THE AUXIN-LIKE PROPERTIES OF POTASSIUM NAPHTHENATES
AND THEIR EFFECT ON INDOLE-3-ACETIC ACID BIOSYNTHESIS
AND DEGRADATION.

by

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B.Agr.Sc. (Hons), (Malaysia), 1970.

A thesis submitted in partial fulfillment
of the requirements for the degree of
MASTER OF SCIENCE
in the Department of
BOTANY

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER,
BRITISH COLUMBIA, CANADA.

June 1972
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Date 22nd August, 1972
ABSTRACT

The auxin-like properties of potassium naphthenates (KNap), and the effect of these compounds on indoleacetic acid (IAA) biosynthesis and degradation were examined.

Chapter I. The auxin-like properties of KNap.

Cucumber seeds were treated with 1000 ppm KNap and a significant (at the 0.05 level) inhibition of root growth (91%), compared with control seedlings was obtained.

The effects of KNap and indolebutyric acid on the initiation of roots by stem cuttings of Phaseolus vulgaris L. were examined. The treatments with 10 and 100 ppm of both compounds significantly stimulated root initiation.

Root initiation of azalea stem cuttings was significantly augmented by 10, 100, and 1000 ppm KNap.

The elongation of dark-grown Alaska pea stem segments was stimulated by 1.0 ppm KNap (279% over the control). Surprisingly, this stimulation did not differ significantly from that caused by 0.1 ppm IAA (339% over the control).

KNap at 100 and 1000 ppm, applied to the distal end of debladed petioles, did not affect abscission. The times required for 50% abscission of petioles treated with 10 and 1000 ppm cyclohexanecarboxylic acid were significantly greater
than that for abscission of control petioles, but not from that required by petioles treated with 100 ppm naphthalene-acetic acid.

Chapter II. The effect of KNap on IAA biosynthesis and degradation.

When the seeds of *Phaseolus vulgaris* L. were soaked for 12 hours in a solution of 100 ppm KNap immediately prior to sowing, there was a significant increase (140% over control plants) in the content of IAA in the apical 5-8 cm of the stems of 14-day-old plants.

The immersion of the root systems of 13-day-old dark-grown bean plants in a solution of 100 ppm KNap for 24 hours resulted in a significant stimulation (4% over the control) of the activity of the IAA oxidase system.

The evidence presented is interpreted as supporting the view that KNap has some auxin-like properties. The validity of this interpretation is discussed.
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<td>HNap</td>
<td>Naphthenic acids</td>
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<tr>
<td>KNap</td>
<td>Potassium naphthenates</td>
</tr>
<tr>
<td>CHCA</td>
<td>Cyclohexanecarboxylic acid</td>
</tr>
<tr>
<td>Sh-8</td>
<td>A compound of the cyclohexylbutanol class</td>
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<tr>
<td>NAA</td>
<td>Naphthaleneacetic acid</td>
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<tr>
<td>IAA</td>
<td>Indoleacetic acid</td>
</tr>
<tr>
<td>IBA</td>
<td>Indolebutyric acid</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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DEDICATION

This thesis is dedicated to my wife, Trudy, for her devotion, assistance, and patience that have made my study possible.
ACKNOWLEDGEMENTS

Grateful thanks are extended to my advisor, Professor D.J. Wort, for counsel, guidance, and critical review of the entire manuscript; to Drs. R.F. Scagel, I.E.P. Taylor, W.B. Schofield, and R. J. Bandoni, for assistance and advice; to Mr. S.M. Smith of the Faculty of Forestry, for advice in statistical analysis; and to Mr. D.R. Peirson, for technical assistance in making photographic prints.
INTRODUCTION

The publication of Went's classical paper "Wuchsstoff und Wachstum" in 1928 in which he described a quantitative method of using Avena seedlings for assaying plant growth substances, opened a new era of research. Kögl et al in 1933 isolated a crystalline growth-inducing substance from human urine. They also determined the chemical nature of the growth substance, and, together with the late F.A.F.C. Went, coined the term "auxin" (from Greek "auxein", to grow).

The frequent use of the term "auxin" by researchers results in the lack of uniformity in nomenclature, and leads to confusion. Many workers use the term "auxin" to refer to a single substance, indole-3-acetic acid (IAA). To add to the confusion, IAA is the substance referred to as "heteroauxin" by many investigators in Russia and Bulgaria. Tukey et al (1954) recommended the term "auxin" be used as a generic term or group-name for "compounds characterized by their capacity to induce elongation in shoot cells". The name "auxin" used here is taken in the generic sense.
The definition of auxin by Tukey et al (1954) requires a revision in view of similar characteristics exhibited by other compounds such as gibberellic acids. A good definition for auxin is lacking.

The general belief that Went's Avena coleoptile curvature test (Went, 1928) is a conclusive bioassay for auxin is questioned by the finding of Huseinov (1960) that "hybberellic acid" (probably gibberellic acid) caused bending of the Avena coleoptile, suggesting polar movement of gibberellic acid. Huseinov's finding has been supported by Jacobs and Kaldeway (1970) who demonstrated polar movement of gibberellic acid (GA₃) in young petioles of Coleus.

The lack of polar movement of IAA in some plants has been shown by Eschrich (1968) using radioactive IAA on Vicia faba.

The bioassays commonly used to demonstrate auxin activity are:

1. Wents Avena coleoptile curvature test (Went, 1928).
2. Split pea stem curvature test (Went, 1934).
4. Straight growth test, using *Avena* coleoptile segments (Bonner, 1933, 1949); using pea stem segments (Galston and Hand, 1949; Galston and Baker, 1951, 1953; Christiansen and Thimann, 1950).


The detection of IAA complexes such as IAA-glucose (Klambt, 1961; Zenk, 1964), and IAA-aspartate (Andreae and Good, 1955; Zenk, 1964) is an important contribution towards the understanding of the mechanism of IAA action in plants. IAA complexes may escape detection because they do not give typical indole colour reactions with the Salkowski and Ehrlich reagents which are commonly used by investigators in auxin research. While workers are becoming increasingly aware that free IAA accounts for only a portion of the auxin activity in plants, and that bound IAA is of "physiological significance", the time has come for a turning point in auxin research. Future research should be geared to isolation, identification, and quantitative determination of IAA complexes.
The fact that application of naphthenic acids (HNap) to plants results in increased growth, and the report that petroleum growth substances caused bending of the Avena coleoptile by Huseinov (1960), raises the question of the qualification of HNap and their salts as auxin. In this investigation, some of the auxin-like properties of naphthenates were determined, as were their effects on the biosynthesis and degradation of IAA.

The first phase of the present investigation deals with the evaluation of the auxin-like properties of potassium naphthenates (KNap), utilising the following bioassays:
1. Growth of intact roots of cucumber (Cucumis sativa L)
2. Adventitious root initiation in succulent and woody stem cuttings.
3. Elongation of pea stem segments.
4. Abscission of petioles of bean (Phaseolus vulgaris) plants.

The second phase of the present investigation is concerned with the effect of KNap on IAA biosynthesis and degradation.

In the pages that follow, the term "significant" implies statistical significant difference at the 0.05 level, unless otherwise stated.
NAPHTHENIC ACIDS

The name, naphthenic acid, was first suggested by Markovnikoff and Oglobin (1883) for the $C_{11}H_{20}O_2$ acids of unknown structure which Hell and Medinger (1874) recovered from Rumanian crude oil. The plant growth stimulators obtained from petroleum have been named and abbreviated differently by various researchers namely: naphthenic growth substances (N.G.S.), petroleum growth substances (P.G.S.), naphthenic acids (H_Nap), potassium naphthenates (K_Nap), oil hormone substances (O.H.S.), naphtha growth matter, oil growth matter, petroleum nutrient, sintovit (an oxidised petroleum product), Sh-8 (a cyclobutanol), petroleum or growth-helping substances (P.R.V. or N.R.V.), H.P.B., Kh.T.I., H.T.I. The latter two are used by investigators in Bulgaria. In the Russian language, the abbreviations R.V. mean "growth-helping substance".

Naphthenic acids are isolated from petroleum by acid-base extraction, and from diesel oil by adsorption. Naphthenic acids are known to be a complex mixture of alkylated alicyclic monocarboxylic acids, some of which are derivatives of cyclopentane, cyclohexane, and cycloheptane. Jolly (1967) noted that cyclopentyl derivatives predominate in the naphthenic acid mixture, followed by cyclohexyl compounds. Recently,
Kazanis (1971) by mass spectrometric, nuclear magnetic resonance and infrared analyses, detected methyl 2-DL-4D-dimethyl heptanoate, methyl octanoate, methyl nonanoate, and cis 1,3-dimethylcyclohexycarboxylate in the naphthenic acid mixture isolated from crude petroleum.

The molecular weights and boiling points of HNap vary with the source of the acids. HNap with an average molecular weights of 214 (Cason and Khodair, 1966); 211, 239, and 303 (Tanchuk, 1971); 216 (Nametkin, 1971) have been reported. A boiling point range from 30° to 150° C was reported by Kazanis (1971), and 45° to 170° by Artamonov (1971). The commercially available naphthenic acids, used in the present investigations, have an average molecular weight of 230.

The carboxyl group of most individual members of the naphthenic acid mixture is not attached directly to the alicyclic ring, but is separated from the ring by an aliphatic side chain containing one to five or more methylene groups. According to Jolly (1967), a general formula may be written as $R(CH_2)_n COOH$, where $R$ is an alicyclic nucleus composed of one or more rings. An exception to this general rule is cyclohexanecarboxylic acid and its K salt which have been
shown to exhibit biological activity (Wort and Patel, 1970; Severson, 1971; Padmanabhan, 1972; Peirson, 1972) and also in the present investigations.

The naphthenic acids obtained from Baku have an acid number of 259 mg KOH/g (Nametkin, 1971).

Naphthenic acids have a characteristic rubbery odour which varies with the acid source, degree of refinement, and content of phenolic and sulphur compounds. The acids are readily soluble in non-polar solvents, and the lower molecular weight members, such as cyclohexanecarboxylic acid and cyclopentanecarboxylic acid are sparingly soluble in water.

In low concentrations (5000 ppm or less), naphthenates have been found to promote vegetative and reproductive growth (e.g. Severson, 1971). There are also reports that when applied at high concentrations naphthenates act as herbicides (Mailov, 1968; Zhukova, 1965). In this respect, the behaviour of naphthenates is similar to that of 2,4-D.

The mode of action by which applied naphthenates influence plant growth and metabolism is obscure. Wort (1969) suggested
the anion, naphthenate was responsible for the stimulation of vegetative and reproductive growth in beans. The glucose ester and aspartic acid amide of naphthenic acids rather than the free acid were responsible for the stimulation of glucose uptake and its metabolism in bean root tips according to Severson (1972). At this time, it is not possible to designate one or more naphthenic acids as specifically responsible for stimulation of plant growth and development but some structural characteristics of effective acids have been suggested (Wort and Patel, 1970). Following their application to bean plants, naphthenates stimulate many physiological and biochemical processes.

The stimulation of photosynthesis and dark respiration (Fattah, 1969; Wort et al., 1970), protein synthesis (Severson, 1972; Wort et al., 1971), and the specific activities of numerous enzymes in crude extracts (Chu, 1969; Fattah, 1969; Fattah and Wort, 1970; Wort et al., 1971) suggest that naphthenate stimulation of plant growth is the result of the action of the chemicals or their metabolites at the genetic and metabolic levels (Wort et al., 1971).

A method for quantitative determination of sodium naphthenates in plants was given by Guseinov (1970).
Responses to naphthenate treatments, including greater vegetative and reproductive growth have been reported for maize, tomato, potato, winter cereals, grapes, and other crop plants (Popoff and Boikov, 1966). The effects of naphthenates on plant growth, yield and composition were summarised by Severson (1971).

Petroleum growth substances are versatile in causing stimulation of growth and metabolism. The stimulatory effects of P.G.S. are not only observed in plants but also in animals. P.G.S. when administered orally at 1.0 mg/kg body weight of castrated rams, increased the serum protein level by 3.64-7.2% with a maximum after 40 days, and increased serum albumin level by 12.92% (Mekhtiev, 1970). Gorshkova (1970) reported proliferation of both inter and intra lobular tissues in the liver of rabbits 10 days after administration of 20 mg P.G.S. per kg body weight. Thirty-day doses of 5 mg/kg increased rabbit body weight by 18-20%, while 20 mg/kg decreased the body weight by 20-22% (Loshmanova, 1970).

The non-biological uses of naphthenic acids and their salts are varied. They are used as lubricants in refrigeration
and air-conditioning, driers in the paint industry, catalysts, preservatives, emulsifiers (Jolly, 1967), and as lead naphthenate, a constituent in a mixture that is resistant to salt water corrosion. They are also used as copper naphthenates in fungicidal preparations.

The information accumulated thus far is sufficient to justify the inclusion of naphthenic acids and their salts as one of the plant growth substances along with the so-called "established" plant growth regulators such as: indole-3-acetic acid, gibberellic acids, cytokinins, 2,4-D, 2,4,5-T, naphthaleneacetic acid, 2,3,6 tri-iodobenzoic acid, maleic hydrazide, cycocel, and phosfon. Naphthenic acids and their salts may be superior to other plant growth regulators due to their dual stimulatory effects on plants and animals.
NAPHTHENIC ACIDS

Cyclopentanecarboxylic acid
\[ \text{C}_6\text{H}_{10}\text{O}_2 \]
molecular weight 114

Cyclohexanecarboxylic acid
\[ \text{C}_7\text{H}_{12}\text{O}_2 \]
molecular weight 128

3-methyl-cyclopentyl-n-propionic acid
\[ \text{C}_9\text{H}_{16}\text{O}_2 \]
molecular weight 156
CHAPTER I

INVESTIGATION OF THE AUXIN-LIKE PROPERTIES OF POTASSIUM NAPHTHENATES.
LITERATURE REVIEW

A survey of the literature on the influence of naphthenates on root growth indicates the scanty nature of research in this area.

The prevalent response of intact roots to exogenous auxin is retardation of elongation. Roots have been reported to respond to low concentrations of indoleacetic acid (IAA) by slight increase in growth rate (e.g. Burstrom, 1951; Larsen, 1961). The use of root measurements of red clover to determine the presence of 2,4-D in soil was described in a paper by Nutman et al (1945). They found that 2,4-D at concentrations of 1.0, 10, and 100 ppm were inhibitory to root growth.

Investigations on the effect of auxins on root growth have shown that, in general, substances which cause growth promotion in the Avena coleoptile, cause inhibition of root growth (e.g. Bentley and Bickle, 1952).

Moewus in 1949 who devised the cress root test as a quantitative method of estimating growth substances, found
that $2.4 \times 10^{-7}$ M IAA was necessary for 50% inhibition of root growth. Audus (1949) obtained 50% inhibition of root growth in cress, radish, garden pea, and maize in $6.8 \times 10^{-7}$ M 2,4-D in solution culture at pH 6.8. On the effect of IAA on root growth using Moewus (1949) cress test, Bentley (1951) showed that IAA inhibited root growth in Lepidium sativum over a range of concentrations from $1.0$ to $10^{-5}$ mg/l. A slight growth promotion at lower concentrations, to a maximum of ca 30% at $10^{-7}$ to $10^{-8}$ mg/l was also found. Ready and Grant (1947) found seedlings of Cucumis sativus to be sensitive to 2,4-D. The growth of primary roots was inhibited to 50% by $2.1 \times 10^{-7}$ M solution of 2,4-D. Using seedlings of Avena sativa grown on filter paper moistened with solutions of growth substances, Lane (1936) found that $1.7 \times 10^{-6}$ M IAA was necessary for 50% inhibition of root growth, while Bonner and Koepfli (1939) reported $3 \times 10^{-7}$ M IAA and $7 \times 10^{-6}$ M NAA were required to bring about the same inhibition.

A reduction of ca 10% in growth of the root system following treatment with sprays of 0.005% P.G.S. on eggplants was demonstrated by Ali-Zade and Guseinov (1965).
Bentley (1958) found an average maximum stimulation of 20% with IAA at $5.7 \times 10^{-3}$ M using Moewus root test (Moewus, 1948, 1949).

The effect of temperature on root growth was described in a paper by Pilet and Went (1956). They observed that IAA at $10^{-4}$ M retarded growth of old roots of *Lens culinaris*, and the degree of retardation increased markedly with temperature, whereas the growth of young roots was accentuated slightly at low temperatures and inhibited at high temperatures. At $1.0 \times 10^{-8}$ M IAA, growth of both old and young roots was stimulated at all temperatures (5-27°C).

Huseinov (1960), utilising similar concentrations of naphthenic growth substances (N.G.S.) and IAA (heteroauxin) (0.1, 0.01, 0.001, and 0.0001%) obtained similar trends in response of root growth of wheat i.e. inhibitory at high concentration (0.1%) and stimulatory at weak concentrations (0.0001% N.G.S., 0.001% IAA). He also employed different periods of seed soak (6, 12, and 24 hours) in 0.005, 0.01, and 0.05% N.G.S., and found that root lengths of winter wheat were greater than that of the controls. Seed wetting with
weak solutions of N.G.S. (0.004 and 0.0004%) prior to sowing, enhanced the root lengths of cotton, onion, cucumber, and winter wheat. The growth of cotton roots was stimulated by soaking the seeds in solutions of 10 mg/l HNap (Babaev, 1966).
MATERIALS AND METHODS

Seeds of cucumber (Cucumis sativus L.) were washed with tap water and fifteen uniform size seeds were placed in each petri dish (diameter 9 cm) containing three pieces of Whatman No. 1 filter paper. Eight ml volumes of each of the following test solutions were separately to each dish:

- KNap 1000 ppm
- KNap 100 ppm
- KNap 10 ppm
- KNap 1.0 ppm
- Distilled water served as control.

The petri dishes were covered and placed in an incubator at a constant temperature of 20° C for 72 hours. At the end of the incubation period, the lengths of the primary roots were determined. The average length of the roots was used to compute the percent inhibition of root elongation:

\[
\frac{\text{Average control root length minus average treated root length}}{\text{Average control root length}} \times 100
\]
Each treatment was repeated four times, and the whole experiment was laid down in a randomised complete block design. The results of the experiment were subjected to statistical analysis and Duncan's New Multiple Range Test (Duncan, 1955).

**Preparation of potassium naphthenates (KNap) aqueous solution from naphthenic acids (HNap)**

Seventeen ml of a 12.3% (w/v) KOH solution (2.1 g KOH in 17 ml distilled water) was added to an Erlenmeyer flask containing 5 g HNap (Practical grade, average molecular weight 230; Eastman Organic Chemicals, Rochester, New York). The flask was shaken for 10-15 minutes and the solution was made to a volume of 25 ml with distilled water. The solution thus prepared was the stock solution containing 250 mg of KNap per ml. By diluting 1.0 ml of the stock solution with 49 ml of distilled, the final concentration of KNap was $2 \times 10^{-2}$ M (5000 ppm or 0.5%). The pH of the diluted solution was adjusted to ca 10 with 1.0 N HCl.
RESULTS

Low KNap concentrations of 1.0 and 10 ppm treatment of cucumber seeds stimulated root growth (ca 3 and 4% respectively). However, this stimulatory effect of KNap was not significant. High KNap concentrations (100 and 1000 ppm) had an inhibitory effect on root growth (ca 21 and 91% respectively). Only the inhibitory effect of 1000 ppm was significant (Table I). The results are presented graphically in figure 1.
Table I. Response of intact roots of cucumber to KNap.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Average length of roots from 60 determinations (mm)</th>
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<tr>
<td>0 (control)</td>
<td>93.18 a</td>
</tr>
<tr>
<td>1.0</td>
<td>96.32 a</td>
</tr>
<tr>
<td>10</td>
<td>97.37 a</td>
</tr>
<tr>
<td>100</td>
<td>73.30 a</td>
</tr>
<tr>
<td>1000</td>
<td>8.37 **</td>
</tr>
</tbody>
</table>

Values followed by the same letter do not differ significantly from one another, according to Duncan's New Multiple Range Test (Duncan, 1955).

** Value differs significantly from the control value at the 0.01 level.
Figure 1. Growth response of intact roots of cucumber.
DISCUSSION

The trend in response of cucumber roots to KNap is typical of auxin effect, i.e. prevalent retardation of elongation in high concentrations and slight promotion in low concentrations (e.g. Burström, 1951; Larsen, 1961).

The inhibition of cucumber root growth by 1000 ppm KNap is similar to that reported by Huseinov (1960) using N.G.S. in wheat. In the present experiment, the treatments with low concentrations of KNap (1.0 and 10 ppm) on cucumber resulted in a slight promotion of root growth. Similar increases have been reported by Huseinov (1960) utilising 5, 10, and 50 ppm N.G.S. seed soak of wheat.

The failure to obtain significant stimulation of root growth in 1.0 and 10 ppm KNap concentrations suggests that the effective property of KNap is predominantly that of inhibition of root growth which is characteristic of auxin (e.g. Burström, 1951; Larsen, 1961).

The retardation of root growth caused by IAA is exhibited in much lower concentrations than KNap, for instance, at ca 0.3 ppm on Avena sativa (Lane, 1936);
at a range from $1.0$ to $10^{-5}$ ppm on *Lepidium sativum* (Bentley, 1951); at $10^{-7}$ M on wheat root (Burström, 1942).

The fact that it requires a relatively high concentration of KNap (1000 ppm) to bring about a significant retardation of root growth as compared to IAA (e.g., $1.0$ to $10^{-5}$ ppm, Bentley, 1951) suggests that the degree of effectiveness of KNap is less than that of IAA.
ib) Adventitious root initiation in succulent and woody stem cuttings.

LITERATURE REVIEW

The auxin-induced formation of roots on stem and leaf cuttings, and on intact plants provides a good bioassay for auxin activity. That the formation of adventitious roots on a cutting always occurs at the physiological base, was first shown in willow by H. Vöchting in 1878. An extensive critical review of the role of hormones in the formation of adventitious roots was published by Libbert (1957a).

The original quantitative method was developed by Went (1934b), utilising etiolated Pisum stem cuttings. Later, Went's method was modified by other investigators for the use with different species of plants (e.g., Libbert, 1957b; Luckwill, 1956).

The participation of auxin in controlling root branching was amply demonstrated by Thimann (1936) in experiments
treated the roots of *Avena* and *Pisum* with IAA. The application of indolebutyric acid in concentrations of 12.5 and 25 mg/l to the base of root cuttings of *Taraxacum* and *Cichorium* stimulated abundant root production (Warmke and Warmke, 1950).

Polikarpova (1963) reported the use of P.G.S. during root formation in green cuttings of cherries, gooseberries, and blackcurrants, favourably affected the formation of roots and subsequent development of the cuttings. The best concentration was found to be 0.01% (100 ppm) P.G.S.

The physiological activities of sodium salts of low and high molecular weight naphthenic acids obtained from different sources were investigated with respect to root formation (Porutskii *et al.*, 1963). They found that root formation decreased with increasing molecular weights of HNap obtained from different sources as indicated in this order:

diesel fuel < transformer oil < lubrication oil < motor oil.

Root formation was stimulated by P.G.S. in cuttings of cranberry, phlox, vine, privet, and the leaves of perilla, but no stimulation in cuttings of spindle-tree and poplar was observed (Yusufov, 1963).

The physiology of root primordia initiation has been extensively discussed following the introduction of the rhizocaline hypothesis by Bouillenne and Went in 1933.
The name, rhizocaline, was first suggested by Bouillenne and Went in 1933 for the rooting substance produced by the leaves in the presence of light. This substance is stored in the cotyledons and buds, and transported basipetally.

According to the rhizocaline hypothesis (Cooper, 1936; Went, 1938), the basipetal transport of auxin causes it to accumulate at the base of a cutting, and the resulting auxin gradient causes a downward movement of rhizocaline to the base of the cutting. Auxin then "activates" or "reacts" with rhizocaline to bring about root formation. The existence of rhizocaline or rhizocaline-like substances was amply demonstrated (Bouillenne and Bouillenne, 1952; Galston, 1948; Hess, 1962). The latest modification of the rhizocaline hypothesis by Bouillenne (1964) describes rhizocaline as a complex of three factors, namely 1) A highly specific, mobile factor with orthodiphenolic groups which is synthesised in the leaves. 2) Auxin. 3) An oxygen-requiring enzyme located in specific cells and tissues.

Hess (1957) and Kawase (1964) added the concept of cofactors. The cofactors were considered to be endogenous substances capable of acting synergistically with IAA in the rooting of cuttings of mung bean and brittle willow.

Haissig (1971) suggested that RNA is necessary for the initiation of root primordia.
MATERIALS AND METHODS

i) Succulent stem cuttings using bean plants.

Uniform size seeds of bush bean plant, Phaseolus vulgaris L. cultivar Top Crop (Buckerfield's Ltd., New Westminster, B.C.) were sown on steam-sterilised composted soil in six wooden flats (30 x 47 x 7 cm). Uniformity of plants was achieved by periodic culling of runts. The plants were grown in a greenhouse with a 14-hour photoperiod under Sylvania Gro-Lux phototubes which gave 10,000 lux light intensity at the top of the plants at $25^\circ \pm 1^\circ$ C.

The stems of 14-day-old seedlings were cut at ca 2 cm above soil level and washed with running tap water. One-half of each of the two primary leaves was removed to reduce transpirational loss of water. The bases of the stem cuttings thus prepared were soaked for six hours to a depth of four cm in one of the following solutions:
1) Distilled water served as control.
2) KNap 10 ppm
3) KNap 100 ppm
4) Indolebutyric acid 10 ppm
5) Indolebutyric acid 100 ppm

A circular Pyrex glass container (diameter 27 cm) sufficient to hold one litre of solution, was placed in a wooden flat. Care was exercised not to allow the leaves to come in contact with the solution.

At the conclusion of the soak period, the cuttings were removed from the solution, and the basal portions of their stems inserted to a depth of four cm in evenly spaced rows in the rooting medium kept in the propagation chamber. The rooting medium consisted of 1:1 (v/v) of fine sand and peat. The sand was sieved successively through ¼-inch mesh and 1/8-inch mesh sieves, and then washed thoroughly.
The peat was wetted with tap water and screened through ¼-inch mesh sieve. The rooting medium was sterilised at 248°C for 15 minutes at a pressure of 15 lb per square inch.

The propagation chamber was covered with a polythene lid to allow a daily 14-hour light of intensity 5200 lux to reach the cuttings. The chamber had air temperatures of 20.5 ± 1°C, and a relative humidity of 90 ± 5%.

Indole-3-butyric acid, a rooting hormone, was included in this experiment to serve as a standard for comparison (Mitchell et al, 1968).

The stem cuttings were allowed to root for five days, then the root systems were thoroughly washed in running water. The number and length of roots were determined.

The experiment was conducted in a randomised complete block design with three blocks. Each treatment consisted of 24 cuttings, with eight cuttings randomly placed in each block. The results were subjected to analysis of variance and Duncan's Multiple Range Test (Duncan, 1955).
ii) Woody stem cuttings using azalea plants.

Stem cuttings of "Chichibu", an evergreen Japanese azalea of the Wadai group (*Rhododendron indicum* var. *eriocarpum* x *kaempferi*, according to Lee et al, 1952), were collected on 20th August, 1971 from several bushes in the Nursery on campus. Uniform stem cuttings were taken from the apical regions (12-15 cm) of the plants. The stem cuttings collected from the field, were washed and were cut under water below the second node. Three to four leaves were left on each cutting. The prepared stem cuttings were surfaces sterilised by immersing in 1.0% Captan fungicide for 15 minutes. Thereafter, the cuttings were soaked for six hours in one of the following solutions in the greenhouse at a light intensity of 6000 lux, and air temperatures of $25^\circ\pm 1^\circ$ C:

1) KNap 1000 ppm
2) KNap 100 ppm
3) KNap 10 ppm
4) Distilled water served as control.
Following the six-hour soak period, the basal ends of the cuttings were inserted into sterilised rooting medium, consisted of 1:1 (v/v) fine sand and peat prepared as described in (i), contained in wooden flats. The cuttings were allowed to root in a greenhouse. The greenhouse provided a 16-hour photoperiod, air temperatures of 22 ± 1° C, and a light intensity of 4,400 lux. The relative humidity of the experimental area was maintained at ca 100% by means of an overhead water spray operated by an automatic humidity sensing device.

The experiment was set up in accordance to the randomised complete block design comprising four treatments arranged in four blocks with ten cuttings of each treatment per block.

The stem cuttings remained in the rooting medium for a total of 35 days. The rooted cuttings were then carefully removed, washed, and the number and length of the roots determined. An analysis of variance and Duncan's New Multiple Range Test (Duncan, 1955) were performed on the results obtained.
RESULTS

i) Rooting of succulent stem cuttings of bean.

The average number of roots on a stem cutting is used to represent the degree of root initiation. This is based on the assumption that the number of roots is analogous to the number of root primordia.

In a preliminary experiment, logarithmic dilutions of KNap concentrations of a range from 1000 to 0.0001 ppm were used to determine the effect of different concentrations of KNap on root initiation and growth, and to ascertain the optimal concentrations for subsequent investigations. It was found that 1000 ppm KNap was injurious to stem cuttings, and caused a profound inhibition of root initiation. The portion of the stem cuttings immersed in 1000 ppm KNap gave a "water soaked" appearance. The greatest stimulatory effect was observed with 10 and 100 ppm.
The induction of roots, and the promotion of root growth following treatments with KNap and indolebutyric acid (IBA) were significantly different from that of control plants at the 0.01 level. The treatments with 10 and 100 ppm KNap gave increases in root initiation of 46.35 and 152.94% respectively over control plants. The cuttings in 10 and 100 ppm IBA had increases of 164.54 and 297.41% respectively over control plants. The stimulation of root induction (152.94%) by 100 ppm KNap did not differ significantly from the stimulation (164.54%) by 10 ppm IBA (Table II).

Greater swelling at the bases of KNap-treated stem cuttings over control cuttings were observed, but no detectable differences between KNap-treated and IBA-treated cuttings.

The stimulation of root initiation as percentage of the control, is represented schematically in figure 2. The effects of the treatments on the stem cuttings can be seen in Plate 1.
Table II. Rooting response of bean stem cuttings to KNap and IBA treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>+Average number of roots per cutting</th>
<th>Increase over control (%)</th>
<th>+Average total length of roots per cutting</th>
<th>Increase over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H₂O (control)</td>
<td>28.20</td>
<td>--</td>
<td>231.33</td>
<td>--</td>
</tr>
<tr>
<td>KNap 10 ppm</td>
<td>41.27</td>
<td>46.35</td>
<td>400.00</td>
<td>72.91</td>
</tr>
<tr>
<td>KNap 100 ppm</td>
<td>71.33 a</td>
<td>152.94</td>
<td>507.40</td>
<td>119.34</td>
</tr>
<tr>
<td>IBA 10 ppm</td>
<td>74.60 a</td>
<td>164.54</td>
<td>722.67</td>
<td>212.40</td>
</tr>
<tr>
<td>IBA 100 ppm</td>
<td>112.06</td>
<td>297.41</td>
<td>1257.33</td>
<td>443.52</td>
</tr>
</tbody>
</table>

+Value from 15 stem cuttings. Values followed by the same letter do not differ significantly from each other, according Duncan's New Multiple Range Test (Duncan, 1955).
Figure 2. Stimulation of root initiation of bean stem cuttings by KNap and IBA treatments.
Plate 1. Rooting response of bean stem cuttings to KNap and IBA treatments.

Legend:

1 Cutting in distilled water.
2 Cutting in 10 ppm KNap.
3 Cutting in 100 ppm KNap.
4 Cutting in 10 ppm IBA.
5 Cutting in 100 ppm IBA.
ii) Rooting of woody stem cuttings of azalea.

The treatments of azalea stem cuttings with KNap (10, 100, and 1000 ppm) stimulated both induction and growth of roots. High concentrations of KNap (100 and 1000 ppm) resulted in a significant increase in number of roots (ca 50 and ca 134% respectively) over the control cuttings at the 0.01 level. However, the low concentration (10 ppm) of KNap had no significant stimulation of root initiation over control values. All concentrations of KNap (10, 100, and 1000 ppm) significantly augmented root growth (ca 36, 66, and 220% respectively) at the 0.01 level. The treatments with 10 and 100 ppm KNap did not differ significantly from each other. A slight "scorching" of the portion of the stems immersed in 1000 ppm KNap was observed.

The results of this investigation are given in Table III and the stimulatory effect of the treatments can be seen in Plate 2. Root initiation calculated as percentage of control values are represented in a histogram in figure 3.
### Table III. Response of stem cuttings of azalea to KNap.

<table>
<thead>
<tr>
<th>Concentration of KNap (ppm)</th>
<th>Average number of roots per cutting +</th>
<th>Increase over control (%)</th>
<th>Average total length of roots per cutting +</th>
<th>Increase over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>29.62 a</td>
<td>--</td>
<td>279.75</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>33.37 a</td>
<td>12.66</td>
<td>382.17 b</td>
<td>36.61</td>
</tr>
<tr>
<td>100</td>
<td>44.46</td>
<td>50.10</td>
<td>464.12 b</td>
<td>65.90</td>
</tr>
<tr>
<td>1000</td>
<td>69.29</td>
<td>133.93</td>
<td>894.58 b</td>
<td>219.77</td>
</tr>
</tbody>
</table>

+ Value from 24 stem cuttings.

Values followed by the same letter do not differ significantly from each other, according to Duncan's New Multiple Range Test (Duncan, 1955).
Figure 3. Stimulation of root initiation in azalea stem cuttings by KNap treatments.
Plate 2. Rooting response of azalea stem cuttings to KNap treatments.

The concentrations of KNap employed as indicated.
DISCUSSION

The rooting of stem cuttings in distilled water is evidence of the presence of endogenous auxin, and the predominance of its basipetal transport. The average number of roots formed per treated stem cutting is taken as a measure of root-forming activity of KNap and IBA treatments. The marked increases in the average number of roots per cutting in KNap- and IBA-treated cuttings suggest that these compounds possess high root-forming activity. The greater number of roots in KNap treated cuttings may be the result of accelerated mitosis which leads to an increased rate of initiation of root meristems. The stimulation of root initiation and the increased swellings at the extreme tips of the bases of KNap-treated stem cuttings over that of the controls may be accounted for on three bases:

1) The possible augmentation of endogenous auxin by KNap.

2) The relatively low mobility, and greater chemical stability of KNap following application to stem cuttings which resulted in the accumulation and retention of KNap near the site of application.

3) A mobilization of root-forming substances or cofactors
for example rhizocaline, to the site of KNap application.

Chemical stability and low mobility are two important properties of root-forming substances (e.g., Audus, 1959).

The stimulation of rooting of azalea cuttings is in agreement with the observations of Polikarpova (1963) using 100 ppm P.G.S. on woody cuttings of cherries, gooseberries, and blackcurrants.

In very high KNap concentration (1000 ppm), a toxic effect on bean cuttings set in and offset stimulation resulting in a "water soaked" appearance in the treated stem portions. The optimum root-forming property of KNap was found to be in the concentrations of 100 ppm in succulent bean cuttings, and 1000 ppm in woody azalea cuttings.

The increases in root growth of treated stem cuttings are interpreted as mainly due to a relatively early root initiation compared with the control cuttings.

The lack of significant difference between the effects of 100 ppm KNap and 10 ppm IBA treatments on bean cuttings indicates that the root-initiation effect of IBA is similar to that of KNap. IBA is an established rooting hormone, and proved to be superior to IAA (e.g., Audus, 1959).

As is typical of the effect of auxin, there is a stimulation of adventitious root initiation by KNap.
ic) Elongation of pea stem segments.

LITERATURE REVIEW

The behaviour of stem segments of *Pisum* under the influence of synthetic auxin in various experimental conditions has been studied by Galston and Hand (1949); Nitsch and Nitsch (1956) and others. The response of such segments has been employed to detect and characterise naturally-occurring auxin (Larsen, 1961). Galston and Hand (1949) who incubated 8-day-old dark-grown Alaska pea stem segments in various concentrations of IAA at 25°C for 24 hours, found that half maximal growth was generally produced by 0.01 mg/l, and maximal growth by 0.1 and 1.0 mg/l. The segments immersed in 10 mg/l IAA had a "water soaked" appearance. Maximum response in the straight growth test was obtained by the use of 0.1 mg/l IAA, pH 6.1 (KH₂PO₄ - Na₂HPO₄) in 2% sucrose, and at 30°C in darkness (Galston and Hand, 1949).
White light retards the elongation of etiolated pea stem segments in varying concentrations of IAA, and this light-induced inhibition of growth could not be explained in terms of photoinactivation of IAA. At low exogenous IAA levels, there is relatively greater efficiency in the utilisation of IAA by the segments. This efficiency is greater in the dark than in light (Galston and Hand, 1949).

The effect of sugar on elongation of stem segments is debatable. Christiansen and Thimann (1950) reported that sucrose has no effect on elongation of pea stem segments, whereas Galston and Hand (1950) reported an increase in elongation with 2% sucrose. However, more investigators include 2% sucrose in their trials than otherwise.

The response of segments to test materials depends greatly on the portion of the stem from which the segments are taken. Purves and Hillman (1958) suggested that segments taken furthest from the apex of the internodes respond well to exogenous IAA. Christiansen and Thimann (1950) suggested the use of segments taken from third internodes.
Nitsch and Nitsch (1956) utilised segments taken from the first internodes.

The lower limit of this test is $10^{-9}$ mg/l IAA (e.g., Bentley, 1958).

To date, no literature is available on the effect of HNap on the growth of stem segments.
MATERIALS AND METHODS

The procedure adopted in this investigation was modified from Christiansen and Thimann (1968). Uniform seeds of *Pisum sativum* L cultivar Alaska were soaked for six hours in distilled water and sown in vermiculite in wooden flats. The plants were grown in darkness at air temperatures of $23^\circ \pm 0.5^\circ$ C, and a relative humidity of $95\% \pm 2\%$. After seven days, uniform plants were selected and 19.5 mm segments were cut from the subapical portion of the third internodes with razor blades mounted 19.5 mm apart on a plastic block. This region has been shown to respond well to auxin (Purves and Hillman, 1958). Immediately following excision the segments were washed in distilled water, blotted dry with paper towels, and divided into groups of five. Each group was weighed and placed in petri dishes containing one of the following solutions:

1) IAA (K salt) 0.1 ppm
2) IAA (K salt) 1.0 ppm
3) CHCA (K salt) 0.1 ppm
4) CHCA (K salt) 1.0 ppm
5) CHCA (K salt) 10.0 ppm
6) KNap 0.1 ppm
7) KNap 1.0 ppm
8) KNap 10.0 ppm
9) Distilled water served as control.

With the exception of the control, the pH of the above solutions was adjusted to 10 with 1N KOH.

The stem segments were incubated for 24 hours in darkness at an air temperature of 29° C.

The experiment was conducted in a randomised complete block design, arranged in three replications, with five segments in each replication.

At the completion of the specified incubation period, the lengths of the segments were measured with a micrometer. All observations and manipulations were performed under two 40-watt red Sylvania lamps.

The results were subjected to analysis of variance and Duncan's New Multiple Range Test (Duncan, 1955) for multiple comparison of treatment means.
RESULTS

The effect of treatment on elongation of stem segments was assessed using this formula:

\[
\frac{\text{Increase of treated over control}}{\text{Increase in Control}} \times 100
\]

The stimulatory effect of the treatments on segment elongation, and on increases in weight of segments was significant at the 0.01 level.

A rise in concentrations of the IAA, CHCA, and KNap treatments resulted in an increase in elongation of the segments, and a corresponding increase in weight of the segments (Table IV).

The elongation of segments in 0.1 ppm IAA (ca 339% increase over control segments) did not differ significantly from that of 1.0 ppm KNap (ca 279% over control segments), and also from the induced elongation by 1.0 and 10 ppm CHCA (ca 327 and 360% increase over control segments). This suggests that the stimulatory effects of CHCA and KNap are similar to that of IAA.

In low KNap concentration (0.1 ppm), there was no significant difference of induced elongation over the control segments.
KNap at 10 ppm had greater stimulatory effect on elongation (ca 473% over control segments) than that of IAA (ca 339% over control segments).

The increases in weights of segments receiving 0.1 ppm IAA (ca 70%), 10 ppm KNap (ca 67%), and 1.0 ppm KNap did not differ significantly from one another, according to Duncan's New Multiple Range Test (Duncan, 1955).
Table IV. Response of dark-grown Alaska pea stem segments to exogenously applied IAA, CHCA, and KNap.

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Average length of 15 segments (mm)</th>
<th>Average increase in length (mm)</th>
<th>Increase in length over control (%)</th>
<th>Average increase in total weight of 5 segments (+mg)</th>
<th>Increase in weight over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>19.83*</td>
<td>0.33</td>
<td>--</td>
<td>21.00</td>
<td>--</td>
</tr>
<tr>
<td>2 IAA 0.1</td>
<td>20.95</td>
<td>1.45</td>
<td>339.39</td>
<td>35.67</td>
<td>69.85</td>
</tr>
<tr>
<td>3 IAA 1.0</td>
<td>22.10</td>
<td>2.60</td>
<td>687.87</td>
<td>74.67</td>
<td>255.57</td>
</tr>
<tr>
<td>4 CHCA 0.1</td>
<td>20.24</td>
<td>0.74</td>
<td>124.24</td>
<td>21.67</td>
<td>3.19</td>
</tr>
<tr>
<td>5 CHCA 1.0</td>
<td>20.91</td>
<td>1.41</td>
<td>327.27</td>
<td>27.00</td>
<td>28.57</td>
</tr>
<tr>
<td>6 CHCA 10</td>
<td>21.02</td>
<td>1.52</td>
<td>360.60</td>
<td>29.67</td>
<td>41.28</td>
</tr>
<tr>
<td>7 KNap 0.1</td>
<td>20.16</td>
<td>0.66</td>
<td>100.00</td>
<td>25.33</td>
<td>20.62</td>
</tr>
<tr>
<td>8 KNap 1.0</td>
<td>20.75</td>
<td>1.25</td>
<td>278.78</td>
<td>31.00</td>
<td>47.61</td>
</tr>
<tr>
<td>9 KNap 10</td>
<td>21.39</td>
<td>1.89</td>
<td>472.72</td>
<td>35.00</td>
<td>66.66</td>
</tr>
</tbody>
</table>

Segment elongation

| Treatment number: | 3 9 6 2 5 8 4 7 1 |

Increase in weight

| Treatment number: | 3 2 9 8 6 5 7 4 1 |

△Corresponds to the treatment in Table V.

Treatments underscored by the same line do not differ significantly, according to Duncan's New Multiple Range Test.

+ Value from three replications.

* Initial length was 19.5 mm for all segments.
The widely held concept of IAA-induced cell elongation is that the primary effect of IAA is on the cell wall, and that IAA regulates cell expansion by loosening the cell walls (Heyn, 1931, 1940; Cleland and Bonner, 1956; Burstrom, 1961; Preston and Hepton, 1960). Burstrom (1942) suggested, in the case of wheat roots, the influence of IAA on root growth was mainly due to its effect on cell elongation rather than cell multiplication. The same concept may apply to the effect of other auxin-like substances on stem segments.

The lack of significant difference in the effects of 0.1 ppm IAA, 1.0 and 10 ppm CHCA, and 1.0 ppm KNap on augmentation of elongation of pea stem segments provides evidence that the behaviour of CHCA and KNap at the said concentrations was analogous to that of IAA. The consequence of this analogy leads to the suggestion that in the promotion of cell elongation, CHCA and KNap first relax the cell walls, and, as a result of this relaxation, water is taken in osmotically. The absence of an active process in water uptake during IAA-induced cell expansion has been amply demonstrated (Cleland and Bonner, 1956;
Ordin et al., 1956; Levitt, 1953; Thimann, 1954). That cell enlargement is largely due to increased water uptake is substantiated in this experiment by marked increases in weight in KNap- and CHCA-treated segments.

From the reports of increases in de novo synthesis and/or activity of certain enzymes in bush beans resulting from KNap and CHCA treatments (Fattah and Wort, 1970; Severson, 1972), and that KNap acts at the genetic level (Wort et al., 1971), it is tempting to speculate that the KNap- and CHCA-augmented increases in cell elongation may be mediated through the stimulation of the synthesis of hydroxyproline-rich protein, and the subsequent incorporation of this protein into the cell wall in order for cell elongation to occur. This explanation is in agreement with the suggestion by Cleland (1967) with respect to IAA-induced elongation of Avena coleoptiles.
id) Abscission of debladed petioles of bean plants.

LITERATURE REVIEW

There is still uncertainty prevailing at the present time with respect to the physiology of abscission. Since 1933, when Laibach found that the abscission of debladed petioles was delayed by the application of auxin-rich orchid pollinia to the cut surfaces of the debladed petioles, it is recognised that auxin plays a regulatory role in abscission. Inhibition of abscission by the application of 5 mg/l IAA to debladed petioles of Coleus blumei Benth. by La Rue (1936) confirmed the classical work of Laibach. Early investigations showed that leaf auxin decreased with age (Avery, 1935; Goodwin, 1937; Went and Thimann, 1937). Working with Coleus, Myers (1940) found positive correlations between age of leaves, amount of diffusible auxin, and times required for abscission of intact or debladed leaves. Shoji et al (1951) reported that free auxin in the mature healthy leaflets of bean (Phaseolus vulgaris L), is about
three times that in the leaf stalk. Immediately before abscission, leaflet auxin fell to a level approximately equal to that of the leaf stalk, while stalk auxin remained unchanged. Similar changes were observed in *Coleus* and cotton (Jacobs, 1955; Carns, 1957, respectively).

The process of abscission is affected by a multitude of compounds and environmental factors:

1) **Auxin**

Auxin retards or promotes abscission depending on the site of application with respect to the abscission zone (e.g., Addicott *et al*., 1955). The regulatory effect of auxin on abscission is governed by the time of its application to petioles after the removal of the leaf blades (Rubinstein and Leopold, 1962, 1963), and by the concentration of auxin (e.g., Gaur and Leopold, 1955). The role of auxin in the regulation of abscission is discussed.

Abscisic acid was thought to be an abscission-regulating hormone based on the fact that the amounts of this hormone increase during periods of ageing, and that ageing is correlated with abscission. The application of abscisic acid to explants accelerates abscission.

3) **Gibberellic acids**

Gibberellic acids promote abscission (Bornman, 1965; Carns *et al.*, 1951; Chatterjee and Leopold, 1964; Greenblatt, 1965).

4) **Ethylene**

Ethylene is a potent accelerant of abscission (e.g., Zimmerman *et al.*, 1931). Its effect on the abscission process has been studied by Gawadi and Avery (1950); Hall (1952). Ethylene accelerates abscission through its inhibitory influence on the basipetal transport of auxin and on the initiation of specific RNA, and protein synthesis (Beyer and Morgan, 1971).

5) **Amino acids and carbohydrates**

Rubinstein and Leopold (1962) reported that alanine and glutamic acid promoted abscission of bean petioles.
Yager and Muir (1958) found that methionine was a potent promoter of abscission.

Carbohydrates have been shown to retard or promote abscission under a wide range of experimental conditions (Biggs and Leopold, 1957; Brown and Addicott, 1950).

6) Environmental factors

An extensive review of the influence of environmental factors on abscission is described in a paper by Addicott (1968). With bean leaflet explants, the rate of abscission is very low below 5°C, rises with temperature to a maximum between 25°C and 30°C, and falls at higher temperatures (Yamaguchi, 1954). This phenomenon may be associated with enzyme activity as cellulase has been found to break down cell walls (e.g., Abeles and Leather, 1971). Exposure to relatively extreme temperatures is often followed by increased abscission of leaves, flowers, and fruits (Chandler, 1925). The autumn leaf abscission of deciduous trees is correlated with shortening of photoperiod, e.g., sugar maple (Acer saccharum) (Wiesner, 1904). Under reduced oxygen tensions, abscission is retarded (Sampson, 1918).

* An explant is a small segment of a leaf containing an abscission zone.
Oxygen acceleration of abscission may be the result of inactivation of auxin by IAA oxidase system (e.g., Morgan and Hall, 1963).

The auxin acceleration of abscission was first observed following applications of 10, 105, and 525 mg/l IAA to the proximal side of the abscission zones of excised leaflets of bean (Addicott and Lynch, 1951). However, applications of a range of IAA concentrations 10-1000 mg/l distal to the abscission zones were shown to retard abscission. They obtained similar results with 2,4-D and 2,4,5-T. Retardation of abscission was found to be in direct proportion to the concentration of IAA applied.

The work of Addicott and Lynch (1951) has been confirmed and extended by several workers. Jacobs (1955) working with Coleus, indicated that auxin moving from the apical bud and from intact leaves accelerated the abscission of the lower debladed petioles. With field cotton, Louie (1960) found that removal of the apical bud retarded abscission, and application of IAA to the stem stump accelerated abscission of debladed petioles of the upper leaves.
The employment of petiole abscission serves a suitable bioassay to evaluate the auxin-like properties of test compounds.

No less than six theories have been proposed in an attempt to elucidate the physiology of abscission.

The more recent theories are:
1) **Auxin gradient** (Addicott, Lynch, and Carns, 1955)

   This theory is formulated on the following principles:
   a) A gradient of auxin exists across the abscission zone.
   b) Typically, the gradient appears to be from relatively high auxin distally, to relatively low auxin proximally.
   c) A lowering of the gradient initiates abscission.
   d) The degree of lowering of the auxin gradient determines the rate of abscission.

   This theory is supported by Terpstra (1956) and Louie (1960).

2) **Two-stage theory** (Rubinstein and Leopold, 1962, 1963)

   This theory apparently supersedes the auxin gradient theory of Addicott et al, (1955). Utilising $5 \times 10^{-4} \text{M}$
radioactive NAA on bean petiole explants, Rubinstein and Leopold (1963) were able to demonstrate the lack of an auxin gradient across the abscission zone. Stage I is the induction stage of about six hours or more, and is inhibited by auxin. Stage II is accelerated by auxin of similar concentration. Gaur and Leopold (1955) reported that low concentrations of NAA (1-10 ppm) promoted abscission and high concentrations (100-1000 ppm) retarded abscission whether the auxin was applied distally or proximally with respect to the abscission zone. The "two-stage" response to NAA is interpreted by Rubinstein and Leopold (1962) as attributable to the "two-stage" effect. They proposed that the promotion of abscission by distal applications of low auxin concentrations is "a consequence of an amount of auxin just low enough to allow the induction stage to proceed to completion yet high enough to stimulate the second stage..." This "two-stage" effect of auxin on abscission is in corroboration with the two-phase scheme of auxin action on growth as proposed by Thimann (1951), whereby low auxin concentration promotes growth, while high auxin concentration is inhibitory.
3) Aging-ethylene theory (Abeles, 1968)

Abeles (1968) noted that ethylene initiates specific RNA and protein syntheses necessary for cell separation. As the tissue ages, it becomes increasingly sensitive to ethylene. Ethylene production by plant tissues in response to wounding was reported by Burg (1962), and suggested that ethylene produced by freshly-cut bean explants (ca 3 microlitres per g per hour) is sufficient to stimulate abscission (Burg, 1968). Beyer and Morgan (1971) indicated that endogenously produced ethylene may function in part to regulate leaf abscission through its inhibitory effect on auxin transport and the induction of the synthesis of the cell wall-degrading enzyme cellulase. Horton and Osborne (1967) reported that cellulase was localised in the separation layer, and that ethylene increased cellulase activity, while 2,4,5-T decreased it.

The latter two theories have substantial bases, and offer satisfactory explanations of the process of abscission. The discrepancies in the findings of various researchers may be due to the use of different species of plants.
Petiolar explant technique is popular with investigators in this field. Since this technique does not account for the correlative influence of other plant parts on the abscission zone in question, the results obtained from such studies may not reflect the true or close-to-true situation in intact plants. In this respect, the employment of intact plants to test the activity of various compounds in an effort to gain insight into the process of abscission may prove to be superior.

No information in the literature has been encountered with regard to the influence of HNap on abscission.
MATERIALS AND METHODS

Four seeds of bush bean (*Phaseolus vulgaris* L. cultivar Top Crop), were sown in composted soil in 15-cm plastic pots. Uniformity of plants was obtained by reducing to two seedlings in each pot seven days after sowing, and finally to one plant, two days before treatment. The plants were arranged in rows of four, and grown in a greenhouse under natural light conditions. The number of sunshine hours for the months of September and October, 1971 were:

September: total 162.8 hours, average 5.43 hours, range 0.00-11.7 hours.

October: total 121.2 hours, average 3.90 hours, range 0.00-9.7 hours. The light intensity varied from 12,000 to 20,000 lux.

The procedure used in this investigation was modified from Mitchell *et al.*, (1968). Ten days from sowing, the blade of a primary leaf of each plant was severed just below the pulvinus. The debladed petioles were treated by applying, with a wooden toothpick, lanolin-Tween 20 containing one of the test compounds namely: KNap, CHCA (K salt), NAA, and lanolin-Tween 20. The concentrations employed in each case, except the lanoline-Tween 20, were 1.0, 10, 100, and 1000 ppm.
The treatments were applied around the debladed petiole as a band of about five mm wide, and at a distance of about five mm from the stem.

The determination of abscission was made by application of a constant five g pressure once each day in a downward direction against the upper surface of each debladed petiole, at a distance of 8-10 mm from the stem. This was accomplished by pressing the metal rod of a pressure applicator against the petiole as indicated in Plate 3.

The number of petioles abscised each day upon application of the five g pressure was recorded. The number of days required for 50% of the petioles in each treatment to abscise was taken for comparison of the treated and control plants.

This investigation was set up in a randomised complete block design involving three blocks. Four plants of each treatment were arranged in a row in each block. Analysis of variance and Duncan's New Multiple Range Test (Duncan, 1955) for multiple treatment comparisons were performed on the results obtained.
Plate 3. Determination of abscission of a petiole by the use of a five gramme pressure applicator.
RESULTS

The application of lanolin containing 1.0 ppm KNap to the distal end of debladed petioles of ten-day-old bean plants resulted in a significant acceleration of abscission (9 days) compared with about 11 days in control plants (Table V).

The times required for 50% abscission in plants which received high KNap concentrations (10, 100, and 1000 ppm) were not statistically different from that of the control plants, or from each other. The times required for 50% abscission in plants treated with 10 and 1000 ppm CHCA (13 and 12.67 days, respectively) were significantly different from that of control plants, but not from plants treated with 100 ppm NAA.

In plants treated with 1000 ppm NAA, the period required for 50% abscission was 25 days which was significantly different from that of control plants at the 0.01 level.
**Table V. Abscission of debladed petioles of bean (Phaseolus vulgaris L) in response to KNap, CHCA, and NAA treatments.**

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>+Average time to 50% abscission (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1   Lanolin alone (control)</td>
<td>10.67</td>
</tr>
<tr>
<td>2   KNap 1.0</td>
<td>9.00</td>
</tr>
<tr>
<td>3   KNap 10</td>
<td>12.00</td>
</tr>
<tr>
<td>4   KNap 100</td>
<td>11.67</td>
</tr>
<tr>
<td>5   KNap 1000</td>
<td>12.00</td>
</tr>
<tr>
<td>6   CHCA (K salt) 1.0</td>
<td>12.00</td>
</tr>
<tr>
<td>7   CHCA (K salt) 10</td>
<td>13.00 *</td>
</tr>
<tr>
<td>8   CHCA (K salt) 100</td>
<td>12.33</td>
</tr>
<tr>
<td>9   CHCA (K salt) 1000</td>
<td>12.67 *</td>
</tr>
<tr>
<td>10  NAA 1.0</td>
<td>12.00</td>
</tr>
<tr>
<td>11  NAA 10</td>
<td>12.33</td>
</tr>
<tr>
<td>12  NAA 100</td>
<td>13.00 *</td>
</tr>
<tr>
<td>13  NAA 1000</td>
<td>25.00 **</td>
</tr>
</tbody>
</table>

ΔTreatment number: 13 12 7 9 8 11 3 5 6 10 4 1 2

+Value from 12 plants.
ΔTreatment number corresponds to treatment in Table VI.
The treatments underscored by the same line do not differ significantly from one another.
* Value significantly different from the control value.
** Value differs from the control value at the 0.01 level.
DISCUSSION

It has been recognised for a long time that auxin regulates abscission. The two-phase theory of abscission proposed by Rubinstein and Leopold (1962) gives a satisfactory elucidation of the process of abscission. According to these two workers, phase I of the abscission process is inhibited by auxin, and phase II is accelerated by auxin of similar concentration.

In recent years, the involvement of ethylene in the abscission process has received considerable interest by researchers. That ethylene is a potent accelerant of abscission has been suggested by, for example, Burg (1962); Pratt and Goeschl (1969). The action of ethylene to promote abscission is indirect. It may act through its effects on auxin level. Ethylene has been shown to inhibit polar transport of auxin (Beyer and Morgan, 1971), to increase auxin destruction (Hall and Morgan, 1964), and to initiate the synthesis of specific RNA and protein for cell separation (Abeles, 1968).
The retardation effect of 10 and 1000 ppm CHCA on abscission may be attributed to the enhancement of auxin action and/or IAA biosynthesis. It was demonstrated in the present investigations, that 100 ppm KNap stimulated IAA biosynthesis in bean epicotyls. The lack of significant difference in delaying abscission by 10 and 1000 ppm CHCA, and 100 ppm NAA suggests that the retardation effect of CHCA is similar to that of auxin. It is possible that CHCA acted in the first phase of the two-phase theory of abscission of Rubinstein and Leopold (1962). Another possibility is that CHCA inhibited ethylene synthesis or action.

The acceleration of abscission by the low KNap concentration (1.0 ppm) may be interpreted as the enhancement effect of KNap on the biosynthesis and/or action of ethylene.
CHAPTER II

EFFECT OF POTASSIUM NAPHTHENATES ON IAA BIOSYNTHESIS AND DEGRADATION.
Effect of KNap on IAA biosynthesis.

LITERATURE REVIEW

IAA is well established as the principal hormone of higher plants. Yet the pathways of IAA biosynthesis in higher plants are obscure, and are still the subject of much investigation and controversy. It appears that the pathways of IAA biosynthesis differ in different plant species. There is ample evidence to establish that tryptophan is the predominant natural precursor of IAA in higher plants as well as in numerous microorganisms (Moore and Shaner, 1967, 1968; Zenk and Scherf, 1963; Sherwin and Purves, 1969; Miura and Mills, 1971; Phelps and Sequira, 1967; Wightman, 1963). The conversion of the D-enantiomer of tryptophan to the L-enantiomer in cell cultures of tobacco was demonstrated by Miura and Mills (1971). Gordon (1961) noted that with several plant preparations, D-tryptophan was equal to or more effective than L-tryptophan as a precursor of IAA. Similarly, Kim
and Rohringer (1969) reported that D-tryptophan-methylene-C\textsuperscript{14} was incorporated more efficiently into IAA-C than L-tryptophan-
\textsuperscript{14} methylene-C by excised wheat leaves. Several researchers question the validity of the claim that tryptophan is the natural precursor of IAA (Libbert \textit{et al.}, 1966; Winter, 1966; Thimann and Grochowska, 1968; Black and Hamilton, 1971).

The conversion of tryptophan to IAA in various plant species has been worked out, and, according to workers in this area, this conversion follows two major proposed pathways:


The following pathway of IAA biosynthesis has been demonstrated in the tips of green pea seedlings (\textit{Pisum sativum}) (Moore and Shaner, 1968; Erdmann and Schiewer, 1971); in oat coleoptiles (Erdmann and Schiewer, 1971);

tryptophan → indolepyruvic acid → indoleacetaldehyde → IAA

Another pathway: tryptophan → tryptamine → indoleacetaldehyde → IAA was found to occur in tomato plants (*Lycopersicum esculentum*) (Wightman, 1963).

The existence of yet another pathway:

tryptophan → indoleacetonitrile → indoleacetaldehyde → IAA was detected in cabbage by Wightman (1962).

Another hypothetical route for IAA biosynthesis in *Avena* coleoptiles is given by Winter (1966):

anthranilonitrile → anthranilic acid → indole → tryptamine → IAA

Tryptamine rather than tryptophan is the precursor of IAA in *Avena* coleoptiles was the conclusion reached by Thimann and Grochowska (1968). These two workers emphasised that the conversion of tryptophan to tryptamine did not occur, and suggested that the conversion of tryptophan to IAA was due to bacterial contamination. This was substantiated by Winter (1966) and Black and Hamilton (1971).
The failure of exogenously supplied tryptophan to promote elongation of *Avena* coleoptiles was the result of its incorporation into protein and consequent unavailability for conversion to IAA under sterile conditions, according to Black and Hamilton (1971). The conversion of tryptophan to IAA in bean (*Phaseolus vulgaris* L) shoot experiments with $^{14}$C tryptophan-C was reported by Black and Hamilton (1971). The findings of Perley and Stowe (1966) with bacterial cultures of *Bacillus cereus* strain KVT, showed that direct decarboxylation of tryptophan to tryptamine is a very rare reaction.

To the best of my knowledge, there is only one publication on the effect of HNap on the content of auxin in plants. Bazanova (1970) found that Sh-8 (or HNap) at 0.005% caused redistribution of endogenous auxin and inhibitors in various organs of fine-fibred cotton, increased the translocation of growth-regulating substances from vegetative to reproductive organs, and enhanced the activity of natural growth-regulating substances and inhibitors in reproductive organs.
MATERIALS AND METHODS

Bush bean seeds (*Phaseolus vulgaris* L, cultivar Top Crop) were surface sterilised by washing with 95% ethanol for ten minutes, then with sterile water. The seeds were soaked for 12 hours in 100 ppm (0.01%) sterilised KNap (Wort and Patel, 1970; Naghibin, 1966; Ejubov, 1966). The seeds used as control were soaked in sterile water for the same length of time. The seeds were sown in rows in sterilised vermiculite ("Terra-lite", Grace Construction Materials Ltd., Vancouver), saturated with 1.0% Captan fungicide. Sterilization was carried out at 248°C for 15 minutes at a pressure of 15 lb per square inch. The plants were allowed to grow for 14 days in the dark, at air temperatures of 22.5 ± 1°C, and 95 ± 2% relative humidity.

Apical regions (5-8 cm) including leaves were excised and washed with cold distilled water. Immediately after excision, 20 g fresh weight of plant tissues was homogenised in a Waring blender at full speed for ten minutes in 40 ml of KH₂PO₄-Na₂HPO₄ buffer in a cold room at 4°C (Moore and
Shaner, 1968). The concentration of the phosphate buffer was 0.1 M at pH 7.4 (Moore and Shaner, 1968) containing 0.1 M sucrose (Wightman, 1968), ten units per ml penicillin G, and 100 units per ml streptomycin sulphate (Valdovinos and Perley, 1966). The crude homogenates were filtered through four layers of cheesecloth and kept in an ice bath throughout the preparative procedures. The filtrate thus obtained, was centrifuged at 10,000 g for 20 minutes at 4°C (Moore and Shaner, 1968) and the resulting supernatant was used as the enzyme extract. Enzyme extracts were dialysed against phosphate buffer for 24 hours at 4°C with constant agitation and one change of external buffer.

Reaction mixtures, modified from Moore and Shaner (1968), contained three ml enzyme extract and three ml of 0.1 M KH$_2$PO$_4$-Na$_2$HPO$_4$ buffer, pH 7.4, containing:

- 30 micromoles alpha-ketoglutaric acid
- 0.6 micromoles pyridoxal phosphate
- 0.6 micromoles thiamine pyrophosphate
- 0.6 micromoles nicotinamide adenine dinucleotide
- 0.03 M D-tryptophan
- 0.0005 M diethyldithiocarbamic acid
The above chemicals were purchased from Sigma Chemical Company, St. Louis, Missouri.

The D-enantiomer of tryptophan was found to be the best precursor of IAA (Kim and Rohringer, 1969). The concentration of D-tryptophan (0.03 M) was adopted from Wightman and Cohen (1968), while the inclusion of 0.0005 M diethyldithiocarbamic acid which is a potent inhibitor of IAA oxidase, was adopted from Wagenknecht and Burris (1950).

The reaction mixtures, held in test tubes, were incubated in darkness for six hours at 35° C (Wightman and Cohen, 1968) in a water bath with thermostatic controls (National Appliance Company, Portland, Oregon). Enzymatic reactions were stopped by lowering the pH to 3.0 with two drops of 85% orthophosphoric acid.

The estimations of protein in enzyme extracts were made with a view of assessing the specific activity of the enzymes involved in IAA biosynthesis. The method of Lowry et al (1951) as modified by Eggstein and Kreutz (1955) was used. To one ml of enzyme extract was added five ml of alkaline copper solution (50 ml of 2% Na₂CO₃ in 0.1 N NaOH plus 1.0 ml of 0.5% CuSO₄.5H₂O in 1.0% sodium citrate). The mixture was thoroughly mixed and allowed to stand for ten minutes at room temperature (23-24° C). Thereafter, 0.5 ml of 1.0 N Folin-Ciocalteau reagent (commercial reagent 2 N diluted with
water to give a solution of 1.0 N in acid) was pipetted rapidly into the mixture with thorough mixing. The optical density was measured at 650 nm after 30 minutes. The amount of protein in the enzyme extracts was calibrated by reference to a standard curve developed using crystalline bovine albumin.

**Extraction and quantitative determination of IAA.**

All manipulations were performed in red light, at room temperature (23-24°C). Prior to extraction, ten microgrammes of authentic IAA were added to each reaction mixture, and each reaction mixture was extracted twice with six ml of methylene chloride by the method of Moore and Shaner (1968). The combined extracts of each reaction mixture were evaporated to dryness in a rotary-film vacuum evaporator at 30°C. The residue was dissolved in two ml of 95% ethanol and a 0.5 ml aliquot of each extract was spotted on Whatman No. 1 chromatographic paper (26 x 36 cm) and co-chromatographed with ten microgrammes of authentic IAA. The chromatograms were developed in 10:1:1 (v/v) 95% isopropanol:28% ammonium hydroxide:distilled water (Kuraishi and Muira, 1963; Artemenko and Chkanikov, 1970) in an ascending manner (Wightman, 1963) for 16 hours, at 4°C in the dark. The spots developed on the chromatograms were located under ultraviolet light, and immediately sprayed with Ehrlich's reagent. Ehrlich's reagent was prepared by mixing 2% p-dimethylaminobenzaldehyde in 10 N
HCl with acetone in the proportion of 1:1 (v/v) immediately before use (Wightman, 1963). Quantitative determinations of IAA were made 15 minutes following treatment with Ehrlich's reagent. The optical density of the spots was determined by means of a densitometer (Photovolt Densitometer, Photovolt Corporation, New York City, Model 501 A). Maximum transmission values of the spots obtained with the densitometer were used to compute the amount of IAA present in plant tissues by calibration with values of a standard curve. Transmission values were employed chiefly because of their linear relationship to the logarithm of IAA concentration (Vlitos and Meudt, 1953).

A standard curve was developed by delivering 1.0, 2, 5, and 20 microgrammes of authentic IAA to a chromatogram with a microlitre syringe. The chromatogram was developed and treated in the same manner as described in the preceding paragraph. The concentrations were adopted from Vlitos and Meudt (1953).

All solvents used in chromatography were redistilled.

A randomised complete block design was used in this investigation involving three blocks with two determinations per treatment in each block. The results obtained were subjected to analysis of variance.
RESULTS

Soaking the bean seeds in a 100 ppm solution of KNap for 24 hours prior to sowing resulted in a 140.5% increase in the content of IAA in the epicotyls of dark-grown bean plants, determined 14 days after treatment. The increase was significant at the 0.01 level (Table VI).

The amount of protein (1.26 mg per g fresh weight) in the epicotyls of KNap-treated bean plants was less than that in the control plants (1.96 mg per g fresh weight). The specific activity of the enzymes responsible for the conversion of tryptophan to IAA, expressed in terms of microgrammes of IAA synthesised per mg protein per hour, was 1.34 in the control plants and 5.03 in the KNap-treated plants (Table VII).
Table VI. IAA content of the 5-8 cm tips of epicotyls of 14-day-old dark-grown *Phaseolus vulgaris* L seedlings following treatment with 100 ppm KNap.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>+Microgrammes of IAA per g fresh weight</th>
<th>Increase over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H₂O (control)</td>
<td>5.28</td>
<td>--</td>
</tr>
<tr>
<td>KNap 100 ppm</td>
<td>12.70**</td>
<td>140.5</td>
</tr>
</tbody>
</table>

*+ Mean of six determinations.*

**Value differs significantly from the control value at the 0.01 level.*

Table VII. Specific activity of the enzymes in the conversion of tryptophan to IAA in the epicotyls of dark-grown bean plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IAA content microgrammes per g fresh weight</th>
<th>+Protein content microgrammes per g fresh weight</th>
<th>Specific activity of enzymes microgrammes IAA per mg protein per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H₂O (control)</td>
<td>5.28</td>
<td>1.96</td>
<td>1.34</td>
</tr>
<tr>
<td>KNap 100 ppm</td>
<td>12.70</td>
<td>1.26</td>
<td>5.03</td>
</tr>
</tbody>
</table>

*+ Mean of six determinations.*
DISCUSSION

In view of the absence of reported pathways on the biosynthesis of IAA from tryptophan in *Phaseolus vulgaris* L plants, it is tempting to speculate that the pathway operating in *P. vulgaris* is similar to that of *Phaseolus aureus* L (mung bean). It was reported that IAA is synthesised in excised *P. vulgaris* shoots via tryptophan (Black and Hamilton, 1971). The pathway of enzymatic conversion of tryptophan to IAA in *P. aureus* has been worked out by Wightman and Cohen (1968), and, according to them, the pathway is in the following sequence:

\[
\text{Tryptophan} \rightarrow \text{Indolepyruvic acid} \rightarrow \text{Indoleacetaldehyde} \rightarrow \text{IAA}
\]

The enzymes responsible for the conversion of tryptophan to IAA have been identified, and the properties of the individual enzymes involved in each step of this biosynthetic pathway were determined by Wightman and Cohen (1968). According to these two investigators, the enzyme catalysing the particular step in the pathway is indicated as follows:
Tryptophan transaminase in step I; Indolepyruvic acid decarboxylase in step II; and Aldehyde dehydrogenase in step III.

The increase in the content of IAA in the KNap-treated plants (140.5%) compared with control plants suggests KNap stimulation of IAA biosynthesis \textit{in vitro}. This KNap-stimulated IAA biosynthesis in \textit{P. vulgaris} epicotyls may be mediated by either the induction of de novo synthesis of the enzymes involved in the conversion of tryptophan to IAA (mentioned in the preceding paragraph), or by the stimulation of the activity of these enzymes, or in combination. This suggestion is made in the light of reports on bush bean plants following KNap treatments, on the stimulation of protein synthesis by Severson (1972) and Wort \textit{et al} (1971); on the augmentation of specific activities of numerous enzymes in crude extracts (Chu, 1969; Fattah, 1969; Fattah and Wort, 1970); and on increases in RNA and DNA content (Wort \textit{et al}, 1971).

The increases in plant growth and crop yield following applications of KNap reported in the literature can be interpreted as due to KNap-induced IAA biosynthesis in these plants.
LITERATURE REVIEW

The metabolism of IAA in plants has been intensively discussed but not well understood, even till today. The starting point of discussion on this subject dates back to 1934 when Thimann found that leaf extracts of *Vicia faba* and *Helianthus* could inactivate IAA. But, he, however, did not demonstrate that the disappearance of IAA was enzymatic. Later, in 1936, Larsen discovered that the inactivation of IAA was catalysed by an oxidising enzyme.

The classical publication of Tang and Bonner in 1947 on some characteristics of the IAA-degrading enzyme, IAA oxidase, in etiolated pea epicotyls was quoted repeatedly by investigators which led readers to believe that IAA oxidase is the only enzyme responsible for the destruction of IAA. The enzyme is designated as IAA oxidase chiefly because inactivation of IAA takes place only in the presence of oxygen, and about one molecule of oxygen is consumed per molecule of IAA inactivated, and one molecule of carbon
dioxide is liberated (Tang and Bonner, 1947). Evidence supporting the claim that IAA oxidase is not a single enzyme but a mixture of enzymes is found in the work of Sequira and Mineo (1966); Lee (1971); Meudt (1967); Ray (1960); Hinman and Lang (1965). That IAA oxidase may consist of a mixture of plant peroxidases, and that IAA oxidase together with peroxidases may exist as aggregates was suggested by Sequira and Mineo (1966). Ray (1960) considered both peroxidase and IAA oxidase activities were due to one enzyme. IAA oxidase has been identified as a heme protein peroxidase by Goldacre (1951). It may be inferred from these findings that IAA oxidase is an isoenzyme of peroxidase. The ability of peroxidases to oxidise IAA has been reported by Meudt (1967), using crystalline horseradish peroxidase; Hinman and Lang (1965); Ray (1960), using peroxidase from the fungus Omphalia flavida. Seven peroxidases isoenzymes were detected in horseradish roots, with no interconversions among the isoenzymes (Shannon et al, 1966). Jermy and Thomas (1954) reported that the isoenzymes of horseradish peroxidase have substrate specificities.
Enzymes of the polyphenol oxidase systems are also known to oxidise IAA (Leopold and Plummer, 1961; Skoog, 1944).

That the IAA oxidase system in etiolated pea epicotyls possessed a considerable degree of substrate specificity, was one of the conclusions reached by Tang and Bonner (1947). They reported that the IAA oxidase system did not attack indoleacetamide, indolebutyric acid, indolepyruvic acid, indolecarboxylic acid or tryptophan.

The products of IAA oxidation have been studied, but no clear-cut conclusions are available. Hinman and Lang (1965) suggested 3-methylene oxindole, and a neutral indole, and emphasised that the product composition is highly dependent on IAA concentrations. That the end product is indolealdehyde, and the intermediates, indole-3-glycollic and indole-3-glyoxylic acids were suggested by Goldacre (1951). A general agreement among investigators is that the indole ring remains intact (Tang and Bonner, 1947; Hinman and Lang, 1965; Meudt, 1967). The oxidation products are inactive in the Avena test or in chemical methods of IAA determination.
Meudt (1967) indicated that the oxidative transformation of IAA leads to the formation of biologically active products, provided prevailing conditions are such to prevent the formation of secondary oxidation products which inactivate IAA. Meudt and Galston (1962) suggested a mechanism by which IAA attains biological activity in plants. The primary product of IAA oxidation is oxindole (Hinman and Lang, 1965), and is spared from destruction by binding to RNA. The oxindole-RNA complexes then stimulate plant growth. These complexes gave positive reactions in Salkowski and Ehrlich reagents (Meudt and Galston, 1962). A support for this hypothesis comes from the study of the distribution pattern of peroxidase activity in plants. Meudt (1967) found that the distribution pattern of peroxidase activity correlates with the growth centres of the plant. Peroxidase activity is higher in actively-growing tissues than in mature and dormant tissues.

In the light of evidence presented, IAA oxidase should not be thought of as a single component, but rather as a
mixture of peroxidase isoenzymes. It is appropriate to consider IAA oxidase as a system of enzymes, hence the term "IAA oxidase system" should be used. The latter term has been used by investigators for example, Goldschmidt, Goren, and Monselise (1967); Lee (1971).

Babaev (1966) reported that soaking cotton seeds in solutions of HNap (10 mg/l), and the application of the acid to the soil (20 mg/kg of dry soil) activated peroxidase activity in the roots.
MATERIALS AND METHODS

Uniform seeds of *Phaseolus vulgaris* cultivar Top Crop were surface sterilised by washing in 95% ethanol for ten minutes. The seeds were sown in sterilised vermiculite, saturated with 1.0% Captan fungicide, contained in wooden flats, and allowed to grow in darkness at an air temperature of 22.5 ± 0.5°C and a relative humidity of 95 ± 2%.

Thirteen-day-old dark-grown seedlings were carefully removed from the vermiculite and washed with tap water. Treatment was effected by immersing the root systems in 100 ppm (0.01%) KNap solution for 24 hours in darkness at room temperature (23-24°C). The root systems of control plants were soaked in distilled water under similar conditions.

Subsequent handling of plant materials was performed in red light at room temperature. Epicotyls (5-8 cm) including leaves, were harvested, washed with ice cold distilled water, and immediately frozen with liquid nitrogen. The following procedure was modified from Sequeira and Mineo (1966). The frozen plant tissues were ground to a fine
powder in a mortar with pestle. The powder was suspended in cold 0.02 M $\text{KH}_2\text{PO}_4-\text{Na}_2\text{HPO}_4$ buffer, pH 6.1, in the proportion of ten g of frozen tissue to 50 ml buffer, and stirred for 1.5 hours in the cold room at $4^\circ$ C. The mixture was centrifuged at 18,000 g for ten minutes at $4^\circ$ C. The supernatant was decanted into an 150-ml Erlenmeyer flask and solid $(\text{NH}_4)_2\text{SO}_4$ was added gently to 35% saturation (Green et al, 1955). The precipitate which was formed at $4^\circ$ C in 24 hours, was removed by centrifugation at 18,000 g for ten minutes. The supernatant was then brought to 70% saturation with solid $(\text{NH}_4)_2\text{SO}_4$, and the precipitate which was formed at $4^\circ$ C in 24 hours, was recovered by centrifugation at 18,000 g for ten minutes, and resuspended in 12.5 ml buffer. The solution was dialysed for 24 hours in the cold room ($4^\circ$ C) against 3000 ml buffer with one change of external buffer. This dialysed solution, which had both IAA oxidase and peroxidase activities, was referred to as crude enzyme. No attempts were made to separate these two enzymes as both were found to degrade IAA (Ray, 1960; Sequeira and Mineo, 1966; Meudt, 1970).
Assay for enzyme activity

The activity of the IAA-destroying enzymes was determined by the Salkowski reaction (Tang and Bonner, 1947; Gordon and Weber, 1951; Gordon and Paleg, 1957). The reaction mixture (five ml) was prepared by the method of Sequeira and Mineo (1966), and consisted of the following:

0.5 ml enzyme extract
0.25 ml 1.0 mM Na salt of 2,4-dichlorophenol
1.00 ml 1.0 mM IAA in 0.5 mM MnCl$_2$ (1:1, v/v)
3.25 0.02 M KH$_2$PO$_4$-Na$_2$HPO$_4$, pH 6.1

The reaction mixture was shaken in a water bath (Dubnoff Metabolic Shaking Incubator, Precision Scientific, Chicago) for two hours at 30° C in darkness, and then 1.0 ml of Salkowski reagent was added. The mixture was shaken for an additional three hours and absorbance at 525 nm was determined with a Bausch and Lomb Spectronic 20 colorimeter using a blue-sensing phototube, type CEA-59RX. The Salkowski reagent was prepared according to Gordon and Weber (1951), by mixing 1.0 ml of 0.5 M FeCl$_3$ to 50 ml of 35% HClO$_4$. 
The residual IAA in the reaction mixtures was determined by reference to a standard curve. The standard curve was developed by mixing a series of two ml known quantities of authentic IAA to four ml Salkowski reagent for 35 minutes. The absorbance was read at 525 nm with a Spectronic 20 spectrophotometer.

The experimental setup used in this investigation was the randomised complete block design, comprising two replications with five determinations per replication in each treatment. The results were subjected to analysis of variance.
RESULTS

When the root systems of 13-day-old dark-grown bean plants were immersed in a 100 ppm solution of KNap for 24 hours, there was a 4% increase in the activity of the IAA oxidase system compared with that of control plants. This stimulatory effect of KNap was significant at the 0.01 level (Table VIII). The activity of the IAA oxidase system was measured in terms of microgrammes of IAA destroyed per g fresh weight of epicotyl tissue.
Table VIII. The activity of the IAA oxidase system in the 14-day-old dark-grown bean epicotyls following treatment with 100 ppm KNap.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial IAA concentration in reaction mixture (ugm/ml)</th>
<th>+Residual IAA concentration in reaction mixture (ugm/ml)</th>
<th>Amount of IAA destroyed (ugm/g fr wt)</th>
<th>Amount of IAA destroyed (% increase over control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>175</td>
<td>26.27</td>
<td>371.82</td>
<td>--</td>
</tr>
<tr>
<td>KNap 100 ppm</td>
<td>175</td>
<td>20.20</td>
<td>386.00**</td>
<td>3.81</td>
</tr>
</tbody>
</table>

+ Mean of 15 determinations.
** Value differs significantly from the control value at the 0.01 level.
DISCUSSION

It is recognised that the IAA oxidase system is involved in the degradation of IAA.

The increase (ca 4% over control plants) in the activity of the IAA oxidase system in bean plants following treatment with 100 ppm KNap supports the report of increases in peroxidase activity in the roots of cotton plants resulted from seed soak with 10 ppm HNap by Babaev (1966). That peroxidase is a component of the IAA oxidase system was suggested by, for example, Sequeira and Mineo (1966).

The stimulation of IAA biosynthesis in bean epicotyls by 100 ppm KNap reported by the author in the present investigation, and the activation of the IAA oxidase system by similar KNap concentration, suggests that the activity of the IAA oxidase system is induced by the substrate, IAA.

It is difficult to resolve the paradox that IAA oxidase system is capable of inactivating IAA; and that the activity of this system is induced by the substrate, IAA, and correlates with the active growth centres of plants i.e.
greater activity in actively growing tissues than in mature and dormant tissues (Meudt, 1967). This poses a fundamental question: how can there be active growth if IAA is continually destroyed? It is possible that in the actively growing plant tissues, IAA is in excess and able to sustain destruction, and, at the same time, maintains active growth. A solution to this question must await further research.
CONCLUSIONS

1) Potassium naphthenates (KNap) exhibited the following auxin-like properties:
   a) **Inhibition of root growth**
      KNap at 1000 ppm inhibited the growth of intact roots of cucumber.
   b) **Stimulation of root initiation**
      The treatment of bean stem cuttings with 10 and 100 ppm KNap resulted in the stimulation of root initiation compared with control cuttings. The treatment of azalea stem cuttings with 100 and 1000 ppm KNap significantly increased root initiation compared with control cuttings.
   c) **Stimulation of elongation of pea stem segments**
      KNap at 10 ppm had a greater stimulatory effect on elongation of pea stem segments than 0.1 ppm IAA.
   d) **Retardation of petiole abscission**
      The applications of 10 and 1000 ppm cyclohexanecarboxylic acid to the distal end of debladed petioles of bean, resulted in retardation of abscission.

2) When applied to the seeds for 12 hours prior to sowing, 100 ppm KNap augmented the biosynthesis of IAA from tryptophan in the 5-8 cm tips of dark-grown epicotyls of bean.

3) When applied to the roots of 13-day-old dark-grown bean plants for 24 hours, 100 ppm KNap stimulated IAA degradation in the 5-8 cm tips of epicotyls.
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