EFFECTS OF COBALT ON THE RESPONSE OF SECTIONS OF ETIOLATED PEA EPICOTYLS TO PLANT GROWTH REGULATORS

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

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August 1964.

ABBREVIATIONS

Co	Cobalt
IAA or I	Indole-3-acetic acid
TCPAA or T	Trichlorophenoxyacetic acid

ACKNOWLEDGEMENTS

I am deeply grateful to Dr. W. G. Boll for introducing the research project to me, and for his generous help, advice and encouragement throughout the research and in the preparation of this thesis.

Sincere thanks are due also to Misses E. Fung-A-Ling and B. Fung-A-Ling for their assistance in the typing of this thesis, to Miss J. Paterson and Mr. A. Lo for the computing of the standard errors, and to all members of the Botany Department who helped in the realization of this work.

Last, but not least, I would like to thank my husband for his assistance in draughting and for his cooperation and understanding throughout this work.

The financial aid for this research has been granted by the Research Council of Canada and is hereby acknowledged.

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INTRODUCTION

Lowther and Boll (1960) in the study of the effect of 2,4,5-TCPAA* on etiolated bean leaf discs observed that inhibition of expansion at low concentrations was relieved at high concentrations where growth was equal to that of the light plus Co control. This means that at appropriate concentrations, TCPAA completely replaced Co in the expansion of light-treated leaf discs.

Some of the questions that arose from the facts revealed by Lowther and Boll (1960) concerned the roles of TCPAA and Co in the stimulation of cell growth and the regulation of the stoppage of growth. Because cell growth involves essentially an increase in area and volume of the cell wall, interest in the roles of Co and TCPAA in growth is particularly directed to their influences in the processes of increase in area and volume of the wall. The question as to the role of TCPAA as compared with the native auxin IAA (See Figure 1) in the regulation of growth also became of interest.

The present research employs the straight growth of etiolated pea epicotyl sections in the study of the above problems. The project consists altogether of 13 studies. Except for Studies 3 and 8, each study consists of one, two or more experiments, each of very

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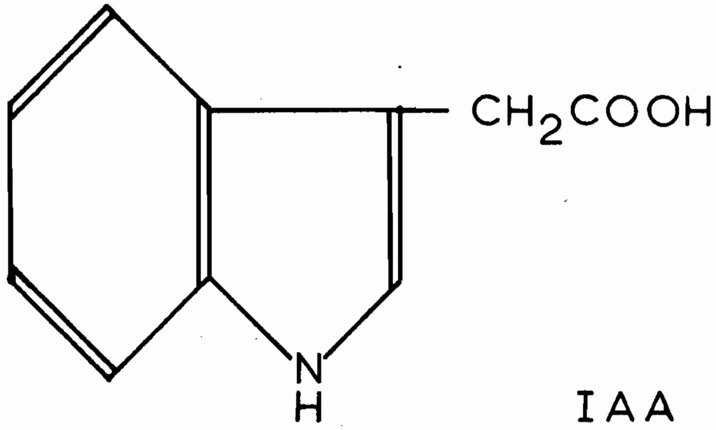
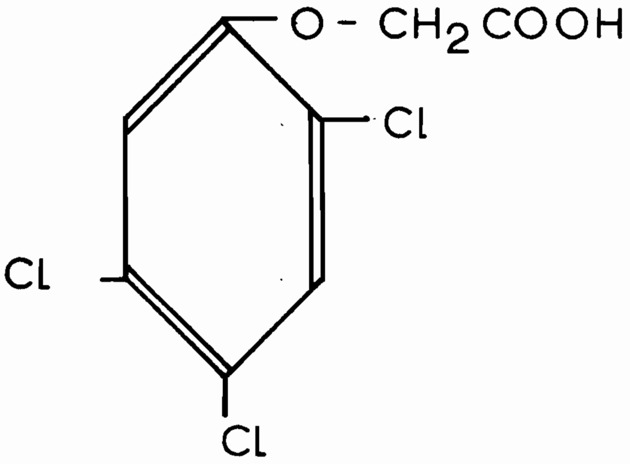
* This particular sample was purchased from Bios Laboratories Inc. and was believed to be 2,4,6-TCPAA. However the sample showed auxin activity although it had so far been reported to be an antiauxin (McRae and Bonner, 1952, 1953; Åberg, 1961). Accordingly, its activity was checked against 2,4,5-TCPAA (Nutritional Biochemicals Corporation) and 2,4,6-TCPAA (Bios Laboratories Inc.) on the elongation of etiolated pea epicotyl sections. The sample from Bios Laboratories of doubtful position showed activity comparable with 2,4,5-TCPAA activity, whereas 2,4,6-TCPAA, an antiauxin showed no growth promoting activity. It was concluded that the sample referred to by Lowther and Boll (1960) as the antiauxin 2,4,6-TCPAA was in fact the auxin 2,4,5-TCPAA.

Figure 1.   Formulae of IAA and 2,4,5-TCPAA.



IAA



2, 4, 5 - TCPAA

similar design, i.e. more or less repeats of the same experiment. The studies are of four types. Studies 1 and 3 undertakes the study of the concentration-response curves using either Co or IAA or TCPAA, to find the optimum concentration for each, and to see if results obtained here are comparable with those previously reported by other workers. Studies 4 to 7 are on the effects on growth of the simultaneous variation in the concentrations of either Co with IAA, or Co with TCPAA, or IAA with TCPAA. Study 8 is on the effects on growth of variation in Co concentration in the presence of different TCPAA concentrations (suboptimal or optimal or supraoptimal) with or without different IAA concentrations (suboptimal or optimal or supraoptimal). Studies 9 to 13 deal with the growth curves of sections when grown in the significant treatments revealed in Studies 1 to 8.

It was anticipated that the data obtained in the experiments would throw some light on the roles of Co, 2,4,5-TCPAA and IAA in growth regulation with particular reference to the processes in the cell wall which lead to expansion of the cell wall and to the stoppage of growth.

I. REVIEW OF LITERATURE

A. THE POSITION OF Co WITH RESPECT TO ANIMALS, LOWER PLANTS AND HIGHER PLANTS, AND OTHER KNOWN PROPERTIES OF Co

1. The Position of Co with Respect to Animals

The heavy metal Co is known as an essential element for animals. Its principal effect in animals is through its conversion to vitamin B_{12} (cyanocobalamin) or related substances. It is also known to protect brain tissue from damage caused by high levels of oxygen, and to protect mice against damage by X-rays (Galston and Siegel, 1954).

2. The Position of Co with Respect to the Lower Plants

There is some evidence that Co may function in the synthesis of vitamin B_{12} factors in algae. The presence of vitamin B_{12} factors in the marine alga *Stichococcus* and the response of *Chrysomonadales* only to vitamin B_{12} itself and not to related compounds have been reported (Pirson, 1955). Holm-Hansen *et al* (1954) found that the addition of minute amounts of vitamin B_{12} replaced the Co requirement in blue-green algae. Ballentine (1953) observed that in the course of growth and metabolism of *Neurospora*, Co was incorporated to a slight extent in protein complexes. Scott and Erison (1955), in their study of Co accumulation from sea water by *Rhodymenia palmata* using Co^{60}, reported the incorporation of Co into a stable organic compound. However he found this compound to differ from vitamin B_{12}.

Promotive and inhibitive effects on growth by Co have been reported in the lower plants. These differences may perhaps be explained by specific enzymatic and metabolic differences in different species. Holm-Hansen et al (1954) reported Co in small quantities as an essential element to blue-green algae. Nordbring-Hertz (1955) found that Co relieved the inhibition of growth by auxin in Candida albicans. Turel (1955) reported that Co in the presence of methyl-p-hydroxybenzoate increased germination of Melampsora occidentalis Jacks. However, Schade (1949) found Co to inhibit growth (increase in size and cell division) of Proteus vulgaris, and that only histidine and cysteine of 17 amino acids employed overcame the growth inhibition. Nickerson and van Rij (1949) found Co to inhibit cell division and to stimulate production of mycelial forms in yeast, and that the effect of Co was antagonised by cysteine.

From the observation that the action of Co is antagonised by cysteine, Nickerson and van Rij (1949) have suggested that the cell division mechanism in yeast is regulated by the amount of sulphydryl (-SH) groups. A maintenance of a high proportion of -SH promotes cell division. The addition of Co in subinhibiting concentrations influences this mechanism in favour of mycelial production by shifting the equilibrium $-SH \rightleftharpoons -S-S$ to the right. Nordbring Hertz (1955) suggests that such a Co effect on the cell division mechanism may also be operational in bacteria.

Healy et al (1955), working with Neurospora crassa observed that Co toxicity produced an enzyme pattern strikingly similar to that of iron deficiency and that this applied not only to the iron enzymes but also to enzymes not known to involve iron directly. The idea that Co competes directly with iron thus interfering with the action of the latter was proved wrong by the fact that the toxic effect of Co could be reversed by higher iron concentrations. However, Co must in some way interfere with iron metabolism which is of some interest in relation to the chlorotic effects of Co in higher plants.

3. The Position of Co with Respect to the Higher Plants

Co has never been proved to be essential to higher plants. The possibility that Co may serve the same purpose in the higher plants as in animals, through its conversion to vitamin B_{12} or related substances is very doubtful because vitamin B_{12} has never been detected nor found functional in the higher plants (Ford and Hutner, 1955; Thimann, 1956).

Studies of the effect of Co in the higher plants have been few mainly because the actual effect of the metal, as expected, is generally obscured by the complex of other processes going on in the living higher plant. However, Glass (1955) in his study of the action of heavy metals on the root tip mitosis of *Vicia faba* observed that Co caused the hardening of chromosome threads in mitosis. Tsao and Youngken (1952) reported that Co inhibited the growth of *Digitalis purpurea* and produced marked inhibition of glycoside production. These two observations suggest that Co may play a part in growth regulation, but more evidence for correlation and substantiation of results are required.

Co in excess interferes with iron metabolism. It is known to induce chlorosis, mainly suggestive of iron deficiency, and necrosis, due to specific effects of the metal (Hewitt, 1953; Vergnano and Hutner, 1953; Forster, 1954).

(a) The Promotive Effects of Co on Growth of Isolated Parts of Higher Plants

The stimulatory effect of Co on the expansion of etiolated bean leaf discs was reported by Miller (1951, 1952) and Lowther and Boll (1960). This stimulatory effect on growth has also been reported in pea stem segment growth (Miller, 1954; Thimann, 1951a, 1956 ; Bertch, 1961, 1963), in the split pea stem (Thimann and Marré, 1954; Thimann, 1956), and in *Avena* coleoptile sections (Thimann,1956 ; Busse, 1959). Howell and Skoog (1955) showed that Co promoted growth of tissue cultures of pea epicotyls. Klein (1959) observed that Co promoted the opening of the hypocotyl hooks of dark-grown bean seedlings.

4. Studies Regarding the Mode of Action of Co

The study of the mechanism of action of Co in plant tissues has been approached from a number of different directions. Most of the approaches are physiological; some are biochemical. None of the approaches has as yet yielded any clearcut explanations. Relevant information is reviewed below.

(a) Some Chemical and Biochemical Properties and Effects of Co

Cobaltous salts, in a weakly alkaline solution on exposure to air, form complexes with cysteine. Among these complexes, one designated cobaltotricysteine is particularly prevalent (Michaelis, 1929; Michaelis and Yamaguchi, 1929). This point is of some significance because it indicates the ability of Co to complex with an amino acid, and gives rise to the idea that Co may perhaps effect growth by combining with the sulphydryl group of an enzyme under physiological conditions. The Co-amino complex formed in the presence of air indicates that a Co-amino acid complex may perhaps be responsible for the antioxidant property attributed to Co (see below).

Galston and Siegel (1954), working with pea root tissues, measured peroxigenesis by oxidation of pyrogallol to purpurgallin, and IAA oxidation, and observed that pea root tissues damaged by incubation in pure O_2 showed increased peroxide production and increased IAA destruction. The presence of Co decreased peroxigenesis and IAA destruction. Galston and Siegel suggested the role of Co to be an antiperoxidative action and protection of IAA from destruction.

Co is also known as an antioxidant in the inhibition of experimental substrate oxidation (Siegel _et al_, 1959 ; Siegel and Porto, 1961). Dedic and Koch (1956) were able to culture _Clostridium tetani_, an obligate anaerobe in air in the presence of Co^{++} salts. They concluded that Co acts as an antioxidant in protecting the organism against ordinary atmospheric levels of oxidant.

Co is known to activate certain enzymes in plants. Activation of phosphoglucomutase from _Phaseolus radiatus_, an enzyme which is not activated by metal-binding agents such as cysteine, was reported by Ramasarma _et al_ (1954). Sison _et al_ (1958) reported that Co activated the cellulase enzyme of _Poria vaillantii_. The element is also known to induce the formation of the adaptive enzyme cellulase in _Trichoderma viride_ (Mandels and Reese, 1957).

Pirson (1955), from a survey of studies on Co, suggested that Co might work by an unblocking, e.g. by making Ca^{++} more available. So far there has been no experimental evidence in support of this.

(b) Co and Light Effects on Isolated Plant Parts

Miller (1952) noted that etiolated plant tissues, the growth of which was promoted by Co, were also acted upon by light. Both Co and light promoted the expansion of bean leaf discs and the two factors were additive over the entire range of growth which could be induced by varying the light dose and Co concentration. These facts led Miller to postulate that the two factors decreased the same growth-limiting condition, although they might decrease it by different pathways.

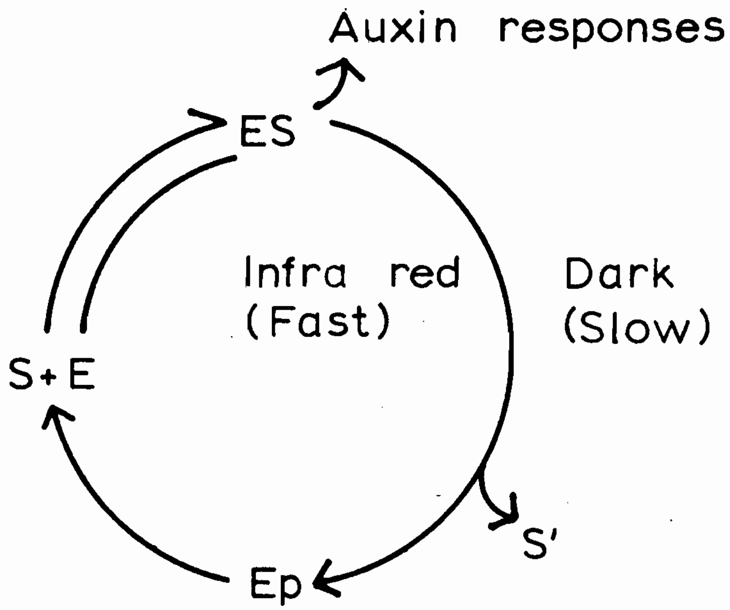
Klein (1959) found Co to be the only additive which would act in a manner similar to red light in the opening of the hypocotyl hooks of dark-grown bean seedlings. IAA, gibberellic acid and kinetin all inhibited the opening of the hook. The Co effect was additive to the red light in the same manner as reported by Miller (1952), and this was taken to indicate that two different sites are affected. Klein believed that Co caused hook-opening via some other pathway than the photoreaction, because the maximum response induced by an optimum concentration of Co was only one third of that induced by red radiant energy.

The unrelated processes of growth of *Avena* coleoptiles, expansion of etiolated bean leaf discs, photoperiodic respiration and seed germination have been observed to be affected by red light and auxin, and the effects of red light are reversed by far-red light (Liverman *et al*, 1955). Liverman (1955), and Liverman and Bonner (1953) stated that these processes may all have their basis in a common mechanism. Their hypothesis (see Figure 2) assumed that red light exerts its effect through generation of an auxin-receptive entity E within the plant and that far-red light acts to decompose the active complex ES into an entity which does not complex with auxin.

Liverman (1955), Liverman and Bonner (1953), Liverman _et_ _al_ (1955) and Klein (1959) conducted studies with tissues in which red light promotes growth, but Bertch (1961, 1963) conducted studies with etiolated pea epicotyl segments in which red light inhibits growth under suitable conditions. Bertch (1961, 1963), using etiolated pea epicotyl sections, found that all growth caused by Co in the dark in the presence of sugar, or by sugar alone in the dark, was inhibited by brief exposure to red light. Growth caused by IAA was however not photosensitive in the pea tissue. Bertch concluded that the sugar-cobalt growth system is distinct from the IAA growth system.

(c) Other Studies on the Mode of Action of Co on Plant Parts

Miller (1954) found sucrose alone sometimes promoted and sometimes inhibited the elongation of etiolated pea epicotyl segments, but that it markedly increased fresh weight. Co in the presence of IAA promoted growth slightly, but Co in the presence of IAA and sucrose increased elongation to a great extent. From these observations Miller postulated that sugar causes water uptake and increase in cell volume, also leading to synthesis of wall materials, and that Co promotes the increase in surface area of cell walls.


Auxin responses
ES
Infra red
(Fast)
Dark
(Slow)
S+E
S'
Ep

Thimann (1956) observed that growth promotion by Co was just as great in the presence of naphthaleneacetic acid (NAA) as in the presence of IAA. Because NAA is not attacked by the enzyme which oxidises IAA, Thimann disagrees with the suggestion put forward by Galston and Siegel (1954) that Co protects IAA from destruction by anti-peroxidative action. He also showed that the promotive effects of Co were not due to increased uptake of solutes from the external medium since growth promotion was effected, to varying extents, in the presence of a number of substrates other than sugar. From the observations that arsenite and dinitrophenol which are known to inhibit coupling of phosphorylation to oxidation, almost completely eliminated the growth promotive effects of Co, and that with energy sources other than sugar the effect of Co is less than with sugar and the optimum Co concentration is much higher than with sugar, Thimann concluded that, "Co must promote some step in oxidative metabolism which normally makes a source of energy (ATP) available for growth, and diverts it from other metabolic roles". The action of Co is considered to be due to a combination of Co with sulphydryl groups. The addition of 2,3-dimercaptopropanol-1 (BAL) to protect sulphydryl groups alleviated the inhibition caused by 10 and 3.10^{-4} Co, and inhibition of growth by excess Co was therefore attributed to the inactivation of a sulphydryl group, i.e. secondary complexing with sulphydryl groups of enzymes.

Busse (1959) believed that Co must act by decreasing the production of wall materials, or in other words, by inhibiting the development of rigidity of the cell wall, because he observed in his studies with coleoptile segments that growth promotion in the presence of Co did not occur until growth of controls had ceased, i.e. after about 24 hours' incubation. Thimann *et al* (1958), however, in their experiments with labelled galactose, found that Co did not interfere with the conversion of sugar to wall materials.

Lowther and Boll (1960) found that Co stimulated expansion of etiolated bean leaf discs both with and without a light treatment, whereas ethionine only stimulated expansion following a light treatment. On this basis, it did not seem likely that the stimulations by Co and ethionine in the bean leaf disc were effected through the same mechanism.

Thimann and Takahashi (1961) observed some parallelism between the action of Co and ethylenediaminetetraacetic acid (EDTA). Co like EDTA shows little or no effect in the absence of auxin, but gave significant increment in the presence of auxin; both Co and EDTA are somewhat more effective on coleoptiles than on pea stems; both are more effective in the presence of sucrose than in its absence. Thimann and Takahashi and Thimann (1963) have suggested that the similarity in action between EDTA and Co is because EDTA chelates a functional metal *in situ* on a catalyst, whereas Co competes for and combines with a particular site on the catalyst, at which site some other functional metal is normally combined.

B. GROWTH-REGULATING ACTIVITIES OF 2,4,5-TCPAA AND ITS MECHANISM OF ACTION FROM STUDIES OF STRUCTURE IN RELATION TO ACTIVITY OF THE SUBSTITUTED PHENOXYACETIC ACIDS

The synthetic growth hormone 2,4,5-TCPAA is widely employed in agriculture. It is known to induce root formation in cuttings, to prevent fruit drop and leaf abscission, to inhibit opening of flower buds, to promote fruit set, to stimulate ovary growth and promote ripening of fruits, and it is also a potent herbicide (Avery *et al*, 1947; Leopold, 1955; Audus, 1959).

Thimann (1951a), Fawcett *et al* (1953), Wain and Wightman (1953) reported the stimulatory effect of 2,4,5-TCPAA in the split pea stem test. Muir *et al* (1949), Hansch and Muir (1950), Fawcett *et al* (1953), Wain and Wightman (1953), Linser (1956), and Åberg (1961) have all reported the stimulatory effect of 2,4,5-TCPAA in the *Avena* coleoptile. Lowther and Boll (1960) reported stimulatory effects of the growth regulator in etiolated bean leaf discs, Åberg (1961) in wheat and flax roots, and Jönsonn (1961) in leaf epinasty.

Koepfli *et al* (1938), pioneers in the study of structure of growth substances in relation to their growth-regulating activities, from the standpoint of organic chemistry of the molecules, postulated from experimental evidence that the minimum structural requirements for auxin activity in the higher plants, were a ring system as nucleus, in which is a double bond, and a carboxyl group or structure readily converted to a carboxyl group on a side chain of the nucleus. However it was Zimmerman and Hitchcock (1942) who discovered that the phenoxyacetic acids can produce responses in plants similar to those produced by IAA.

1. PHENOXYACETIC ACID AND THE GENERAL EFFECTS OF SUBSTITUENTS IN THE AROMATIC RING OF THE PHENOXYACETIC ACID MOLECULE

Phenoxyacetic acid shows negligible auxin activity in a number of standard tests. However it has also been reported to show antiauxin activity. Zimmerman and Hitchcock (1942) reported it to be inactive in inducing curvature in stems and leaves; Thimann (1951a) reported it to be inactive in the split pea test and Fawcett et al (1953) in the split pea and *Avena* coleoptile. Muir and Hansch (1953) obtained slight auxin activity in the *Avena* coleoptile, and Jönsonn (1961) reported it as a root auxin in the wheat root elongation. Linser (1956) reported inhibitory activity. Åberg (1952, 1961) and Audus and Shipton (1952) have reported phenoxyacetic acid as an auxin antagonist in flax and cress roots.

However, when appropriate substituents are introduced into the ring structure of the phenoxyacetic acid molecule, the resulting compounds are potent auxins. The halogens fluorine, chlorine and bromine are effective substituents and the introduction of methyl groups is quite effective. Substitution with the above substituents in any one of the 2-, 3-, and 4- positions in the aromatic ring of the phenoxyacetic acid molecule enhances activity. Thus 2-chlorophenoxyacetic acid was reported active by Zimmerman and Hitchcock (1942), Thimann (1951), Fawcett et al (1953), Hansch and Muir (1953), and Wain and Wightman (1953); 3-chlorophenoxyacetic acid was reported active by Hansch and Muir (1953) and confirmed by Wain and Wightman (1953); 4-chlorophenoxyacetic acid and 4-bromophenoxyacetic acid reported active by Zimmerman and Hitchcock (1942), and the former by Fawcett et al (1953) and Hansch and Muir (1953). A second substituent may increase activity, e.g. 2,4-dichlorophenoxyacetic acid. The activity of disubstituted compounds, however, is generally not increased by further substitution.

2. THE EFFECTS OF SUBSTITUTION IN THE DIFFERENT POSITIONS OF THE AROMATIC RING OF THE PHENOXYACETIC ACID MOLECULE

Åberg (1954) observed that the substitution of a methyl group in the 4 position of the ring structure of the phenoxyacetic acid molecule (para substitution) imparted slight auxin activity to the originally antiauxin phenoxyacetic acid. Increasing the size of the 4-alkyl substituent gradually eliminated the auxin activity and increased antiauxin activity. Åberg (1956) concluded that the effect of increasing the size of a 4-substituent was to decrease the intrinsic activity of the growth regulator molecule, but not the affinity of the molecule to the growth sites. The affinity between the growth regulator and growth site remains high enough to result in antiauxin activity.

Åberg (1956) observed that the effects of 4-chlorination generally increased the auxin activity of weak auxins in the phenoxyacetic acid series. The exception was 2,6-dichlorophenoxyacetic acid. The effect of 4-chlorination of 2,6-dichlorophenoxyacetic acid to 2,4,6-trichlorophenoxyacetic acid increased antiauxin activity. Åberg suggested that increased auxin activity was due mainly to increased affinity to the growth centres and in the intrinsic activity. Increase in antiauxin activity was due to higher affinity and decreased intrinsic activity.

Åberg (1957, 1961) reported that the substitution of the phenoxyacetic acid molecule in the 2 or 6 position (ortho substitution) and in the 3 or 5 position (meta substitution) with the substituents chlorine, bromine, methyl and nitro groups, produced compounds which behave as antiauxins at low concentrations and auxins at high concentrations, depending on the type of system in which they are acting. By assuming that 2- or 6- substituted and 3- or 5- substituted phenoxyacetic acids as substances with considerably lowered, but not totally eliminated intrinsic activity, Åberg explained their ability to show auxin and antiauxin effects.

Åberg (1954, 1956, 1957) supports the hypothesis that growth regulator molecules are characterized by their "affinity" for sites active for growth regulation or for "growth centres" (probably a receptor of protein nature) and by their "intrinsic auxin activity" when properly bound to these sites. A typical competitive auxin antagonist or antiauxin is then a substance which is bound to the auxin receptor in the same place as the auxin, but which gives a complex unable to initiate the usual growth responses.

3. THE EFFECTS OF SUBSTITUTION AT BOTH THE 2 AND 6 POSITIONS IN THE AROMATIC RING OF THE PHENOXYACETIC ACID MOLECULE

The substitution at both the 2 and 6 positions (di-ortho substitution) of the phenoxyacetic acid molecule with groups such as chlorine, bromine or methyl was shown to drastically decrease auxin activity or cause inactivity (Muir _et al_, 1949; Hansch and Muir, 1950; Muir and Hansch, 1951, 1953, 1955; Hansch _et al_, 1951; Leaper and Bishop, 1951). McRae and Bonner (1952, 1953) also stressed the antiauxin activity of the 2,6-substituted 2,6-dichlorophenoxyacetic acid and 2,4,6-trichlorophenoxyacetic acid in the presence of IAA and 2,4-dichlorophenoxyacetic acid. The observers of the effects of substitution at both the 2 and 6 positions in the phenoxyacetic acid molecule therefore supported the postulation that active phenoxyacetic acids become attached at two points to a specific substrate within the cell, the points of attachment being the carboxyl group and either the 2 or 6 position in the aromatic ring.

There is however some evidence against the above theory. Wain and Wightman (1953) reported that the 2,6-dichloro- and 2,3,6-trichlorophenoxyacetic acids were significantly active in the pea test and that 2,4,6-trichlorophenoxyacetic acid showed slight activity. Osborne _et al_ (1954, 1955) investigated a number of compounds, substituted at both the 2 and 6 positions in the pea and _Avena_ cylinder test with respect to the variation in growth rate with time. They found that during the first few hours, 2,6-dichlorophenoxyacetic acid caused a conspicuous increase in the growth rate in the _Avena_ cylinders, which was later followed by shrinkage due to toxic effect, so that the total effect after 24 hours was almost nil or even negative. No such stimulation was observed with 2,6-dimethyl-, 2,4,6-trichloro- and 2,4-dichloro-6-methyl phenoxyacetic acids.

Toothill _et al_ (1956) demonstrated that 2,4-dichloro-6-fluoro- and 2,4-dibromo-6-fluoro-phenoxyacetic acids were highly active in the _Avena_ cylinder and pea test, and in the tomato epinasty test. They found 2-chloro-6-methyl phenoxyacetic acid to be slightly active in both the pea and _Avena_ tests, but 2,6-dichloro-, 2,4,6-tribromo- and 2,4,6-trichlorophenoxyacetic acids were inactive in the _Avena_ cylinder and tomato epinasty tests and only slightly active in the pea test, and that a number of other 2,6-substituted phenoxyacetic acids with bulky groups were entirely inactive in all the three tests. This has been interpreted to mean that it is not the presence of the 2,6-substituents themselves, but their size which interferes with the activity of the compound. If at least one of the substituent groups is small enough, e.g. fluorine, then activity persists.

Toothill _et al_ (1956) also found that introduction of a suitable alkyl group at a position α to the side chain brings about activity in 2,6-substituted phenoxyacetic acids.

4. THE EFFECTS OF SUBSTITUENTS AT BOTH THE 3 AND 5 POSITIONS IN THE AROMATIC RING OF THE PHENOXYACETIC ACID MOLECULE

Inactivity brought about by substitution of both the 3 and 5 positions in the aromatic ring of phenoxyacetic acid has been demonstrated by Leaper and Bishop (1951), Thimann (1952), Muir and Hansch (1953), Wain and Wightman (1953), Toothill (1956), and Åberg (1961). They showed that 3,5-dichlorophenoxyacetic acid was inactive. Leaper and Bishop (1951) concluded that unless the compound contained an open 2 or 6 position, and in addition two free 3 and 5 positions, the molecule was inactive or of very low activity. He supposed that the two unsubstituted 3 and 5 positions enabled quinone formation.

Wain and Wightman (1953) disagreed with Leaper and Bishop (1951) because they found 2,3-, 2,3,4-, 2,3,5-, and 3,4,5-substituted acids active in the _Avena_ cylinder test. These compounds did not possess free 3 and 5 positions. Toothill _et al_ (1956) has found that the introduction of further substituents into the nucleus of 3,5-dichlorophenoxyacetic acid could bring about activity.

5. THE POSSIBLE MECHANISM OF ACTION OF 2,4,5-TCPAA AND THE ACTIVE PHENOXYACETIC ACIDS

Activation and deactivation of certain ring positions brought about by nuclear substituents have been considered by Muir and Hansch (1951, 1953), Hansch _et al_ (1951), and Thimann (1952). The fact that phenoxyacetic acid and pentachlorophenoxyacetic acid are almost inactive would appear to indicate that at least one free activated ring position is necessary for auxin activity. The inactivity of 2,3,4,6- and 2,3,5,6-tetrachlorophenoxyacetic acids (Toothill _et al_, 1956) suggest that the 4,5 positions are not involved in attachment with a substrate. However, 2,3,4,5-tetrachlorophenoxyacetic acid has been reported active (Toothill _et al_, 1956), and this may indicate that the 6 position might be important in reacting with a substrate to bring about auxin activity.

C. IAA ACTION AND GROWTH OF THE CELL WALL

The study and discussion in this thesis deal in effect with the extension growth of cell walls under the influence of growth regulators. It is therefore pertinent to briefly review some relevant aspects of our knowledge of the growth of plant cell walls.

Because the growth of the cell is essentially the increase in area and volume of the cell wall, the proper reaction between an auxin and a "growth enzyme" will be expected to somehow affect the wall in such a way that the resulting processes include expansion of the cell wall and the deposition of new wall material.

Chemical analyses of the cell wall have shown that the main constituents of the cell wall are: cellulose, non-cellulosic polysaccharide, pectic substances, polyuronide hemicellulose and protein. The cellulose component is known to form the fibrillar phase of the wall, and the other components are known to form the amorphous matrix in which the fibrillar phase is embedded.

A comparison of the cell walls of organs of various plants show differences in the proportions of the above constituents (see Table A). Physical methods using optical methods involving X-rays and electron microscopy have shown that in the fibrillar phase of the cell wall elementary fibrils come together to form microfibrils or micelles, that the microfibrils aggregate to form the fibres, and that between the microfibrils are spaces occupied by paracrystalline cellulose.

A common form of orientation of the microfibrils in the cell wall is the orientation parallel to the fibre axis with some random scattering of the microfibrils. In the meristematic cells, however, the orientation of the microfibrils are generally at right angles to the fibre axis of the cell.

The study of IAA action in relation to growth of the cell wall has given evidence that auxin induces the plasticization of the cell wall and that there are differential effects of IAA on the elastic and plastic components of the loosening of the cell wall (Heyn, 1940; Thimann, 1951b; Burström, 1957; Tagawa and Bonner, 1957; and Cleland, 1958). The idea that an auxin may regulate the plasticization of the cell wall by its influence on the amount of substances has not been substantiated by strong evidence. Although Wilson and Skoog (1954) and Buffel and Carlier (1955) have reported auxin to increase the pectic substances, Burström (1958) has found auxin to have no effect on the relative con-

Component	Maize Coleoptile	Sunflower Hypocotyl	Maize Straw	Maize Cob	Hop Flower	Bamboo Stem	Pine Sapwood
Cellulose	36	38	46.5	38.3	30.8	41	53.6
Non-cellulosic polysaccharide	30	8	8.3	0.5	5.4	10	8.8
Pectic substances	13	46	0.3	0.5	-	-	1.0
Polyuronide hemicellulose	-	-	33.4	42.4	9.7	14	3.0
Lignin	-	8	19.5	16.7	54.1	32	26.4
Cuticular substance	21	-	-	-	-	-	-
Protein	-	-	1.9	3.2	-		-
Ash	-	-	3.5	1.4	-		-

Table A. Percentage cell wall composition in various plant organs. (From Siegel, 1962).

centrations of the wall components.

The discoveries that auxin induces the methyl esterification of the pectic substances of the wall (Ordin et al, 1955, 1957; Albersheim and Bonner, 1959; Jansen et al, 1960b), that auxin stimulates the activity of methylesterase activity (Neely et al, 1950; Bryan and Newcomb, 1954) and later that auxin induces the binding of the methylesterase to the cell wall (Glaziou, 1957a, b; Glaziou and Inglis, 1958) indicated that the methyl esterification of the pectic substances and the protection of the pectin from deesterification may be important in maintaining the plastic state of the cell wall. However, Cleland (1963a) reported that the elongation induced by auxin can occur even when the methylation induced by the auxin is blocked by ethionine. Cleland (1963b) reported the restricted occurrence in some plants of the induced methylation of the pectin by auxin. Jansen et al (1960a) reported that the binding of methylesterase to the cell wall is non-specific and of little importance in the maintenance of the plastic state.

Very little is known of the effects of auxin on the hemicellulose component of the cell wall. The hemicellulose component generally forms a big part of the total wall material. This indicates that the hemicellulose may be an important factor in the plasticization of the cell wall. The basis of the plasticization and later the development of rigidity of the cell wall may lie either in the quantitative or qualitative changes of the hemicellulose component.

It is known that there is an overall increase in the wall material during growth. Siegel (1962), contrary to Busse (1958), reported changes in the proportions of wall components in growth.

It is also known that as maturation proceeds, lignin is incorporated into the amorphous matrix of the cell wall. However, little is known as to how IAA regulates the synthesis of wall components. In the _Avena_ coleoptile IAA depresses the incorporation of acetate into pectates, polyuronides and cellulose, but increases the incorporation of sucrose into protopectin and cellulose. IAA is also known to prevent the action of peroxidase in lignification. In view of the lack of knowledge of IAA action on the synthesis of wall components and whether IAA influences rigidification, it is reasonable to believe that the incorporation of wall material into those parts already present will lead to rigidification of the wall.

The growth of the cell wall may then be attributed to two sets of reactions. First, those reactions which tend to effect plasticization in the cell wall and reduce the strength of the binding between the microfibrils. Secondly, those reactions which are synthetic in nature and tend to build up the cell wall. The relative intensities of the two sets of reactions determine whether growth or consolidation will occur. For normal growth of the cell wall to occur there must be a balance between the two sets of reactions. The growth regulator such as IAA in combining with the "growth enzyme" is presumably important in regulating this balance. A fundamental question is whether it regulates directly, both the plasticization process and the opposite rigidification process through the same receptor or different receptors.

II. GENERAL MATERIALS AND METHODS

A. Growth Room

A light-tight dark room, kept at 26° ± 1°C by cooling the steam heated room with an air-conditioner, was used for the growing of the etiolated peas used in the project. The peas were maintained in this dark room after treatment for the required period.

All manipulations in the dark room were carried out in green light. The light source was a Kodak Utility Safe Lamp (Model C) containing a 100 watt, tungsten bulb and a green colour filter (Corning glass No. 4-64, 6" x 6").

B. Germination and Growth of Seedlings

Vermiculite ("Terra Lite") used for the planting of the etiolated peas was manufactured by F. Hyde and Company Ltd. - F. Hyde (Ontario) Ltd.

Two enamel tubs (17" at widest dimension, 12½" midway across widest dimension, 8½" high) were used for soaking the vermiculite. One tub was used for water-saturated (previously soaked) vermiculite and the other for moist vermiculite (soaked for about 6 hours).

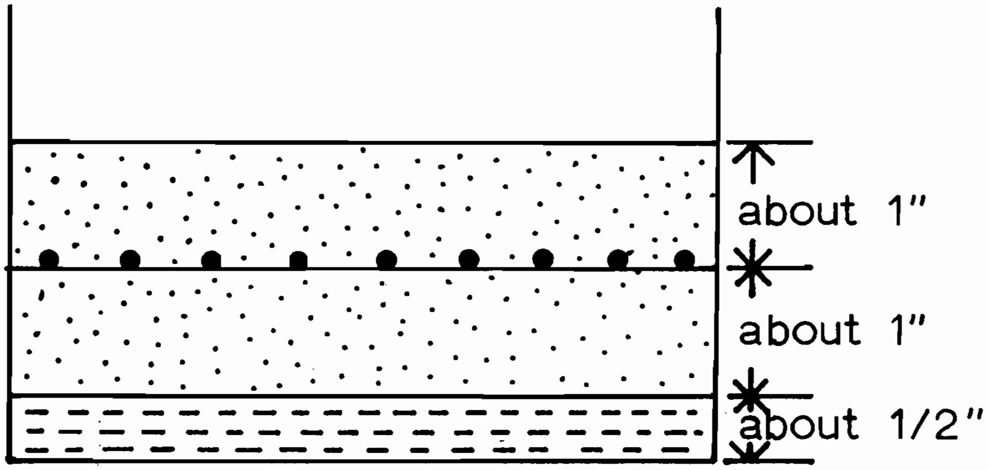
Rectangular polyethylene pans perforated at the bottom were used for the planting of the peas. White enamel trays placed underneath the pans were used to collect excess water which drained off from the vermiculite into the trays. Pieces of level stones were placed between the bottom of each pan and tray to allow easy drainage of water.

Inverted light-tight boxes, weighted down by bricks, and light-tight cupboards were used for covering and accommodating the peas in darkness after planting and during their growth. Four of the inverted light-tight boxes were of plywood, 16" x 21½" x 16½" high, with edges banded with black felt; one was of heavy cardboard, 17" x 37" x 16" high. Each of the former was used for covering one pan of peas. The latter was used for covering two pans of peas. One of the light-tight cupboards was wooden, 20" x 29" x 52" high, with two shelves, the lower shelf raised about 1' above ground level. The other light-tight cupboard was an old refrigerator, 21" x 30" x 5' high, with three shelves, the lowest shelf raised about 1' above ground level. Each of these cupboards was used to accommodate three pans of peas. Tests with photographic paper proved all the boxes to be light-tight.

The pans of peas during growth were placed on wooden tables about 3' above ground level.

About 2,000 peas were placed in a glass trough and rinsed first in tap water, then in single distilled water, and further rinsed 2-3 times in single distilled water. They were then covered with one inch single distilled water and put under a light-tight box in the dark room for 3½-4 hours. After soaking for 3½-4 hours, about 500 peas were sown closely together in each of the four polyethylene pans used in a single planting. Each pan contained about a one-inch layer of moist vermiculite soaked about 6 hours, with about a half-inch layer of water-saturated (previously soaked) vermiculite at the bottom of the pan. The seeds were then covered with about a one-inch layer of moist vermiculite (see Figure 3). The pans were then kept in the appropriate set of light-tight boxes and the seeds allowed to grow for 8 days.

Figure 3. The germination and planting of pea seedlings.



---Water saturated vermiculte

•••• Moist vermiculite

● ● Peas.

C. Glassware and Cleaning of Glassware

All glassware used was pyrex, unless otherwise stated. All glassware except pipettes was washed by the following method:

1) dipped in chromic acid for at least 10 minutes; 2) rinsed in tap water to get rid of acid; 3) washed in detergent; 4) rinsed well in tap water; 5) rinsed twice in single distilled water; 6) rinsed twice in double distilled water; 7) shaken free of excess water; 8) allowed to air dry on trays on paper towels.

Pipettes were immersed in a chromic acid bath for at least 10 minutes, rinsed with tap water to remove acid, then placed in a syphon pipette washer for at least an hour. They were rinsed similarly with single distilled and double distilled water, and shaken free of excess water. They were allowed to air dry, inverted, within the rims of a tripod stand, on paper towels. Forceps and spatulae were washed in detergent and rinsed similarly with single and double distilled water as in the washing of glassware.

D. Distilled Water

Single distilled water used was from a block tin still. Double distilled water used was obtained by redistilling the single distilled water in a pyrex glass still.

E. The Preparation of Test Media

Reagent grade chemicals and double distilled water were used to make up all stock solutions. Stock solutions of IAA, 2,4,5-TCPAA were kept in the refrigerator at $4^{o}C$. The remainder was discarded if not used after three weeks.

IAA	-	California Foundation for Biochemical Research.
2,4,5-TCPAA	-	Nutritional Biochemicals Corp., Cleveland, Ohio.
$CoCl_2$, KCl, KOH, HCl	-	Highest purity available, Fisher Scientific Company, U.S.A.

The preparation of the test media involved the preparation of stock solutions, the basal medium, and the making up of the final test solution.

Because the final volume of each test medium was to be 50 ml (a convenient volume for working) stock solutions were made so that the amount of stock solution added to the basal medium to make up the final test medium, was a convenient volume to handle, and such that the final test medium would not exceed 50 ml. The stock solutions made were as follows:

$CoCl_2$	-	0.1189 gm/100 ml (0.1 ml used for 10^{-5} M)
IAA	-	5 mg/100 ml (1 ml for 1 mg/l)
TCPAA	-	10 mg/50 ml (2.5 ml for 10 mg/l)
	-	10 mg/50 ml diluted 10 times (2.5 ml for 1 mg/l)
KCl	-	1.865 gm/500 ml (1 ml for 50 ml test medium)
KOH	-	0.1 N solution
HCl	-	0.05 N solution

The basal medium contained 2% sucrose and 0.001 M KCl. Double strength basal medium (5 gm sucrose, 5 ml stock KCl/125 ml basal medium) was made. For each 50 ml of test medium, 25 ml of basal medium was used.

To make up the test medium, the required volumes of Co and/or IAA, TCPAA to make up the right concentrations, were added to the 25 ml of basal medium delivered first into a 50 ml volumetric flask, and the solution was made up to 50 ml with double distilled water.

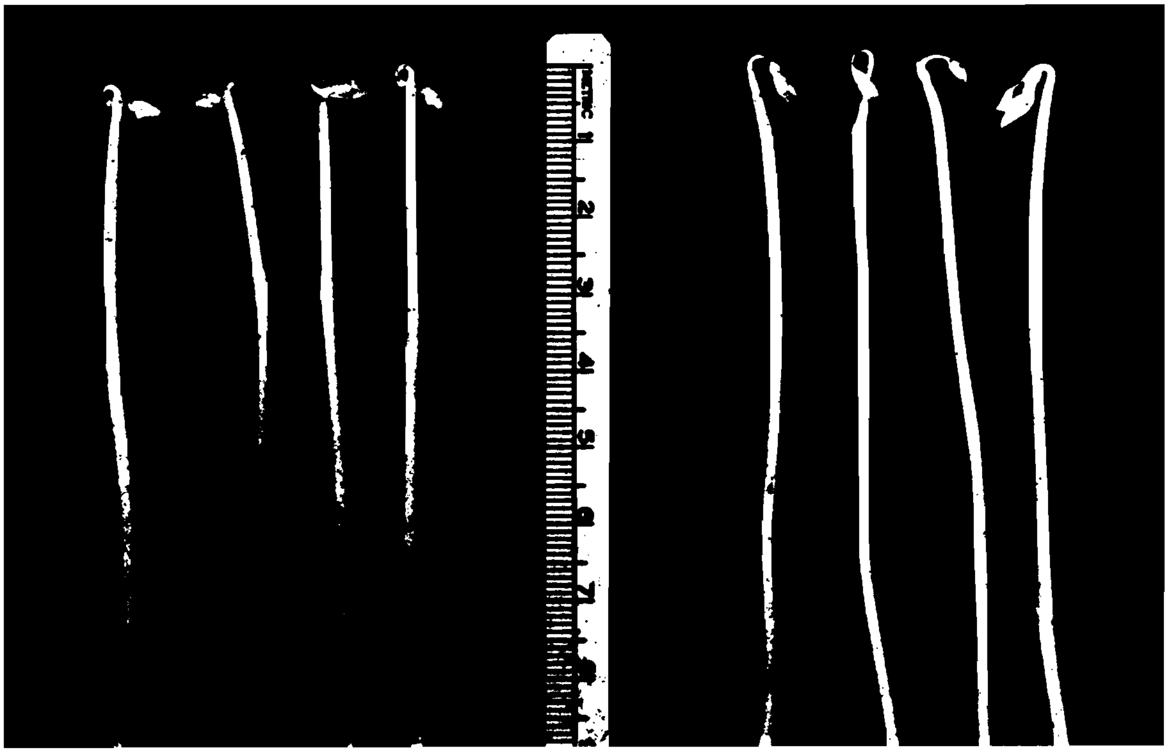
Each 50 ml of test medium was poured into a 100 ml beaker and covered. All test media were adjusted to a pH of 5.5 ± 1 with either 0.05 N HCl and 0.1 N KOH using a glass electrode. When pH adjustment was complete 5 ml of each test medium was pipetted into 6 cm. petri dishes.

F. The Cutting of Pea Epicotyl Sections and Inoculation into Test Media

After 8 days of growth the third internode of the pea seedlings had developed and for most of the seedlings ranged in size from 30-60 mm. The seedlings then were selectively harvested. The method of selection of seedlings (see Figure 4) is comparable to that employed by Bertch (1961). For uniformity seedlings with recurved hooks and third internode within the range of 20-65 mm. were picked above the vermiculite by hand. They were then placed on Kleenex tissues wetted with double distilled water in a glass trough until 10 mm. sections were taken by cutting just below the apical hooks with a device which consisted of a metal block with removable razor blades placed 10 mm. apart. Cutting was performed on the cover of a 9 cm. petri dish on a piece of filter paper moistened with double distilled water.

The 10 mm. epicotyl sections were placed by means of a spatula and a pair of forceps in a 9 cm. petri dish containing double distilled water until the required number of sections were obtained.

Figure 4. The method of selection of the etiolated pea seedlings. Those to the left would be considered acceptable. (From Bertch and Hillman, 1961).



When sectioning was completed, the sections in the petri dish were randomized by stirring with the spatula. Using the spatula and forceps, sections were then taken out of the petri dish, surface moisture removed with filter paper, and transferred into the petri dishes of test media. Unless otherwise stated, 10 sections were placed in each petri dish, which was then gently shaken to provide mixing of sections and test medium. The dishes containing the epicotyl sections were placed in a light-tight box and incubated for the required period.

G. The Measurement of Straight Growth

In the initial experiments, sections were allowed to incubate in test media for 20-24 hours and growth was measured at the end of the 20-24 hour period. In the later experiments where growth curves were involved, samples of 10 sections were measured at the required intervals.

Sections were measured in daylight. Each 10-section lot was removed from its test medium by means of a spatula, surface dried by means of filter paper, placed in double distilled water, redried, and then measured. Measurement was done against a plastic scale rule to the nearest 0.5 mm. Forceps were used to place sections against the scale. Gentle bending by the fingers was employed to straighten some sections that were slightly curved. Results are expressed as the average growth of 10 sections in the different test media.

III. EXPERIMENTAL AND RESULTS

A. Study 1. The Concentration-response Curve of Co and its Optimum Concentration

This study consisted of six experiments. The results of all the experiments are given in Table 1 in the Appendix. These results were variable within limits from one experiment to another. Figure 5 illustrates the set of results considered to be representative of the data (Experiment 1a in Appendix Table 1). Increase in the concentration of Co gave an increase in growth with an optimum concentration of about 3.10^{-5}M. Further increase in Co concentrations beyond the optimum inhibited growth. Co at 3.10^{-5}M was chosen as the working optimum concentration for subsequent experiments. Thimann (1956), Bertch (1963) also found that the optimum Co concentration for growth of etiolated pea epicotyl sections was about 3.10^{-5}M. Miller (1954) obtained an optimum at 8.10^{-5}M, but his manipulations were carried out in dim red light.

In subsequent experiments, Co at stimulatory concentrations (2.10^{-5} to 10^{-4} in Figure 5) sometimes promoted growth but at other times did not. This is in agreement with some other reports (Thimann, and Takahashi, 1961).

Study 2. The Concentration-response Curve of IAA and its Optimum Concentration

This study consisted of four experiments. The results are given in Table 2 in the Appendix, and illustrated in Figure 6 These results were variable within limits from one experiment to another. Experiment 3 in Figure 6 is the most representative. The low IAA concentrations (0.01 to 0.1 mg/l) promoted growth but

Figure 5. The effects of increasing Co concentrations on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.

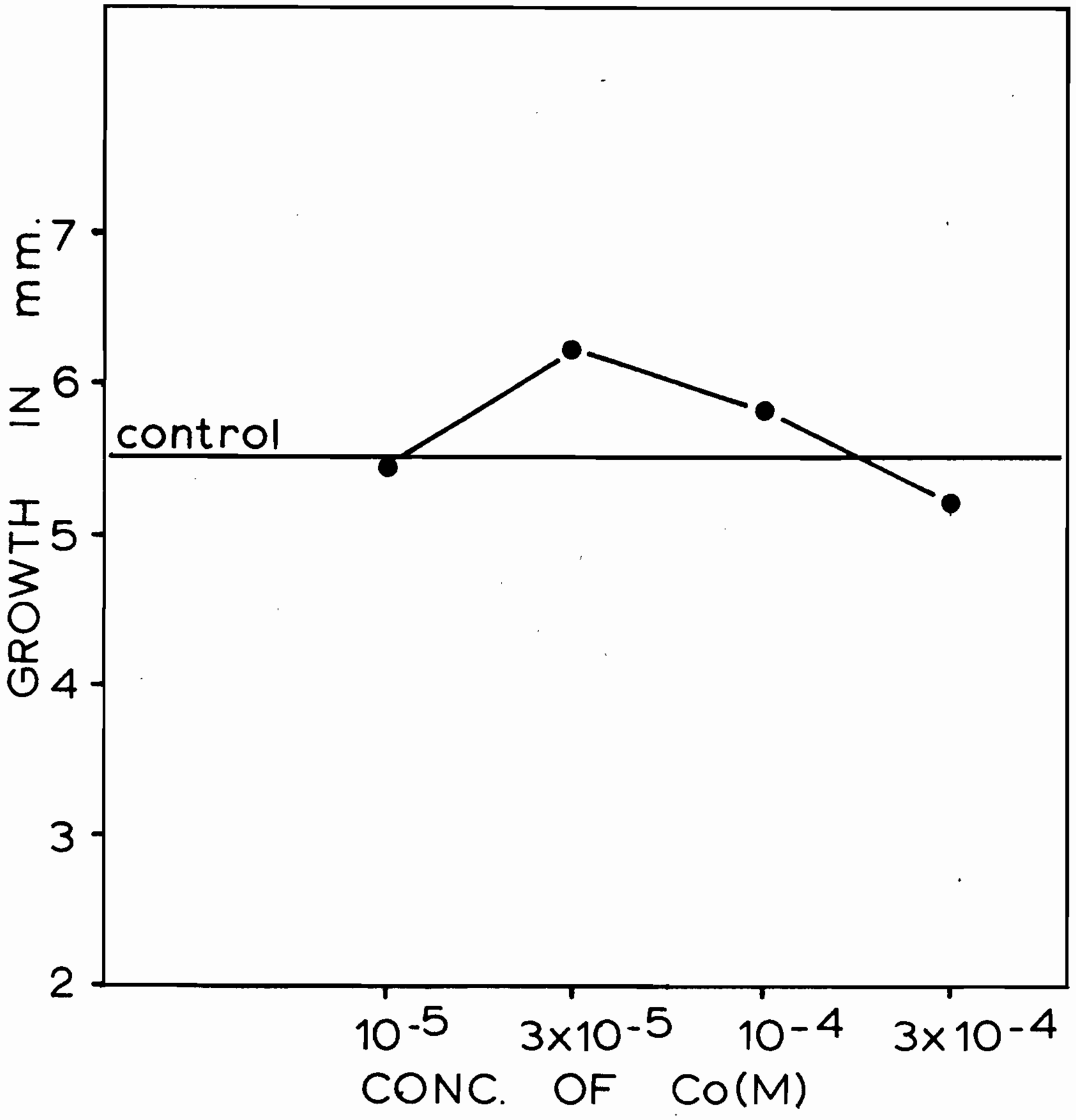
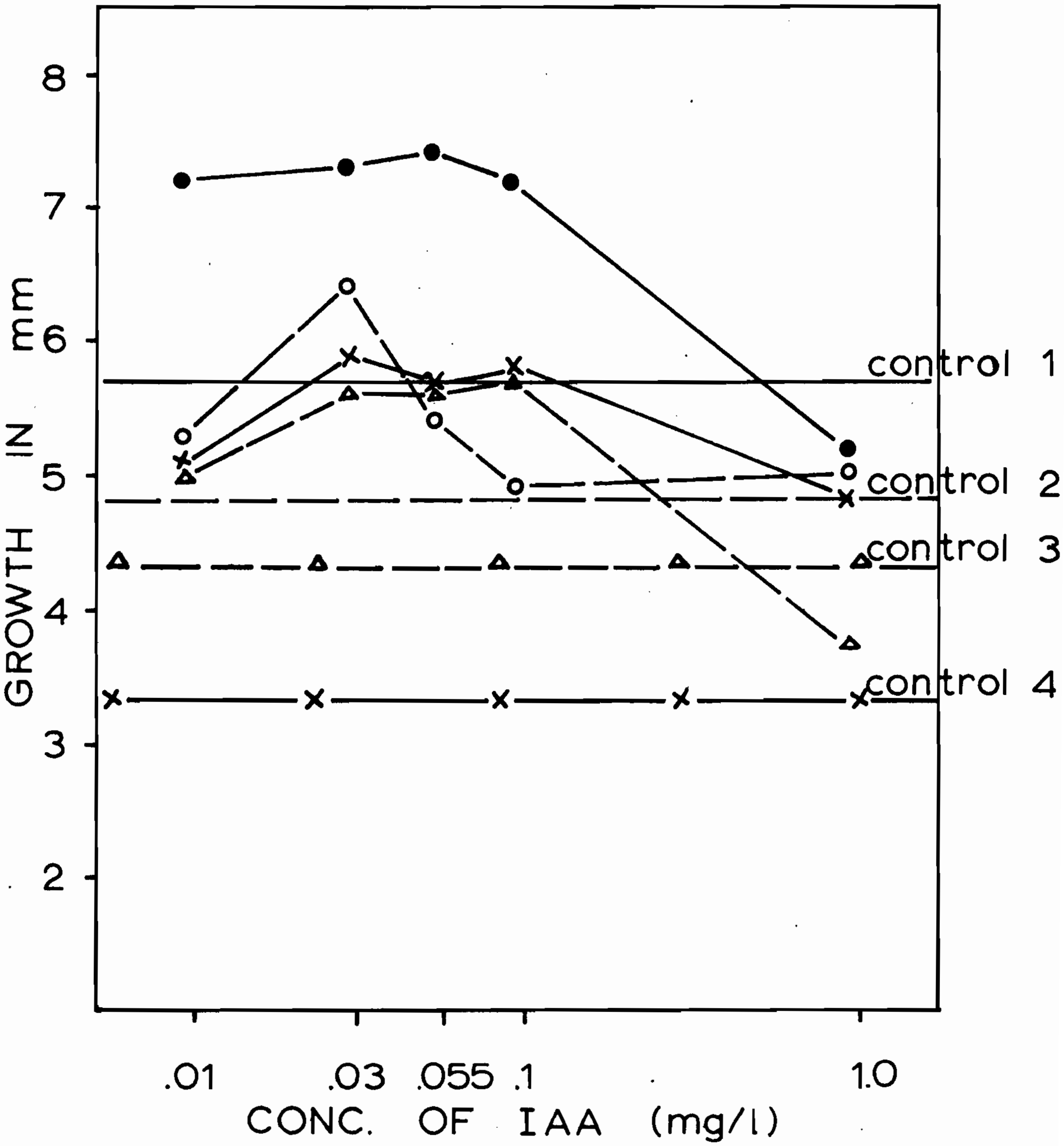

GROWTH IN mm.
7
6
5
4
3
2
control
10^{-5}
3×10^{-5}
10^{-4}
3×10^{-4}
CONC. OF Co(M)

Figure 6. The effects of increasing IAA concentrations on the growth in length of etiolated pea epicotyl sections.


8
7
6
5
4
3
2
GROWTH IN mm
control 1
control 2
control 3
control 4
.01
.03
.055
.1
1.0
CONC. OF IAA (mg/l)

the high IAA concentration (1.0 mg/l) inhibited growth. The optimum concentration was about 0.1 mg/l IAA.

Miller (1954) using bean leaf disks and Thimann (1956) using pea epicotyl sections used 1 mg/l IAA in their experiments with Co. Lowther and Boll (1960) using pea epicotyl sections found a variation of 0.1 to 1 mg/l as the optimum. The optimum of 0.1 mg/l IAA obtained here however proved to be valid in subsequent experiments.

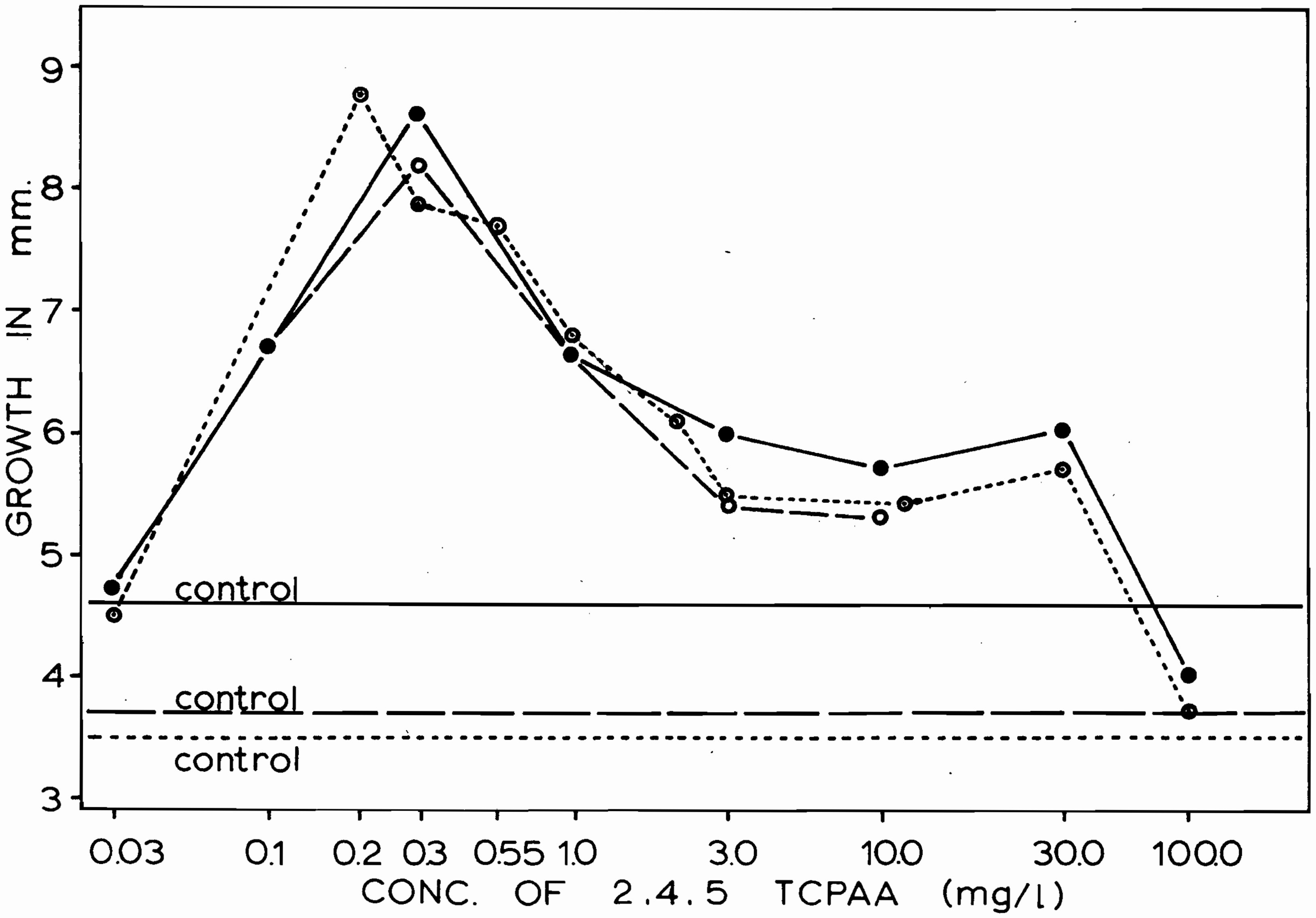
C. Study 3. The Concentration-response Curves Using Different TCPAA Samples and Their Optima

The TCPAA samples used were authentic 2,4,5-TCPAA, a sample labelled TCPAA used by Lowther and Boll (1960) and authentic 2,4,6-TCPAA.

The results of the experiments performed are given in Tables 3, 4 and 5 in the Appendix. The concentration-response curves using authentic 2,4,5-TCPAA are shown in Figure 7 , and those for authentic 2,4,6-TCPAA are shown in Figure 9 . The comparable results using the sample of TCPAA used by Lowther and Boll (1960) are shown in Figure 8.

Figure 7 shows that growth promotion was obtained at all concentrations used except at the high concentration of 2,4,5-TCPAA of 100 mg/l. The lowest concentration of 2,4,5-TCPAA at which growth promotion was obtained was 0.03 mg/l. The optimum concentration was about 0.3 mg/l. Further increase in the TCPAA concentration beyond the optimum concentration and up to 10 mg/l progressively decreased growth. Above 10 mg/l, there was a slight increase in growth promotion at a concentration of about

Figure 7. The effects of increasing 2,4,5-TCPAA concentrations on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.



GROWTH IN mm.
9
8
7
6
5
4
3
control
control
control
0.03
0.1
0.2
0.3
0.55
1.0
3.0
10.0
30.0
100.0
CONC. OF 2.4.5 TCPAA (mg/l)

Figure 8. The effects of increasing TCPAA* concentrations on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.

TCPAA* denotes the sample used by Lowther and Boll, 1960.

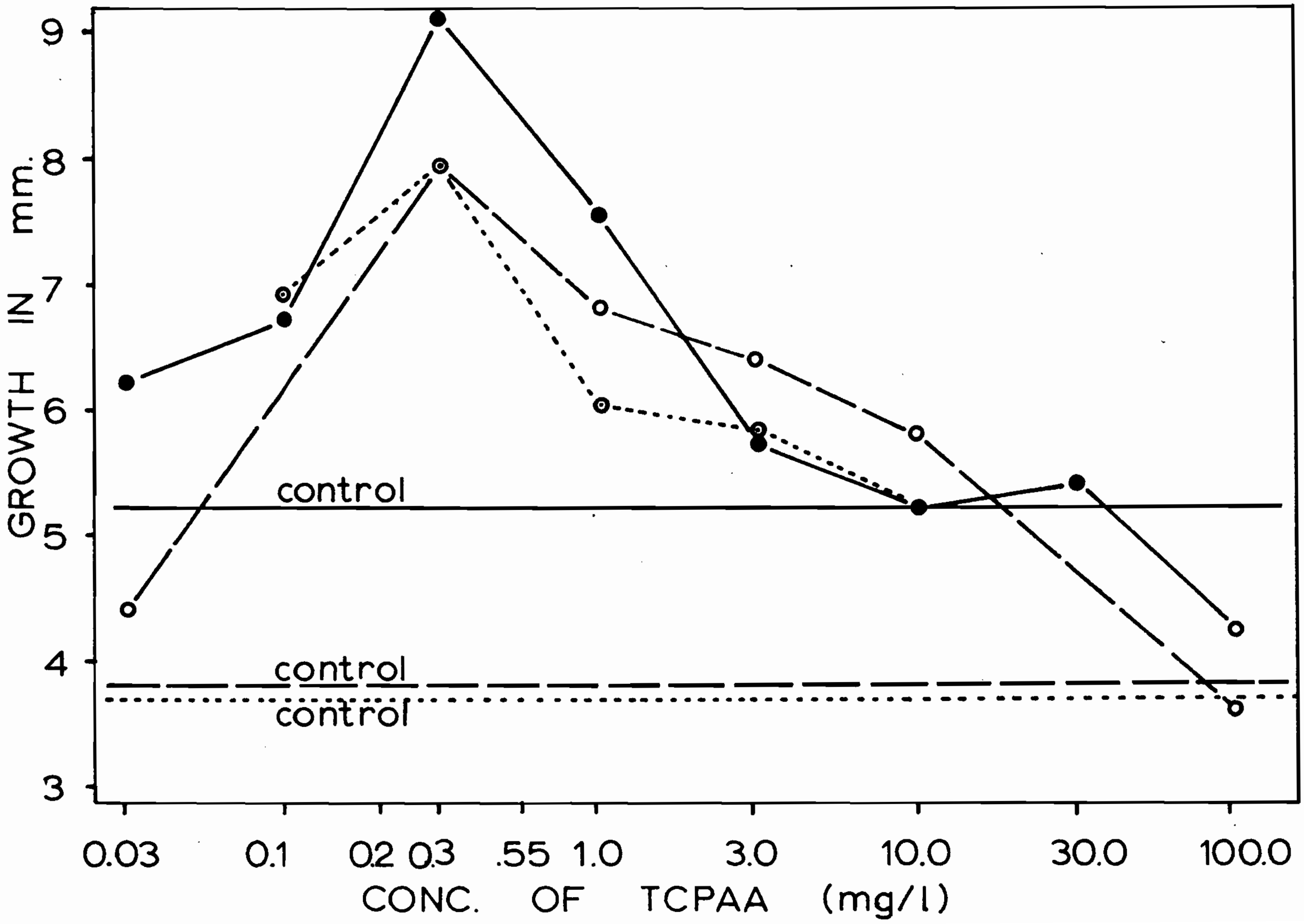
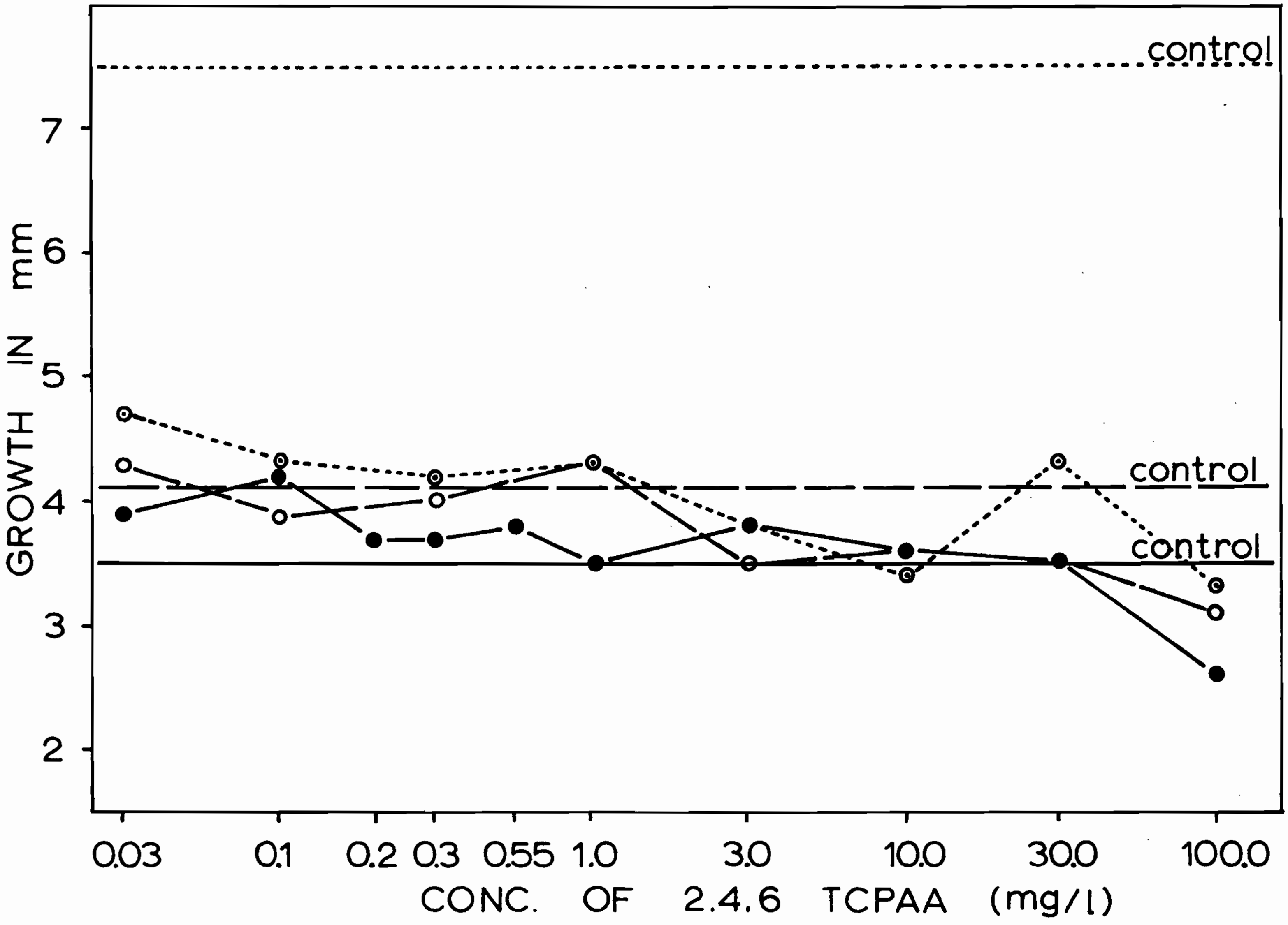

9
8
7
6
5
4
3
GROWTH IN mm.
control
control
control
0.03
0.1
0.2
0.3
.55
1.0
3.0
10.0
30.0
100.0
CONC. OF TCPAA (mg/l)

Figure 9. The effects of increasing 2,4,6-TCPAA concentrations on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.


control
control
control
GROWTH IN mm
7
6
5
4
3
2
0.03
0.1
0.2
0.3
0.55
1.0
3.0
10.0
30.0
100.0
CONC. OF 2.4.6 TCPAA (mg/l)

30 mg/1. At 100 mg/1 growth was inhibited.

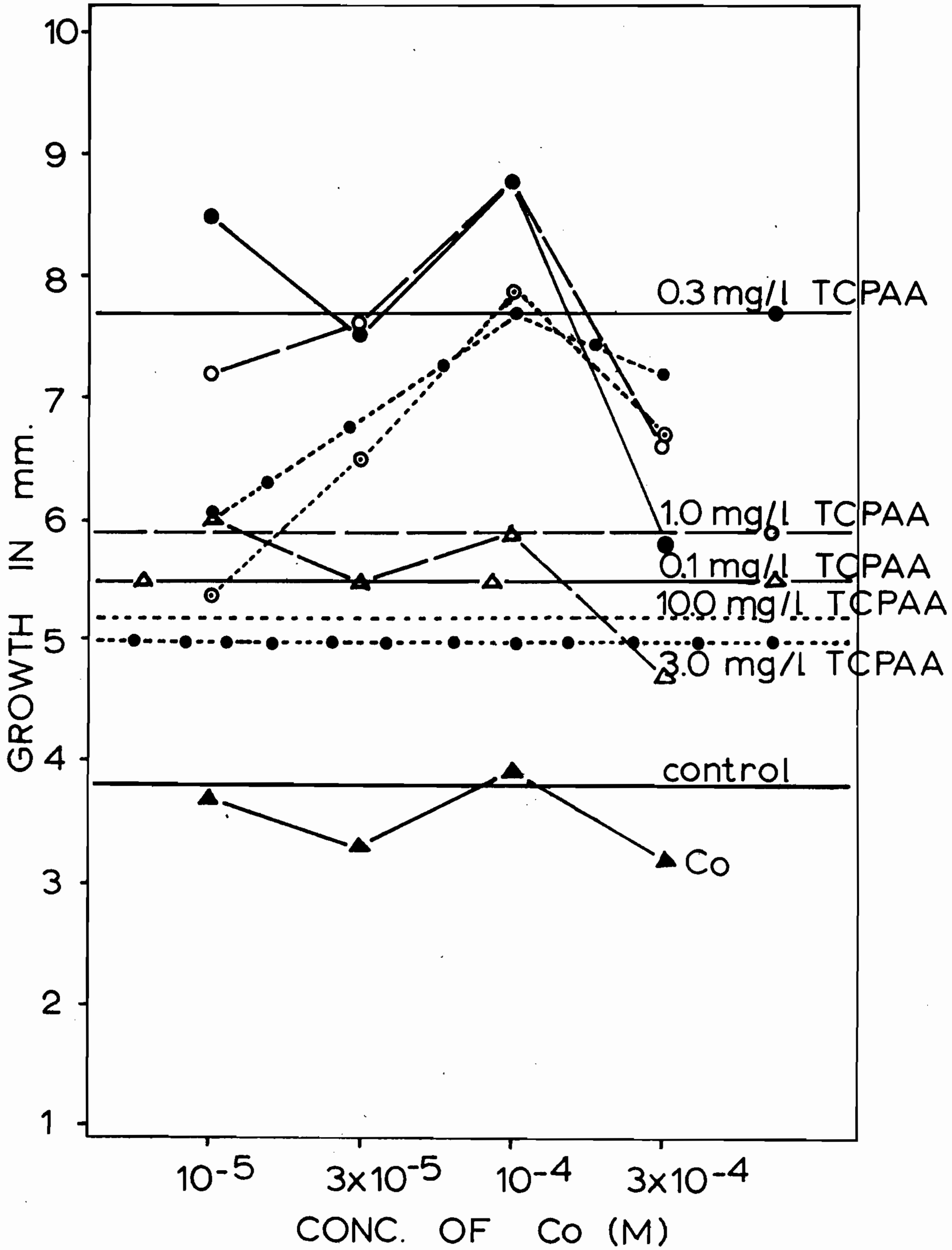
A concentration of 0.3 mg/1 of 2,4,5-TCPAA was chosen as the working optimum. It proved to be valid in many subsequent experiments.

The complete absence of growth promoting activity by 2,4,6-TCPAA is shown in Figure 9 . A comparison of Figure 8 and Figure 9 shows that the sample of TCPAA used by Lowther and Boll (1960) was, undoubtedly, 2,4,5-TCPAA.

D. Study 4. The Effects of Co Concentrations in the Presence of Different Concentrations of 2,4,5-TCPAA

Results of the five experiments performed are given in Table 6 in the Appendix. They were somewhat variable but tended to show the same general facts. Experiment 5 gave results which appeared to be the most representative (Figure 10). In the minus-Co controls the greatest growth promotion was with 0.3 mg/1 TCPAA. At higher concentrations to 10 mg/1, the growth promotion was progressively decreased. This was in agreement with previous results obtained in the study of the concentration-response curve using 2,4,5-TCPAA. Synergistic effects between Co and 2,4,5-TCPAA were shown at various concentrations. Synergism was not as obvious at low TCPAA concentrations (0.1, 0.3 mg/1) as at high TCPAA concentrations (1.0, 3.0, 10.0 mg/1). Looking at this in another way, at high supraoptimal TCPAA concentrations Co reversed the inhibitory effect of TCPAA. Reversal of inhibition by Co at 1.0 mg/1 TCPAA gave a growth level comparable to that in Co plus 0.3 mg/1 TCPAA. The depression of the level of growth by Co plus 1.0 mg/1 TCPAA at

Figure 10. The effects of Co concentrations in the presence of increasing 2,4,5-TCPAA concentrations on the growth in length of etiolated pea epicotyl sections. Growth is the average of 8 or 9 or 10 sections.


10
9
8
7
6
5
4
3
2
1
GROWTH IN mm.
0.3 mg/l TCPAA
1.0 mg/l TCPAA
0.1 mg/l TCPAA
10.0 mg/l TCPAA
3.0 mg/l TCPAA
control
Co
10^{-5}
3×10^{-5}
10^{-4}
3×10^{-4}
CONC. OF Co (M)

3.10^{-5}M Co is probably not representative, because this did not occur in two other cases.

E. Study 5. The Effects of Co in the Presence or Absence of Optimum IAA (0.1 mg/1)

The results of the three experiments performed are shown in Table 7 in the Appendix. These results were variable within limits, but gave the same general indications. Experiment 2 is considered to be the most representative and is shown in Figure 11. In the absence of IAA Co promoted growth slightly at 3.10^{-5} and 5·5.10 M. However, in the presence of optimal (0.1 mg/1) IAA growth promotion by Co was much greater. In other words, the effects of Co and IAA were synergistic. However, this only applied at lower Co concentrations (10^{-5} to 10^{-4}M), but did not apply at the high Co concentration (3.10^{-4}M). At 3.10^{-4}M, there was inhibition of growth obtained in the IAA control. This also occurred in Experiment 1 of the study. In Experiment 3, inhibition of growth at the higher Co concentration was to the level of the IAA control.

F. Study 6. The Effects of Co Concentrations in the Presence of Suboptimal (0.01 mg/1), Optimal (0.1 mg/1), and Supraoptimal (1.0, 3.0 mg/1) IAA Concentrations

Two experiments were performed, the results of which are shown in Table 8 in the Appendix. Within limits, the results were variable, but they indicated the same facts. Figure 12 shows the representative experiment. Co, at concentrations 10^{-5}, 3.10^{-5} and 3.10^{-4}M, inhibited growth in the presence of suboptimal (0.01 mg/1) IAA. Inhibition was to a level about the level of the Co controls. Figure 12 is not representative in this aspect in that the 0.01 mg/1

Figure 11. The effects of Co concentrations in the presence or absence of optimum (0.1 mg/l) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.

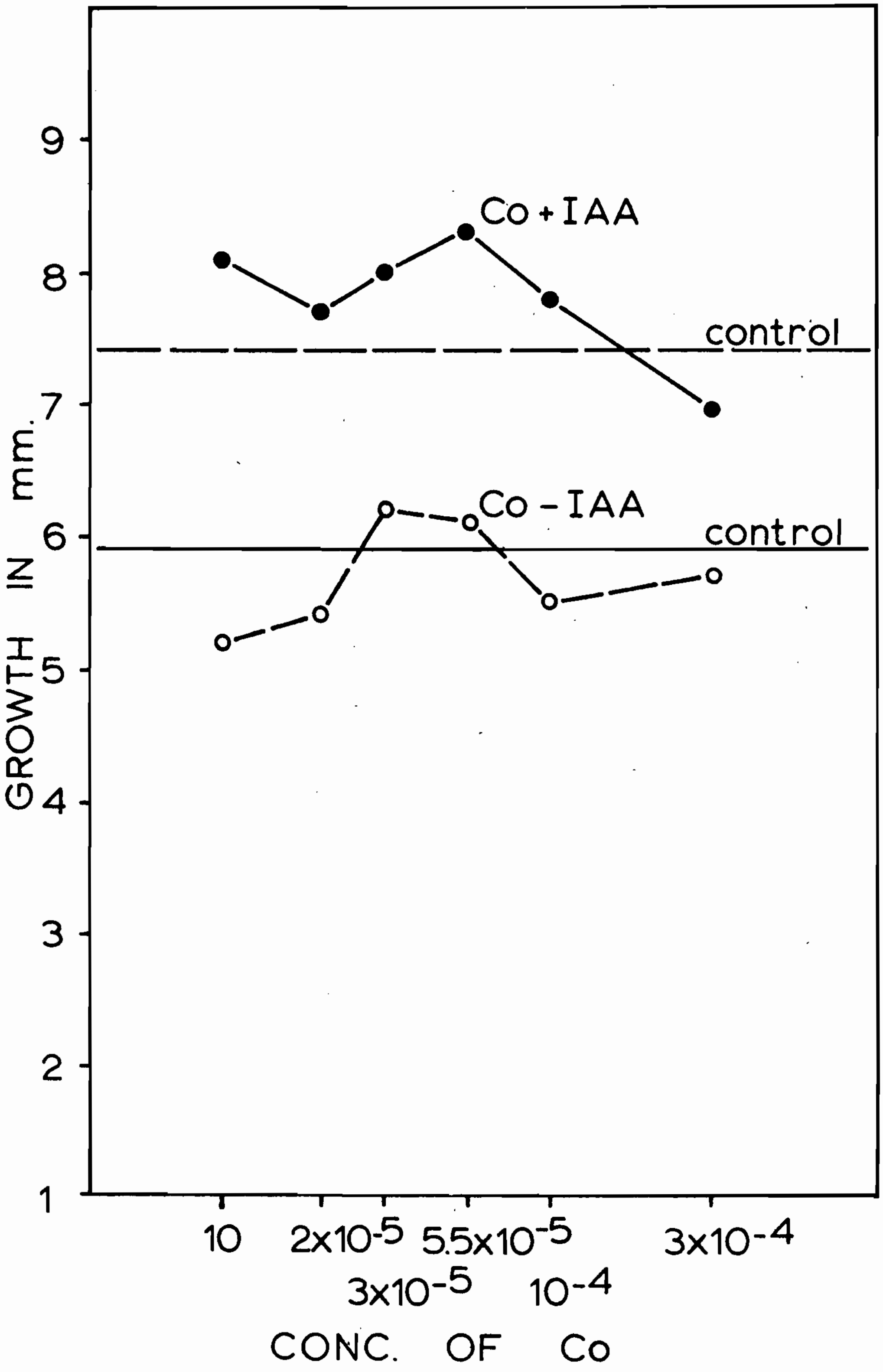
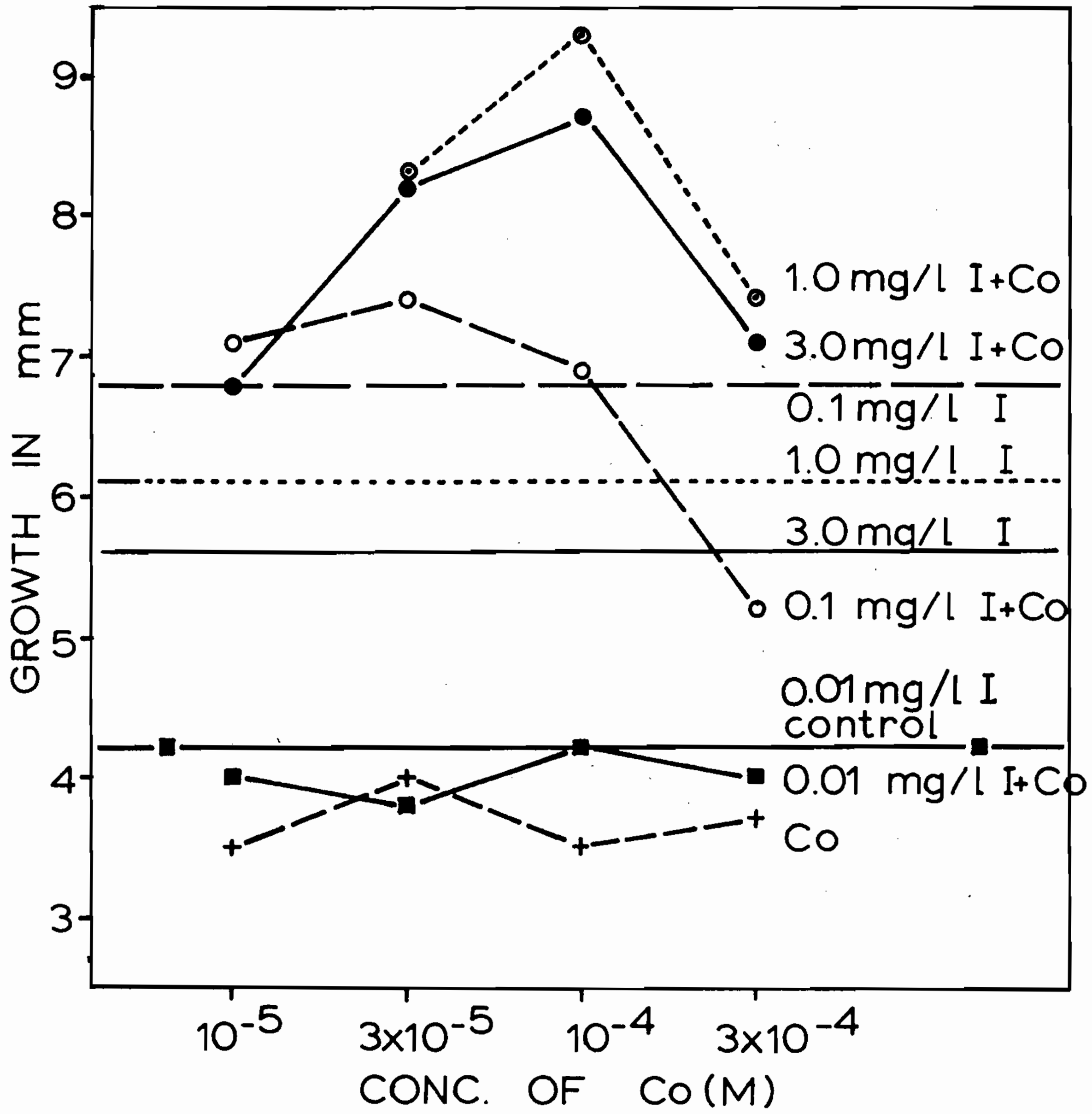

GROWTH IN mm.
9
8
7
6
5
4
3
2
1
Co + IAA
control
Co - IAA
control
10
2x10^{-5}
5.5x10^{-5}
3x10^{-4}
3x10^{-5}
10^{-4}
CONC. OF Co

Figure 12. The effects of Co concentrations in the presence of suboptimal (0.01 mg/l), optimal (0.1 mg/l), and supraoptimal (1.0 mg/l, 3.0 mg/l) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.


GROWTH IN mm
9
8
7
6
5
4
3
1.0 mg/l I+Co
3.0 mg/l I+Co
0.1 mg/l I
1.0 mg/l I
3.0 mg/l I
0.1 mg/l I+Co
0.01 mg/l I
control
0.01 mg/l I+Co
Co
10^{-5}
$3x10^{-5}$
10^{-4}
$3x10^{-4}$
CONC. OF Co (M)

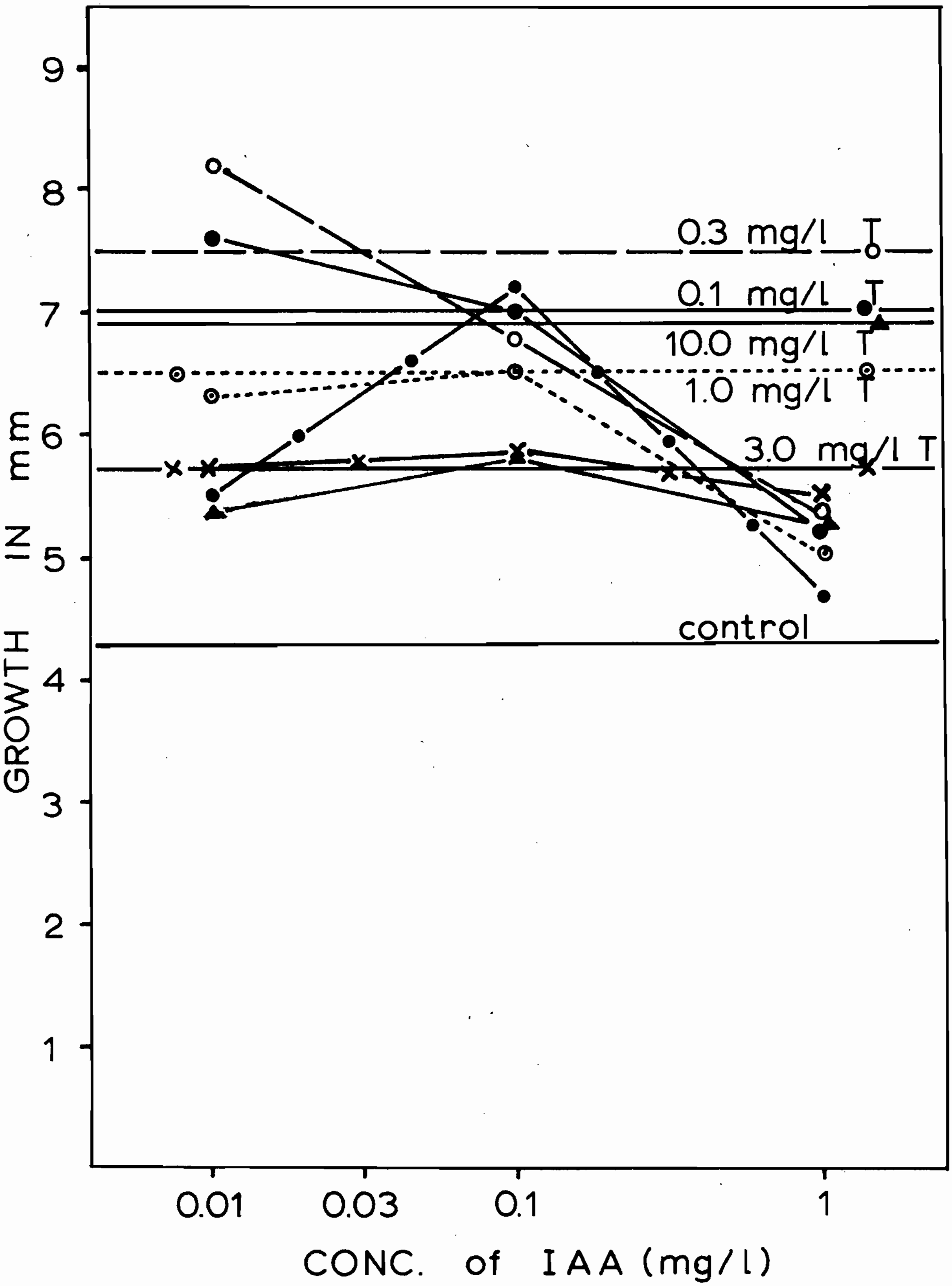
IAA control did not promote growth above the basal medium control. Results of the other experiment however showed this promotion and furthermore it did not show synergism with Co. Thus Co does not act synergistically with IAA at low or suboptimal (0.01 mg/l) IAA. However Co in the presence of optimum (0.1 mg/l) IAA showed synergistic effects and promoted growth above that in the optimal (0.1 mg/l) IAA control except at the highest concentration (3.10^{-4}M). The high Co concentration of 3.10^{-4}M inhibited growth in presence of optimal (0.1 mg/l) IAA. One of the most important observations is that Co in the presence of supraoptimal (1.0, 3.0 mg/l) IAA completely reversed the growth inhibition caused by the supraoptimal IAA. This reversal of inhibition occurred at all Co concentrations used and is comparable to the Co effect at supraoptimal TCPAA concentrations, where there was also reversal of the inhibitions due to supraoptimal TCPAA.

G. Study 7. The Effects of Concentration of IAA at Various Concentrations of TCPAA

The results of the two experiments performed are given in Table 9 in the Appendix. They were variable within limits, with both giving the same general picture. Experiment 1 (Figure 13) was chosen to illustrate the results obtained. The TCPAA controls (0.1 to 10 mg/l) and IAA concentration curve (with 0.01 to 1 mg/l) obtained were in agreement with previous results. The optimum concentrations for TCPAA and IAA at 0.3 mg/l and 0.1 mg/l respectively were confirmed.

It was shown that TCPAA together with IAA with some exceptions gave growth inhibition at all concentrations. The exceptions

Figure 13. The effects of concentration of IAA at various concentrations of TCPAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 8 sections.


9
8
7
6
5
4
3
2
1
GROWTH IN mm
0.3 mg/l T
0.1 mg/l T
10.0 mg/l T
1.0 mg/l T
3.0 mg/l T
control
0.01
0.03
0.1
1
CONC. of IAA (mg/l)

are suboptimal (0.1 mg/l) TCPAA together with suboptimal (0.01 mg/l) IAA; suboptimal (0.1 mg/l) TCPAA together with suboptimal (0.03 mg/l) IAA; and optimal (0.3 mg/l) TCPAA together with suboptimal (0.01 mg/l) IAA. These combinations show synergistic growth promotion. However this was not shown in the other experiment performed. A later experiment (see Figure 25) confirmed the growth promotion by suboptimal (0.1 mg/l TCPAA) plus suboptimal (0.01 mg/l) IAA. Figure 13 also shows that in general the high or supraoptimal (1.0 mg/l) IAA concentration was more effective than the low or suboptimal (0.01 mg/l, 0.03 mg/l) IAA concentrations in causing growth inhibition when supplied together with TCPAA. It should be noted that the TCPAA concentration which is more effective in promoting growth by itself is in turn the concentration which, when together with supraoptimal (1.0 mg/l) IAA, brings about greater growth inhibition. In other words, the effectiveness of a TCPAA concentration in growth promotion is correlated with its effectiveness in causing growth inhibition when supplied together with supraoptimal (1.0 mg/l) IAA.

H. Study 8. The Effects of Co Concentrations in the Presence of Different Concentrations of TCPAA with or without IAA

Altogether fourteen experiments were performed in Study 8.

Experiment	Aim
1-4	To study the effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without either supraoptimal (1.0 mg/l) IAA, or optimal (0.1 mg/l) IAA.

Experiment	Aim
3 and 6	To study the effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without either suboptimal (0.01 mg/l) IAA or optimal (0.1 mg/l) IAA.
5	To check the effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without suboptimal (0.01 mg/l) IAA.
10	To confirm the effects of Co concentrations in the presence of optimal (0.3 mg/l) TCPAA, with or without either suboptimal (0.01 mg/l) or optimal (0.1 mg/l) IAA.
11	To confirm the effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with supraoptimal (1.0 mg/l) IAA.
13	To confirm the effects of Co concentrations in the presence of suboptimal (0.1 mg/l) TCPAA, with or without either suboptimal (0.01 mg/l) IAA or optimal (0.1 mg/l) IAA.
7 and 8	To study the effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without suboptimal (0.01 mg/l)

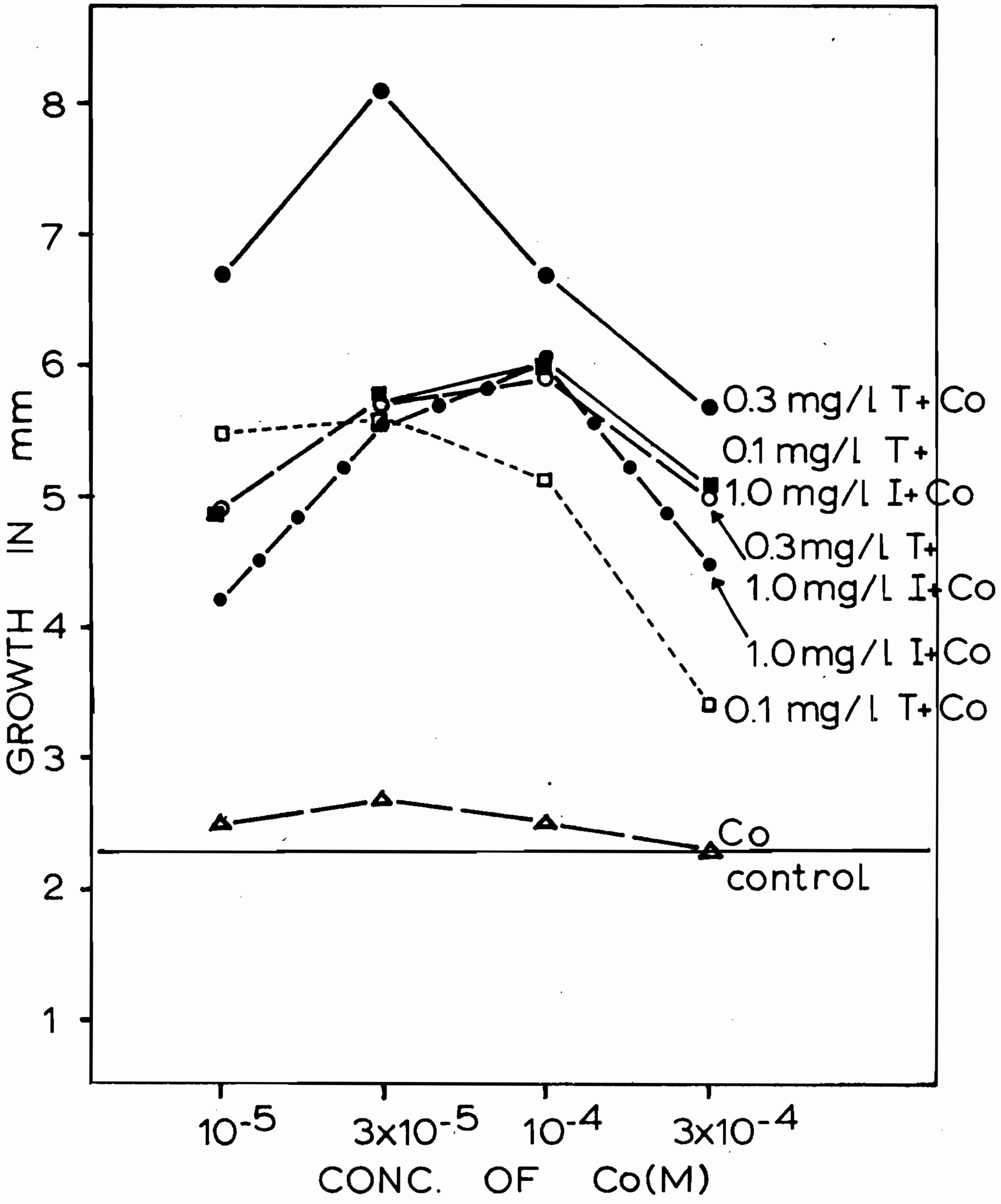
Experiment	Aim
7 and 8	IAA, optimal (0.1 mg/1) IAA and supraoptimal (1.0 mg/1) IAA. The supraoptimal concentration of 3.0 mg/1 IAA was also studied in Experiment 8.
9	To check the effects of Co concentrations in the presence of supraoptimal (1.0 mg/1) TCPAA, with or without suboptimal (0.01 mg/1) IAA.
12 and 14	To check the effects of Co concentrations in the presence of supraoptimal (1.0 mg/1) TCPAA, with optimal (0.1 mg/1), supraoptimal (1.0, 3.0 mg/1) IAA. The suboptimal (0.01 mg/1) IAA was included in Experiment 14.

The effects of Co concentrations in the presence of suboptimal (0.1 mg/1) or optimal (0.3 mg/1) TCPAA, with or without supraoptimal (1.0 mg/1) IAA were observed in four experiments. The results are given in Table 10 in the Appendix. They were variable, but the results of Experiment 4 (Figure 14) best illustrates the results obtained.

At the suboptimal TCPAA concentration, the supraoptimal IAA concentration inhibited growth at the low Co concentration (10^{-5}M) but the supraoptimal IAA overcame the inhibition caused by high Co concentrations (10^{-4} to 3.10^{-4}M) added to suboptimal (0.1 mg/1) TCPAA.

At the optimal TCPAA concentration, IAA inhibited growth

Figure 14. The effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA with or without supraoptimal (1.0 mg/l) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 8, 9 and 10 sections.


GROWTH IN mm
8
7
6
5
4
3
2
1
10-5
3x10-5
10-4
3x10-4
CONC. OF Co(M)
0.3 mg/l T+Co
0.1 mg/l T+
1.0 mg/l I+Co
0.3mg/l T+
1.0mg/l I+Co
1.0mg/l I+Co
0.1 mg/l T+Co
Co
control

at all concentrations of Co but this inhibition was much more drastic at low Co concentration (10^{-5}M) than at high Co concentrations ($3.10^{-5}$to 3.10^{-4}M). Hence the observations with suboptimal and optimal TCPAA are in general agreement.

The effects of Co concentrations in the presence of suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without optimal (0.1 mg/l) IAA may be observed in seven of the experiments performed in Study 8. These results are given in Table 11 in the Appendix. They were variable but Experiment 3 (Figure 15) is probably the most representative.

At the suboptimal TCPAA concentration, the addition of optimal IAA concentration increased growth and showed synergism between TCPAA and IAA at all Co concentrations. This synergism is not shown at 3.10^{-4} Co in Figure 15 but it was shown in most of the experiments.

At the optimal TCPAA concentration, the addition of IAA had no effect on growth at any concentration of Co. In only one experiment was inhibition shown at all Co concentrations.

The effects of Co in the presence of suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without suboptimal (0.01 mg/l) IAA are shown in several of the experiments performed in Study 8. (Table 12 in the Appendix). These results were variable within limits. The results in Experiment 6 (Figure 16) are the most representative.

At the suboptimal TCPAA concentration, the suboptimal IAA concentration increased growth at all the Co concentrations.

At the optimal TCPAA concentration, the suboptimal IAA

Figure 15. The effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA with or without optimal (0.1 mg/l) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 8, 9 and 10 sections.

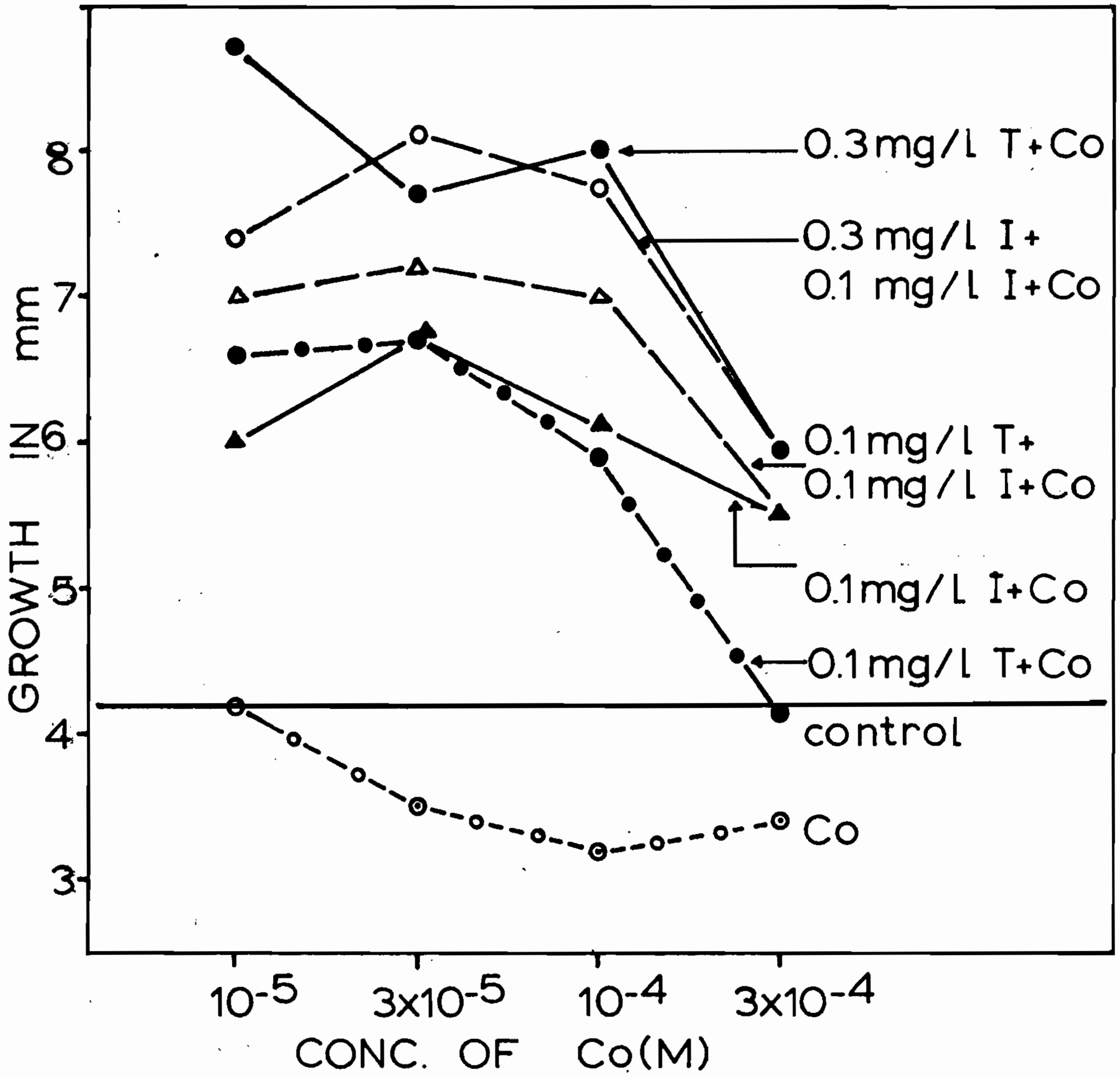
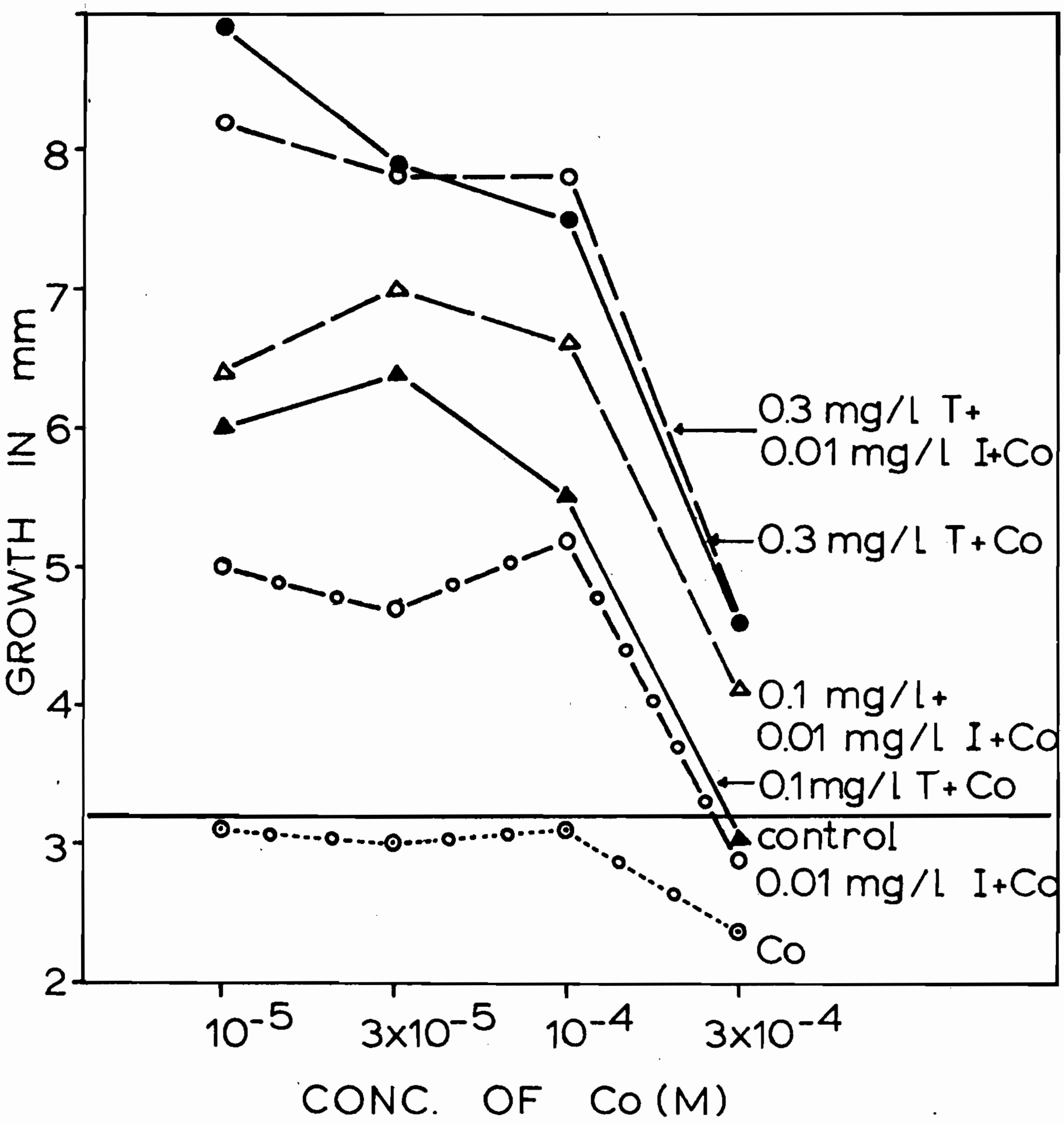

GROWTH IN mm
3
4
5
6
7
8
10-5
3x10-5
10-4
3x10-4
CONC. OF Co(M)
0.3mg/l T+Co
0.3 mg/l I+
0.1 mg/l I+Co
0.1mg/l T+
0.1mg/l I+Co
0.1mg/l I+Co
0.1mg/l T+Co
control
Co

Figure 16. The effects of Co concentrations in the presence of either suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA with or without suboptimal (0.01 mg/l) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 8, 9 and 10 sections.


GROWTH IN mm
2
3
4
5
6
7
8
0.3 mg/l T+
0.01 mg/l I+Co
0.3 mg/l T+Co
0.1 mg/l+
0.01 mg/l I+Co
0.1 mg/l T+Co
control
0.01 mg/l I+Co
Co
10^{-5}
3×10^{-5}
10^{-4}
3×10^{-4}
CONC. OF Co (M)

concentration had no effect on growth at any of the Co concentrations, except in one of the experiments which is considered to be unrepresentative, and in which IAA increased growth at all the Co concentrations.

The effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without either suboptimal (0.01 mg/l) IAA, or optimal (0.1 mg/l) IAA or supraoptimal (1.0, 3.0 mg/l) IAA are shown in five of the experiments performed in Study 8. The results of these five experiments are given in four tables in the Appendix. Table 13 gives results regarding the effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without suboptimal (0.01 mg/l) IAA. Table 14 gives results regarding the effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without optimal (0.1 mg/l) IAA. Table 15 gives results regarding the effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without supraoptimal (1.0 mg/l) IAA. Table 16 gives results regarding the effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with suboptimal (0.01 mg/l), optimal (0.1 mg/l), and supraoptimal (1.0, 3.0 mg/l) IAA.

The results were variable within limits. Experiment 8 (Figure 17) and Experiment 14 (Figure 18) are chosen to represent the data obtained.

Figure 17 shows that at the supraoptimal TCPAA concentration, the suboptimal IAA concentration gave growth promotion at lower Co concentrations (10^{-5} to 10^{-4}M). However, this growth promotion was not obtained in all experiments. Figure 17 also shows that at the highest Co concentration (3.10^{-4}M), the addition of IAA decreased

Figure 17. The effects of Co concentrations in the presence of supraoptimal (1,0 mg/1) TCPAA with or eithout either suboptimal (0.01 mg/1) or optimal (0.1 mg/1) or supraoptimal (1.0 mg/1) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 8, 9 and 10 sections.

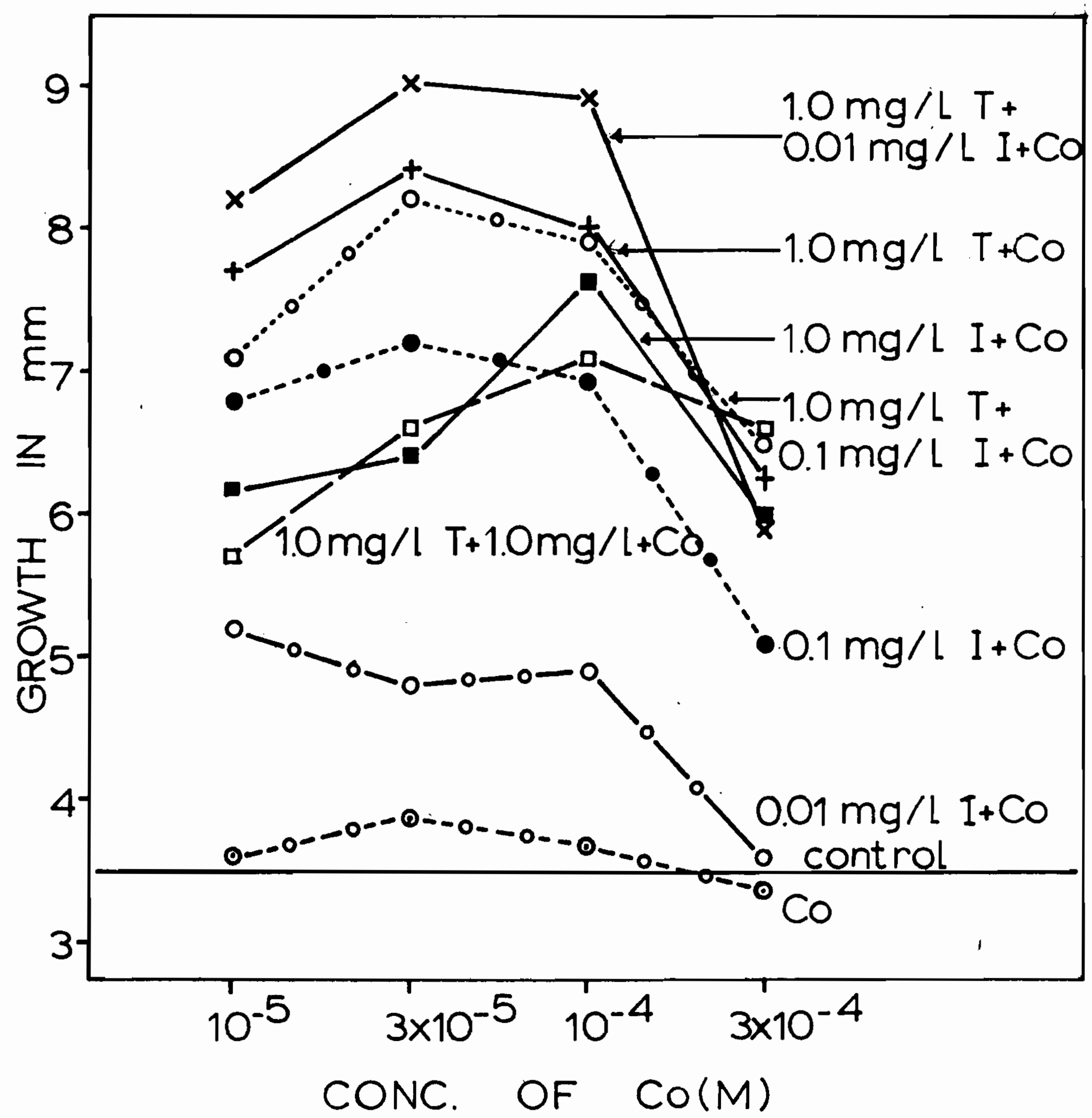
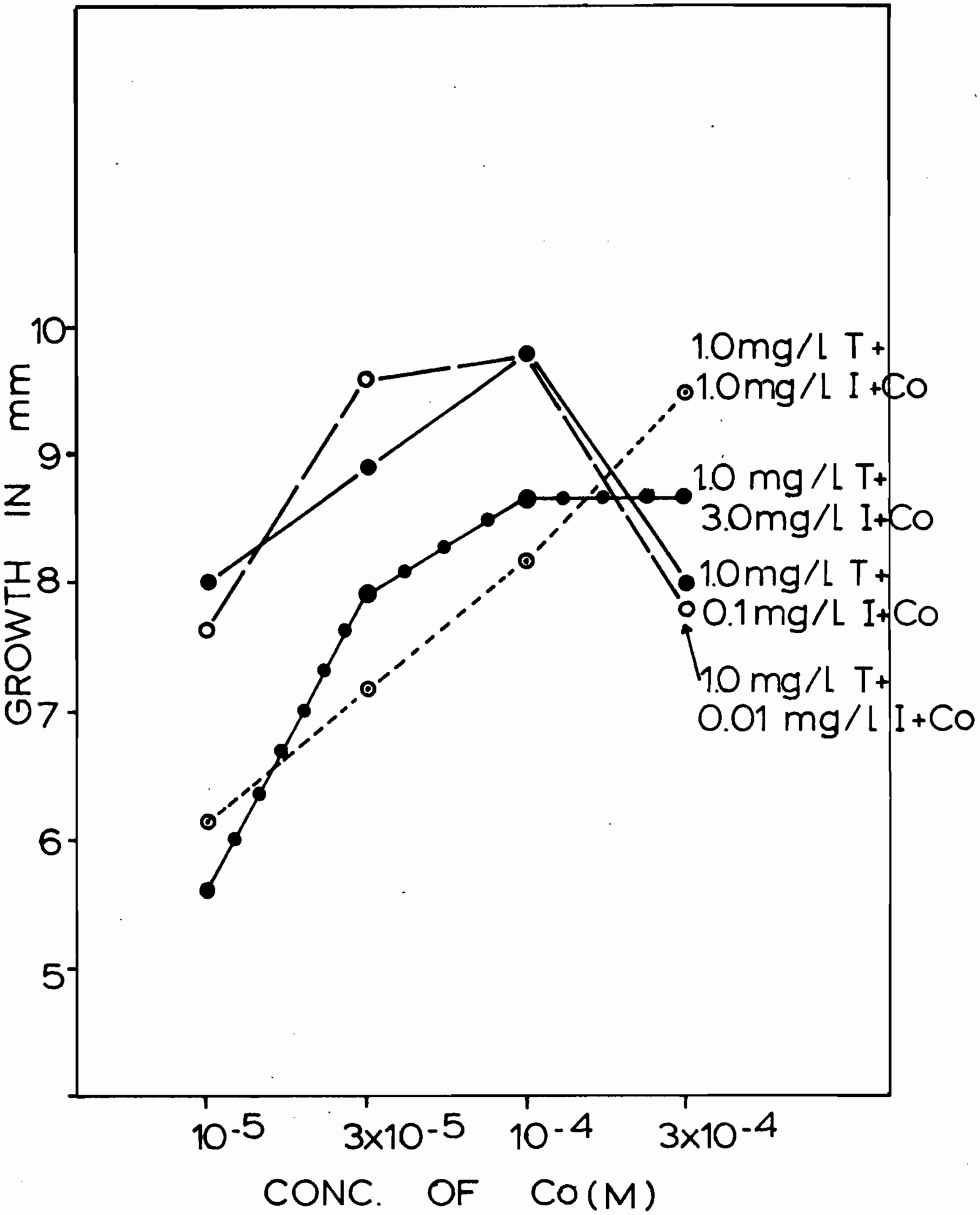

1.0 mg/L T+ 0.01 mg/L I+Co
1.0 mg/L T+Co
1.0 mg/L I+Co
1.0 mg/L T+ 0.1 mg/L I+Co
1.0 mg/L T+1.0 mg/L+Co
0.1 mg/L I+Co
0.01 mg/L I+Co
control
Co
GROWTH IN mm
3
4
5
6
7
8
9
10^{-5}
3×10^{-5}
10^{-4}
3×10^{-4}
CONC. OF Co(M)

Figure 18. The effects of Co concentrations in the presence of supraoptimal (1.0 mg/1) TCPAA with either suboptimal (0.01 mg/1) or optimal (0.1 mg/1) or supraoptimal (1.0 mg/1, 3.0 mg/1) IAA on the growth in length of etiolated pea epicotyl sections. Growth is the average of 10 sections.


GROWTH IN mm
10
9
8
7
6
5
10-5
3x10-5
10-4
3x10-4
CONC. OF Co(M)
1.0mg/l T+
1.0mg/l I+Co
1.0 mg/l T+
3.0mg/l I+Co
1.0mg/l T+
0.1mg/l I+Co
1.0 mg/l T+
0.01 mg/l I+Co

growth. This was also shown in the other three experiments.

In addition, Figure 17 shows that at the supraoptimal TCPAA concentration, the optimal IAA concentration decreased growth except at the high Co concentrations (10^{-4} and 3.10^{-4}M) Co. The inhibitory effect of IAA added to supraoptimal TCPAA at low concentrations of Co can also be deduced from Figure 18.

Figure 18 also shows that at the supraoptimal TCPAA concentration, the supraoptimal IAA decreased growth except at the high Co concentration of 3.10^{-4}M, where inhibition by Co was relieved. This can also be deduced from Figure

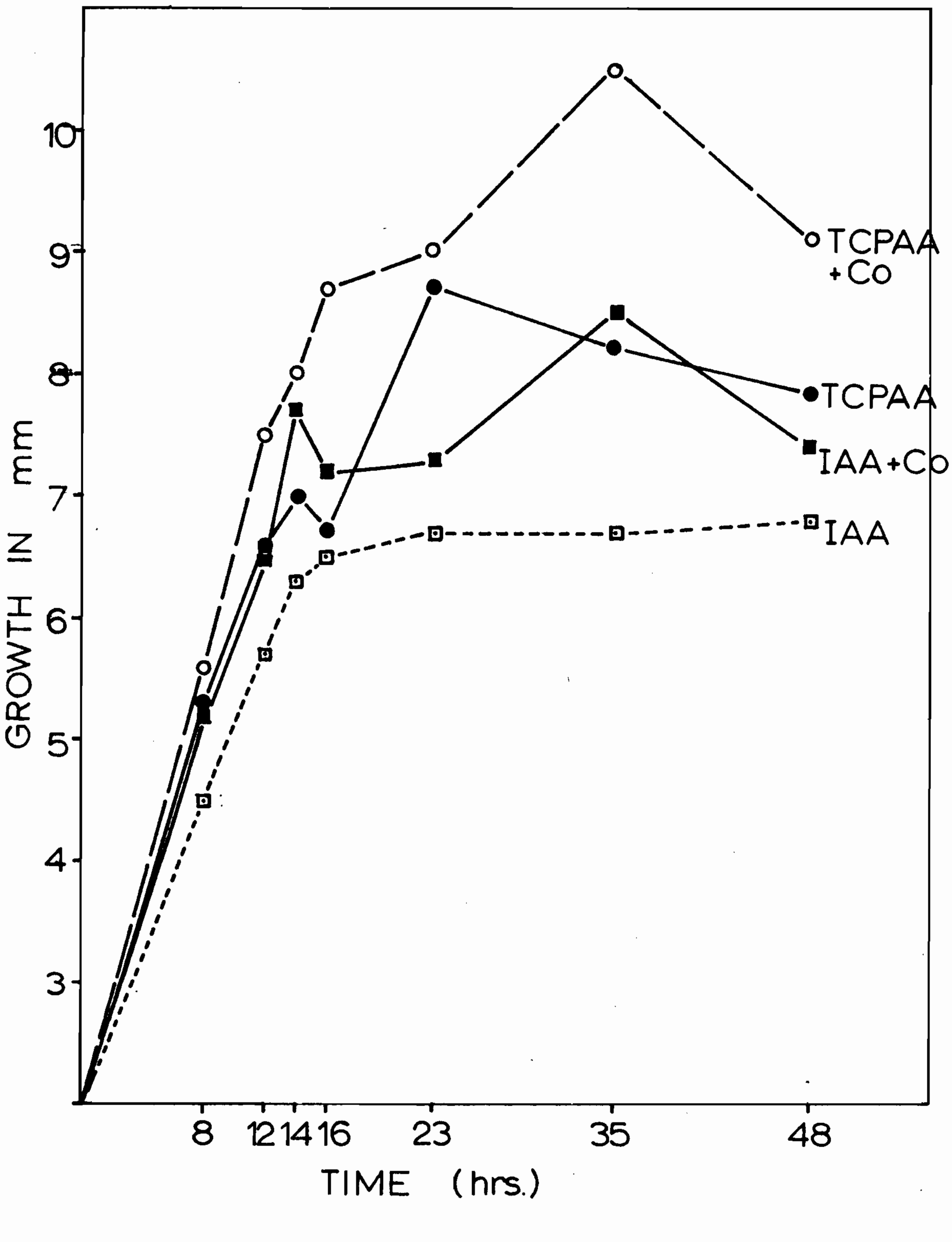
At the supraoptimal TCPAA concentration, the supraoptimal (3.0 mg/1) IAA relieved the inhibition by the high Co concentration of 3.10^{-4}M (Figure 18). Relief of inhibition was however less than with IAA at a supraoptimal concentration of only 1.0 mg/1. It was noted that at lower Co concentrations of 3.10^{-5} and 10^{-4}M, the inhibition by IAA at the supraoptimal (1.0 mg/1) concentration was relieved by the higher supraoptimal (3.0 mg/1) concentration of IAA.

I. Study 9. The Growth Curves in Optimal (0.3 mg/1) TCPAA, Optimal TCPAA plus 3.10^{-5}M Co, Optimal (0.1 mg/1) IAA, and in Optimal IAA plus 3.10^{-5}M Co

One experiment was performed to study the growth curves in the first 48 hours. The results of this experiment are given in Table 17 in the Appendix. Figure 19 illustrates the results graphically.

An initial growth phase during which linear growth occurred at a more or less steady rate was shown to take place in the first 8 hours in every treatment. The second phase of growth, during which there was a fall in rate of growth, leading to the completion

Figure 19. The growth in length of etiolated pea epicotyl sections in TCPAA, TCPAA plus Co, IAA, IAA plus Co in 48 hours. TCPAA, IAA and Co are all in optimum concentrations of 0.3 mg/l, 0.1 mg/l, 3.10^{-5}M respectively.


GROWTH IN mm
3
4
5
6
7
8
9
10
8
12
14
16
23
35
48
TIME (hrs.)
TCPAA +Co
TCPAA
IAA+Co
IAA

of growth, occurred during the 8th to 16th hour period in every case. Growth was almost complete at the 16th hour of incubation in the different test media. The irregularities in the curves for IAA, IAA plus Co, TCPAA, and TCPAA plus Co were probably caused by a fall in temperature due to breakdown of temperature control which occurred about the 12th hour onwards during this experiment. However, the curves clearly show that growth at the 23rd hour in every case was about the same as the growth attained at the 48th hour of incubation in test medium. This indicates that at the 23rd hour growth was more or less complete in the etiolated pea epicotyl sections.

Figure 19 shows that the principal stimulatory effect of Co added to either IAA or TCPAA occurred after the 8th hour and was complete by about the 16th hour with IAA, and probably after the 23rd hour with TCPAA.

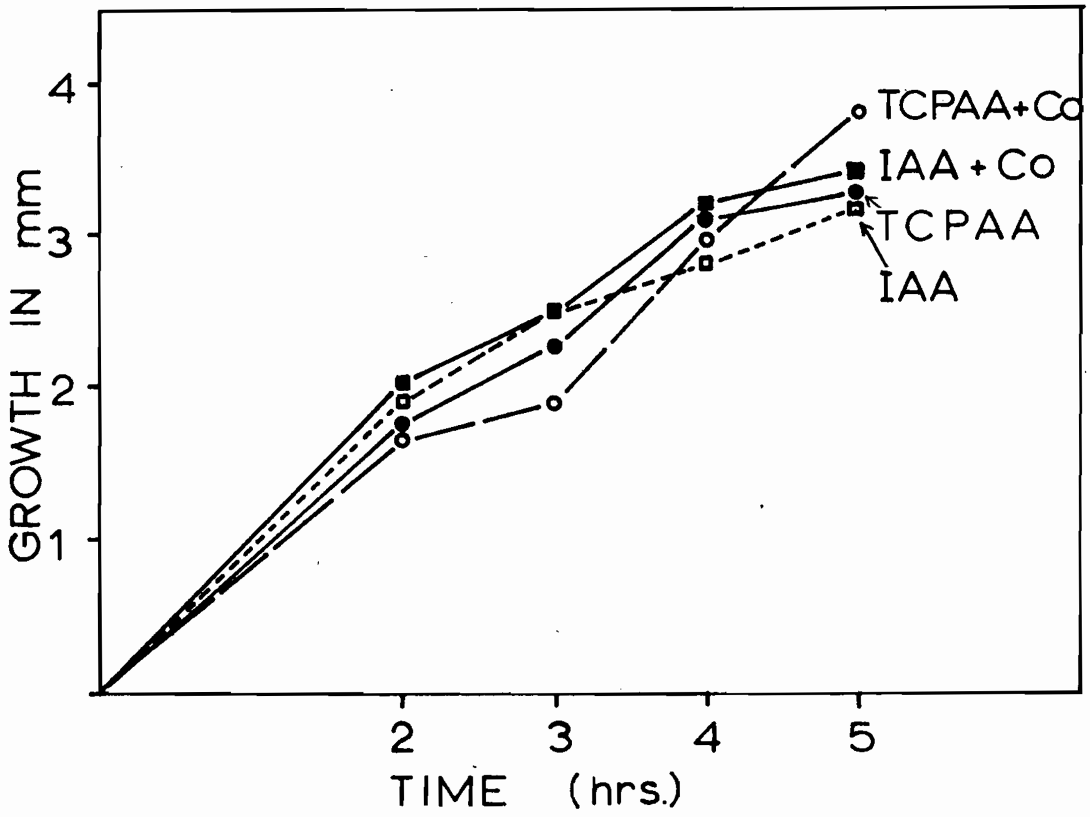
Figure 19 also shows that the greater final growth in TCPAA, as compared with that in IAA, is mainly a consequence of differences arising after the 8th hour.

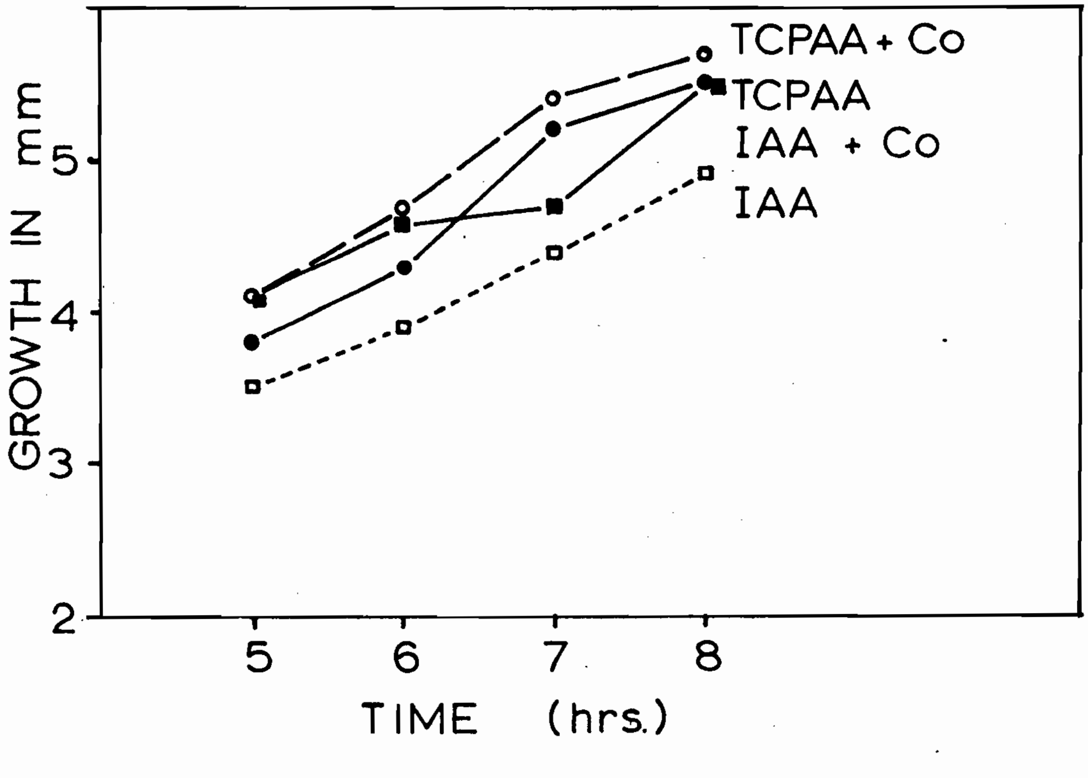
Three other experiments were performed to study the onset of Co effect in the initial steady growth phase during the first 8 hours. The results of these experiments are recorded in Table 18 in the Appendix. The results of Experiments 3 and 4 in this study best represent the data obtained (Figures 20 and 21).

The growth in optimal IAA was higher than that in the optimal TCPAA in the initial 3 hours, but this situation was reversed about the 4th hour onwards. Likewise the growth in TCPAA plus Co overtook the growth in IAA plus Co about the 5th hour. The first manifestation of an effect of Co in the presence of TCPAA occurred

Figure 20. The growth in length of etiolated pea epicotyl sections in optimal (0.3 mg/1 TCPAA, optimal TCPAA plus Co (3.10^{-5}M), optimal (0.1 mg/1) IAA, Optimal IAA plus Co (3.10^{-5}M) in the first four hours. Growth is the average of 10 sections.

Figure 21. The growth in length of etiolated pea epicotyl sections in optimal (0.3 mg/1) TCPAA, optimal TCPAA plus Co (3.10^{-5}M), optimal (0.1 mg/1) IAA, optimal IAA plus Co (3.10^{-5}M) from the 5th to the 8th hour. Growth is the average of 8 and 9 sections.


GROWTH IN mm
TIME (hrs.)
1
2
3
4
2
3
4
5
TCPAA+Co
IAA + Co
TCPAA
IAA


GROWTH IN mm
TIME (hrs.)
2
3
4
5
5
6
7
8
TCPAA + Co
TCPAA
IAA + Co
IAA

at about the 5th hour. The first manifestation of an effect of Co in the presence of IAA occurred at about the 4th hour. It must be mentioned that Experiment 2 of Study 9 (Table 18) indicated that the Co prevented the fall off in growth from the 5th hour onwards. This is seen from a comparison of the curve for Co plus IAA with that for IAA only.

J. Study 10. The Growth Curves of Etiolated Pea Epicotyl Sections in Optimal (0.3 mg/l) TCPAA, Supraoptimal (1.0 mg/l) TCPAA, Optimal (0.1 mg/l) IAA, Supraoptimal (1.0 mg/l) IAA

The results of the experiment are given in Table 19 in the Appendix, and illustrated graphically in Figure 22. The growth in the optimal TCPAA, supraoptimal TCPAA, optimal and supraoptimal IAA concentrations was approximately the same in the initial 5 hours, but at the 5th hour, fall off in growth commenced in the supraoptimal TCPAA and the supraoptimal IAA. In contrast, decrease in growth rate started at about the 8th hour in optimal IAA and optimal TCPAA. It appears that the lower final growth in supraoptimal IAA than in optimal IAA, and in supraoptimal TCPAA than in optimal TCPAA is, in both cases, a consequence of both an earlier fall off in growth rate and a faster decrease in growth rate during the period of decreasing growth rate.

K. Study 11. The Growth Curves of Etiolated Pea Epicotyl Sections in Optimal (0.3 mg/l) TCPAA, Supraoptimal (1.0 mg/l) TCPAA, and Supraoptimal (1.0 mg/l) TCPAA plus 3.10^{-5}M Co; Optimal (0.1 mg/l) IAA, Supraoptimal (1.0 mg/l) IAA, and Supraoptimal (1.0 mg/l) IAA plus 3.10^{-5}M Co

The results are given in Table 20 and illustrated graphically in Figure 23. They were in agreement with the previous results

Figure 22. The growth of etiolated pea epicotyl sections in optimal (0.3 mg/l) TCPAA, supraoptimal TCPAA, optimal (0.1 mg/l) IAA, supraoptimal (1.0 mg/l) IAA in 30 hours. Growth is the average of 10 sections.

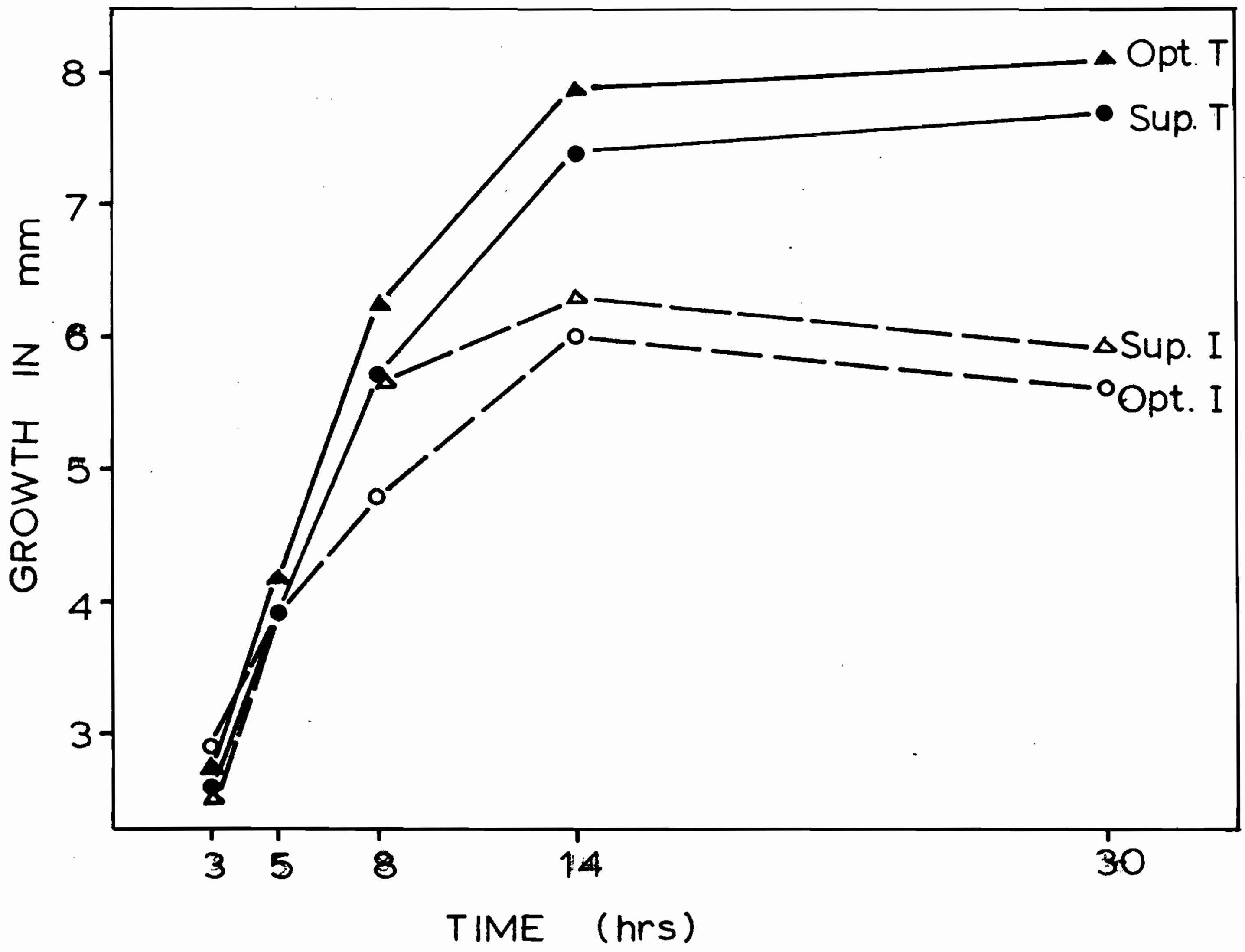
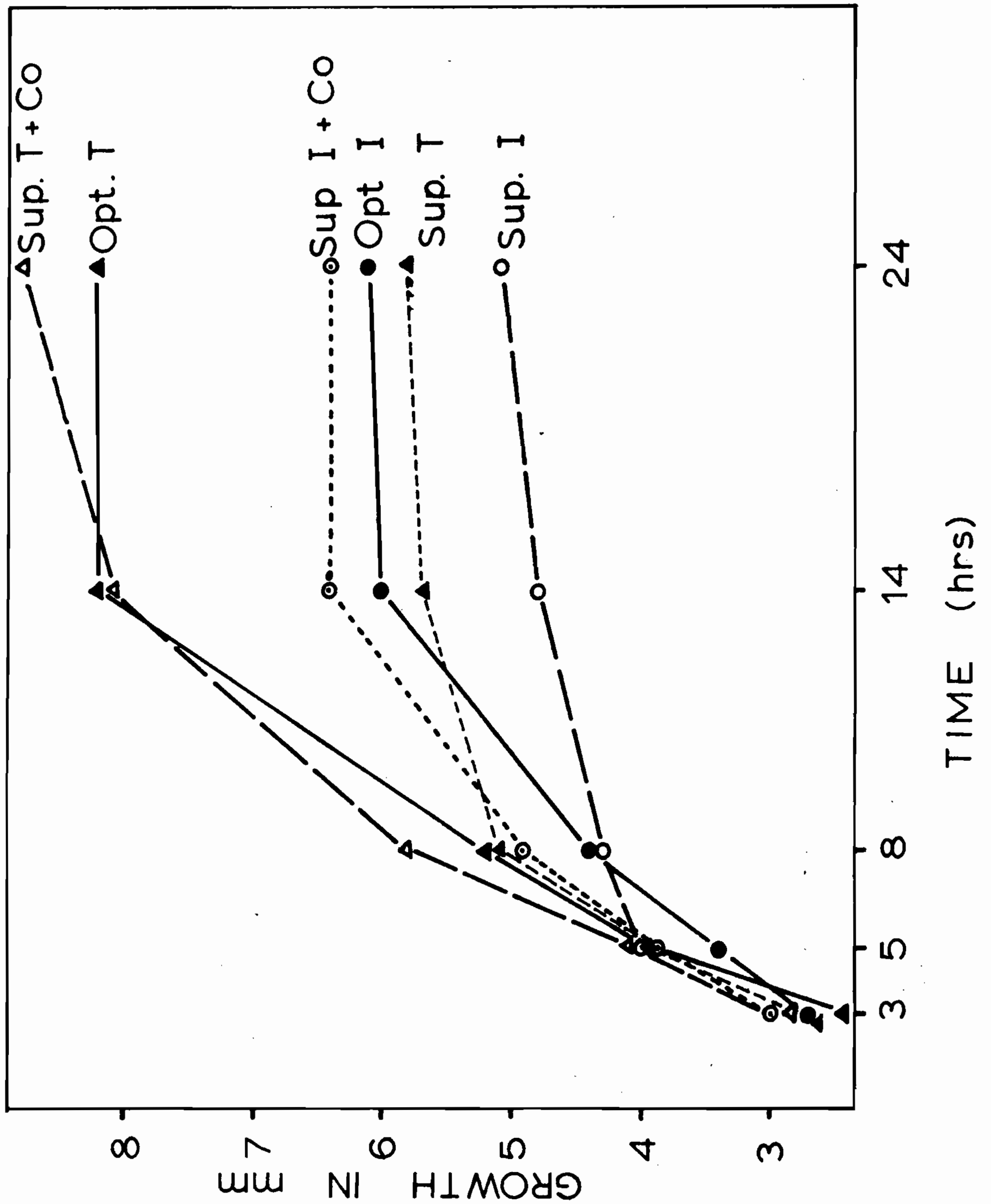

Opt. T
Sup. T
Sup. I
Opt. I
GROWTH IN mm
8
7
6
5
4
3
3
5
8
14
30
TIME (hrs)

Figure 23. The growth in length of etiolated pea epicotyl sections in optimal (0.3 mg/1) TCPAA, supraoptimal (1.0 mg/1) TCPAA, supraoptimal TCPAA plus Co (3.10^{-5}M), optimal (0.1 mg/1) IAA, supraoptimal (1.0 mg/1) IAA, supraoptimal IAA plus Co (3.10^{-5}M) in 24 hours. Growth is the average of 10 sections.



GROWTH IN mm
3
4
5
6
7
8
TIME (hrs)
3
5
8
14
24
Sup. T + Co
Opt. T
Sup I + Co
Opt I
Sup. T
Sup. I

obtained (Figure 22) except for the growth curve in optimal IAA. The latter gave lower growth than the optimal and supraoptimal TCPAA, and the optimal IAA in the initial 5 hours. This was probably an experimental error because no fall off in growth rate prior to the 5th hour is shown in Figure 22 nor with optimal (0.1 mg/l) IAA in a subsequent experiment (Figure 25).

Co in the supraoptimal TCPAA and supraoptimal IAA prevented fall off in growth rate at the 5th hour, which occurred in the supraoptimal TCPAA or supraoptimal IAA alone. Co, by raising the growth in the supraoptimal TCPAA and the supraoptimal IAA at the two phases, i.e. the initial steady growth in the first 8 hours, and the phase from 8 hours onwards where decrease in growth rate occurred, promoted the final growth to a level above that in optimal TCPAA and optimal IAA alone.

L. Study 12. The Growth Curves in Suboptimal (0.1 mg/l) TCPAA plus 3.10^{-5}M Co; Suboptimal TCPAA plus Suboptimal (0.01 mg/l) IAA plus 3.10^{-5}M Co; Suboptimal TCPAA plus Optimal (0.1 mg/l) IAA plus 3.10^{-5}M Co; and Optimal (0.3 mg/l) TCPAA plus 3.10^{-5}M Co

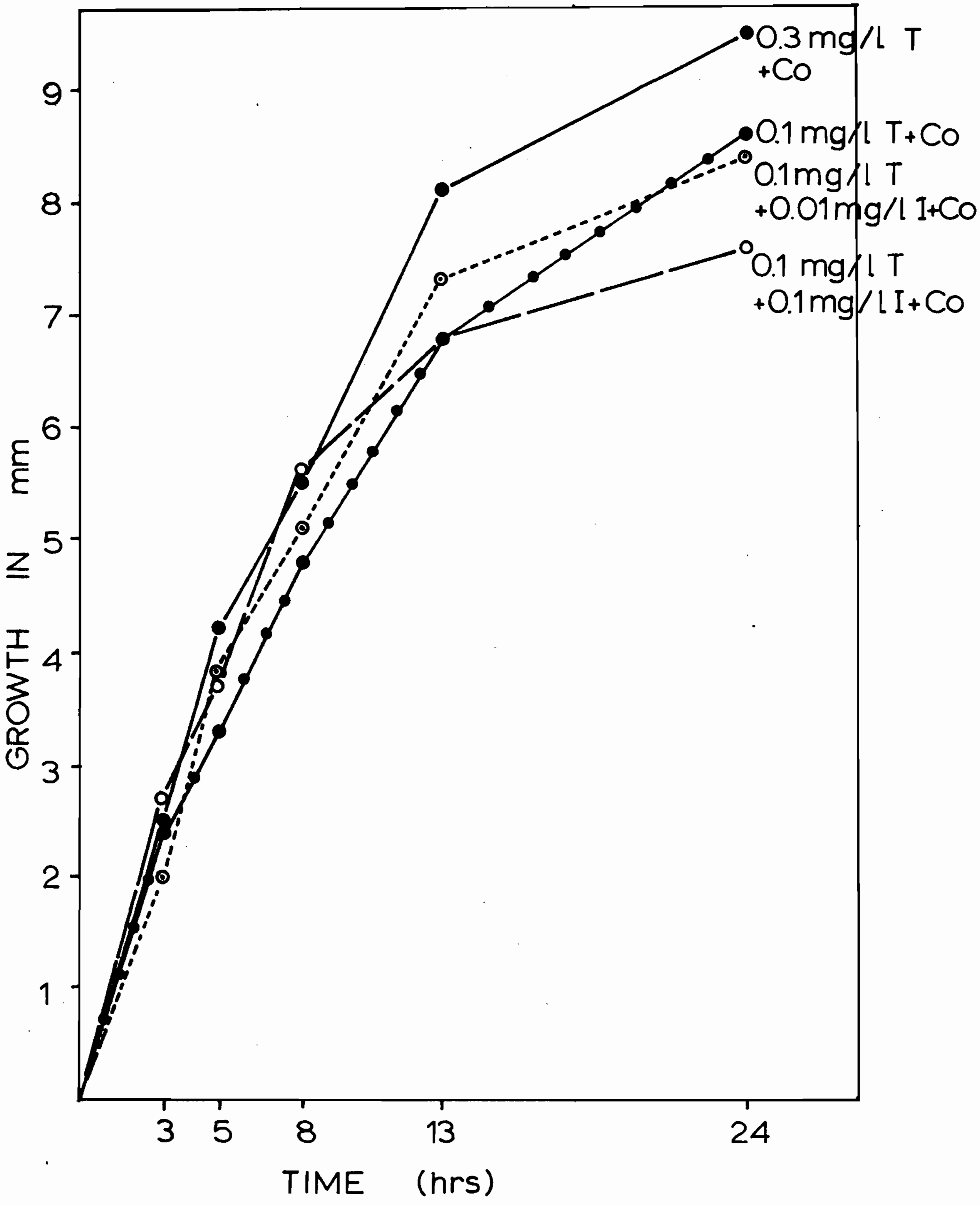
The results of the experiment are given in Table 21 in the Appendix and presented graphically in Figure 24.

In the initial 5 hours growth in the different test media was about the same. From the 5th hour onwards there was fall off in growth in the suboptimal TCPAA plus Co, when compared with the growth in the optimal TCPAA plus Co.

The suboptimal IAA, in the presence of suboptimal TCPAA plus Co, raised the growth in the initial steady phase of growth to a higher growth level than that attained in the suboptimal TCPAA

Figure 24. The growth in length of etiolated pea epicotyl sections in suboptimal TCPAA plus Co, suboptimal TCPAA plus suboptimal IAA plus Co, suboptimal TCPAA plus optimal IAA plus Co, and optimal TCPAA plus Co.

Suboptimal TCPAA = 0.1 mg/l, optimal TCPAA = 0.3 mg/l, suboptimal IAA = 0.01 mg/l, optimal IAA = 0.1 mg/l. Co concentration used = 3.10^{-5} M.


GROWTH IN mm
1
2
3
4
5
6
7
8
9
3
5
8
13
24
TIME (hrs)
0.3 mg/l T +Co
0.1 mg/l T+Co
0.1mg/l T +0.01mg/l I+Co
0.1 mg/l T +0.1mg/l I+Co

plus Co. In other words, the suboptimal IAA could replace the TCPAA in the initial steady growth phase.

The results at the 24th hour are not consistent with those obtained in earlier experiments (see Study 8, Figure 16 on Page 31b) in that suboptimal IAA in the presence of suboptimal TCPAA and Co did not promote growth at the 24th hour above that in suboptimal TCPAA plus Co. This throws some doubt upon the experiment as a whole, although if the assumed experimental error is confined to the final stages of growth, it means that suboptimal IAA replaces TCPAA in the second growth phase.

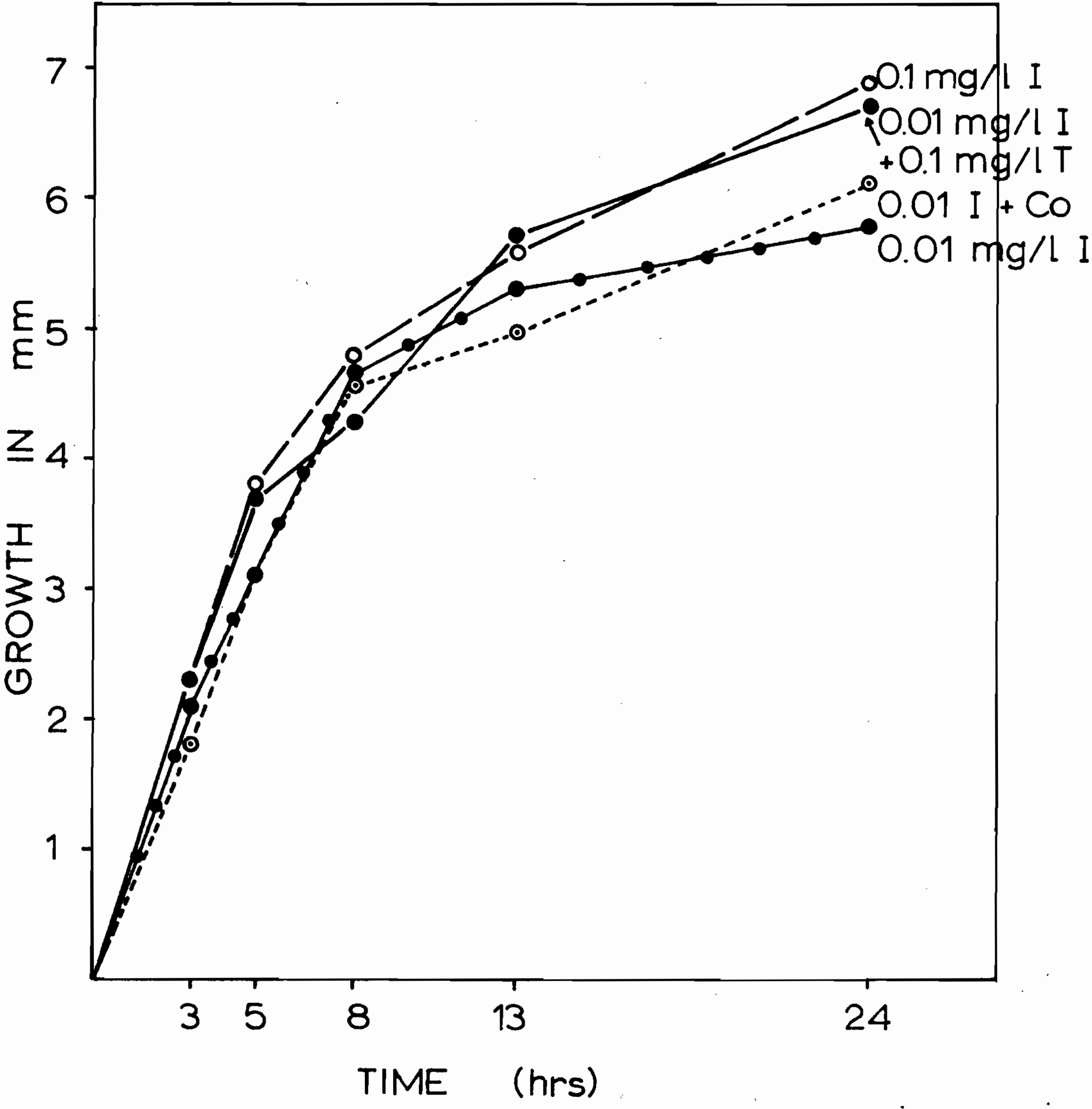
The optimal IAA, in the presence of suboptimal TCPAA plus Co, gave growth inhibition by the 24th hour to a level much below the growth given by the suboptimal TCPAA plus Co. This was not in agreement with previous data (see Study 8, Figure 15 on Page 36a) and it is felt that the results for the latter part of the growth are invalid. However the results do show that IAA replaced TCPAA at least in a big part of the initial growth phase.

M. Study 13. The Growth Curves in Suboptimal (0.01 mg/1) IAA; Optimal (0.1 mg/1) IAA; Suboptimal IAA plus 3.10^{-5}M Co; Suboptimal IAA plus Suboptimal (0.1 mg/1) TCPAA minus Co

The results of the two experiments performed are given in Table 22. Figure 25 shows the representative set of results of Experiment 2.

Suboptimal TCPAA in the presence of suboptimal IAA promoted growth above that in the suboptimal IAA control at all intervals except one. The results indicate, therefore, that the suboptimal TCPAA could replace IAA in the two phases of growth in promoting

Figure 25. The growth in length of etiolated pea epicotyl sections in suboptimal IAA, optimal IAA, suboptimal IAA plus Co, suboptimal IAA plus suboptimal TCPAA.
Suboptimal IAA = 0.01 mg/1, optimal IAA = 0.1 mg/1, Co concentration = 3.10^{-5}M, suboptimal TCPAA = 0.1 mg/1.


7
6
5
4
3
2
1
GROWTH IN mm
3
5
8
13
24
TIME (hrs)
0.1 mg/l I
0.01 mg/l I
+0.1 mg/l T
0.01 I + Co
0.01 mg/l I

growth to the level obtained in the optimal IAA.

The lower growth in the suboptimal IAA plus suboptimal TCPAA as compared with the growth in the suboptimal IAA control, at the 8th hour, was probably an experimental error because the depressed growth at the 8th hour is not consistent with the growth curve in suboptimal IAA and suboptimal TCPAA.

The addition of Co to suboptimal IAA did not cause any clear increase in growth at any stage. This is consistent with the results of two other experiments in which Co was added to suboptimal IAA and growth was measured at about 24 hours (see Study 6, Figure 12 on Page 31b). The Co did not stimulate growth.

IV. DISCUSSION

There have been many articles and reviews on the effects of auxin in plants and in the plant cell, and the mode of action of auxin in the regulation of growth (Leopold, 1955; Muir and Hansch, 1955; Åberg, 1957a; Bentley, 1958; Audus, 1959; Galston and Purves, 1960; Fawcett, 1961; Thimann, 1963). Despite all the studies on growth regulators in relation to growth, no definite conclusions have yet been reached as to their exact mode of action.

The most obvious physiological process involved in growth or elongation of the cell is the increase in area and volume of the cell wall, i.e. the expansion of the cell wall and the deposition of new wall material. This has given rise to the idea that the mechanism for the control of cell growth will be found in or contiguous with the wall in those processes which lead to the plasticization of the existing wall material and later to the formation of substances which lead to rigidity of the wall. The question then arises as to the role of auxin or growth regulators in both the plasticization process and the development of rigidity.

The discussion here will centre around the plasticization and rigidification processes of the wall in growth, and also the question of the attachment of auxin to a receptor following which growth is initiated.

The data presented here show that the curve of growth in length of etiolated pea epicotyl sections grown in an optimal concentration of IAA consisted mainly of two phases. In the first phase the curve of growth is more or less linear up to about the 8th hour of incubation. In other words, growth rate decreases only slightly up to about the 8th hour of incubation. In the second phase of growth, from about the 8th hour onwards, there is fall in rate of growth leading to completion of growth by about the 24th hour. It should be pointed out that different workers have reported different forms of this growth curve.

Bonner and Foster (1955) reported the growth rate of Avena coleoptile sections in the presence of IAA to be constant with time over a wide range of time intervals (up to 18 hours) and IAA concentrations.

Bennet-Clark and Kefford (1954) found that the curve of growth of Avena coleoptile sections grown in 0.1 mg/1 IAA was linear up to about 15 hours after which growth rate declined.

Osborne (1958) however has shown that in etiolated sections of pea internode, the low concentrations of IAA of 0.01 to 3.0 p.p.m. gave an initial steady growth, then a fall off in growth after 6 to 10 hours of incubation.

Van Overbeek (1956) has demonstrated a steady initial growth of maize coleoptile sections in 0.05 p.p.m. IAA and that the increase in the IAA concentration to 5 p.p.m. at the 5th hour increased the initial steady rate until the 8th hour.

Using Avena coleoptile sections incubated either in IAA or in IAA plus Co, Thimann and Takahashi (1961) obtained two phases of growth, i.e. an initial steady phase followed by a phase of declining growth rate.

The results obtained here are therefore more or less in agreement with those of Osborne (1958), Van Overbeek (1956) and Thimann and Takahashi (1961).

One of the theories of the mode of auxin action postulates that the auxin reacts with an enzyme receptor of protein nature. The reaction initiates a process which leads to growth. A large part of the evidence for this view comes from the many studies on the relation between structure and activity of plant growth regulators.

Some evidence that a part of the molecule of an auxin may react with the sulphydryl group of an enzyme are briefly summarised as follows. Wildman and Gordon (1942) reported the isolation of an auxin-protein complex from the leaves of spinach. Siegel and Galston (1953) and Galston (1956) reported the coupling of IAA to protein in vivo in excised pea roots. Pilet (1957) found that in the Lens root high sulphydryl content was correlated with high auxin and low IAA oxidase contents. Price and Leopold (1956) observed that the sulphydryl content in old pea epicotyl sections was correlated with a proportional inhibition of the growth rate. Galston and Kaur (1959 a,b) found some evidence that auxin affects the protein component of the non-particulate phase of the cytoplasm, and that it decreases the heat coagubility of this protein component, which may indicate the binding of the auxin to the protein molecules. Substances known to react with sulphydryl groups, e.g. iodoacetate, arsenite, maleimides, etc. are known to inhibit growth (Commoner and Thimann, 1941; Thimann and Bonner, 1948; 1949; Van Overbeek et al, 1955), although it is of course possible that the effects of these substances is because they complex with the sulphydryl groups of the metabolic process other than those of the growth process. Finally the study of structure in relation to growth- regulating activity of auxins also indicates interaction between the auxin molecule and a sulphydryl group on the receptor molecule (Galston and Purves (1960).

The concentration response curve of IAA (Study 2) was more or less typical of such curves and showed a single optimum about 0.1 mg/1. Little is known about the reasons for the form of this dose-response curve and particularly of the reasons for the inhibition of growth by supraoptimal IAA. However, in this connection, attention should be drawn to the two-point attachment theory of auxin action (McRae and Bonner, 1952, 1953; Foster _et al_, 1952, 1955; Bonner and Foster, 1956). Within this theory is the view that auxin activity only results after the auxin is attached to a receptor site at two points, and that at supraoptimal concentrations there is interference between molecules such that auxin molecules are only attached at one point and hence auxin activity is not manifested. Hence there is an apparent inhibition by the supraoptimal auxin.

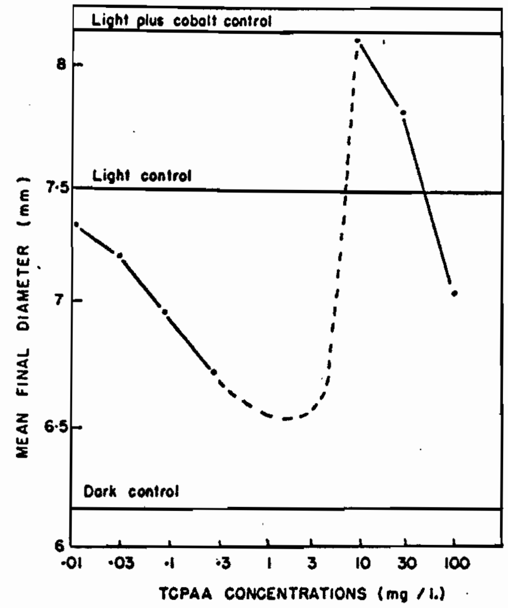
The only other explanation of the inhibitory effect of supraoptimal IAA known to the author is from a note by Bennet-Clark (1956) in which he mentions some of his own work, and from the work of Marinos (1957). In this the inhibition is attributed to injury by the high auxin concentration. The concentrations used by Bennet-Clark and Marinos were considerably higher than those used here and these conclusions are probably not applicable to the work done here.

With TCPAA the concentration-response curve (Study 3) was not of the same form as that for IAA. It showed an optimum at 0.3 mg/1. Above this concentration TCPAA was inhibitory but there was a subsidiary peak of activity at 30.0 mg/l. This two-peaked curve is reminiscent of that shown with etiolated bean leaf discs given a light treatment and grown in TCPAA (Lowther and Boll, 1960; see Figure 26). With these bean leaf discs, a strong inhibition of growth beginning at about 0.003 mg/l with maximum inhibition at about 1 mg/l TCPAA was followed by a high growth promotion with a peak at about 30 mg/l. Thus from these observations alone, it is apparent that TCPAA has a growth regulatory action involving processes other than those affected by IAA.

Explanations of the effects of Co on the IAA-induced growth of excised tissues are reviewed in the literature survey in this thesis. An important aspect of the work on this topic are those studies which show the effects of Co on the kinetics of auxin-induced growth.

In the work reported here it was found that the curve of growth of the sections in the presence of optimal IAA approached linearity up to the 8th hour after which growth rate steadily declined. This decline in rate was very marked after the 12th hour. The detailed study of linear growth up to the 5th hour showed that even during this phase there was a slight decrease in rate of growth with time. The effects of the addition of Co were clear. There was no obvious effect during the first three hours but by the 5th hour growth was clearly higher with Co. However the main effect of Co came later. Although because of technical difficulties it was not possible within the time available to obtain a perfect comparison of the growth curves in IAA only and IAA plus Co for the period from the 12th to the 24th hours of growth, it appears that the main difference in section length

Figure 26. The effect of TCPAA on the expansion of etiolated bean leaf discs. In concentrations 1.0 and 3.0 mg/1 the discs were too distorted for accurate measurement. (From Lowther and Boll, 1960).


Light plus cobalt control
Light control
Dark control
MEAN FINAL DIAMETER (mm)
8
7·5
7
6·5
6
·01
·03
·1
·3
1
3
10
30
100
TCPAA CONCENTRATIONS (mg / l.)

between the two treatments is established between about the 10th and 18th hours of growth. In other words, the increased growth due to Co which becomes manifested during the first 8 hours of growth is then exaggerated by the more prolonged growth in the presence of Co.

These results using pea sections are more or less in agreement with those of Thimann and Takahashi (1961) using *Avena* coleoptile sections and are not in agreement with those of Busse (1959) who also used *Avena* sections but who recorded that the main stimulation due to Co was because it permitted growth to continue for many hours after growth in IAA had stopped.

The results with the addition of Co to TCPAA are similar to those using IAA. Co clearly increased the growth by the 8th hour and counteracted the fall off in growth which occurred in TCPAA alone. The results as a whole showed that Co produced a greater stimulation of growth with TCPAA than with IAA.

It is apparent that the curve of growth of the epicotyl section treated with growth regulator is the integration of two curves. One curve is that representing the process of cell elongation which is presumably caused by the plasticization of the wall, and the second is that curve which represents the onset and gradual preponderance of the rigidification process which leads to the slowing down and stoppage of cell growth. The lack of absolute linearity in the growth curve even before the 5th hour indicates that the process of rigidification has started even before this time. The interpretation of the effects of Co or growth regulators must consider the effects on both the processes of plasticization and rigidification and not on a single hypothetical growth process. Further discussion will be based on these considerations.

The growth-promoting effect of the addition of Co to optimal IAA or TCPAA is thus interpreted to mean that Co inhibits the rigidification process in cell growth. This is in agreement with Busse's (1959) interpretation of the effects of Co on the growth of *Avena* sections. However the implications of the results obtained here go beyond this simple statement.

The comparison of either the growth curves in supraoptimal IAA with that from optimal IAA, or the growth curves from supraoptimal TCPAA with that from optimal TCPAA, showed clearly the reason for the inhibition of growth by the supraoptimal concentration of growth regulator. The supraoptimal concentration causes an earlier and a faster slowing down in the rate of growth. In other words, supraoptimal growth regulator speeds up the onset of the rigidification process and has little if any effect upon the plasticization process. An important question which now arises is whether the effect of growth regulator on the two processes of plasticization and rigidification is because of an action of the regulator at one receptor site or whether the regulator affects two distinct systems. It is argued below that the growth regulator affects the two processes separately, even if simultaneously, and therefore that two receptor sites for growth regulator action may be involved in the overall control of cell growth.

Some of the most significant observations to this discussion are those from the experiments in which Co concentration was varied at different concentrations of growth regulator. Addition of Co to optimal IAA or TCPAA increased growth as measured at 24 hours. However this is not the case when Co was added to suboptimal IAA and the addition of Co to suboptimal TCPAA gave only a slight and variable increase in growth. The addition of Co in the absence of exogenous growth regulator did not consistently promote growth and in any case, such promotion was slight. A comparison of the growth curves obtained with suboptimal IAA only, and suboptimal IAA plus optimal Co, showed that Co did not significantly affect the growth at any time during the growth period. In contrast Co completely reversed the inhibition caused by supraoptimal IAA or TCPAA. In fact in Study 6 it was shown quite clearly that the growth in supraoptimal IAA plus Co greatly exceeded the growth obtained with optimal IAA plus Co. This was not consistently the case with supraoptimal TCPAA but it occurred in two out of five experiments, and in the others growth in supraoptimal TCPAA plus Co was at least equal to that in optimal TCPAA plus Co.

These facts, together with the view that the inhibitory effect of supraoptimal growth regulator is because it speeds up the onset of the rigidification process, are interpreted as indicating that the effects of Co on cell growth is that it counteracts the promotion of the rigidification process by the growth regulator, and that Co does not promote the effect of the growth regulator on the plasticization process. These interpretations mean that when sections are grown in optimal IAA there is a dual effect of the IAA. On the one hand it is promoting growth by an effect on the plasticization process; on the other hand it is inhibiting growth by an effect on the rigidification process. The addition of Co counteracts the promotion of the rigidification process by IAA, leading to increased final growth and at the same time, it permits the addition and the action of a much higher level of exogenous IAA.

An important conclusion from these interpretations is that the inhibitory effect of supraoptimal IAA is not a consequence of molecular interference at a two-point receptor site as postulated by Bonner and Foster (1956), but is a consequence of action in two separate processes. In other words, the present interpretations argue against the use of supraoptimal inhibition to support the idea of two-point attachment. At the same time the interpretations argue in favour of two separate sites of growth regulator action. One of these sites mediates in the plasticization process and is not sensitive to Co, whereas the other mediates in the rigidification process and is sensitive to Co.

The general results of the experiments in which concentration of IAA and TCPAA was varied simultaneously with or without simultaneous variation in the concentration of Co are summarised in Table B. The interpretation of these results in terms of the hypothesis of growth regulator and Co action given above is not easy because of the many components in such multiple interactions. If it is correct that the growth regulator acts separately on the two processes of plasticization and rigidification and that Co only counteracts the stimulatory effect of the growth regulator on the rigidification process, then the results of adding two growth regulators simultaneously and in the presence of cobalt will be dependent upon differences between the growth regulators in their affinity for the separate receptor sites, possible differences in

IAA Cocn.	TCPAA Cocn.	-Co	+Co
Suboptimal	Suboptimal	Synergistic	Synergistic at low and high Co.
Optimal	Suboptimal	- same as TCPAA alone. - slightly below IAA alone.	Low Co. - synergistic. High Co - equal to IAA control, much higher than TCPAA control.
Suboptimal	Optimal	- higher than or same as TCPAA alone. - much higher than IAA alone.	Low Co - equal to TCPAA control, much higher than IAA control. High Co - equal to TCPAA control, much higher than IAA control.
Optimal	Optimal	- inconsistent, lower than or slightly higher than TCPAA alone. - inconsistent, lower than or much higher than IAA alone.	Low Co - lower or equal to TCPAA control, higher than IAA control. High Co - equal to TCPAA control, higher than IAA control.
Supraoptimal	Suboptimal	- much lower than TCPAA alone. - slightly higher than IAA alone.	Low Co - higher than IAA control, lower than TCPAA control. High Co - higher than IAA control, much higher than TCPAA control.

Table B. The effects of simultaneous variation of IAA and TCPAA in the presence or absence of Co. (From Studies 7 and 8).

(Continued on next page)

IAA Cocn.	TCPAA Cocn.	-Co	+Co
Suboptimal	Supraoptimal	- same as TCPAA alone. - greater than IAA alone.	Low Co - inconsistent, equal to or higher than TCPAA control, much higher than IAA control. High Co - slightly lower than TCPAA control, much higher than IAA control.
Optimal	Supraoptimal	- same as TCPAA alone. - lower than IAA alone.	Low Co - lower than TCPAA control, slightly higher than IAA control. High Co - slightly higher than TCPAA control, much higher than IAA control.
Supraoptimal	Optimal	- much lower than TCPAA alone. - about equal or slightly higher than IAA alone.	Low Co - same as in -Co High Co - same as in low Co.
Supraoptimal	Supraoptimal	- same as above, i.e. as in supraoptimal IAA and optimal TCPAA.	Low Co - much lower than TCPAA control, about equal to IAA control. High Co - about equal to TCPAA control, higher than IAA control.

Table B. (Continued)

their effectiveness when bound to the sites, possible differences in rates of movement to the sites, and rates of destruction, all of which, in turn, will be related to concentration of the regulators. Furthermore it is possible that the growth regulators may have chelating properties which interfere with the action of Co in the system. Conversely the presence of the metal ion might interfere in part with the action of the growth regulator with which it chelates. Thus at this point a detailed interpretation of all the results of the experiments of interaction of IAA, TCPAA and Co is of little value. However it is felt that none of the results are inconsistent with the hypothesis of growth regulator and Co action presented here.

It should be pointed out that if the hypothesis regarding the effect of Co in the rigidification process and the existence of a receptor site for this process is correct, and further if the published views relating the action of cobalt to sulphydryl are correct, then it follows that this sulphydryl is involved in the receptor site for the rigidification process.

A comparison of the effects of IAA and TCPAA gives some indication of a difference between the growth regulatory actions of these two substances. The first difference was mentioned above and is that the concentration-response curve of TCPAA is two-peaked, whereas that of IAA shows a single optimum. Further, the growth after 24 hours caused by optimal TCPAA is consistently greater than that caused by optimal IAA. This is true both in the absence and presence of cobalt. An examination of the growth curves shows that this difference is manifested by the 8th hour and that growth in TCPAA continues for a somewhat longer time than is the case in IAA. The synergism between suboptimal IAA and suboptimal TCPAA (Study 13) is manifested as an increase in growth rate during the first phase of growth. In other words, TCPAA appears to be less potent than IAA in promoting the rigidification process in cell growth. The results of the experiments (Figures 14 and 17) in which Co concentration was varied in supraoptimal TCPAA or in supraoptimal IAA show that a lower concentration of Co is required to relieve inhibition by supraoptimal TCPAA than is required to relieve inhibition of supraoptimal IAA. If it is correct that Co prevents the action of the growth regulator in promoting the rigidification process, these results are consistent with the view that TCPAA is less potent than IAA in promoting rigidification.

The paths in which research could be profitably pursued may be listed as follows: 1) IAA and plasticization; 2) the influence of IAA on the synthesis of wall materials, and its influence on the rigidification process; 3) the effects of Co in preventing the rigidification process.

In the study of IAA and plasticization the effect of IAA in inducing the plasticization process must be checked. Biochemical studies on how IAA induces the plasticization process will prove interesting. An important point to be investigated is the actual relation between the plasticization and cell elongation with respect to time.

In the study of the influence of IAA on the synthesis of wall material, and the effects of Co in preventing rigidification, the use of tracers, refined methods of fractionation to give minimum contamination or artefact formation in the separation of wall components, and effective qualitative and quantitative measurements, will prove worthwhile. Results from these experiments will provide some clues as to whether IAA regulates the rigidification process, and whether Co counteracts the rigidification process induced by the auxin.

V. SUMMARY

1. The effects of Co, IAA or TCPAA in the growth of etiolated pea epicotyl sections were studied mainly in connection to their roles in growth regulation in those processes which lead to the growth of the cell wall and the stoppage of growth.

2. A review of the literature pertaining to this work was completed in April, 1964. The general contents of this review involve the position of Co with respect to animals, lower plants, higher plants and other known properties of Co; growth-regulating activities of 2,4,5-TCPAA and its mechanism of action from studies of structure in relation to activity of the substituted phenoxyacetic acids; IAA action and growth of the cell wall.

3. Studies which dealt with the concentration-response curves of either Co or IAA or TCPAA revealed an optimum of about 3.10^{-5}M for Co, an optimum of about 0.1 mg/l for IAA, and an optimum of about 0.3 mg/l for TCPAA, but with a subsidiary peak of activity at about 30.0 mg/l.

4. Experiments on the effects on growth of the simultaneous variations of either Co with TCPAA, or Co with IAA, or IAA with TCPAA gave the following results.

Co in the presence of either the optimal TCPAA or optimal IAA showed synergistic effects. However Co in the presence of either the supraoptimal TCPAA or supraoptimal IAA concentrations completely reversed the inhibitions by these supraoptimal concentrations of growth regulators. Co, in the presence of the suboptimal (0.01 mg/l) IAA does not promote growth, and in the presence of the suboptimal (0.1 mg/l) TCPAA gave only slight and variable growth.

Simultaneous variation of IAA and TCPAA concentrations in the absence of Co showed that IAA and TCPAA were synergistic only when both were supplied in suboptimal concentrations.

5. The effects of variation in Co concentration in the presence of different TCPAA concentrations (suboptimal or optimal or supraoptimal) with or without different IAA concentrations (suboptimal or optimal or supraoptimal) were also studied.

6. The study of the growth curves in optimal IAA, optimal TCPAA, and each plus Co showed that the growth curve in the IAA control medium was more or less linear up to about the 8th hour after which growth decreased gradually until about the 24th hour when growth was completed. There is slight decrease in rate of growth with time even within the first 5 hours. The growth curve in optimal TCPAA is similar except that the fall off in growth rate is more gradual than in IAA.

The effect of Co in optimal IAA or TCPAA becomes apparent at about the 5th hour and becomes exaggerated with time. Co promoted more growth in the optimal TCPAA than in the optimal IAA.

7. Experiments which dealt with the growth curves in optimal IAA, optimal TCPAA, supraoptimal IAA and supraoptimal TCPAA, and those which dealt with the effects of Co at these concentrations of growth regulators revealed that the inhibition of growth at the supraoptimal IAA or TCPAA concentration was due to an earlier and faster fall off in growth rate, and that the effect of Co was to counteract this effect.

8. The study of the growth curves in suboptimal (0.1 mg/l)

TCPAA plus Co, optimal (0.3 mg/l) TCPAA plus Co, suboptimal (0.1 mg/l) TCPAA plus suboptimal (0.01 mg/l) IAA plus Co, suboptimal (0.1 mg/l) TCPAA plus optimal (0.1 mg/l) IAA plus Co, and a comparison of the growth curves in the suboptimal TCPAA plus suboptimal IAA plus Co with those in the suboptimal TCPAA plus Co, and the optimal TCPAA plus Co indicated that IAA is able to replace TCPAA at least in a great part of the initial growth.

9. The study of the growth curves in suboptimal (0.01 mg/l) IAA, optimal (0.1 mg/l) IAA, suboptimal (0.01 mg/l) IAA plus Co, and suboptimal (0.01 mg/l) IAA plus suboptimal (0.1 mg/l) TCPAA showed that Co does not act synergistically with the suboptimal IAA throughout the growth period, and that TCPAA is able to replace IAA throughout the growth period.

10. The results presented here are interpreted as showing that the use of the two-point attachment theory to explain the inhibition of growth by supraoptimal growth regulator is invalid.

The overall results are considered to support a hypothesis that an auxin type growth regulator affects both a plasticization and a rigidification process in cell growth via two separate receptor sites. It is postulated that the regulator promotes both plasticization and rigidification and that the stimulatory effect of Co on auxin-induced growth is because it counteracts the promotion of the rigidification process caused by the regulator.

Evidence is presented to show that IAA and TCPAA differ in growth regulatory action, and particularly that TCPAA is less potent than IAA in promoting the rigidification process in cell growth.

11. The question of how the present research may be continued profitably is discussed.

12. Statistical calculations of the standard errors of the mean final lengths of the etiolated pea epicotyl sections in the experiments are included in the Appendix.

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APPENDIX

The following are included in this Appendix:

(1) Tables showing the results of the experiments. These results are given in terms of the mean growth or average increase in length of the etiolated pea epicotyl sections.

(2) Tables showing the standard errors of the final lengths of the etiolated pea epicotyl sections in the experiments taken to be representative of the data. The figure in brackets, after the standard error, shows the number of replicates in the sample.

Expt. No.	Co Concentrations (M)						
	0	10^{-5}	2.10^{-5}	3.10^{-5}	$5{\cdot}5.10^{-5}$	10^{-4}	3.10^{-4}
1a	5.5	5.4	-	6.2	6.0	5.8	5.2
1b	4.2	4.0	-	4.7	4.0	4.0	3.3
2a	5.8	5.3	-	5.8	5.4	5.8	5.0
2b	4.1	3.2	-	4.5	3.8	4.2	3.9
3	5.5	5.3	6.4	6.0	6.8	5.6	5.9
4	5.5	5.8	6.1	5.7	5.9	4.9	5.1

Appendix Table 1. The effects of increasing Co concentrations on the growth (mm.) of etiolated pea epicotyl sections. Growth is the average of 10 sections. Experiments a and b were carried out at the same time.

Expt. No.	Co Concentrations (M)					
	0	10^{-5}	3.10^{-5}	$5.5.10^{-5}$	10^{-4}	3.10^{-4}
1a	15.5 ± 0.20 (10)	15.4 ± 0.30 (10)	16.2 ± 0.47 (10)	16.0 ± 0.37 (10)	15.8 ± 0.22 (10)	15.2 ± 0.25 (10)

Appendix Table 1a. Standard errors of the final lengths of the sections in Experiment 1a of Appendix Table 1.

Expt. No.	IAA Concentrations (mg/l) 0	0.01	0.03	0.055	0.1	1.0
1	5.7	7.2	7.3	7.4	7.2	5.2
2	4.8	5.3	6.4	5.4	4.9	5.0
3	4.3	5.0	5.6	5.5	5.7	3.7
4	3.3	5.1	5.9✡	5.7✡	5.8	4.8

Appendix Table 2. The effects of increasing IAA concentrations on the growth (mm.) of etiolated pea epicotyl sections. Growth is the average of 10 sections except for Experiment 3 where growth is the average of 9 sections from 0 - 0.1 mg/l IAA.

✡ Denotes the mean of two 10-section averages.

Expt. No.	IAA Concentrations (mg/1)					
	0	0.01	0.03	0.055	0.1	1.0
3	14.3 ± 0.22 (9)	15.0 ± 0.30 (9)	16.6 ± 0.30 (9)	15.5 ± 0.24 (9)	15.7 ± 0.07 (9)	13.7 ± 0.18 (10)

Appendix Table 2a. Standard errors of the final lengths of the sections in Experiment 3 of Appendix Table 2.

Expt. No.	2,4,5-TCPAA Concentrations (mg/l)										
	0	0.03	0.1	0.2	0.3	0.55	1.0	3.0	10.0	30.0	100.0
3	4.6	4.7	6.7	-	8.6	-	6.6	6.0	5.7	6.0	4.0
4	3.7	-	6.9	-	8.2	-	6.6	5.4	5.3	-	-
5	3.5	4.5	7.2	8.8	7.9	7.7	6.8	5.5	5.4	5.7	3.7

Appendix Table 3. The effects of increasing 2,4,5-TCPAA concentrations on the growth (mm.) of etiolated pea epicotyl sections. Growth is the average of 10 sections.

Expt. No.	*TCPAA Concentrations (mg/l)								
	0	0.03	0.1	0.3	1.0	3.0	10.0	30.0	100.0
1	5.2	6.2	6.7	9.1	7.5	5.7	5.2	5.4	4.2
2	3.8	4.4	6.0	7.9	6.8	6.4	5.8	-	3.6
4	3.7	-	6.9	7.9	6.0	5.8	5.2	-	-

Appendix Table 4. The effects of increasing TCPAA concentrations on the growth (mm.) of etiolated pea epicotyl sections. Growth is the average of 10 sections.

* TCPAA denotes the sample used by Lowther and Boll (1960).

Expt. No.	2,4,5-TCPAA Concentrations (mg/1)										
	0	0.03	0.1	0.2	0.3	0.55	1.0	3.0	10.0	30.0	100.0
5	13.5 ± 0.17 (9)	14.5 ± 0.22 (9)	17.2 ± 0.41 (10)	18.8 ± 0.42 (10)	17.9 ± 0.36 (10)	17.7 ± 0.24 (10)	16.8 ± 0.27 (10)	15.5 ± 0.22 (10)	15.4 ± 0.18 (10)	15.7 ± 0.26 (10)	13.7 ± 0.21 (10)

Appendix Table 3a. Standard errors of the final lengths of the sections in Experiment 5 of Appendix Table 3.

Expt.	2,4,6-TCPAA Concentrations (mg/l)										
No.	0	0.03	0.1	0.2	0.3	0.55	1.0	3.0	10.0	30.0	100.0
5	3.5	3.9	4.2	3.7	3.7	3.8	3.5	3.8	3.6	3.5	2.6
6	4.1	4.3	3.9	-	4.0	-	4.3	3.5	3.6	3.5	3.1

Appendix Table 5. The effects of increasing 2,4,6-TCPAA concentrations on the growth (mm.) of etiolated pea epicotyl sections. Growth is the average of 10 sections.

TCPAA (mg/1)	0					0.1				
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	4.8	4.4	4.8	4.3	7.5	4.2	5.5	5.8	4.9	7.2
" 2	3.8	3.7	4.4	3.9	3.9	4.5	5.6	5.3	6.1	4.3
" 3	6.0	3.6	4.3	3.3	3.5	8.1	4.6	4.4	4.4	3.7
" 4	3.5	3.5	3.0	3.5	3.4	4.7	6.0	5.5	5.3	4.3
" 5	3.8	3.7	3.3	3.9	3.2	5.5	6.0	5.5	5.9	4.7

TCPAA (mg/1)	0.3				
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	4.7	9.0	8.0	7.0	7.7
" 2	6.8	7.2	7.5	6.1	5.6
" 3	9.8	7.1	7.2	7.1	5.3
" 4	6.3	7.7	8.2	7.4	5.8
" 5	7.7	8.5	7.5	8.8	5.8

Appendix Table 6. The effects of Co concentrations in the presence of different concentrations of 2,4,5-TCPAA on the growth (mm.) of etiolated pea epicotyl sections. Growth is the average of 8, 9 and 10 sections.

(Continued on next page)

TCPAA (mg/1)	0					0.1				
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 5	13.8 ± 0.19 (10)	13.7 ± 0.22 (9)	13.3 ± 0.14 (9)	13.9 ± 0.20 (9)	13.2 ± 0.25 (9)	15.5 ± 0.53 (8)	16.0 ± 0.32 (8)	15.5 ± 0.29 (9)	15.9 ± 0.63 (9)	14.7 ± 0.18 (8)

TCPAA (mg/1)	0.3					1.0				
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 5	17.7 ± 0.39 (8)	18.5 ± 0.54 (8)	17.5 ± 0.31 (9)	18.8 ± 0.17 (9)	15.8 ± 0.39 (8)	15.9 ± 0.27 (8)	17.2 ± 0.33 (8)	17.9 ± 0.32 (9)	18.8 ± 0.42 (9)	16.6 ± 0.39 (9)

Appendix Table 6a. Standard errors of the final lengths of the sections in Experiment 5 of Appendix Table 6.

(Continued on next page)

TCPAA (mg/1)	3.0					10.0				
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 5	15.0 ± 0.27 (8)	16.0 ± 0.19 (8)	16.8 ± 0.28 (9)	17.7 ± 0.30 (9)	17.2 ± 0.52 (8)	15.2 ± 0.23 (8)	15.4 ± 0.19 (8)	16.5 ± 0.33 (9)	17.9 ± 0.26 (8)	16.7 ± 0.38 (8)

Appendix Table 6a. (Continued)

Expt. No.	IAA (mg/l)	Co (M)						
		0	10^{-5}	2.10^{-5}	3.10^{-5}	$5.5.10^{-4}$	10^{-4}	3.10^{-4}
1	0	5.1	4.7	5.0	4.4	4.6	4.9	4.4
	0.1	7.0	6.8	6.4	6.5	7.1	7.6	6.2
2	0	5.9	5.2	5.4	6.2	6.1	5.5	5.7
	0.1	7.4	8.1	7.7	8.0	8.3	7.8	7.0
3	0	5.0	4.9	4.8	5.3	5.3	4.9	5.2
	0.1	6.7	7.5	8.9	7.7	8.3	7.8	6.8

Appendix Table 7. The effects of Co concentrations in the presence or absence of optimum (0.1 mg/l) IAA on the growth (mm.) of etiolated pea epicotyl sections. Growth is mostly the average of 10 sections, and sometimes 9 sections.

Expt. No.	IAA (mg/1)	Co(M)						
		0	10^{-5}	2.10^{-5}	3.10^{-5}	$5.5.10^{-5}$	10^{-4}	3.10^{-4}
2	0	15.9 ± 0.21 (10)	15.2 ± 0.44 (10)	15.4 ± 0.29 (10)	16.2 ± 0.31 (10)	16.1 ± 0.14 (9)	15.5 ± 0.39 (10)	15.7 ± 0.41 (10)
	0.1	17.4 ± 0.12 (10)	18.1 ± 0.41 (10)	17.7 ± 0.36 (10)	18.0 ± 0.60 (9)	18.3 ± 0.66 (10)	17.8 ± 0.63 (9)	17.0 ± 0.26 (10)

Appendix Table 7a. Standard errors of the final lengths of sections in Experiment 2 of Appendix Table 7.

IAA (mg/l)	0					0.01	0.1	1.0	3.0	0.01			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	0				10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	4.2	4.7	4.6	4.5	3.4	5.0	8.0	5.5	-	4.4	4.7	4.3	4.3
" 2	4.2	3.5	4.0	3.5✡	3.7	4.2	6.8	6.1	5.6	4.0	3.8	4.2	3.8

IAA (mg/l)	0.1				1.0				3.0			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	8.4	8.7	8.6	5.4	7.3	8.0	8.8	6.8	-	-	-	-
" 2	7.1	7.4	6.9	5.2✡	-	8.3	9.3	7.4	6.8	8.2	8.7	7.1

Appendix Table 8. The effects of Co concentrations in the presence of suboptimal (0.01 mg/l), optimal (0.1 mg/l), and supraoptimal (1.0 mg/l, 3.0 mg/l) IAA concentrations on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 10 sections.

✡ Growth is the average of 9 sections.

IAA (mg/l)	0					0.01	0.1	1.0	3.0	0.01			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	0				10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 2	14.2 ± 0.13 (10)	13.5 ± 0.16 (10)	14.0 ± 0.17 (10)	13.5 ± 0.22 (9)	13.7 ± 0.18 (10)	14.2 ± 0.11 (10)	16.8 ± 0.36 (10)	16.1 ± 0.22 (10)	15.6 ± 0.18 (10)	14.0 ± 0.16 (10)	13.8 ± 0.18 (10)	14.2 ± 0.24 (10)	13.8 ± 0.17 (10)

IAA (mg/l)	0.1											
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 2	17.1 ± 0.36 (10)	17.4 ± 0.39 (10)	16.9 ± 0.32 (10)	15.2 ± 0.31 (9)	–	18.3 ± 0.42 (10)	19.3 ± 0.41 (10)	17.4 ± 0.22 (10)	16.8 ± 0.29 (10)	18.2 ± 0.31 (10)	18.7 ± 0.34 (10)	17.1 ± 0.33 (10)

Appendix Table 8a. Standard errors of the final lengths of sections in Experiment 2 of Appendix Table 8.

TCPAA (mg/l)	0					0.1					0.3				
IAA (mg/l)	0	0.01	0.03	0.1	1.0	0	0.01	0.03	0.1	1.0	0	0.01	0.03	0.1	1.0
Expt. 1	4.3	5.5	-	7.2	4.7	7.0	7.6	-	7.0	5.2	7.5	8.2	-	6.8	5.6
" 2	3.9	5.0	5.7	5.8	4.5	6.6	6.3	6.3	6.5	4.7	7.5	7.4	6.8	7.8	5.0

TCPAA (mg/l)	1.0					3.0					10.0				
IAA (mg/l)	0	0.01	0.03	0.1	1.0	0	0.01	0.03	0.1	1.0	0	0.01	0.03	0.1	1.0
Expt. 1	6.5	6.3	-	6.5	5.0	5.7	5.7	-	5.8	5.5	6.9	5.4	-	5.8	5.2
" 2	5.7	5.8	5.7	5.1	4.2	5.2	4.9	5.3	5.1	4.1	5.2	5.0	4.4	4.4	4.0

Appendix Table 9. The effects of concentration of IAA at various concentrations of TCPAA on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 8 sections.

TCPAA (mg/l)	0				0.1				0.3			
IAA (mg/l)	0	0.01	0.1	1.0	0	0.01	0.1	1.0	0	0.01	0.1	1.0
Expt. 1	14.3 ± 0.14 (8)	15.5 ± 0.23 (8)	17.2 ± 0.32 (8)	14.7 ± 0.18 (8)	17.0 ± 0.38 (8)	17.6 ± 0.32 (8)	17.0 ± 0.34 (8)	15.2 ± 0.23 (8)	17.5 ± 0.38 (8)	18.2 ± 0.28 (8)	16.8 ± 0.29 (8)	15.6 ± 0.17 (8)

TCPAA (mg/l)	1.0											
IAA (mg/l)	0	0.01	0.1	1.0	0	0.01	0.1	1.0	0	0.01	0.1	1.0
Expt. 1	16.5 ± 0.32 (8)	16.3 ± 0.34 (8)	16.5 ± 0.16 (8)	15.0 ± 0.42 (8)	15.7 ± 0.94 (8)	15.7 ± 0.73 (8)	15.8 ± 0.38 (8)	15.5 ± 0.19 (8)	16.9 ± 0.07 (7)	15.4 ± 0.29 (8)	15.8 ± 0.13 (8)	15.2 ± 0.51 (8)

Appendix Table 9a. Standard errors of the final lengths of sections in Experiment 1 of Appendix Table 9.

TCPAA Cocn. (mg/l)	0	0				0.1				0.3			
IAA Cocn. (mg/l)	0	0				0				0			
Co Cocn. (M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	4.3	7.0	7.5	7.3	8.3	7.0	5.4	7.4	6.5	5.9	6.4	5.6	4.5
" 4	3.3	3.5	3.7	3.5	3.3	6.5	6.6	6.1	4.4	7.7	9.1	7.7	6.7
" 11	4.3	6.7	8.3	7.0	4.1	9.2	9.9	7.7	7.1	6.5	6.4	7.0	6.0
" 15	-	7.9	7.2	7.5	4.1	-	-	-	-	5.4	6.6	6.7	5.8

Appendix Table 10. The effects of Co concentrations in the presence of suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA with or without supraoptimal (1.0 mg/l) IAA, on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 8, 9 and 10 sections.

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TCPAA Cocn. (mg/l)	0				0.1				0.3			
IAA Cocn. (mg/l)	1.0				1.0				1.0			
Co Cocn. (M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	7.7	8.1	7.1	5.4	9.0	8.5	7.4	6.0	-	-	-	-
" 4	5.2	6.5	7.0	5.5	5.9	6.2	7.0	6.1	-	6.7	6.9	6.0
" 11	6.4	6.6	8.0	6.4	6.2	6.2	8.7	6.2	4.3	3.9	4.4	3.2
" 15	4.5	4.5	4.5	4.5	-	-	-	-	-	-	-	-

Appendix Table 10. (Continued)

TCPAA (mg/1)	0	0				0.1				0.3			
IAA (mg/1)	0	0				0				0			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 4	13.3	13.5	13.7	13.5	13.3	16.5	16.6	16.1	14.4	17.7	19.1	17.7	16.7
	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.22	0.16	0.19	0.30	0.20	0.38	0.38	0.24	0.14	0.29	0.58	0.57	0.34
	(10)	(10)	(10)	(10)	(10)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)

TCPAA (mg/1)	0				0.1				0.3			
IAA (mg/1)	1.0				1.0				1.0			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 4	15.2	16.5	17.0	15.5	15.9	16.2	17.0	16.1	-	16.7	16.9	16.0
	±	±	±	±	±	±	±	±		±	±	±
	0.26	0.38	0.87	0.36	0.24	0.24	0.32	0.25		0.48	0.33	0.26
	(9)	(9)	(9)	(9)	(9)	(9)	(10)	(9)		(9)	(9)	(9)

Appendix Table 10a. Standard errors of the final lengths of sections in Experiment 4 of Appendix Table 10.

TCPAA (mg/l)	0	0				0.1				0.3			
IAA (mg/l)	0	0				0				0			
Co (M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	4.3	-	-	-	-	7.0	7.5	7.3	8.3	7.0	5.4	7.4	6.5
" 3	4.2	4.2	3.5	3.2	3.4	6.6	6.7	5.9	4.1	8.7	7.7	8.0	5.9
" 4	3.3	3.5	3.7	3.5	3.3	6.5	6.6	6.1	4.4	7.7	9.1	7.7	6.7
" 6	3.2	3.1	3.0	3.1	2.4	6.0	6.4	5.5	3.0	8.9	7.9	7.5	4.6
" 10	-	4.2	4.0	4.4	4.2	-	-	-	-	8.5	7.4	7.6	5.7
" 13	3.9	3.7	4.1	3.8	4.1	7.4	7.4	7.0	4.8	-	-	-	-
" 15	-	-	-	-	-	7.9	7.2	7.5	4.1	-	-	-	-

Appendix Table 11. The effects of Co concentrations in the presence of suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without optimal (0.1 mg/l) IAA, on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 8, 9 and 10 sections.

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TCPAA (mg/l)	0				0.1				0.3			
IAA (mg/l)	0.1				0.1				0.1			
Co (M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 1	5.5	5.0	4.5	3.6	7.1	7.6	6.5	4.5	7.7	8.9	4.9	7.4
" 3	6.0	6.7	6.1	5.5	7.0	7.2	7.0	5.5	7.4	8.1	7.7	5.9
" 4	6.8	6.6	6.8	5.1	6.5	7.5	7.1	5.6	7.2	7.8	7.1	5.6
" 6	6.2	6.2	5.7	4.2	6.3	6.4	6.6	4.0	7.6	8.2	7.5	4.6
" 10	-	-	-	-	-	-	-	-	7.6	8.6	8.3	6.0
" 13	7.2	8.6	7.3	5.9	8.4	8.2	8.3	5.7	-	-	-	-
" 15	7.5	7.3	7.1	5.8	6.2	8.3	7.9	5.6	-	-	-	-

Appendix Table 11. (continued)

TCPAA (mg/l)	0	0				0.1				0.3			
IAA (mg/l)	0	0				0				0			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 3	14.2 ± 0.22 (10)	14.2 ± 0.26 (10)	13.5 ± 0.27 (10)	13.2 ± 0.23 (10)	13.4 ± 0.23 (10)	16.6 ± 0.47 (9)	16.7 ± 0.23 (8)	15.9 ± 0.22 (9)	14.1 ± 0.22 (9)	18.7 ± 0.47 (9)	17.7 ± 0.33 (9)	18.0 ± 0.33 (9)	15.9 ± 0.19 (9)

TCPAA (mg/l)	0				0.1				0.3			
IAA (mg/l)	0.1				0.1				0.1			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 3	16.0 ± 0.31 (10)	16.7 ± 0.29 (9)	16.1 ± 0.22 (10)	15.5 ± 0.26 (10)	17.0 ± 0.38 (10)	17.2 ± 0.31 (9)	17.0 ± 0.30 (9)	15.5 ± 0.44 (10)	17.4 ± 0.41 (10)	18.1 ± 0.30 (9)	17.7 ± 0.28 (10)	15.9 ± 0.36 (9)

Appendix Table 11a. Standard errors of the final lengths of sections in Experiment 3 of Appendix Table 11.

TCPAA (mg/l)	0	0				0.1				0.3			
IAA (mg/l)	0	0				0				0			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 3	4.2	4.2	3.5	3.2	3.4	6.6	6.7	5.9	4.1	8.7	7.7	8.0	5.9
" 5	3.9	3.9	3.7	3.8	3.9	5.2	8.0	6.5	5.1	8.3	8.7	8.2	6.2
" 6	3.2	3.1	3.0	3.1	2.4	6.0	6.4	5.5	3.0	8.9	7.9	7.5	4.6
" 10	-	4.2	4.0	4.4	4.2	-	-	-	-	-	-	-	-
" 13	3.9	3.7	4.1	3.8	4.1	7.4	7.4	7.0	4.8	-	-	-	-

Appendix Table 12. The effects of Co concentrations in the presence of suboptimal (0.1 mg/l) or optimal (0.3 mg/l) TCPAA, with or without suboptimal (0.01 mg/l) IAA, on the growth of etiolated pea epicotyl sections.

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TCPAA (mg/l)		0				0.1				0.3			
IAA (mg/l)		0.01				0.01				0.01			
Co(M)		10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt.	3	4.8	4.3	5.1	3.7	6.5	6.9	6.1	4.7	8.4	8.1	7.9	6.5
"	5	5.0	4.7	4.9	4.1	7.4	6.5	6.3	6.8	8.9	8.8	7.9	4.1
"	6	5.0	4.7	5.2	2.9	6.4	7.0	6.6	4.1	8.2	7.8	7.8	4.6
"	10	-	-	-	-	-	-	-	-	9.1	9.2	8.2	6.3
"	13	4.2	4.1	4.3	3.9	7.6	7.7	7.0	5.4	-	-	-	-

Appendix Table 12. (Continued)

TCPAA (mg/l)	0	0				0.1				0.3			
IAA (mg/l)	0	0				0				0			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 6	13.2	13.1	13.0	13.1	12.4	16.0	16.4	15.5	13.0	18.9	17.9	17.5	14.6
	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.17	0.14	0.13	0.18	0.16	0.26	0.31	0.23	0.17	0.34	0.37	0.27	0.35
	(9)	(10)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)

TCPAA (mg/l)	0				0.1				0.3			
IAA (mg/l)	0.01				0.01				0.01			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 6	15.0	14.7	15.2	12.9	16.4	17.0	16.6	14.1	18.2	17.8	17.8	14.6
	±	±	±	±	±	±	±	±	±	±	±	±
	0.28	0.48	0.34	0.32	0.52	0.32	0.34	0.25	0.24	0.34	0.35	0.27
	(8)	(8)	(8)	(8)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)

Appendix Table 12a. Standard errors of the final lengths of sections in Experiment 6 of Appendix Table 12.

TCPAA (mg/l)	0	0				1.0				0			
IAA (mg/l)	0	0				0				0.01			
Co (M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 7	4.1	4.2	4.2	4.5	3.7	7.5	8.5	9.1	6.5	6.3	6.9	6.1	4.4
" 8	3.5	3.6	3.9	3.7	3.4	7.7	8.4	8.0	6.2	5.2	4.8	4.9	3.6
" 9✡	4.3	4.5	4.2	4.1	4.1	8.5	10.2	9.2	7.1	6.1	5.4	4.7	5.6

TCPAA (mg/l	1.0			
IAA (mg/l	0.01			
Co (M)	10^{-5}	3.10^{-5}.	10^{-4}	3.10^{-4}
Expt. 7	8.4	8.7	8.3	5.9
" 8	8.2	9.0	8.9	5.9
" 9✡	8.7	9.3	9.0	6.5

Appendix Table 13. The effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without suboptimal (0.01 mg/l) IAA, on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 8, 9 and 10 sections.

✡ Commercial sucrose was used in the Basal Medium.

TCPAA (mg/l)	0	0				1.0				0			
IAA (mg/l)	0	0				0				0.01			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 8	13.5 ± 0.24 (9)	13.6 ± 0.15 (10)	13.9 ± 0.22 (9)	13.7 ± 0.22 (9)	13.4 ± 0.30 (9)	17.7 ± 0.31 (9)	18.4 ± 0.26 (9)	18.0 ± 0.35 (9)	16.2 ± 0.30 (9)	15.2 ± 0.21 (9)	14.8 ± 0.31 (8)	14.9 ± 0.35 (8)	13.6 ± 0.18 (9)

TCPAA (mg/l)	1.0			
IAA (mg/l)	0.01			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 8	18.2 ± 0.95 (9)	19.0 ± 0.27 (9)	18.9 ± 0.54 (9)	15.9 ± 0.44 (9)

Appendix Table 13a. Standard errors of the final lengths of sections in Experiment 8 of Appendix Table 13.

TCPAA (mg/l)	0	0				1.0				0			
IAA (mg/l)	0	0				0				0.1			
Co (M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 7	4.1	4.2	4.2	4.5	3.7	7.5	8.5	9.1	6.5	6.6	7.7	6.7	5.5
" 8	3.5	3.6	3.9	3.7	3.4	7.7	8.8	8.0	6.2	6.8	7.2	6.9	5.1

TCPAA (mg/l)	1.0			
IAA (mg/l)	0.1			
Co (M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 7	6.4	8.1	7.7	6.7
" 8	7.1	8.2	7.9	6.5

Appendix Table 14. The effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA, with or without optimal (0.1 mg/l) IAA, on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 8, 9 and 10 sections.

TCPAA (mg/1)	0	0				1.0				0			
IAA (mg/1)	0	0				0				0.1			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 8	13.5 ± 0.24 (9)	13.6 ± 0.15 (10)	13.9 ± 0.22 (9)	13.7 ± 0.22 (9)	13.4 ± 0.30 (9)	17.7 ± 0.31 (9)	18.8 ± 0.26 (9)	18.0 ± 0.35 (9)	16.2 ± 0.30 (9)	16.8 ± 0.23 (9)	17.2 ± 0.29 (9)	16.9 ± 0.49 (9)	15.1 ± 0.16 (9)

TCPAA (mg/1)	1.0			
IAA (mg/1)	0.1			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 8	17.1 ± 0.36 (9)	18.2 ± 0.31 (9)	17.9 ± 0.46 (9)	16.5 ± 0.47 (9)

Appendix Table 14a. Standard errors of the final lengths of sections in Experiment 8 of Appendix Table 14.

TCPAA (mg/1)	0	0				1.0				0			
IAA (mg/1)	0	0				0				1.0			
Co (M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 7	4.1	4.2	4.2	4.5	3.7	7.5	8.5	9.1	6.5	5.5	6.5	6.3	6.3
" 8	3.5	3.6	3.9	3.7	3.4	7.7	8.8	8.0	6.2	6.2	6.4	7.6	6.0

TCPAA (mg/1)	1.0			
IAA (mg/1)	1.0			
Co (M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 7	5.4	6.9	6.9	7.9
" 8	5.7	6.6	7.1	6.6

Appendix Table 15. The effects of Co concentrations in the presence of supraoptimal (1.0 mg/1) TCPAA, with or without supraoptimal (1.0 mg/1) IAA, on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 8, 9 and 10 sections.

TCPAA (mg/l)	0	0				1.0				0			
IAA (mg/l)	0	0				0				1.0			
Co(M)	0	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 8	13.5 ± 0.24 (9)	13.6 ± 0.15 (10)	13.9 ± 0.22 (9)	13.7 ± 0.22 (9)	13.4 ± 0.30 (9)	17.7 ± 0.31 (9)	18.8 ± 0.26 (9)	18.0 ± 0.35 (9)	16.2 ± 0.30 (9)	16.2 ± 0.17 (9)	16.4 ± 0.34 (9)	17.6 ± 0.33 (9)	16.0 ± 0.32 (9)

TCPAA (mg/l)	1.0			
IAA (mg/l)	1.0			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 8	15.7 ± 0.21 (9)	16.6 ± 0.34 (8)	17.1 ± 0.20 (9)	16.6 ± 0.21 (9)

Appendix Table 15a. Standard errors of the final lengths of sections in Experiment 8 of Appendix Table 15.

TCPAA (mg/l)	1.0				1.0				1.0			
IAA (mg/l)	0.01				0.1				1.0			
Co (M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 12	-	-	-	-	8.1	8.4	9.4	7.5	6.7	7.7	8.6	7.2
" 14	7.7	9.6	9.8	7.8	8.0	8.9	9.8	8.0	6.2	7.2	8.2	9.5

TCPAA (mg/l)	1.0			
IAA (mg/l)	3.0			
Co (M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 12	6.8	8.6	8.9	7.8
" 14	5.6★	7.9	8.7	8.7

Appendix Table 16. The effects of Co concentrations in the presence of supraoptimal (1.0 mg/l) TCPAA with suboptimal (0.01 mg/l), optimal (0.1 mg/l) and supraoptimal (1.0 mg/l, 3.0 mg/l) IAA on the growth of etiolated pea epicotyl sections. Growth (mm.) is the average of 10 sections.

★ Growth is the average of 9 sections.

TCPAA (mg/l)	1.0				1.0				1.0			
IAA (mg/l)	0.01				0.1				1.0			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 14	17.7 ± 0.26	19.6 ± 0.33	19.8 ± 0.33	17.8 ± 0.31	18.0 ± 0.25	18.9 ± 0.31	19.8 ± 0.46	18.0 ± 0.31	16.2 ± 0.24	17.2 ± 0.22	18.2 ± 0.27	19.5 ± 0.28

TCPAA (mg/l)	1.0			
IAA (mg/l)	3.0			
Co(M)	10^{-5}	3.10^{-5}	10^{-4}	3.10^{-4}
Expt. 14	15.6 ± 0.14 (9)	17.9 ± 0.32	18.7 ± 0.31	18.7 ± 0.41

Appendix Table 16a. Standard errors of the final lengths of sections in Experiment 14 of Table 16.

Time in Hours	8	12	14	16	23	35	48
TCPAA	5.3	6.6	7.0	6.7	8.7	8.2	7.8
TCPAA + Co	5.6	7.5	8.0	8.7	9.0	10.5	9.1
IAA	4.5	5.7	6.3	6.5	6.7	6.7	6.8
IAA + Co	5.2	6.5	7.7	7.2	7.3	8.5	7.4

Appendix Table 17. The growth (mm.) of etiolated pea epicotyl sections in TCPAA, TCPAA plus Co, IAA, IAA plus Co in 48 hours. TCPAA, IAA and Co are all in optimum concentrations of 0.3 mg/1, o.1 mg/1, 3.10^{-5}M respectively. Growth is the average of 8 sections.

Time in Hours	8	12	14	16	23	35	48
TCPAA	15.3 ± 0.15	16.6 ± 0.18	17.0 ± 0.50	16.7 ± 0.43	18.7 ± 0.25	18.2 ± 0.36	17.8 ± 0.39
TCPAA + Co	15.6 ± 0.32	17.5 ± 0.16	18.0 ± 0.37	18.7 ± 0.51	19.0 ± 0.69	20.5 ± 0.54	19.1 ± 0.85
IAA	14.5 ± 0.19	15.7 ± 0.25	16.3 ± 0.31	16.5 ± 0.29	16.7 ± 0.29	16.7 ± 0.29	16.8 ± 0.26
IAA + Co	15.2 ± 0.23	16.5 ± 0.14	17.7 ± 0.23	17.2 ± 0.28	17.3 ± 0.46	18.5 ± 0.79	17.4 ± 0.58

Appendix Table 17a. Standard errors of the final lengths of sections in the experiment shown in Appendix Table 17.

Time in Hours	2	3	4	5	6	7	8	Expt. No.
TCPAA	1.6	2.3	3.2	3.4	4.2	4.5	5.2	2
"	1.7	2.5	3.4	4.0	-	-	-	3a
"	-	-	-	3.8	4.3	5.2	5.5	3b
"	1.8	2.3	3.1	3.3	-	-	-	4
TCPAA + Co	1.7	2.6	2.9	4.4	4.3	5.2	5.4	2
"	1.8	2.4	3.5	4.0	-	-	-	3a
"	-	-	-	4.1	4.7	5.4	5.7	3b
"	1.7	2.4	3.0	3.8	-	-	-	4

Appendix Table 18. The growth (mm.) of etiolated pea epicotyl sections in optimal (0.3 mg/l) TCPAA, optimal TCPAA plus Co (3.10^{-5}M), optimal (0.1 mg/l) IAA, optimal IAA plus Co (3.10^{-5}M) in the first 8 hours.
Experiments a and b were performed at the same time.

(Contined on next page)

Time in Hours	2	3	4	5	6	7	8	Expt. No.
IAA	1.9	2.7	3.0	3.8	3.8	4.3	4.5	2
"	-	-	-	-	-	-	-	3a
"	-	-	-	3.5	3.9	4.4	4.9	3b
"	1.9	2.5	2.8	3.2	-	-	-	4
IAA + Co	2.0	2.6	3.0	3.9	4.2	4.7	5.3	2
"	-	-	-	-	-	-	-	3a
"	-	-	-	4.1	4.6	4.7	5.5	3b
"	2.0	2.3	3.1	3.4	-	-	-	4

Appendix Table 18. (Continued)

Time in Hours	2	3	4	5	6	7	8	Expt. No.
TCPAA	—	—	—	13.8 ± 0.16 (9)	14.3 ± 0.18 (9)	15.2 ± 0.13 (8)	15.5 ± 0.20 (8)	3b
TCPAA	11.8 ± 0.07 (10)	12.3 ± 0.15 (9)	13.1 ± 0.16 (10)	13.3 ± 0.16 (10)	—	—	—	4
TCPAA + Co	—	—	—	14.1 ± 0.18 (9)	14.7 ± 0.16 (9)	15.4 ± 0.27 (8)	15.7 ± 0.26 (8)	3b
TCPAA + Co	11.7 ± 0.08 (10)	12.4 ± 0.15 (10)	13.0 ± 0.16 (10)	13.8 ± 0.22 (10)	—	—	—	4

Appendix Table 18a. Standard errors of final lengths of sections in Experiments 3b and 4 of Appendix Table 18.

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Time in Hours	2	3	4	5	6	7	8	Expt. No.
IAA	—	—	—	13.5 ± 0.15 (9)	13.9 ± 0.18 (9)	14.4 ± 0.13 (9)	14.9 ± 0.23 (8)	3b
IAA	11.9 ± 0.11 (10)	12.5 ± 0.13 (10)	12.8 ± 0.10 (10)	13.2 ± 0.15 (10)	—	—	—	4
IAA + Co	—	—	—	14.1 ± 0.30 (9)	14.6 ± 0.18 (9)	14.7 ± 0.22 (9)	15.5 ± 0.23 (8)	3b
IAA + Co	12.0 ± 0.07 (10)	12.3 ± 0.10 (10)	13.1 ± 0.11 (10)	13.4 ± 0.13 (10)	—	—	—	4

Appendix Table 18a. (Continued)

Time in hours	3	5	8	14	30
Opt. TCPAA	2.7	4.1	6.2	7.9	8.1
Supraopt. TCPAA	2.5	4.2	5.6	6.3	5.9
Opt. IAA	2.6	3.9	5.7	7.4	7.7
Supraopt. IAA	2.9	3.9	4.8	6.0	5.6

Appendix Table 19. The growth (mm.) of etiolated pea epicotyl sections in Optimal (0.3 mg/l) TCPAA, Supraoptimal (1.0 mg/l) TCPAA, Optimal (0.1 mg/l) IAA, Supraoptimal (1.0 mg/l) IAA in 30 hours. Growth is the average of 10 sections.

Time in Hours	3	5	8	14	30
Opt. TCPAA	12.7 ± 0.13	14.1 ± 0.53	16.2 ± 0.21	17.9 ± 0.29	18.1 ± 0.29
Supraopt. TCPAA	12.5 ± 0.19	14.2 ± 0.22	15.6 ± 0.26	16.3 ± 0.54	15.9 ± 0.19
Opt. IAA	12.6 ± 0.53	13.9 ± 0.12	15.7 ± 0.25	17.4 ± 0.24	17.7 ± 0.42
Supraopt. IAA	12.9 ± 0.10	13.9 ± 0.24	14.8 ± 0.13	16.0 ± 0.15	15.6 ± 0.12

Appendix Table 19a. Standard errors of final lengths of sections in the experiment shown in Appendix Table 19.

Time in hours	3	5	8	14	24
Opt. TCPAA	2.4	3.9	5.2	8.2	8.2
Supraopt. TCPAA	3.0	4.0	5.1	5.7	5.8
Supraopt. TCPAA + Co	2.8	4.0	5.8	8.1	8.9
Opt. IAA	2.7	3.4	4.4	6.6	6.1
Supraopt. IAA	3.0	4.0	4.3	4.8	5.1
Supraopt. IAA + Co	3.0	3.9	4.9	6.4	6.4

Appendix Table 20. The growth (mm.) of etiolated pea epicotyl sections in Optimal (0.3 mg/l) TCPAA, Supraoptimal (1.0 mg/l) TCPAA, Supraoptimal TCPAA plus Co (3.10^{-5}M), Optimal (0.1 mg/l) IAA, Supraoptimal (1.0 mg/l) IAA, Supraoptimal IAA plus Co (3.10^{-5}M) in 24 hours. Growth is the average of 10 sections.

Time in Hours	3	5	8	14	24
Opt. TCPAA	12.4 ± 0.14	13.9 ± 0.13	15.2 ± 0.30	18.2 ± 0.27	18.2 ± 0.38
Supraopt. TCPAA	13.0 ± 0.09	14.0 ± 0.18	15.1 ± 0.15	15.7 ± 0.20	15.8 ± 0.17
Supraopt. TCPAA + Co	12.8 ± 0.07	14.0 ± 0.12	15.8 ± 0.20	18.1 ± 0.24	18.9 ± 0.36
Opt. IAA	12.7 ± 0.13	13.4 ± 0.23	14.4 ± 0.20	16.6 ± 0.26	16.1 ± 0.16
Supraopt. TCPAA	13.0 ± 0.07	14.0 ± 0.10	14.3 ± 0.21	14.8 ± 0.13	15.1 ± 0.22
Supraopt. IAA + Co	13.0 ± 0.09	13.9 ± 0.20	14.9 ± 0.15	16.4 ± 0.15	16.4 ± 0.27

Appendix Table 20a. Standard errors of the final lengths of sections in the experiment shown in Appendix Table 20.

Time in hours	3	5	8	13	24	Expt. No.
Subopt. TCPAA + Co	2.2	2.3	5.1	7.5	9.6	1
" "	2.4	3.3	4.8	6.8	8.6	2
Subopt. TCPAA + Subopt. IAA + Co	2.2	3.3	5.1	6.9	9.6	1
" "	2.0	3.8	5.1	7.3	8.4	2
Subopt. TCPAA + Opt. IAA + Co	2.3	3.7	5.4	8.4	9.9	1
" "	2.7	3.7	5.6	6.8	7.6	2
Optimal TCPAA + Co	2.7	4.5	6.5	7.9	8.9	1
" "	2.5	4.2	5.5	8.1	9.5	2

Appendix Table 21. The growth (mm.) of etiolated pea epicotyl sections in suboptimal TCPAA plus Co, suboptimal TCPAA plus suboptimal IAA plus Co, suboptimal TCPAA plus optimal IAA plus Co, and optimal TCPAA plus Co. Suboptimal TCPAA = 0.1 mg/l, optimal TCPAA = 0.3 mg/l, suboptimal IAA = 0.01 mg/l, optimal IAA = 0.1 mg/l. Co concentration = 3.10^{-5}M. Growth is the average of 10 sections.

Time in Hours	3	5	8	13	24	Expt. No.
Subopt. TCPAA + Co	12.4 ± 0.14	13.3 ± 0.21	14.8 ± 0.22	16.8 ± 0.28	18.6 ± 0.26	2
Subopt. TCPAA + Subopt. IAA + Co	12.0 ± 0.09	13.8 ± 0.13	15.1 ± 0.31	17.3 ± 0.29	18.4 ± 0.43	
Subopt. TCPAA + Opt. IAA + Co	12.7 ± 0.15	13.7 ± 0.31	15.6 ± 0.29	16.8 ± 0.43	17.6 ± 0.44	
Opt. TCPAA + Co	12.5 ± 0.13	14.2 ± 0.22	15.5 ± 0.25	18.1 ± 0.31	19.5 ± 0.41	

Appendix Table 21a. Standard errors of the final lengths of sections in Experiment 2 of Appendix Table 21.

Time in hours	3	5	8	13	24	Expt. No.
Subopt. IAA	-	-	-	-	-	1
" "	2.1	3.1	4.7	5.3	5.8	2
Opt. IAA	2.4	3.3	4.2	6.7	-	1
" "	2.3	3.8	4.8	5.6	6.9	2
Subopt. IAA + Co	1.8	3.0	4.0	5.8	-	1
" "	1.8	3.1	4.6	5.0	6.1	2
Subopt. IAA + Subopt. TCPAA	1.9	2.6	5.0	7.1	-	1
" "	2.3	3.7	4.3	5.7	6.7	2

Appendix Table 22. The growth (mm.) of etiolated pea epicotyl sections in suboptimal IAA, optimal IAA, suboptimal IAA plus Co, suboptimal IAA plus suboptimal TCPAA. Suboptimal IAA = 0.01 mg/l, optimal IAA = 0.1 mg/l, Co concentration = 3.10^{-5}M, suboptimal TCPAA = 0.1 mg/l. Growth is the average of 10 sections.

Time in Hours	3	5	8	13	24	Expt. No.
Subopt. IAA	12.1 ± 0.06	13.1 ± 0.23	14.7 ± 0.24	15.3 ± 0.22	15.7 ± 0.27	2
Opt. IAA	12.3 ± 0.15	13.8 ± 0.12	14.8 ± 0.18	15.6 ± 0.87	16.9 ± 0.31	
Subopt. IAA + Co	11.8 ± 0.18	13.1 ± 0.31	14.6 ± 0.16	15.0 ± 0.35	16.1 ± 0.25	
Subopt. IAA + Subopt. TCPAA	12.3 ± 0.13	13.7 ± 0.20	14.3 ± 0.19	15.7 ± 0.39	16.7 ± 0.46	

Appendix Table 22a. Standard errors of the final lengths of sections in Experiment 2 of Appendix Table 22.