 2

Effects of camphorsulfonic acid (CSA) and indoleacetic acid (IAA) on respiration rates of potato tuber slices in 0.03 M. barbiturate buffer, pH 6.0. Temperature: 30°C.



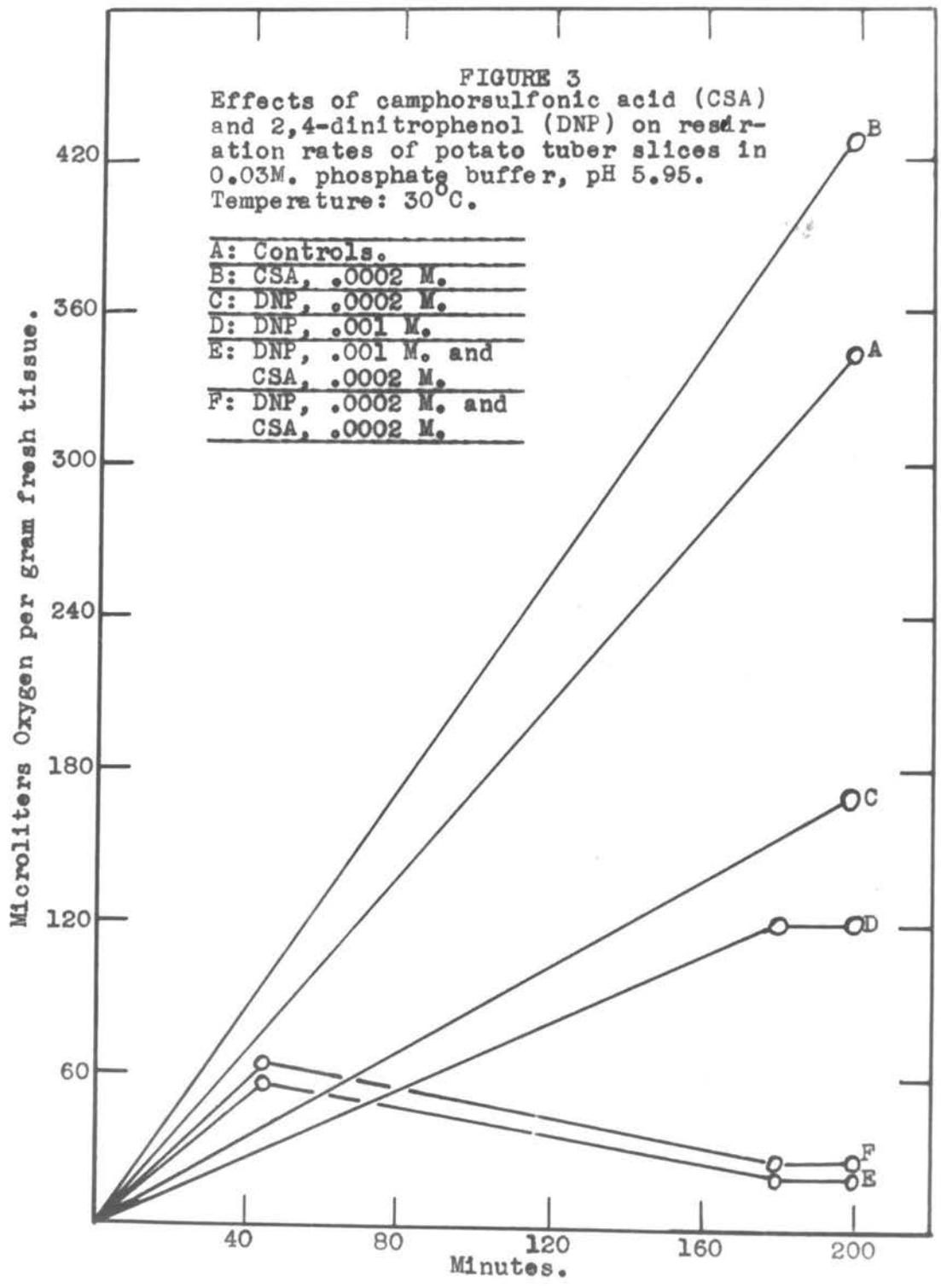
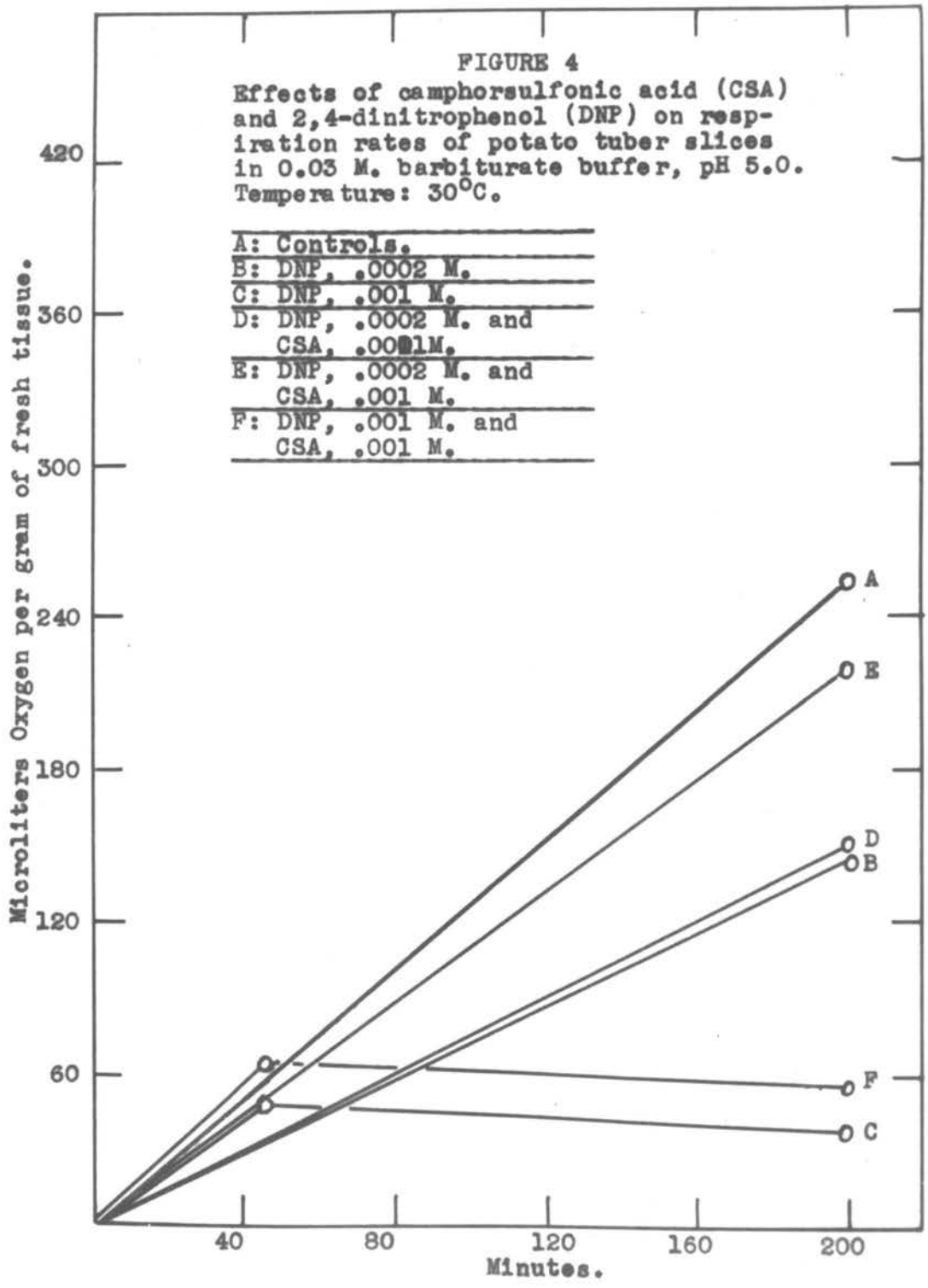


FIGURE 3
Effects of camphorsulfonic acid (CSA) and 2,4-dinitrophenol (DNP) on respiration rates of potato tuber slices in 0.03M. phosphate buffer, pH 5.95. Temperature: 30°C.

FIGURE 4

Effects of camphorsulfonic acid (CSA) and 2,4-dinitrophenol (DNP) on respiration rates of potato tuber slices in 0.03 M. barbiturate buffer, pH 5.0. Temperature: 30°C.

A: Controls.
B: DNP, .0002 M.
C: DNP, .001 M.
D: DNP, .0002 M. and CSA, .0001M.
E: DNP, .0002 M. and CSA, .001 M.
F: DNP, .001 M. and CSA, .001 M.



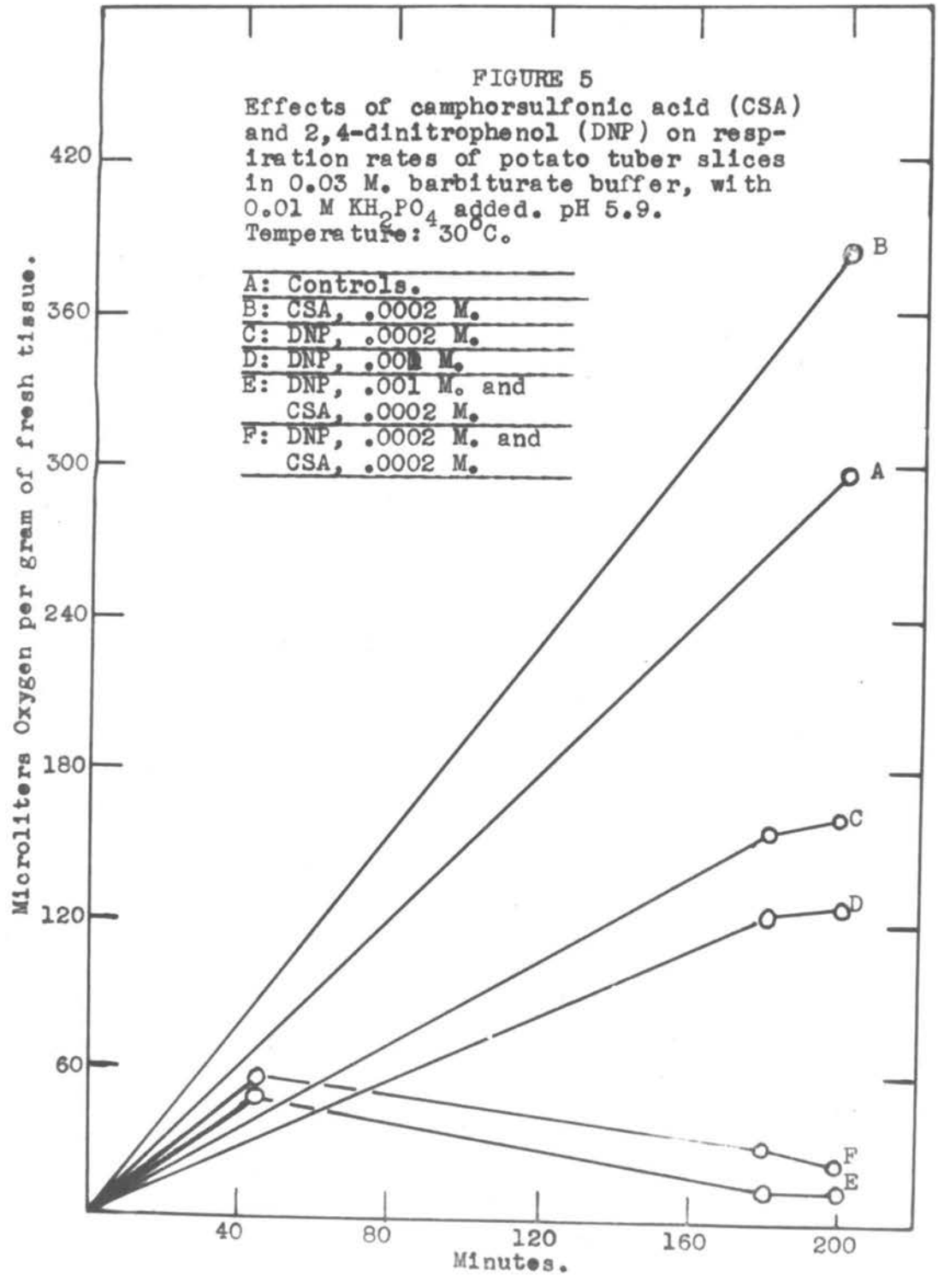


FIGURE 5

Effects of camphorsulfonic acid (CSA) and 2,4-dinitrophenol (DNP) on respiration rates of potato tuber slices in 0.03 M. barbiturate buffer, with 0.01 M KH_2PO_4 added. pH 5.9. Temperature: 30°C.

A: Controls.
B: CSA, .0002 M.
C: DNP, .0002 M.
D: DNP, .00[illegible] M.
E: DNP, .001 M. and CSA, .0002 M.
F: DNP, .0002 M. and CSA, .0002 M.

5.Phosphate uptake.

Figures 6 through 13 present graphically the results of radioactive phosphate uptake studies with potato tuber slices and Elodea shoots. Each figure is a profile of the percentages of total radioactivity at points along paper chromatograms of tissue extracts.

Phosphate uptake in tissues receiving no test substances.

Figure 6 is a chromatogram profile of radioactivity of an extract of potatoslices incubated in barbiturate buffer. The total radioactivity taken up by the tissue slices in 10 hours was 269,000 cpm. At Rf 0.0 to 0.1 there is high activity, presumably from inorganic phosphate taken up and not incorporated into organic compounds. An average of 3% of the total activity is in the region of Rf 0.1 to 0.2, where ATP and 2-phosphoglyceric acid represent the principal components. There is a slight peak at Rf 0.15, attributable to ATP. Complete separation of ATP and 2-PGA was not obtained in the chromatograms resulting from this study. Longer solvent migration paths or the use of two-dimensional separation of the organic phosphates might have proven more effective in separation of these compounds. At Rf 0.25 there is a slight peak in the activity, presumably caused by radioactive Fructose-1,6-diphosphate or free orthophosphate. Activity thereafter is low until a slope starts at Rf 0.5 and rises to a significant peak at 0.63. Glucose-1-phosphate is

presumably responsible for this peak, amounting to 12% of the total activity. Activity appears to be particularly high on glucose-1-phosphate. This distribution of compounds appears to be a normal pattern for a carbohydrate storage organ such as potato tuber.

Figure 10 represents the distribution of radioactivity in an extract of untreated Elodea shoot incubated in .01 M. barbiturate buffer for 12.5 hours. Total radioactivity of the extract was 292,000 cpm. A high proportion of the total activity falls in the Rf range 0.0 to 0.11. This represents the accumulation of a large amount of radioactive inorganic phosphate with concomitant low phosphorylative activity. No significant peaks occur in the chromatogram before Rf 0.60-0.64, where glucose-1-phosphate is presumed to occur. Apparently, the carbohydrate metabolism of Elodea is not as active as that of potato tuber tissue. A generally low activity in the regions of the chromatogram occupied by organic phosphates indicates that the phosphate exchange in Elodea is relatively inactive compared with potato tissue.

Phosphate uptake in tissues treated with 2,4-dinitrophenol.

Figure 7 represents the pattern of phosphate accumulated by potato tuber slices incubated with .0002 M. DNP in barbiturate buffer for 10 hours. A high proportion of the total radioactivity is represented by the inorganic

phosphates the activity of which amounts to 32 to 38% of the total activity between Rf 0.0 and 0.03. A lowered relative activity between Rf 0.1 and 0.2 suggests that ATP synthesis has been inhibited in the DNP-treated slices. Lower activity at Rf 0.24 - 0.25 indicates that fructose-1, 6-diphosphate may be reduced in this sample. Activity at Rf 0.58 to 0.63 is down to 2% of the total activity compared with 12% for the corresponding region in the chromatogram of the control samples (Figure 6). The total radioactivity of the sample is markedly lower than that of the control samples: 45,300 cpm. in contrast to 269,000 cpm. This indicates that total uptake of phosphate was markedly inhibited by DNP. Not only is phosphate uptake reduced to about 20% of the control sample , but a much smaller proportion of this phosphate is incorporated into organic phosphate.

A radioactivity profile similar to that shown in extracts of DNP-treated potato slices is shown by extracts of DNP-treated Elodea. Figure 11 illustrates this similarity. Lower activities in regions representing organic phosphate, particularly Rf 0.60 - 0.65, suggest that phosphorylation has been inhibited in the DNP-treated Elodea tissues. The total activity in the sample is approximately one-fifth that of the control Elodea samples, indicating strong inhibition of phosphate uptake by DNP. Inorganic phosphates represented by Rf 0.0 to 0.1 possess

a percentage of the total radioactivity similar to that shown for inorganic phosphate in the potato slices receiving DNP. This suggests that the phosphate which is taken up in the presence of DNP is not converted to organic esters in Elodea.

Phosphate uptake in tissues receiving 2,4-dinitrophenol and camphorsulfonic acid.

Potato tissues incubated in barbiturate buffer for 10 hours with 0.0002 M. DNP and .0002 M. CSA yielded an alcoholic extract whose radioactivity distribution is illustrated in the chromatogram profile of Figure 8. In the region Rf 0.0 to 0.1 the inorganic phosphate activity is in a proportion similar to the percentage of total activity shown for this region in Figure 7. Total radioactivity of the sample is 57,400 cpm., 12,100 counts higher than that of potato slices receiving DNP alone. From Rf 0.1 to 0.2 there is a slightly lower activity in Figure 8 than in Figure 7, but the difference is so small as to be insignificant. Between Rf 0.2 and 0.3, however, there is diminished activity in the DNP and CSA-treated sample. This region, showing an average of 2% of the total activity in the DNP-treated sample, is from ½ to 1% of the total activity in the sample receiving both CSA and DNP. Activity is negligible from Rf 0.3 to 0.47, where a small peak representing 2% of total activity appears. This is presumably caused by radioactive Glucose-6-phosphate.

There is low activity at Rf 0.60 - 0.65, the percentage of total activity in this region being approximately half that of the corresponding region in the chromatogram of the DNP-treated sample (Figure 7). This suggests that CSA has caused a shift in the proportionate amount of labelling in Glucose-1-phosphate and Glucose-6-phosphate in the presence of DNP. There is an increase in the activity of Glucose-6-phosphate in the DNP-CSA sample that does not appear in the DNP sample.

Phosphate uptake in tissues receiving camphorsulfonic acid alone.

Figure 9 represents the distribution of radioactivity in potato tuber slices receiving .0002 M. CSA in barbiturate buffer for 10 hours. Total activity in the CSA-treated slices is 14% greater than that in the control samples (306,000 cpm. in comparison with 269,000 cpm.). This suggests that CSA stimulates the uptake of phosphate in potato tuber tissue. Corroboration is given this observation by respiratory findings (Figures 1, 3 and 5) which show that CSA stimulates respiration rate of potato tuber slices in phosphate-containing buffer.

Distribution of the phosphate esters in the CSA-treated slices is shown in Figure 9. From Rf 0.0 to 0.1, the relative amount of inorganic phosphates is approximately equal to that in the control slices (Figure 6). From Rf 0.1 to 0.2, the activity in Figure 9 is distributed differently than in the controls. A higher proportion of the

activity in this region is between Rf 0.1 and 0.13 than in the remainder of the region. It is possible that this is attributable to imperfect separation of inorganic phosphates, ATP and 2-PGA. Duplicate chromatograms of this extract showed a similar distribution in this region, however. Significantly lower activity is evident in the CSA-treated sample in the region Rf 0.2 to 0.3 than in the control sample. This is apparently caused by lowered activity of Fructose-1,6-diphosphate or orthophosphate in the CSA-treated sample. Activity remains at a negligible level from Rf 0.3 to 0.4, where a gradual rise in activity occurs up to 0.5. This peak is presumed to be caused by increased labelling in Fructose-6-phosphate, Glucose-6-phosphate, and phosphoenol pyruvate. There is a drop in activity at Rf 0.53 and a sharp rise at Rf 0.54, with a continuing rise up to Rf 0.60, where the amount of activity is 12% of the total activity, indicating heavy labelling in Glucose-1-phosphate. The fluctuation in activity between Rf 0.53 and 0.54 cannot be explained on the basis of published Rf values for organic phosphate esters, for there are no compounds reported for this Rf region (1, p. 408). Heavy labelling in the Glucose-1-phosphate fraction may, however, cause spreading in the chromatogram region closely adjacent to the area of highest activity, and this will obscure the separation of compounds having narrowly-separated Rf values.

Figure 13 represents a profile of a chromatogram of an extract of Elodea incubated for 12.5 hours with 0.0002 M. CSA in barbiturate buffer. Total activity in this extract is 325,000 cpm. in contrast to 292,000 cpm. in the control samples. The difference of 11% may indicate that CSA has stimulated phosphate uptake in Elodea High concentration of the activity between Rf 0.0 and 0.3 in the CSA-treated sample is exhibited in Figure 13. This difference from the control pattern was also observed in duplicate chromatogram strips. Camphorsulfonate has apparently caused a redistribution of the radioactivity into organic phosphate compounds, as evidenced by increased labelling in compounds of Rf 0.1 to 0.3. Poor separation of these compounds is probably explained by concentration of high activity within a short region on the chromatogram acting to obscure the boundaries between closely adjacent areas on the chromatogram. Activity in the CSA-treated samples falls to a negligible level between Rf 0.3 and 0.5, where a slight rise in activity is apparent between Rf 0.5 and 0.55. The identity of this peak is not known, and its presence is probably not caused by high activity in a closely-adjoining region. The peak at Rf 0.5 is a fairly discrete one, amounting to 2% of the total activity. No other peaks occur between it and Rf 0.65, where a similar rise in activity is noted again, amounting to about 2% of the total activity. The activity

of this last peak is about twice that of the peak at a similar place in the chromatogram of the control samples. This would suggest that in Elodea CSA favors the accumulation of organic phosphate esters.

The significance of these observations may lie in the fact that CSA is able to stimulate the incorporation of inorganic phosphate into organic esters as well as stimulate total uptake of phosphate. Camphorsulfonate is apparently able to cause a small increase in phosphate uptake in DNP-poisoned tissues, but its effect on phosphorylation is probably not significant.

That DNP affects phosphate metabolism strongly is indicated by the marked lowering of relative amounts of organic phosphates in potato tuber slices incubated with DNP. Total uptake is also diminished by DNP, as evidenced by the attenuated total radioactivity in samples of Elodea and potato tuber treated with DNP.

The results discussed in the foregoing pages suggest that CSA has the following activities in the plant tissues studied:

1. The property of stimulating growth in Avena coleoptile sections and to a lesser extent, the property of stimulating curvature of slit pea internodes. The compound is active in stimulating growth of Elodea stem sections in concentrations of .0001 M. CSA is toxic to Elodea at .001 M.

2. CSA stimulates respiration rate of potato tuber tissues in phosphate buffer.
3. The stimulation of respiration rate is similar to that of IAA, i.e., is auxin-like.
4. Camphorsulfonic acid stimulates uptake of inorganic phosphate in Elodea and potato tuber slices.
5. Camphorsulfonic acid appears to stimulate phosphorylation in intact tissues. To establish this effect as a real one, critical experiments should be performed to determine the actual amounts of individual phosphate esters formed in CSA-treated tissues.
6. Camphorsulfonate appears to favor the appearance of Glucose-6-phosphate in extracts of plants tested with it. Again, this effect should be checked with experiments designed to measure the actual amount of Glucose-6-phosphate present in CSA-treated tissues.
7. The metabolic effects described are in line with growth effects described in the early part of this thesis.

Table VI

Rf values of organic phosphate esters developed with a 3:6:1 water-methanol-ammonia solvent system. (1, p.408)

Rf value	Compound
0.05	Pyrophosphate
0.06	Tripolyphosphate
0.15	Adenosinetriphosphate
0.18	2-phosphoglycerate
0.24	Fructose-1,6-diphosphate
0.28	Orthophosphate
0.32	Adenosine-3-phosphate
0.35	3-phosphoglycerate
0.44	Fructose-6-phosphate
0.46	Phosphoenolpyruvate
0.48	Glucose-6-phosphate
0.60	Glucose-1-phosphate

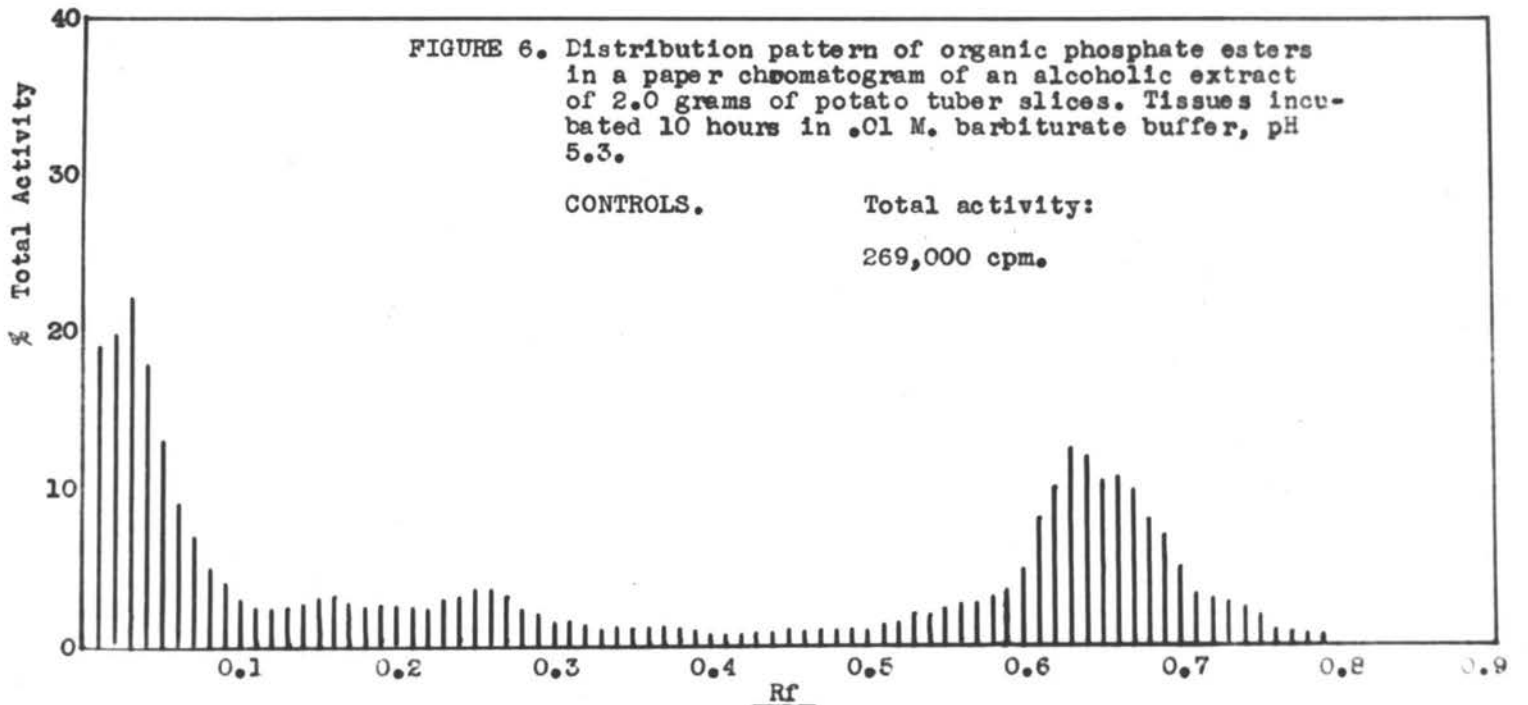


FIGURE 6. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 2.0 grams of potato tuber slices. Tissues incubated 10 hours in .01 M. barbiturate buffer, pH 5.3.

CONTROLS. Total activity:

269,000 cpm.

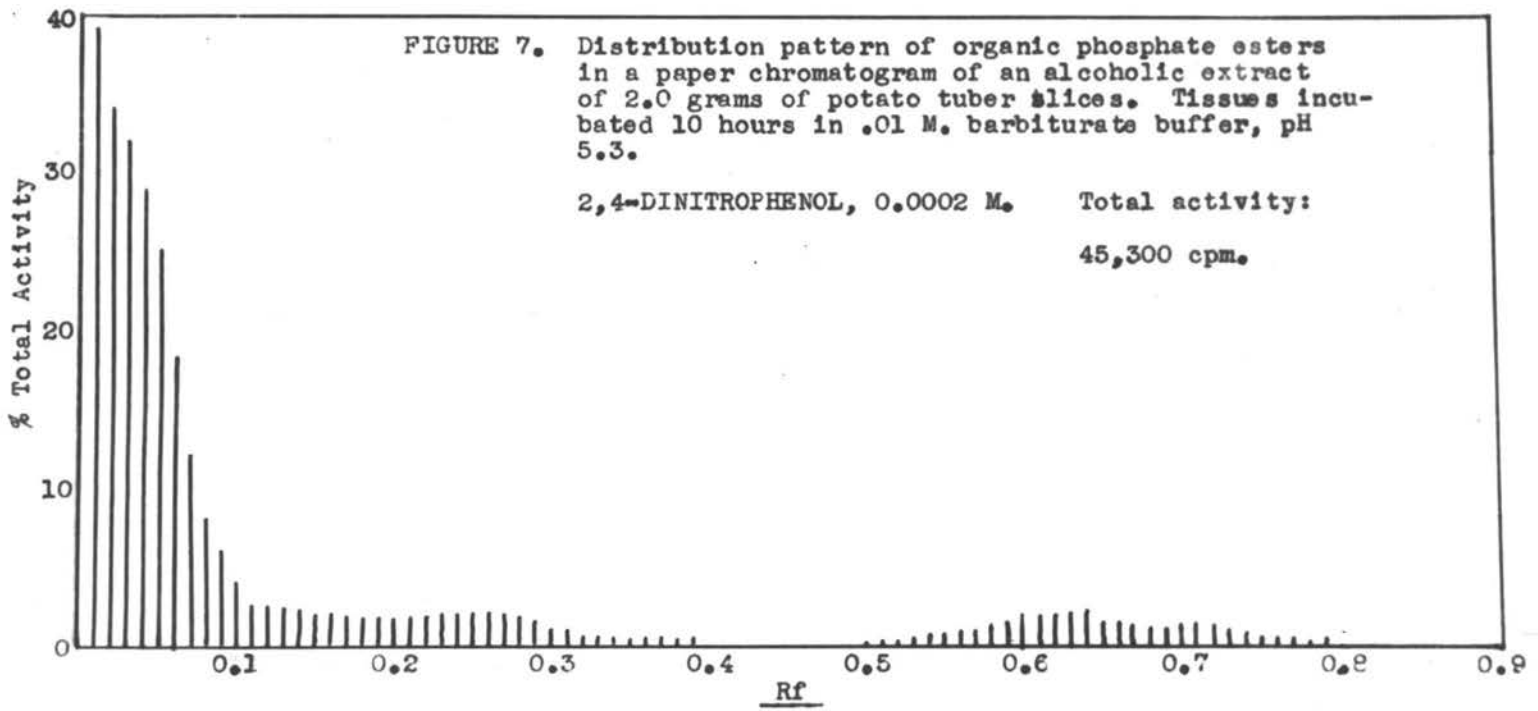


FIGURE 7. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 2.0 grams of potato tuber slices. Tissues incubated 10 hours in .01 M. barbiturate buffer, pH 5.3.

2,4-DINITROPHENOL, 0.0002 M. Total activity:

45,300 cpm.

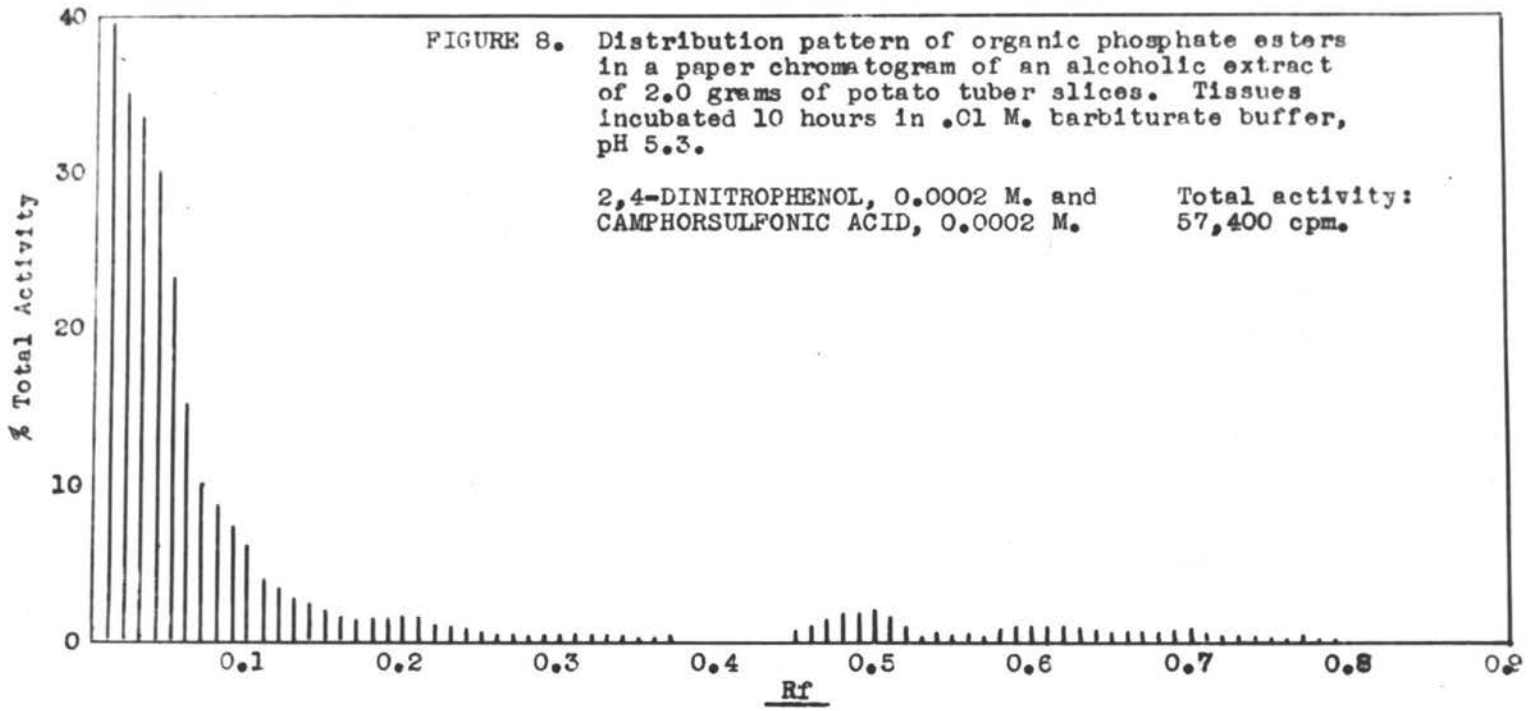


FIGURE 8. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 2.0 grams of potato tuber slices. Tissues incubated 10 hours in .01 M. barbiturate buffer, pH 5.3.

2,4-DINITROPHENOL, 0.0002 M. and
CAMPHORSULFONIC ACID, 0.0002 M.

Total activity:
57,400 cpm.

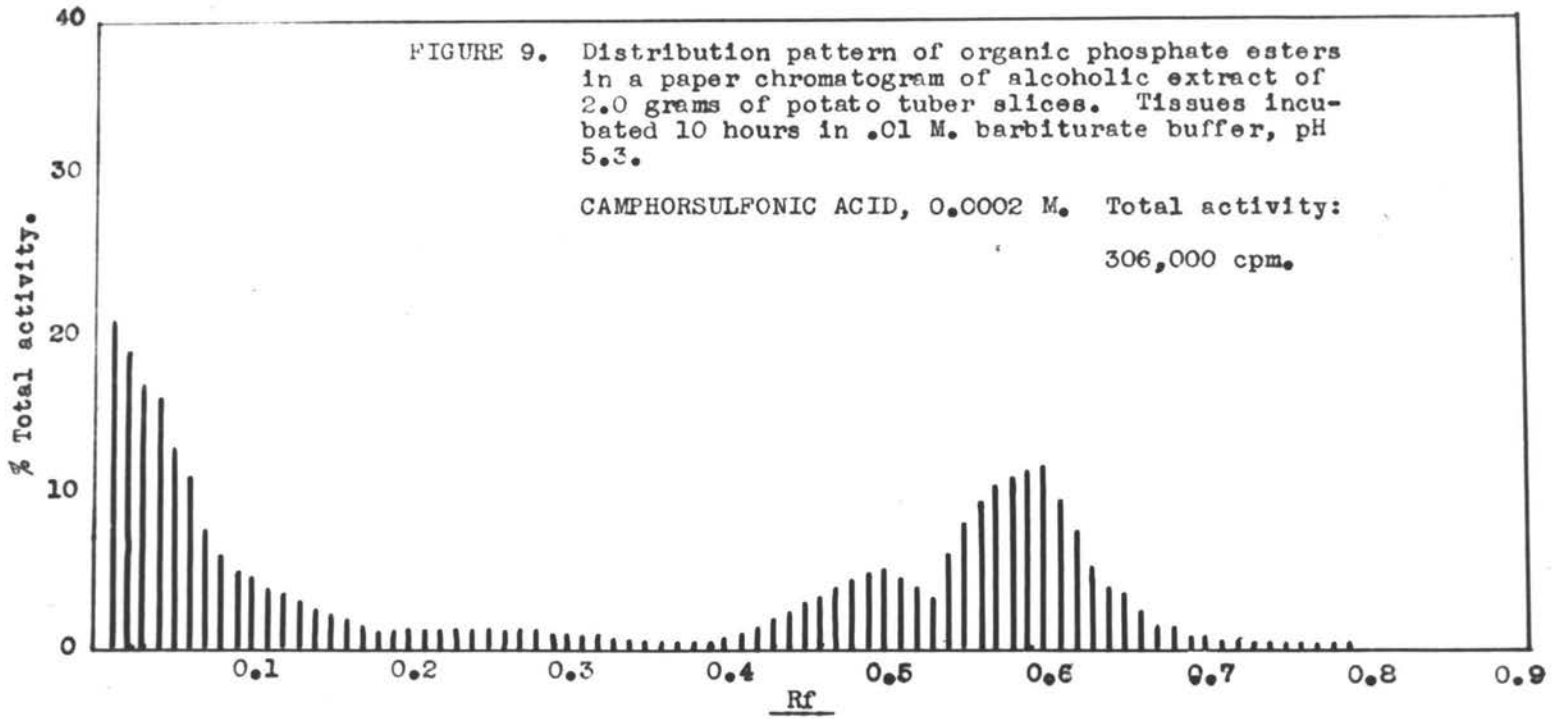


FIGURE 9. Distribution pattern of organic phosphate esters in a paper chromatogram of alcoholic extract of 2.0 grams of potato tuber slices. Tissues incubated 10 hours in .01 M. barbiturate buffer, pH 5.3.

CAMPHORSULFONIC ACID, 0.0002 M. Total activity: 306,000 cpm.

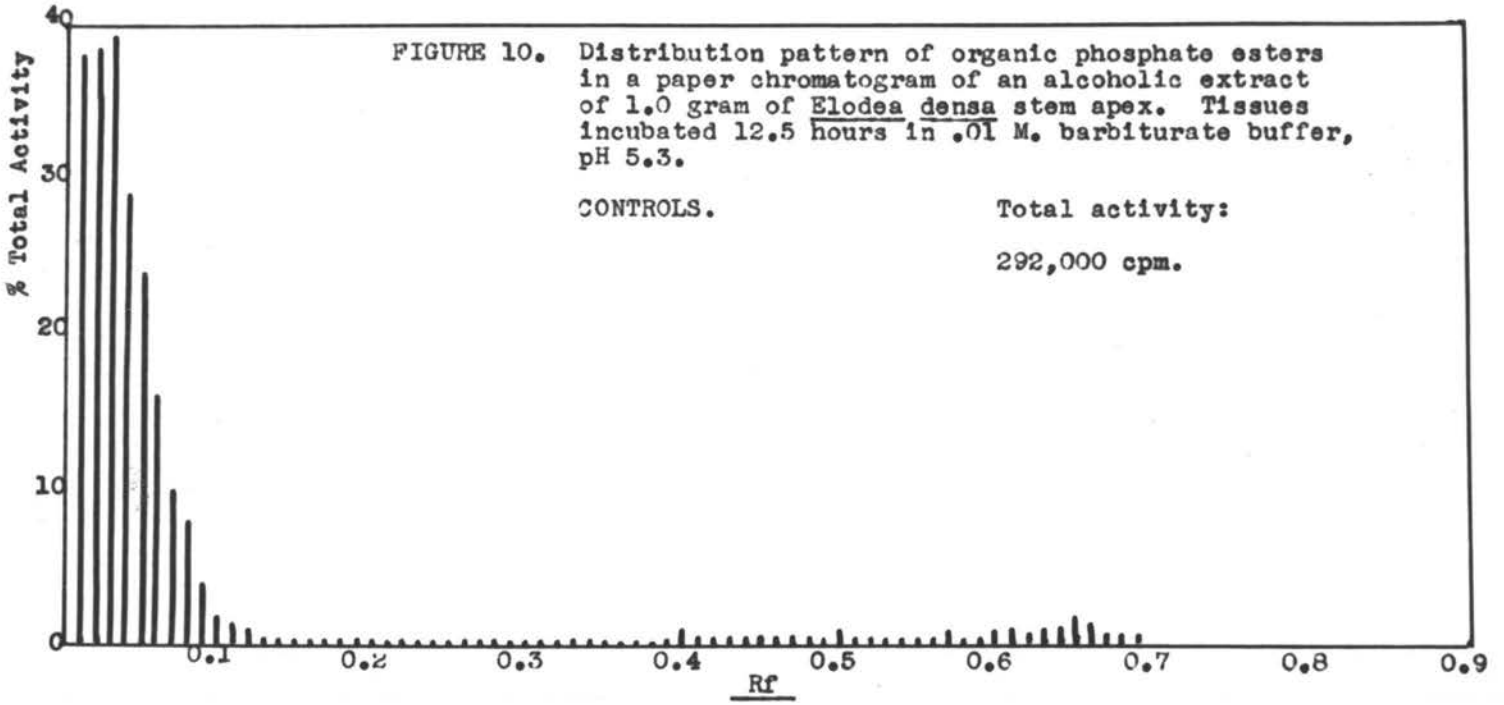


FIGURE 10. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 1.0 gram of Elodea densa stem apex. Tissues incubated 12.5 hours in .01 M. barbiturate buffer, pH 5.3.

CONTROLS.

Total activity:

292,000 cpm.

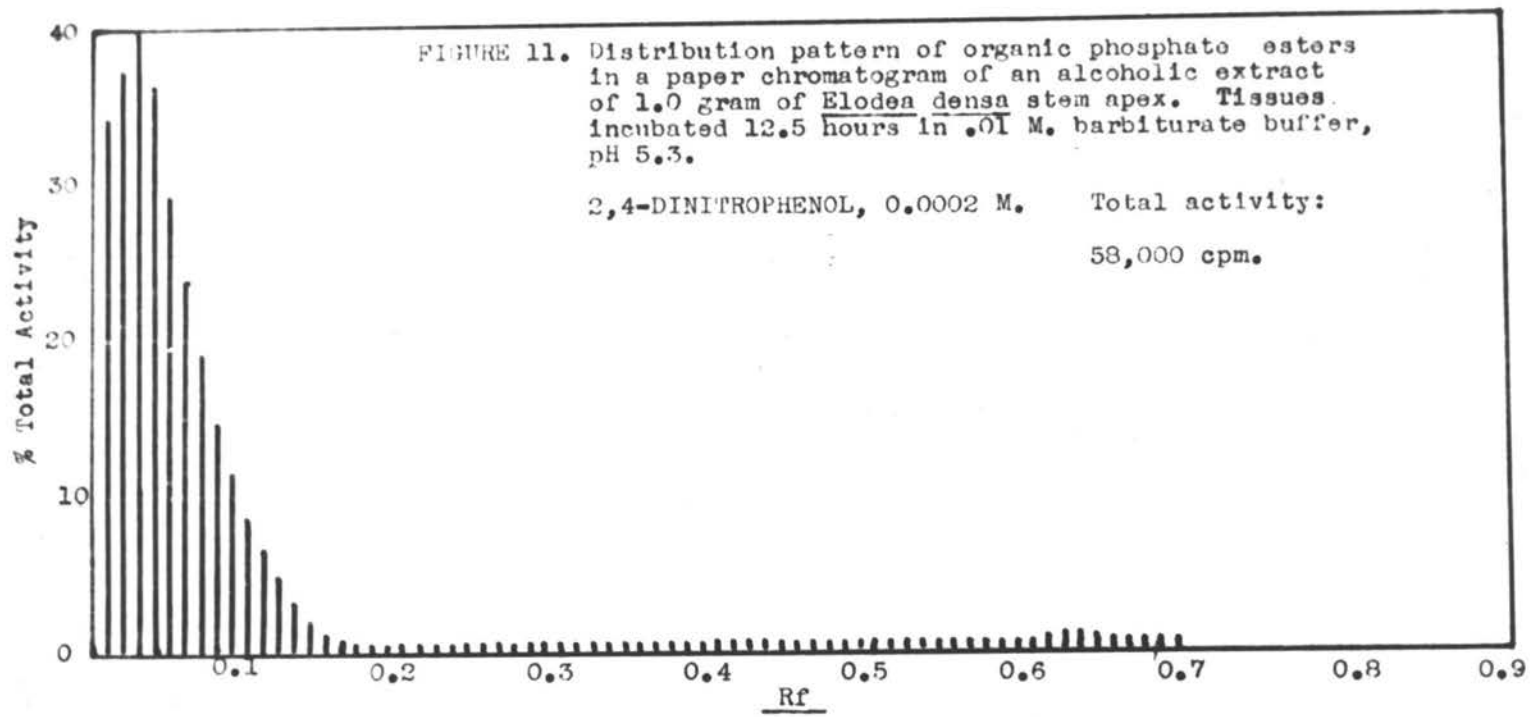


FIGURE 11. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 1.0 gram of Elodea densa stem apex. Tissues incubated 12.5 hours in .01 M. barbiturate buffer, pH 5.3.

2,4-DINITROPHENOL, 0.0002 M. Total activity: 58,000 cpm.

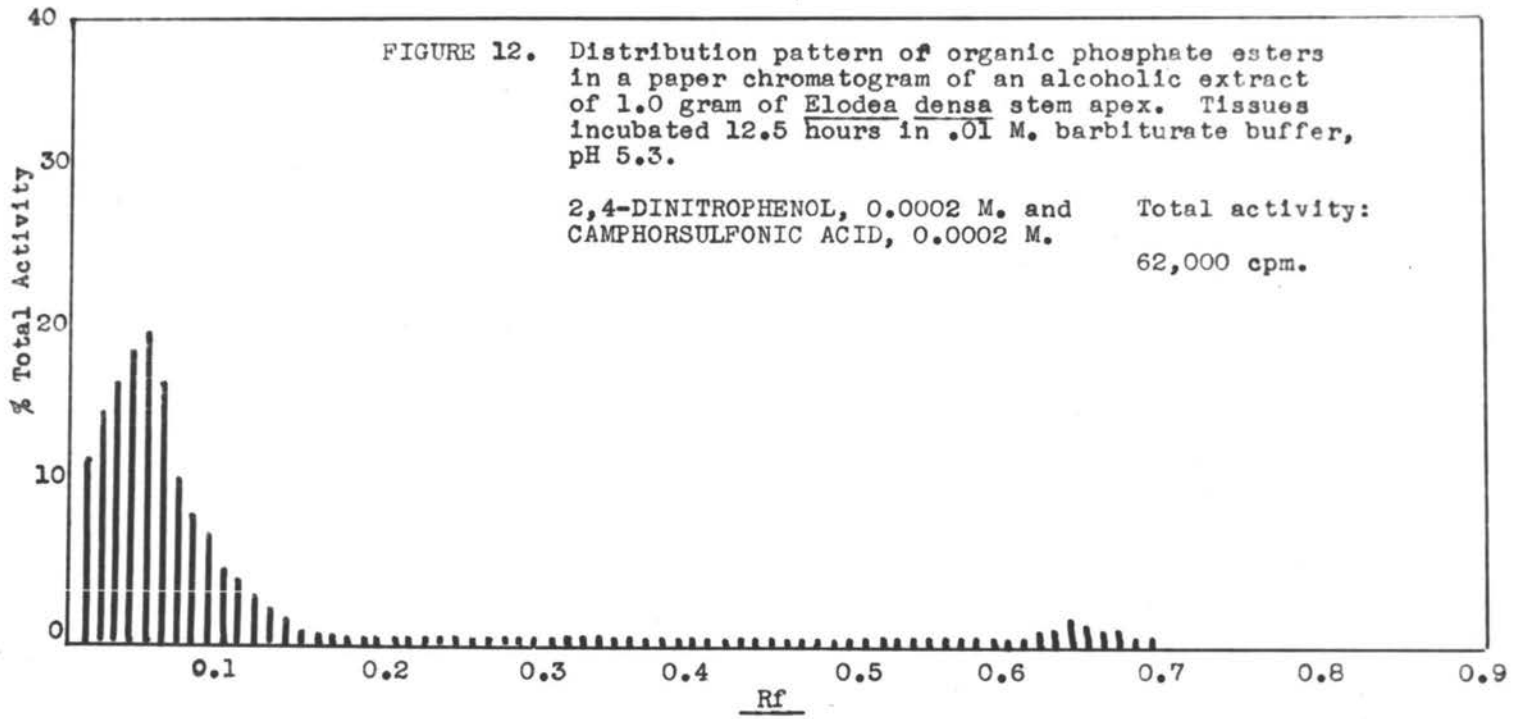


FIGURE 12. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 1.0 gram of Elodea densa stem apex. Tissues incubated 12.5 hours in .01 M. barbiturate buffer, pH 5.3.

2,4-DINITROPHENOL, 0.0002 M. and CAMPHORSULFONIC ACID, 0.0002 M.

Total activity: 62,000 cpm.

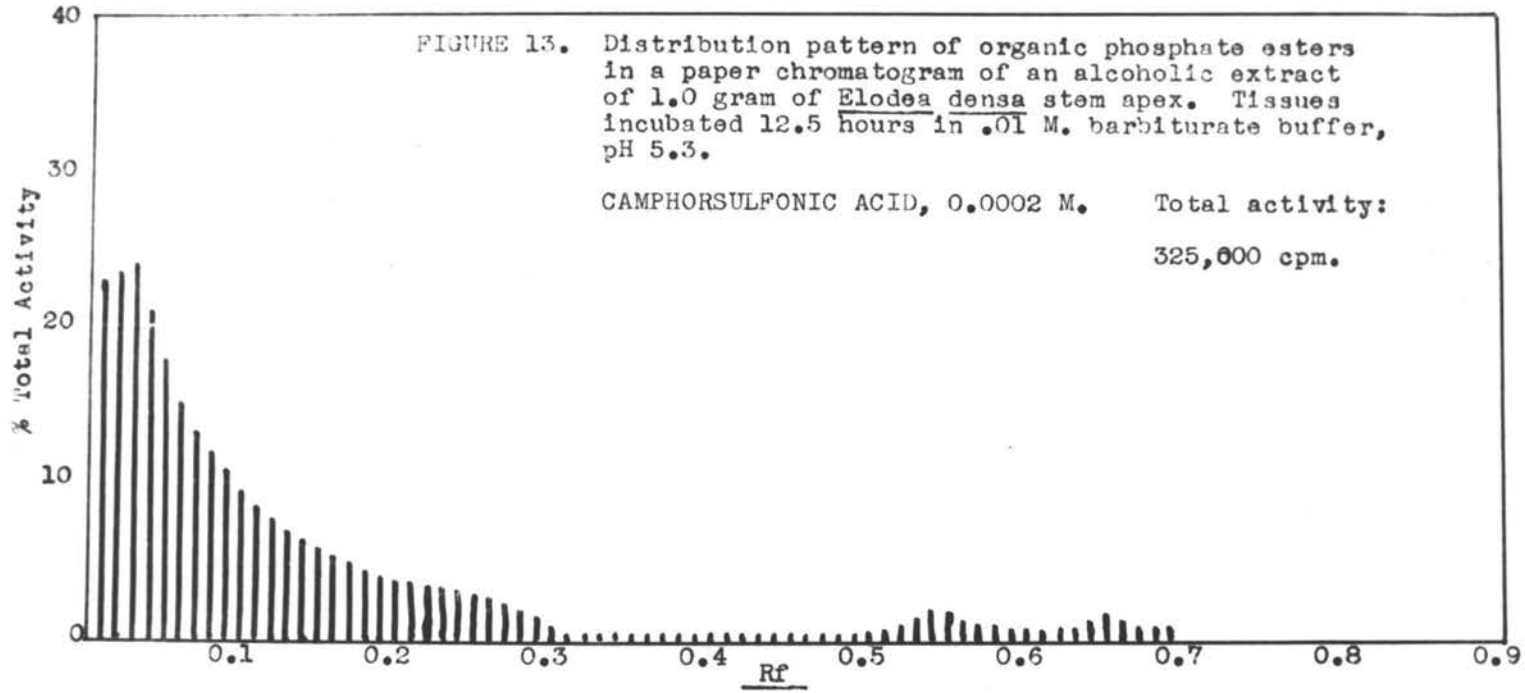


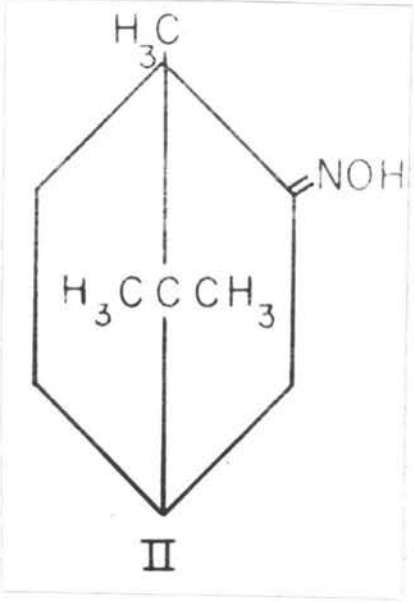
FIGURE 13. Distribution pattern of organic phosphate esters in a paper chromatogram of an alcoholic extract of 1.0 gram of Elodea densa stem apex. Tissues incubated 12.5 hours in .01 M. barbiturate buffer, pH 5.3.

CAMPHORSULFONIC ACID, 0.0002 M. Total activity: 325,600 cpm.

DISCUSSION

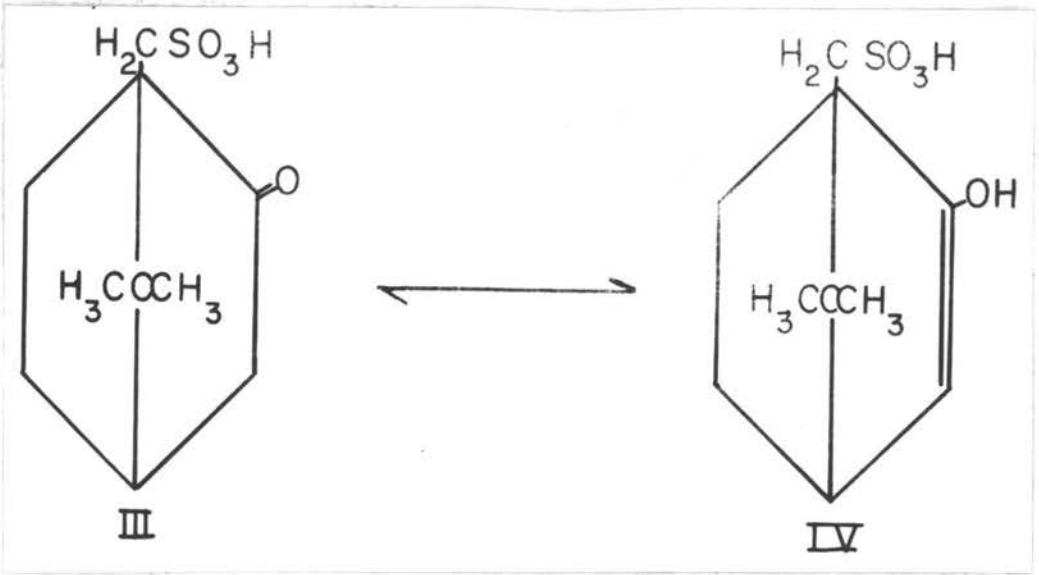
Classical theory demands that a growth substance have the following characteristics: a ring system in the molecule, a double-bond in the ring, a polar group removed from the ring by at least one carbon atom, and a definite spatial relationship between the ring and side-chain (21, p.179). Camphorsulfonic acid appears to lack the essential requirement of having unsaturation at some point within its ring.

Plant growth activity has been reported for the camphor skeleton in at least one instance, that of camphoroxime (37, p.491). Camphorsulfonic acid, while different from camphoroxime (II) could possess similar activity if the specificity of action were not wholly dependent upon the existence of an oxime group on No.2 carbon atom in the ring.



II

Camphorsulfonic acid (III) lacks a double bond in its ring nucleus, which would preclude its inclusion among the substances which theoretically ought to have growth activity. Tautomerization of the No. 2 keto group, however, to yield an enolized form with one double-bond between carbons 2 and 3 would satisfy this requirement (IV).



Alkaline conditions favor formation of the enol form but the amount of this tautomer existing at physiological pH ranges is probably quite small (38, p.369). If the possibility that this form may exist at all under physiological conditions is granted, then the activity of CSA might be related to that of compounds which possess unsaturation in their ring nuclei. Enol derivatives of CSA might be tested for activity to investigate this hypothesis. No such compounds were available for study

during these investigations, and time was not available in which to prepare them.

It has been demonstrated that the optical rotation of growth-active substances may affect their degree of activity. In general, the (+) enantiomorphs of active compounds have been shown to be more active than the (-) forms or their racemates (41, p.174-179). The rotatory power of d-CSA is assured under all normal physiological conditions by its possession of two centers of assymetry at carbon atoms 1 and 4. Racemization of the camphor ring can occur only under drastic conditions, e.g., treatment with fuming sulfuric acid (38, p.351).

Veldstra has proposed that to be active, a compound must be oriented upon adsorption in such a way that the polar group is perpendicular to the plane of adsorption. This would be difficult for CSA to meet because of the unhindered rotation of the sulfonic group. If the activity possessed by CSA residues resides in its ability to adsorb in a specific manner to an interface, the active points of adsorption would probably be the number 2 keto group and the sulfonic group. Work with molecular models may reveal some of the possibilities in this respect. The exact spatial relations of specific adsorption in biological systems are so incompletely understood at present that more than conjecture is not possible.

It is apparent from the data presented that camphor-

sulfonic acid possesses some degree of biological activity in plant tissues. This compound has the property of stimulating elongation in oat coleoptile sections and, to a lesser extent, of causing curvature of slit pea internodes. The concentrations in which CSA is active are somewhat higher than concentrations of IAA which produce the same response. Camphorsulfonic acid at .0001 M. (ca. 23 ppm.) causes elongation of coleoptile segments in a degree only slightly higher than that caused by 1 ppm. IAA (ca. .000057 M.). At .001 M., CSA inhibits growth and may exhibit toxicity. The ethyl ester of CSA appears to be more active in inhibiting coleoptile elongation and in producing marked straightening of pea internode halves. This higher activity of the ester would be expected if the assumption is made than the ester enters tissues more readily than the free acid, which probably exists as an anion at the pH values reported here.

Results of Elodea growth studies are less conclusive but suggest that CSA and Et-CSA exhibit growth activity in this plant. In Avena coleoptile elongation tests and in Elodea experiments there appears to be a synergism between indolyl-type growth regulators and CSA. The application of CSA together with auxin elicits greater responses in coleoptiles or Elodea than auxin or CSA alone. The same relation applies to inhibitory concentrations of CSA. Because indoleacetic acid and indolebutyric acid have

growth activity similar to that of CSA in the tissues studied, it appears that the action of the compounds may be similar.

That CSA stimulates the active uptake of anions is suggested by the results of radioactive phosphate uptake experiments (Figures 9 and 13). Other workers (16, p.558; 23, p.274; 28, p.825) have studied the increase in water-uptake by potato tissue induced by auxin, and their findings point to a stimulation of "active" (i.e., more than osmotic) water-uptake mediated by auxins. The behavior of CSA in stimulating active water-uptake should be checked before more than a suggestion can be made regarding the ability of CSA to stimulate an "active" uptake.

Respiration data indicate that CSA stimulates oxygen uptake in potato slices in a manner similar to that of IAA. It is of interest to note than a phosphate buffer was necessary for the stimulatory effects of CSA and IAA to be exerted. In a barbiturate buffer, both compounds inhibited respiration rate. This observation is interesting in view of the fact that most auxin respiratory studies reported in the literature have involved the use of phosphate buffers or whole tissue parts not immersed in buffer. As far as the writer is able to determine, no auxin respiration experiments have been made with the use of barbiturate or other inert buffers. The dependence of the auxinal supply of inorganic phosphate is suggested by results of the

the present experiments. A comparison of Figures 1, 3 and 5 suggests that barbiturate itself was not affecting the respiratory responses to CSA. These figures show that phosphate alone or phosphate in the presence of barbiturate allowed respiratory increase in the presence of CSA or IAA.

The ability of CSA to stimulate respiration rate in the presence of DNP and in the absence of external inorganic phosphate (Figure 4) suggests a nonauxin-like activity for CSA. It has been reported (7, p.434) that IAA fails to elicit responses from coleoptile sections inhibited by DNP. If the action of CSA were similar to that of IAA, it might be expected that no respiratory response would be promoted by CSA in the presence of DNP. Bonner (loc.cit.) reported that dichloroanisole was antagonistic to the action of DNP. When tissues were inhibited by high concentrations of DNP, addition of dichloroanisole brought respiration rate up to a value higher than that of the control tissues. It may be that the action of CSA is similar.

Consistently higher activity in the Rf 0.4 to 0.65 region in chromatograms of extracts from CSA-treated potato slices and Elodea, suggests that CSA may favor the accumulation of organic phosphate esters (glucose-1-phosphate, glucose-6-phosphate and phosphoenol pyruvate). This may arise in two ways: CSA is inhibiting the utilization of the phosphate esters or it is stimulating their

formation; or, it stimulates or prevents use of a phosphate ester not usually in high concentration. It is not possible to determine from the present experiments which of these mechanisms is responsible for the increase in organic phosphates.

Further work is indicated by the experiments embodied in this thesis. It should be determined whether CSA has any effects on oxidation of Krebs cycle acids by mitochondrial suspensions and it should be determined whether CSA has any effect on the relative rates of glycolysis and other mechanisms related to it. Quantitative measurements of the amounts of phosphorylated intermediates present in CSA-treated tissues and in normal tissues might shed light on the site of action of CSA.

SUMMARY

This thesis embodies experimental work which demonstrates the activity of camphorsulfonic acid in influencing the growth of isolated plant parts. The activity of the ethyl ester of CSA in inhibiting elongation of oat coleoptile sections and in causing curvature of pea stem internodes has been described.

Respiration experiments were performed which show that CSA stimulates respiration rates of potato tuber slices in phosphate buffer systems and inhibits respiration in the absence of phosphate anions. Some interrelations between CSA and 2,4-dinitrophenol are described and discussed.

The effects of CSA on the uptake of radioactive phosphate by potato tuber slices were studied. Results of these studies suggest that CSA may stimulate phosphate uptake and pointed to a possible stimulatory influence of CSA on phosphorylation in potato tuber slices and Elodea.

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APPENDIX

APPENDIX

Data points for respiration rate figures.

Averaged values for duplicate flasks.

Figure 1.

Minutes	Microliters of oxygen per gram of fresh tissue					
	Controls (A)	IAA .0001 M. (B)	CSA .0001 M. (E)	CSA .001 M. (D)	IAA .0001 M. CSA .0001 M (C).	IAA .0001 M. CSA .001 M (F)
30	26	46	38	44	69	56
60	69	102	88	101	144	126
90	95	154	135	144	201	194
120	108	186	164	194	238	250
150	134	236	227	264	300	328
190	164	300	296	356	366	380

APPENDIX, contd.

Figure 2.

Microliters of oxygen per gram of fresh tissue

Minutes	Controls (A)	IAA .0001 M. (B)	IAA .0001 M. CSA .0001 M. (C)	IAA .0001 M. CSA .001 M (F)	CSA .0001 M. (E)	CSA .001 M. (D)
30	56	42	42	36	40	38
60	110	78	78	70	86	79
125	214	134	140	120	174	142
155	258	152	160	134	214	174
190	300	168	188	154	254	192

Figure 3.

Minutes	Controls (A)	CSA .0002 M. (B)	DNP .0002 M. (C)	DNP .001 M. (D)	DNP .001 M. CSA .0002 M (E)	DNP .0002 M. CSA .0002 M. (F)
30	50	63	27	25	35	35
45	72	74	38	36	55	68
120	215	265	120	70	28	34
180	302	385	157	120	21	26
200	360	428	172	118	22	26

APPENDIX, contd.

Figure 4.

Microliters of oxygen per gram of fresh tissue

Minutes	Controls (A)	DNP .0002 M. (B)	DNP .001 M. (C)	DNP .001 M. CSA .001 M. (F)	DNP .0002 M. CSA .0001 M. (D)	DNP .0002 M. CSA .001 M. (E)
45	62	20	44	64	18	40
165	210	138	41	63	124	150
200	256	153	40	60	148	250

Figure 5.

Minutes	Controls (A)	CSA .0002 M. (B)	DNP .0002 M. (C)	DNP .001 M. (D)	DNP .001 M. CSA .0002 M. (E)	DNP .0002 M. CSA .0002 M. (F)
15	25	25	22	12	18	14
30	55	36	28	20	30	45
45	72	100	40	40	45	60
180	242	350	142	112	20	35
200	295	390	144	120	20	30