STUDIES ON THE CONTROL OF SECONDARY THICKENING IN EXCISED ROOTS OF *LYCOPERSICUM ESCULENTUM* MILL.

By

SUNITA SINHA.

A thesis

submitted for the degree

of

Doctor of Philosophy,

University of Glasgow.

February , 1968.

ACKNOWLEDGEMENTS

I am obliged to Prof. P.W.Brian , F.R.S. for providing the facilities necessary for this work.

I wish to express my sincere gratitude to Dr. D.N.Butcher for suggesting the problem and his constant guidance, inspiration and encouragement.

I also wish to thank Dr. U.K.Sinha for his help during the preparation of this thesis.

The receipt of an Edwina Mountbatten grant for the preparation of this thesis is gratefully acknowledged.

CONTENTS

Page

Page

LIST OF ABBREVIATIONS

6-benzylaminopurine	BA.
Cambium	ca.
Cortex	co.
2,4-dichlorophenoxyacetic acid	2,4-D.
Endodermis	en.
Gibberellic acid	GA_3.
Indoleacetic acid	IAA.
β-indolylbutyric acid	IBA.
Length of the main axis	L.M.A.
1-naphthalene acetic acid	NAA.
Number of laterals	N.L.
Parts per million	ppm.
Phloem	ph.
Total length of laterals	T.L.L.
Xylem	xy.

INTRODUCTION

Secondary growth is characteristic of roots of dicotyledons and gymnosperms. The roots of intact plants increase in diameter through the division and enlargement of the meristematic cells of the vascular cambium and of the phellogen. The cambium is first initiated near the root base in association with and continuous with the vascular cambium of the shoot and proceeds acropetally towards the root tip. In contrast it has been reported that isolated roots cultured continuously in a synthetic medium usually lack or at the most have only a fragmentary secondary cambium (Weintraub, 1940; White, 1943; Torrey, 1963; Street, 1966). Isolated roots, therefore, appeared to provide a suitable system for studies of cambial activity, since under culture conditions they appear to be deficient of factors necessary for normal cambium development.

Factors influencing cambial activity in intact plants.

The first observations concerning the control of cambial activity were made by Jost (1891; 1893). He showed that the activity of the stem cambium of dicotyledons is greatly stimulated immediately below the growing leaves. He found that the effect was transmitted only in the morphologically downward direction, since no activity was observed above the leaf. The effect was also seen in etiolated plants. Much later in 1910 Keeble provided evidence that this transmission of cambial activity is due to 'chemical stimulators' and Kastens (1924) elaborated this further and suggested that growth hormones produced in the growing parts were transmitted in a downward direction.

Subsequently Coster (1927; 1928) came to the conclusion that the cambial activity in tropical trees was stimulated by

hormones which were produced in young developing buds and to a lesser extent in the leaves. His observationsled him to conclude that the hormones are produced shortly before the first visible signs of bud development can be detected.

For several tree species it has been shown that cambial activity begins in the branches in the spring at about the same time the buds begin to develop (Priestley, 1930; Snow, 1932; Gill, 1933).

Snow in 1933 produced further experimental evidence that cambial growth is dependent on a hormone. He split the stems of Pisum and Helianthus seedlings longitudinally for several centimetres and severed half the Pisum stem at the lower end of the split and half the Helianthus stem at the upper end. The downward pointing half of the former was then brought into contact with the upward pointing half of the latter, the two halves were bound firmly together, and after 22 days the regions of contact were sectioned and examined. Many cambial layers had formed in and between the bundles in the Helianthus hypocotyl although the tissues had not grown together. Controls not in contact showed no such effect. It was concluded that the stimulus from the Pisum seedlings had passed across a protoplasmic discontinuity from one plant to another of a different species. In other experiments upward and downward portions were both from the same plant, Vicia faba. In such experiments the stimulus passed across a piece of linen inserted in the stem. Thus it appeared that the factor responsible for cambium activity could be transferred from one species to another and that protoplasmic continuity was not essential for the transport of the stimulus.

Later Snow and Le Fanu (1935) activated the cambium in the Helianthus hypocotyl by applying urine or the other extract

of urine and finally succeeded in initiating cambial activity with indole-3-acetic acid (IAA). The cambium was activated for 2-3 cms. below the point of application.

In trees the activation of cambium divisions by auxins has been studied by Söding (1936)a) who showed that insertion of a crystal of IAA into the cambium of woody twigs gives rise to a rapid growth of new secondary wood which in the willow was up to 1 mm. wide. The effect which was due entirely to cambium activation was visible down to about 3 cms. below the point of application.

Another observation made by several workers (Coster, 1927; 1928, and Gouwentak and Hellinga, 1935) was that wounding alone without auxin addition produces some cambial activity. This indicates that auxin or some other hormone-like substance is liberated by wounding.

Avery, Burkholder and Creighton (1937) determined the distribution of auxin in twigs following bud development and found a correlation between the downward spread of auxin and that of cambial activity. At about the same time it was discovered that moderate concentrations of auxins caused a thickening of roots. This was largely due to enlargement of the cortical cells but it was usually accompanied by cell divisions either in the cambium (Jost, 1935)a) or in the pericycle (Thimann, 1936) or both. Divisions in these layers gave rise to lateral roots which may have been produced directly as a result of auxin application.

Went and Thimann (1937) tentatively suggested that the cell division results from the interaction of several factors of which one is auxin; the distribution of these factors differs

for different tissues, and those tissues such as stem and root cambium and root pericycle, which divide readily on the application of auxin, do so because they already contain the other factors, and auxin is therefore limiting.

Although IAA at the appropriate concentrations can often stimulate the production and multiplication of cambium cells, it does not always have a corresponding effect on the subsequent differentiation of the cells so produced into conducting elements. This has been shown to be the case in young stems of beet (Beta vulgaris), and it has therefore been suggested that another essential growth factor, presumably a hormone and supplied by the apex of the shoot, controls this differentiation (Winter, 1954).

Kunning (1950), working with seedlings of Phaseolus multiflorus, P.vulgaris and Helianthus annuus, produced evidence that cambial activity in shoot systems may be stimulated not only by auxins but also by other specific factors. He found that thiamin, ascorbic acid and yeast extract increased the activity of the vascular cambium, their effects however were considerably less than that of auxins.

With the discovery of the gibberellins and cytokinins it was soon found that these growth hormones could influence the development of the cambium. Bradley and Crane (1957) reported that gibberellin stimulated cambial activity in apricot spur shoots. Xylem development was greatly stimulated by the treatment whereas phloem development appeared to be unaffected. Wareing (1958) showed that gibberellic acid (GA_3) had an effect on the initiation of cambial activity in disbudded shoots of Acer pseudoplatanus, Populus nigra v. indica and Fraxinus excelsior. Compared with the controls the gibberellin treated

shoots had a distinct zone of new tissue on the inner side of the cambium. These cambial derivatives were not differentiated however and consisted of very thin walled fusiform cells which still retained their protoplasmic contents. No lignification was observed. In contrast when IAA was applied alone to the upper end of a sycamore shoot a narrow zone of new xylem with lignified vessels was formed, whereas the control shoots treated with lanolin only, produced no wood. In experiments where both IAA and GA_3 were applied, a wide zone of new wood was formed. The width of this zone was much greater than that produced either by IAA or GA3 alone and consisted of fully lignified vessels with intervening fibrous tissue. It resembled the structure of normal wood.

Sorokin, Mathur and Thimann (1962) made a detailed study of the effects of IAA, 2,4-dichlorophenoxyacetic acid (2,4-D), GA_3 and kinetin on the cambial growth in isolated segments from etiolated 'Alaska' pea epicotyls. Treatment with IAA or 2,4-D activated the fascicular cambium and initiated some interfascicular cambium resulting in the abundant production of secondary xylem and in the formation of hyperplastic tissue. It caused the partial or even total occlusion of proto and metaxylem. The secondary xylem formed consisted of short vessel members with scalariform, reticulate or pitted walls which are often interrupted by nonlignified cells. When IAA was used the hyperplastic growth mainly took the form of root primordia whereas 2,4-D initiated the formation of callus. Treatment with kinetin alone or in combination with auxin caused a completely different development. It led to the initiation of a much more active cambium completely surrounding the core of the internode and it formed several layers of secondary xylem. Kinetin produced more normal xylem consisting

mainly of long vessel members with pitted walls. Hyperplastic growth was completely absent and the xylem did not become occluded.

There is a certain amount of evidence which suggests that cambial activity is often dependent on the photoperiod to which the shoot is exposed. Thus root thickening in several species has been reported to be dependent upon the relative lengths of day and night in the shoot environment. Garner and Allard (1920) reported that radish (Raphanus sativus) forms thick roots when in short day conditions and in long day conditions only fibrous roots are produced. Similarly Garner and Allard (1923) presented evidence that secondary thickening in roots of Phaseolus multiflorus and Dioscorea alata was dependent on the day length to which the shoot was exposed - being stimulated by short days.

More recently Wareing (1949) has shown that cambial activity in the shoots of first year seedlings of Pinus sylvestris can be prolonged in the Autumn if they are exposed to long day conditions. Similarly it was found that cambial activity in Robinia pseudoacacia seedlings was dependent upon a long day photoperiod (Wareing and Roberts, 1956). Seedlings were grown in short day conditions for several weeks until the stem extension (growth) had ceased. When such plants were transferred to long day conditions it was found that in half the plants cambial activity was not accompanied by extension growth. It was suggested that the cambial stimulus arose in the mature leaves under long day conditions and that meristematic activity at the shoot apices was not a necessary condition for cambial activity in the root.

Although these reports suggest that cambial activity is often determined by the photoperiod, very little is known about the mechanism and the factors involved.

There have been few recent investigations of the factors which regulate the growth of the cambium in roots. The work of Digby and Wangermann (1965) suggests that the cambial activity in the roots of pea seedlings is stimulated by auxin coming from the shoot apices and not from the root apices. Apices were excised either from the shoot or from the root of 3 or 5 day old seedlings when the roots were 2 cm. long. The apices were replaced by 0.1 M or 0.01 M IAA in lanolin paste. The apices were then re-excised and treated with IAA paste daily and samples for anatomical investigations were taken either every day orevery two days after treatment. They found that IAA treatment of excised shoot apices had a prolonged effect on the secondary growth in the roots. The count for lignified xylem elements was significantly greater and the count for cambium cells and their undifferentiated derivatives was very much less in the root of excised shoot seedlings than in controls. By contrast, excising the root apices had very little effect on secondary growth in the root, there being no difference in the number of cambium cells or of lignified xylem elements between treated plants and controls. It was concluded that cambial activity in the root was stimulated by auxin moving down into the root from the plumule and auxin from the root apex either does not reach the older part of the radicle or moves in tissues where it can have no effect on cambial activity.

<u>The formation of cambium-like layers in callus tissues and tissue explants</u>

Callus cultures are often initiated from tissue explants which include a vascular cambium (Gautheret, 1957; Torrey and Shigemura, 1957; White 1963) and it is often claimed that such cultures are derived from the cambium cells. However, as was

pointed out by Bailey (1943), with such cultures the cambial origin is uncertain and it is difficult if not impossible to decide whether the cultures originate from the cambium itself or from the adjacent maturing xylem and phloem tissues. Also the cells are unlike those of the intact cambium in their cellular structure and functional activities. It is therefore unlikely that investigations with these tissues will contribute directly to the understanding of the activities of the intact vascular cambium.

Although studies on the so called cambial tissue cultures are unlikely to have any special significance, callus cultures in general have provided some interesting results which may be important to an understanding of cambial development.

Gautheret (1957) has studied the types of vascularisations which occur in the callus formed on the initial tissue explants and in established cultures. In certain cultures he found that vascular cambia often develop at the boundary between two different tissues. For example if a piece of xylem tissue of carrot is isolated, the surface cells will proliferate to give a homogenous parenchyma and this cambium forms xylem towards the original explant and phloem away from the explant. From this and other evidence he suggested that the particular orientation of the cambium appears to be conditioned by the nature of the pre-existing tissue i.e. a gradient of differentiation is set up, one end of which will be situated at the pre-existing tissue and of the same nature and the other at the exterior and of a nature opposed to that of the pre-existing tissue. In the established cultures he found that the vascularisation was more anomalous and that organised cambia were less common.

In a series of experiments by Camus (1949) it has been shown that auxin can determine the initiation of a vascular cambium in cultured tissue pieces of storage root of chicory, (Cichorium intybus). Buds were grafted onto root fragments and it was observed that after a few days a cambium was formed which subsequently gave rise to vascular tissues. In certain tissues only isolated conducting cells were produced whereas in others cambium was produced between the conducting system of the stock and that of the grafted bud. In the latter cases xylem was formed on one side and phloem on the other. The chemical nature of the stimulation was demonstrated by its passage through a cellophane film inserted between bud and root stock. Subsequently IAA and β-indolylbutyric acid (IBA) applied to such root tissues produced a response identical with that caused by buds although other auxins, e.g. NAA and 2,4-D produced only slight effects.

Wetmore and Sorokin (1955) have succeeded in inducing vascularisation and cambium formation in homogeneous parenchymatous callus tissues of Syringa vulgaris. They grafted apical portions of the shoot of the same species or introduced agar containing auxin, sucrose or other growth factors into V-shaped incisions made on the top surface of the homogeneous callus tissue. In their earlier experiments they found that vascular tissues including xylem and phloem developed below the grafted shoot. Later they found that agar containing appropriate concentrations of IAA and sucrose was equally effective in inducing the vascular tissues. When the callus tissues were sectioned and examined in detail it was discovered that the vascular arrangement was comparable to that of a stem. Further experiments showed that a low concentration of auxin induced, as seen in cross-section, a small circle of vascular strands, while a higher concentration caused the development of a larger circle

of more widely spaced strands. The vascular strands in these circles became connected by a cambial layer composed of fascicular and interfascicular portions as in a stem. Moreover, each of the nodular strands had phloem externally and xylem internally. They also showed that the sucrose concentration supplied in the agar determined the proportion of xylem to phloem produced. Higher concentrations were needed for phloem than for xylem development. Intermediate concentrations of 2.5-3.5% sucrose favoured the presence of both tissues usually with a cambium-like layer between them. These observations implicate both sugar supply and auxin level as important factors as inducers of cambial activity and differentiation of vascular elements. It was suggested that a particular auxin concentration in a diffusion gradient from the point of application interacts with endogenous growth regulatory substances in the callus to induce the formation of a vascular cambium. Thus it seems, that in this callus tissue auxin is a limiting factor preventing vascularisation. More recently Wetmore and Rier (1963) have been able to repeat these experiments with other woody and herbaceous species, e.g. Fraxinus americana, Ligustrum vulgare, Salix purpurea, Parthenocissus tricuspidata and Helianthus tuberosus and obtained similar results. It has also been shown that NAA can be substituted for IAA and that glucose can replace sucrose.

In their later experiments Wetmore and Rier (1963) used an upright sterile pipette of narrow aperture to supply IAA and sucrose. When the pipette was embedded to a depth of 0.5 cm. or so below the upper surface of a piece of Syringa callus a quite different pattern of vascular tissue developed. Instead of the characteristic nodules of vascular tissue a complete ring

of xylem or xylem and phloem was induced around the pipette. The proportions of xylem to phloem depended on the concentration of sugar and auxin. In the callus treated with NAA and sucrose in appropriate concentrations, vascular tissues were differentiated in a circular band of cells composed of six-seven rows of xylem elements laying towards the pipette. Peripheral to but oriented away from the pipette were three to four rows of phloem cells. Between the two there was a row of cambium cells. The circular band of xylem elements in these tissues was arranged in distinctt radial rows separated by frequent single rows of parenchymatous cells in the pattern characteristic of vascular rays in the secondary body of woody plants. The cells of xylem and phloem were clearly derived from the cells of the cambium.

Rier and Beslow (1967) have studied the vascularisation in cubes of callus from Parthenocissus tricuspidata var. veitchi when grown on a synthetic liquid medium with continuous shaking. Under these conditions the tissue pieces became spherical and some xylem was formed, the amount depending on the sucrose concentration present in the medium. At sugar concentrations of 2.5% and above the xylem elements were organised in clearly defined arcs. The arcs with the higher concentrations of sucrose had correspondingly more tracheary members. The xylary arcs were composed of adjoining radial rows of tracheary cells and were bordered by an internal cambium. This pattern was similar to that induced in callus supplied with sucrose and NAA via a micropipette (Wetmore and Rier, 1963) except that in the latter case the cambium was located externally. It was considered significant that in tissues where the growth factors were supplied externally the cambium was formed on the inner

faces of the individual arcs, whereas in tissues fed at the centre by a micropipette the cambium wasfound on the outer face of the xylem ring. It was suggested that the direction of flow of the inducing agents was responsible for the position of the cambium. Xylem formation occurs first nearest the highest concentration and the cambium forms in a position away from the source of the inducers.

Secondary growth in excised roots

In his successful pioneer studies of the culturability of isolated roots White noted that roots in culture did not exhibit secondary thickening. Even in those plants which characteristically produce a much thickened root in nature, such as carrot, beet and radish, the root when excised and allowed to develop in isolation in a nutrient medium, failed to initiate a vascular cambium and showed no secondary thickening (White, 1938; Bonner and Devirian, 1939; Bonner, 1940; Levine, 1951).

Weintraub (1940) briefly reported that a root tip excised from the white moonflower (Calonyction acutatum) developed a root system with a more or less normal secondary thickening. However few experimental details were given and it was not clear as to whether this root system was developed from a root tip excised directly from the seedling or from isolated roots in continuous culture.

Dormer and Street (1948) examined isolated roots of Lycopersicum esculentum which had been cultured for five to six months without transferring on White's synthetic medium. They observed small amounts of secondary xylem, but the secondary phloem was very poorly developed. The secondary

xylem had a more or less normal distribution in some roots, but in others there was an anomalous condition in which a ring of lignified tissue containing vessels enclosed a central mass of thin walled cells, the whole clearly formed by the activity of a cambium-like layer. Roots grown on a yeast medium produced some sections with normal secondary xylem while others showed pronounced abnormality. Some sections showed repeated disorderly cell divisions on one side of the diarch primary xylem while there was no such development on the other side. Dormer and Street concluded that secondary xylem does occur in excised tomato roots, but that the cambial activity is frequently seriously disorganised and results in the production of conspicuously abnormal structures. From their illustrations it is clear that these roots did not possess a continuously active cambium comparable with that found in the seedling roots of tomato.

The most detailed studies of cambium development in excised roots have been carried out by Torrey and his co-workers. Torrey (1951) found vascular cambium initiation in decapitated roots of Pisum after several weeks in culture following lateral root initiation. The primary root showed a considerable increase in diameter through the entire region of lateral root origin. Serial sections of such roots indicated that root enlargement was due chiefly to the differentiation of secondary xylem resulting from cambium formation. Cambial division occurred in the interstitial region of the triarch vascular cylinder. Discontinuous cambial layers were evident within one week following decapitation in the primary root proximal to the point of origin of the first lateral root. After a month the vascular cylinder showed up to a twofold increase in diameter due to the production of vascular tissue by cambial

cells. The differentiation of cambial layers proceeds basipetally from the point of its first appearance proximal to the first lateral root.

Following up these observations Torrey carried out three distinct types of experiments with isolated pea roots. The first experiments were with isolated pea root tips excised from the germinating seeds which were designated 'initial tips'. Their behaviour in response to auxin treatment is contrasted to that of 'first transfer tips' which were excised from isolated seedling roots grown in culture for a week.

A second type of experiment involved culturing 'first transfer tips' by feeding them nutrients and hormones via the root base; thus simulating the intact plant in which the usual pathway whereby the root is provided with essential nutrients is from the shoot.

The third type of experiment involved the ~~decapitation~~ growth of 0.5 mm. tips of isolated roots grown in culture and following the pattern of root regeneration on different media.

Torrey and Shigemura (1957) and Torrey (1959) observed that 5 mm. root tips excised from 48 hour germinated pea seeds and placed on a modified Bonner medium containing 10^{-5} M IAA are inhibited from elongation about 90% as compared to the control tips on an auxin-free medium. Such treatment produced lateral roots and the initiation of a vascular cambium in the root base. First transfer roots when placed on the same auxin containing medium were also inhibited in their elongation but showed no vascular cambium initiation. It was

concluded from these experiments that the initial tips having been freshly excised from a direct supply of materials stored in the cotyledons were rich in substances essential for vascular cambial initiation when stimulated by auxin, but that these tips when cultured for a week on a control medium used up the materials or had them so diluted in the elongation process that the first transfer tips exposed to auxin treatment were no longer capable of responding. In transverse sections the elements of primary as well as extensive secondary xylem (6-7 radial cell layers) formed during the treatment were evident. At alternate radii primary and secondary phloem fibres were seen. Vascular cambium in initial tips treated with auxin was initiated at the root base, proximal to and independent of the site of lateral root initiation, and cambium activity progressed towards the root tip with time. This situation was similar to that in seedling roots, but was compressed in space by the marked inhibition of root elongation produced by the auxin treatment. In contrast the central cylinder region of first transfer root tips cultured on an auxin medium for one week had the mature primary triarch xylem with incompletely mature elements in the centre, the primary phloem bundles with the phloem fibres at alternate radii and the complete lack of any vascular cambium initiation. From the data on a number of initial root tips treated with auxin it was found that the 'cambial front' moved towards the tip from the base at about 400 μ per day during the seven days. From counts of the number of rows of secondary xylem elements in the radius of root base, it was apparent that about one new row of xylem elements was added radially each day during treatment. Thus Torrey concluded that an actively dividing vascular cambium

was initiated and maintained in the initial tips with hormonal stimulus moving acropetally with time. Torrey did not however state how long this activity persisted and it seems reasonable to suppose that it was of a limited duration.

Torrey (1963) next attempted to provide first transfer tips with factors essential for the induction of cell division leading to secondary vascular tissue formation. He supplemented the IAA medium with many different growth factors. A modification of the technique devised by Raggio and Raggio (1956) was used. First transfer tips, 15 mm. long, were cultured in petri dishes with 5 mm. of base inserted into a separate nutrient medium contained in a small glass vial. By changing the contents of the vial or the dish it was possible to provide inorganic or organic nutrients to the roots either through the base or via the absorbing surface of the root growing on the medium in the dish or both at once. When sugar was added together with a relatively small amount of IAA, a vascular cambium was initiated at the base and proceeded acropetally. With higher sugar concentrations the longitudinal extent of the vascular cambium was increased. The most successful combination of culture conditions for both root elongation and extent of vascular cambium initiation involved the provision of sugar at the base (8%) and in the plate medium (4%) and the provision via the base of IAA and a mixture of additional factors (adenosine sulphate 10^{-4} M and L(+)-aspartic acid, L(+)-arginine HCl, L(+)-glutamic acid, glycine, asparagine and urea all at 10^{-3} M). It was also found that α-naphthaline acetic acid could substitute for

IAA but that 2,4-D and GA_3 were not effective in initiating cambial activity. GA_3 added together with IAA did not enhance the initiation of cambial activity.

In further experiments Torrey (1963) found that 0.5 mm. decapitated roots treated with 10^{-5} M IAA and 5×10^{-6}M kinetin regenerated slow growing root tips in which there was a vascular cambium producing a typical concentric secondary vascular pattern instead of the normal hexarch pattern. It was suggested that the auxin-kinetin treatment led to the activation of cell division in a ring of cells in the primary root rather than the more normal discontinuous ring of cells.

Summarising the results of his investigations Torrey concluded that both primary and secondary vascular tissue formation is determined by internal hormonal gradients and that auxins are particularly important.

More recently Loomis and Torrey (1964) have attempted to induce cambial activity in the isolated roots of radish - a root which is much thickened in the intact plant. Excised seedling roots were cultured for three days to deplete them of possible endogenous cambial regulators. First transfer tips were then cultured with their cut ends placed in media containing substances to be tested for their influence on cambial initiation (Raggio and Raggio technique). Their experiments showed that IAA at various concentrations did not cause visible thickening. A variety of chemical substances including casein hydrolysate, L-asparagine, L-glutamine, gibberellic acid, 6-benzylamino purine and various phenolic acids were tested as vial additives with and without IAA and

were found to be without influence on visible thickening. In contrast, when meso-inositol was added together with a cytokinin and auxin, the roots showed marked visible thickening. Root thickening was apparent within 7 days after transfer. The largest roots were nearly 3 mm. in diameter. Thickening was usually confined to the length of the transfer piece, i.e. it extended only 5-15 mm. along the root from the base. Auxin, cytokinin and inositol all appeared to be necessary for the response. Serial transverse sections cut from the base showed that when IAA was added to the vial medium, cambial activity was limited to one or two radial rows of cell divisions. With both auxin and cytokinin the amount of thickening was much more and as many as nineteen cells aligned in radial rows could be recognised in the secondary xylem. As the roots thickened the epidermis and primary cortex was sloughed off and an exoderm developed apparently from the pericycle and secondary phloem. Although the cambial activity persisted through the second passage it did not continue beyond 10-15 days after transfer. Root thickening was obtained with various cytokinins including 6-phenylaminopurine, 6-benzylamino purine and 6-furfurylamino purine (Kinetin) at 0.1-1.0 mg/l or when coconut milk, which probably contains natural cytokinins, constituted 10% of the vial medium. NAA at 10^{-6}M was also effective as an auxin in stimulating cambial division in the presence of inositol and cytokinin. Gibberellin at concentrations 1-100 ppm. had no effect on the system. The thickening response seemed to be independent of the position and number of lateral roots.

Clearly Torrey and co-workers, by feeding growth factors to the root base have been partially successful in

inducing cambial activity in isolated roots of both pea and radish. However, with both species they have been unable to maintain the activity for an indefinite period. Also their success in initiating cambial activity was confined to initial tips or first transfer tips; they did not use root tips which were maintained in continuous culture. It is possible, therefore, that activity was influenced by substances originating in the seed or seedling shoot system.

Summary

The literature reviewed above can be summarised briefly as follows :-

1. The evidence from the earlier work with intact root systems implicated the auxins as important factors in controlling cambial activity. Subsequently other growth factors, particularly the more recently discovered gibberellins and cytokinins, have often been shown to enhance the auxin effects on cambial growth.

2. Investigations with callus cultures have suggested that concentration gradients of growth substances and nutrients such as sucrose determine the location, orientation and activity of cambium-like cell layers.

3. Studies with 'first transfer' pea and radish root tips have shown that maximum cambial activity is obtained when an auxin, a cytokinin and inositol are fed via the cut end. From this Torrey suggested that this system most nearly resembled the intact plant and that decreasing concentration gradients from the base to the apex are important in initiation and activity of the cambium.

4. There have been few recent reports of secondary thickening occurring in isolated roots which have been maintained in continuous culture for long periods. A certain amount of secondary growth was observed in tomato roots left in culture media for four to five months, but even then the amount was very small and the appearance was abnormal.

The results presented in this thesis have indicated that a normal vascular cambium is initiated in isolated tomato roots in continuous culture if the roots are allowed to grow for two weeks in the same culture medium. However, the activity of this cambium is limited in the White's standard root culture medium and of the growth factors tested only meso-inositol prolonged the duration of activity. The significance of these results has been discussed.

GENERAL TECHNIQUES

The general techniques for the culture of excised tomato roots have been described by Street, McDonagle and Lowe (1951) and Hannay and Street (1954).

Preparation of the culture medium

The standard culture medium was modified from that of White (1943) as advocated by Boll and Street (1951) except that copper sulphate was omitted and ferric-ethylenediamine-tetra-acetate (Fe-EDTA) was substituted for ferric chloride as the iron source (Sheat, Fletcher and Street, 1959).

The composition of the complete standard medium is shown in Table 1. All the constituents of the standard medium excepting the ferric chloride were obtained from British Drug House Limited (B.D.H.). The inorganic salts were of the 'Analar' grade except for the calcium nitrate which was of the 'Laboratory Reagent' grade. The ferric chloride used in the preparation of Fe-EDTA stock solution was in the form of a 10% spectrophotometrically standardised solution obtained from Johnson Matthy and Company Limited.

The inorganic constituents excepting the iron source were stored as a 10 times concentrated stock solution at 4°C. The stock solutions of vitamins and glycine were stored at -15°C at 1,000 times the concentration in the medium. Sucrose was weighed out separately for each batch of the medium.

Except where stated the excised roots were cultured in 100 ml. Erlenmeyer flasks which were closed with nonabsorbent cotton wool plugs covered with muslin cloth. Aluminium caps were placed over the plugs to protect them from condensed water during autoclaving.

Table 1

The composition of the standard culture medium

Calcium nitrate	$Ca(NO_3)_2.4H_2O$	288 mg.
Potassium nitrate	KNO_3	80 mg.
Potassium chloride	KCl	65 mg.
Sodium dihydrogen phosphate	$NaH_2PO_4.2H_2O$	21.5 mg.
Manganese chloride	$MnCl_2.4H_2O$	6 mg.
Zinc sulphate	$ZnSO_4.7H_2O$	2.65 mg.
Potassium iodide	KI	0.75 mg.
Sodium sulphate	$Na_2SO_4.10H_2O$	226.7 mg.
Magnesium sulphate	$MgSO_4.7H_2O$	370 mg.
Boric acid	H_3BO_3	1.5 mg.
Molybdic acid	H_2MoO_4	0.0017 mg.
Ferric chloride	$FeCl_3$	3.1 mg.
Diamino-ethane-tetra-acetic acid		8 mg.
Aneurin hydrochloride		0.1 mg.
Pyridoxine hydrochloride		0.1 mg.
Nicotinic acid		0.5 mg.
Glycine		3 mg.
Sucrose		20 g.

Single distilled water to make 1,000 ml.

Boro-silicate glassware was used throughout. Before use the glassware was cleaned by soaking in 'Pyroneg' overnight, washing thoroughly with tap water and finally rinsed twice with distilled water.

All the culture solutions were made with single glass distilled water from a Loughborough 4 litre per hour water still. The method adopted to make up the medium was to add Fe-EDTA to a solution of sucrose and vitamins. This was thendiluted to about 2 times the final strength before adding the inorganic salt solution. Finally it was made up to volume before adjusting the pH to 4.8 by addition of a few drops of 0.1 N NaOH. On autoclaving the pH fell to approximately 4.6. Aliquots of 50 ml. of the culture medium were distributed to the Erlenmeyer flasks before autoclaving at 15 lbs. per sq.in. for 10 minutes.

In the experimental media in which the added substances were not thermolabile (e.g. 1-naphthalene acetic acid (NAA), 3-indoleacetic acid (IAA) and Kinetin) the supplements were added after the inorganic salts but before the final adjustment of the volume. For the modified Raggio and Raggio technique (see page 25) these substances were added before adding the agar. Gibberellic acid which has been shown to be partially inactivated when autoclaved in White's medium (Butcher, 1960) was sterilised by an alternative method using diethyl ether (see page 25).

1-Naphthalene acetic acid (NAA), gibberellic acid (GA_3), meso-inositol and D-pantothenic acid were obtained from B.D.H.; 3-indoleacetic acid and 6-furfurylaminopurine (kinetin) from Koch Light Lab. Limited; sorbitol, phytic acid and 6-benzylaminopurine from Sigma Chemical Company. Yeast extract

and acid hydrolysed casein (Bacto-casamino acid) were supplied by Difco Laboratories. The coconut milk was obtained from mature coconuts and was stored at -15°C. The agar when used was Oxoid Agar No. 3.

Plant Material and manipulation of the cultures

A clone of excised roots of Lycopersicum esculentum Mill. variety Sutton's 'Best of All' was initiated from seeds in March 1965 and was used throughout the investigation. The procedure for initiating the clone was as follows :-

The seeds were sterilised with 0.5% bromine water, thrice washed with sterile distilled water, and finally placed in sterile petri dishes containing moistened filter papers. They were then incubated for 5 days at 25°C. Root tips approximately 20 mm. in length were excised from the germinating seeds and transferred into 100 ml. flasks containing 50 ml. of standard medium. After 7 days the roots were approximately 70 mm. in length and had several laterals. At this stage a root was selected to start the clone. This was achieved by cutting the main axis into 10 mm. sections each bearing 2-4 laterals (sectors). After a further period of 7 days these sectors were then used to inoculate the further cultures. The stock root material was subcultured through alternate tip and sector passages (growth periods) each lasting 7 days. A tip culture was initiated from a 10 mm. root tip derived from a main lateral of a 7 days old sector culture. A sector culture was initiated from a 7 days old tip culture by transferring a section of the main axis (not including the main apex) 10 mm. long and bearing 4-6 primary laterals each 10-15 mm. in length.

Fig. 1.

Culture flask as used for the modified Raggio and Raggio technique.

For experiments, the tips (10 or 20 mm.) excised from the primary laterals of 7 day old sector cultures, were used as inocula (one to each flask).

Method used for feeding growth factors via the cut end of the excised roots

Raggio and Raggio (1956) described a method whereby the basal ends of excised bean roots could be separately fed with the organic constitutents of the culture medium. Torrey (1963) and Loomis and Torrey (1964) used a similar method for studying the influence of various growth substances on the initiation of cambial activity in excised roots of pea and radish. A modification of the Raggio and Raggio technique was used in these investigations and is described below.

A 100 ml. Erlenmeyer flask was set up as shown in Fig. 1. The liquid medium in the flask contained the inorganic constituents, vitamins and sucrose while the appropriate organic constituents were contained in 1% agar in a 15 x 2 cm. test tube. The latter was wedged into the neck of a flask by means of a cotton wool collar. The mouth of the test tube and the neck of the flask were covered with aluminium foil and the whole 'set up' was autoclaved at 15 lbs. pressure for ten minutes. The sucrose and vitamins were added to both the liquid medium and the agar at the same concentration*? When gibberellic acid was added as a supplement a different procedure was followed. Gibberellic acid crystals we e immersed in diethyl ether in a small specimen tube. The tube was covered with cotton wool and the ether was allowed to evaporate overnight at room temperature. Then under aseptic conditions the crystals were

* In preliminary experiments it was found that when sucrose was supplied only via base the growth was very much impaired and for this reason it was decided to add 2% sucrose both to the agar medium and in the liquid medium. These were similar to the findings of Torrey (1963) with isolated pea roots.

Fig. 2.

The apparatus used for the aseptic culture of tomato seedlings.

dissolved in 200 ml. of distilled water. This solution was added to a previously autoclaved agar solution containing the other organic constituents when the latter was at about 40°C. Immediately this agar solution was poured aseptically into sterile test tubes. Finally the tubes were wedged into previously sterilised flasks containing the liquid medium and covered with aluminium foil as shown in Fig. 1.

Method used for the aseptic culture of tomato seedlings

Seedlings were grown in either 250 ml. Erlenmeyer flasks (100 ml. medium) or 2 litre Penicillium flasks depending on the duration of the experiment (Fig. 2). With both types of flasks the seedlings were supported by muslin tied over the end of a glass tube. The latter was held in place with a cotton wool collar. Tomato seeds were sterilised with 0.5% bromine water and were rinsed thrice with sterile distilled water. They were then transferred aseptically into the muslin support. Finally the tube was adjusted so that the muslin just touched the surface of the medium.

Anatomical investigations

The root material for anatomical investigations was prepared according to the methods described by Johansen (1940) and Sass (1959).

Fixation and dehydration

The roots were first fixed in FAA (formalin:glacial acetic acid:50% ethyl alcohol = 5:5:90) for 24 hours. The roots were then washed with water to remove the fixing fluid and dehydrated by passing through a graded series of ethyl alcohol and water (70%, 85%, 90%, and 100%). Next the absolute alcohol was replaced by chloroform through a graded series of alcohol and

chloroform mixture (2/3 absolute alcohol + 1/3 chloroform; 1/2 absolute alcohol + 1/2 chloroform; 1/3 absolute alcohol + 2/3 chloroform and 100% chloroform).

Embedding

The stoppered test tubes containing the root material in chloroform were kept on a hot plate at 37-40°C. while small chips of paraffin wax (B.D.H. Paraffin wax with ceresin, congealing point about 60°C) were gradually added and dissolved in the chloroform. After 4-5 hours some more wax was added and tubes were unstoppered and left overnight in an oven at 62°C. to remove the chloroform. Next the old wax was replaced with fresh melted wax and finally the infiltrated root material was transferred to a mould containing hot wax.

After cooling the blocks were trimmed and then sectioned using a rotary microtome. The sections, 8 or 10 μ thick were placed on a slide smeared with Haupt's adhesive. After floating the sections on 4% formalin the slides were placed on a hot plate at about 35°C to allow the sections to spread. When the formalin had evaporated, wax was removed by dissolving in xylol.

Staining

Reagents :-

(1)	Safranin 0.1% in cellosolve	50 ml.
	Alcohol 95%	25 ml.
	Distilled water	25 ml.
	Sodium acetate	1 g.
	Formalin	2 ml.
(2)	Crystal violet 1% aqueous	
(3)	Alcohol 95%	25 ml.
	Cellosolve	25 ml.
	Tertiary butyl alcohol	25 ml.

(4)	Fast green FCF saturated in equal parts of clove oil and cellosolve	20 ml.
(5)	Alcohol 95%	60 ml.
	Tertiary butyl alcohol	60 ml.
	Glacial acetic acid	0.6 ml.
(5)	Alcohol 95%	30 ml.
	Tertiary butyl alcohol	30 ml.
	Glacial acetic acid	0.15 ml.
(6)	Orange G saturated in cellosolve	20 ml.
	Cellosolve	20 ml.
	Alcohol 95%	20 ml.
(7)	Clove oil	10 ml.
	Absolute alcohol	10 ml.
	Xylol	10 ml.

Procedure :-

1. Slides were brought down to 70% alcohol.
2. Stained in methyl cellosolve-50% alcohol-safranin solution for 24-48 hours.
3. Rinsed in tap water.
4. Stained in 1% aqueous crystal violet for 10-15 minutes.
5. Rinsed in tap water.
6. Rinsed in a mixture of equal parts of 95% alcohol, cellosolve and tertiary butyl alcohol for 15 seconds.
7. Immersed in fast green FCF solution for 10-15 minutes.
8. Rinsed briefly in a mixture of equal parts of 95% alcohol, tertiary butyl alcohol and 0.5% glacial acetic acid.

9. Immersed in orange G solution for 3 minutes.

10. Rinsed briefly in a solution of one part each of clove oil, cellosolve and 95% alcohol.

11. Rinsed in a wash made up of equal parts of clove oil, absolute alcohol and xylol.

12. Finally rinsed twice in xylol and mounted in balsam.

In many experiments roots were sectioned by hand and stained temporarily either with 50% glycerin + a few crystals of safranin O or with phloroglucinol and HCl.

Standard errors

In most experiments the standard errors for the mean values of numerical data have been calculated from the formula :-

$$S\bar{x} = \sqrt{\frac{Sx^2 - \frac{(Sx)^2}{n}}{n(n-1)}}$$

where $S\bar{x}$ = Sum of all observations,

$(Sx)^2$ = Sum of the squares of each observation, and

n = Number of replicates.

In the tables the standard errors for the treatment means are shown after the mean values preceded by a $\pm$ sign.

Table 2

2 week and 4 week old excised and seedling roots grown in darkness

Mean of 10 roots	Seedling root			Excised root		
2 week	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
	158 ± 4	32	620	196.1 ± 9	51	955
4 week	233 ± 5	51	1103	260.2 ± 2	62	1230

SECTION 1

A COMPARISON OF EXCISED AND SEEDLING ROOTS OF TOMATO

The anatomy of excised and seedling roots of tomato was compared and contrasted as a preliminary to the subsequent investigations where the factors controlling cambial activity were studied. Seedling and excised root cultures were set up in standard medium as described in the materials and methods (page 26). For each comparison, i.e. 2 week seedling vs. 2 week excised or 4 week seedling vs. 4 week excised roots, the flasks were incubated for the appropriate time at 25°C in darkness.

Two week old seedling roots and excised roots

The growth of the roots was recorded as :-

(a) the length of the main axis (in mm.),

(b) the number of primary laterals, and

(c) The total length of the laterals (in mm.).

The roots for anatomical investigations were tested as follows :- Five replicates of each treatment were used. Segments were taken at 0-10, 25, 50, 75, 100, 125, 150, 175 and 200 mm. behind the apex. They were fixed, embedded and transverse sections were cut at 10 μ thickness. The sections were stained in Johansen's quadruple stain as previously described (page 27).

The growth measurements in Table 2 show that 2 week old excised roots were longer than 2 week old seedling roots. The seedling roots differed from the excised roots in that the laterals were not initiated in acropetal order. Also the lateral roots were initiated at the transition region of the seedling.

The observations and measurements made on the serial sections of the apical region showed that there were few differences between the

Table 3A

Anatomy of two week old seedling roots

Distance from promeristem (mm.)	25	50	75	100	125	150
Diameter of the root (μ)	470 ± 19	485 ± 30	462.5 ± 33	397.5 ± 8	350 ± 14	325 ± 31
Diameter of the stele (μ)	142.5 ± 8.4	190 ± 14.3	185 ± 10	184 ± 7.4	171	212.5 ± 4.6
Thickness of cortical layer (μ)	175 ± 19.6	152.5 ± 11.2	155 ± 10.7	105 ± 9.5	82.5	55 ± 5
Number of xylem cells	7 ± 0.034	10 ± 0.088	11 ± 0.057	20 ± 2.9	33 ± 1.2	43 ± 4.4
Cambium	-	-	-	-	-	-

Table 3B

Anatomy of two week old excised roots

Distance from promeristem (mm.)	25	50	75	100	125	150	175
Diameter of the root (μ)	515	452.5	433	415.7	442.5 ± 17.2	440 ± 18	387.5 ± 15.2
Diameter of the stele (μ)	152.5 ± 8.1	166 ± 21.8	185.5 ± 14	220 ± 22.8	225	230.8	235
Thickness of cortical layer (μ)	180 ± 12.25	109 ± 14.4	110 ± 13.8	110	100 ± 2.4	97.5 ± 10.6	85 ± 7.4
Number of xylem cells	10.6 ± 1.8	15.3 ± 0.88	21.3 ± 1.46	27.6 ± 1.46	36.6 ± 3.7	47.3 ± 0.88	66 ± 17.5
Cambium	-	-	-	-	One complete row	Two rows	Two rows

Table 4A

Anatomy of four week old seedling roots

Distance from promeristem (mm.)	25	50	75	100	125	150	175	200
Diameter of the root (μ)	707.5 ± 42	655 ± 38	591.5 ± 29	505 ± 40	510 ± 33	592.5	612.5	666.6
Diameter of the stele (μ)	202 ± 9.2	210 ± 39	232.5	220.8	270 ± 16.9	325 ± 33	345 ± 31	372.5 ± 29
Thickness of cortical layer (μ)	242.5	217.5 ± 9.2	185	153	117.8 ± 3.76	135	135 ± 25	150
Number of xylem cells	6.6 ± 0.21	8.6 ± 0.76	13.6 ± 0.76	21 ± 0.43	40.6 ± 3.38	68 ± 9.9	72.6 ± 8.05	75 ± 2.3
Cambium	-	-	-	-	1 row	2 rows	3 rows	4 rows

Table 4B

Anatomy of four week old excised roots

Distance from promeristem (mm.)	25	50	100	125	150	175	200
Diameter of the root (μ)	439 ± 18	492.5	447.5	438	397.8 ± 19	390 ± 34	392.5
Diameter of the stele (μ)	155.8 ± 19.8	152.5 ± 23	167.5 ± 18.6	182.5 ± 17.6	175 ± 15.5	209 ± 8.98	211 ± 14.9
Thickness of cortical layer (μ)	121 ± 15.8	138 ± 37.7	137 ± 16.6	125 ± 16.7	103 ± 11.3	90 ± 11.6	55 ± 13.8
Number of xylem cells	9 ± 0.214	11.5 ± 0.67	21 ± 1.17	23 ± 1.18	37 ± 3.98	51 ± 1.52	55 ± 2.28
Cambium	-	-	-	-	1 row	1 row	2 rows

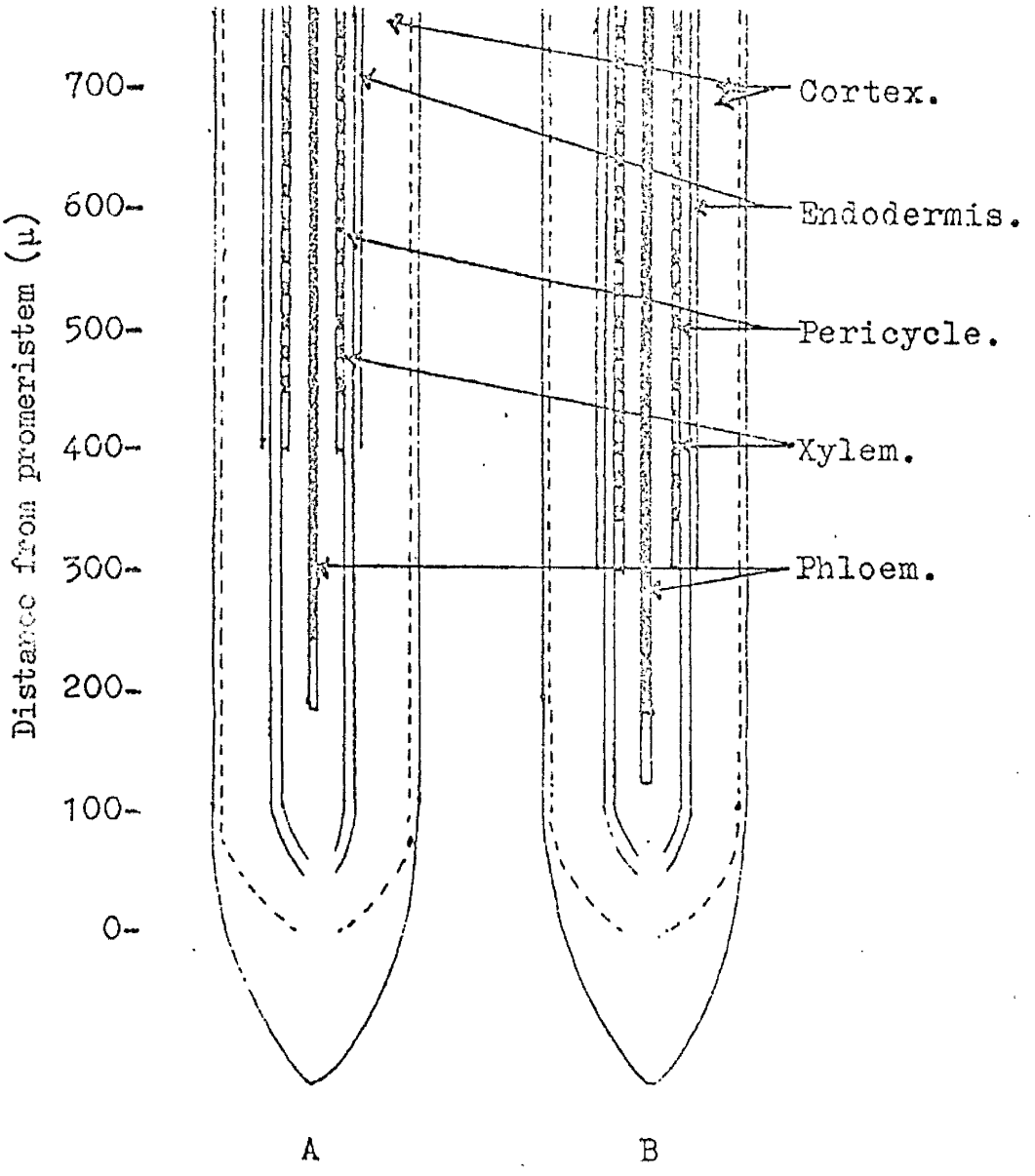


Fig. 3. Diagramatic representation of two week (A) and four week (B) old root apices.

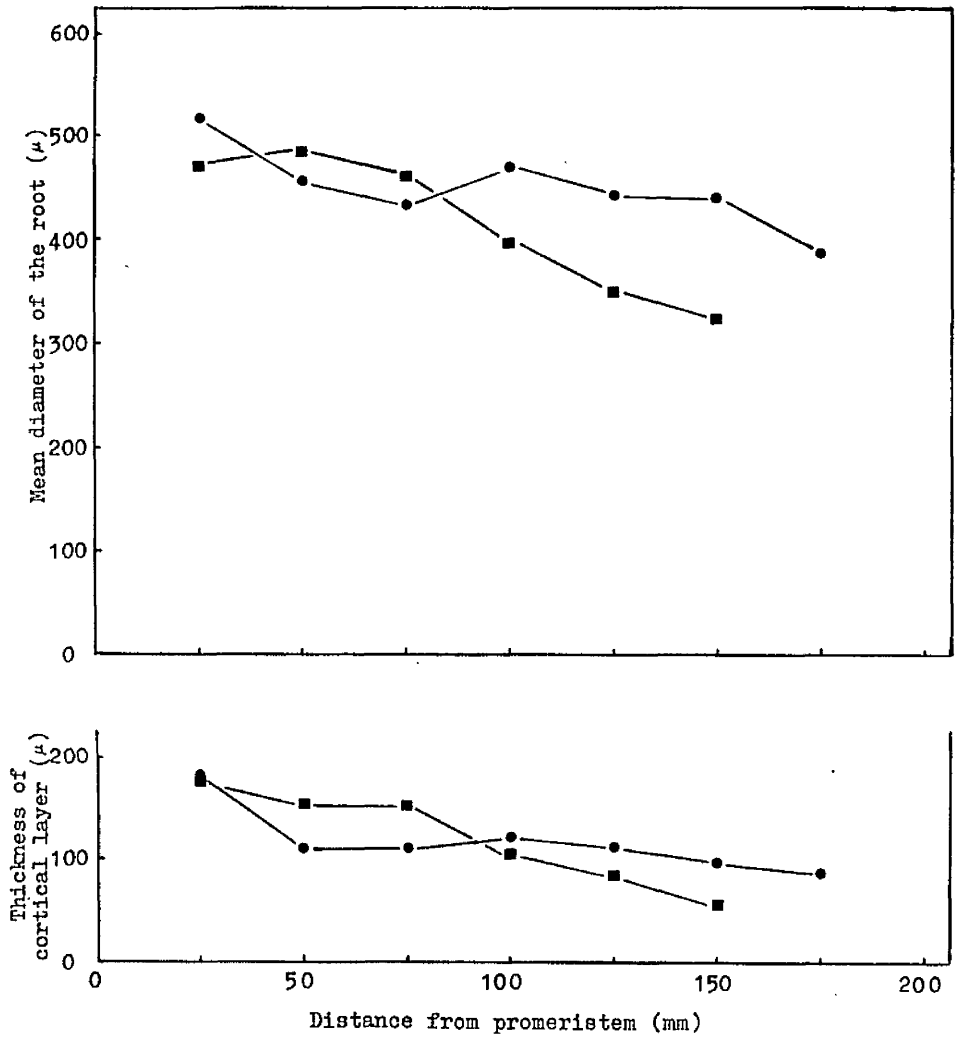


Fig. 4. A comparison of root diameters and thickness of cortical layers in two week old excised (●) and seedling (■) roots.

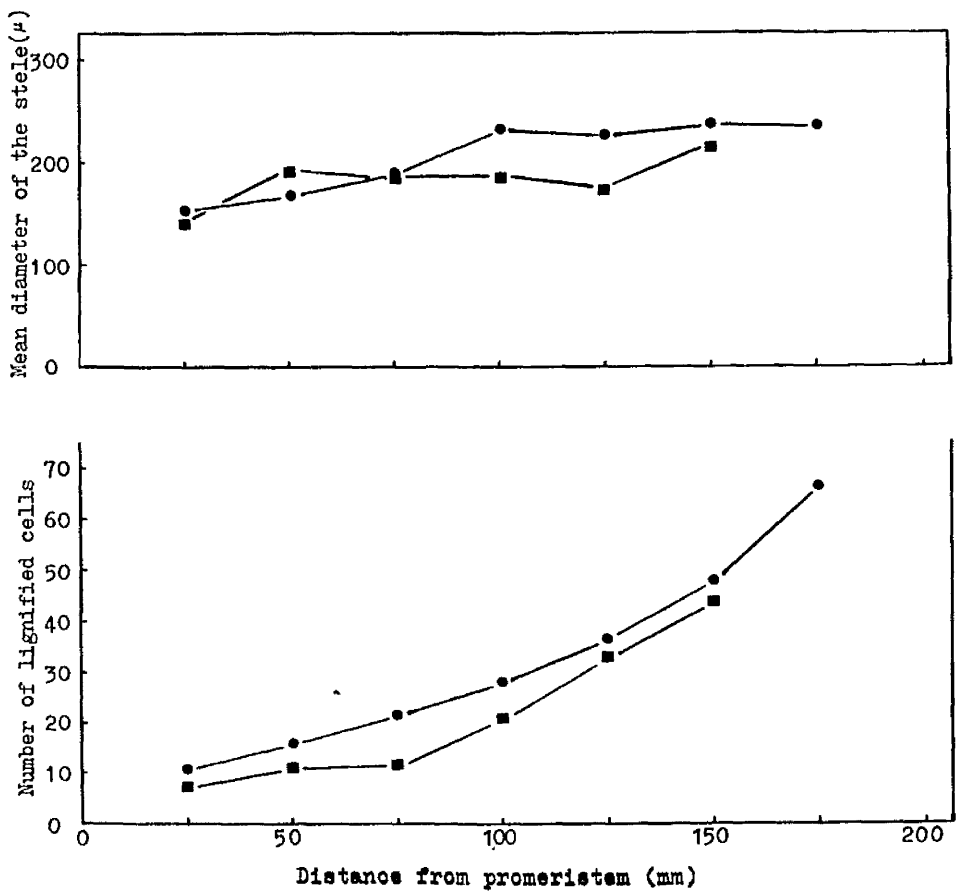
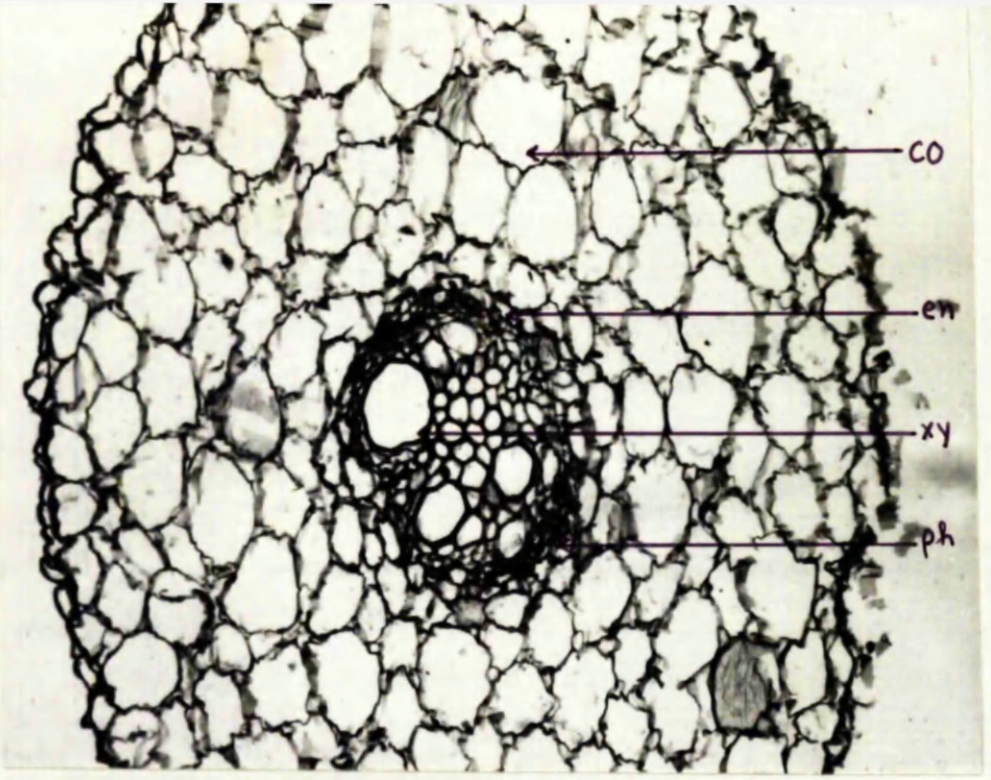
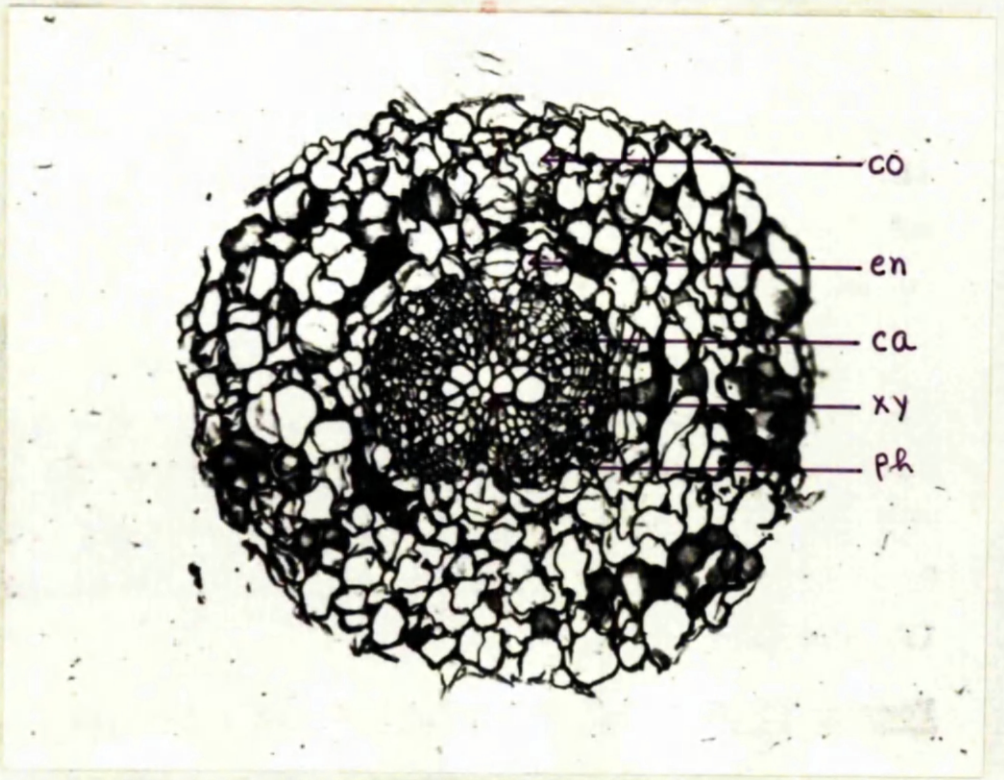


Fig. 5. A comparison of the diameter of stele and the number of lignified cells in two week old excised (●) and seedling (■) roots.



A



B

Fig. 6. Photomicrographs of transverse sections of two week old seedling (A)(X400) and excised (B)(X160) roots, 150 mm and 175 mm from the apices respectively.

co = cortex en = endodermis ca = cambium
xy = xylem ph = phloem

seedling and excised root apices. The xylem and phloem derivatives of the meristem were first detected at approximately the same distances behind the promeristems and there were approximately the same number of xylem and phloem cells in the primary tissues. A composite diagram based on the measurements obtained from the serial sections is presented in Fig. 3A.

Measurements of sections taken from segments at different distances from the promeristem are given in Tables 3A and 3B and Figures 4 and 5. The differences between the excised and seedling roots were as follows :-

1. The diameter of the seedling root decreases as the distance from the promeristem increases and this reflects a decrease in the thickness of the cortex. This was less marked in the excised roots.

2. At a distance of 100 mm. or more from the promeristem the diameter of the stele of the excised root was more than that of the seedling root. Also the number of xylem cells in the stele was greater for excised than for seedling roots.

3. No vascular cambium was observed in any part of the two week old seedling roots (Figure 6A). However, in excised roots a vascular cambium was clearly seen in segments taken at 175 mm. or further back from the promeristem. The cambium was sometimes seen as a complete ring but often it was incomplete (Figure 6B).

Four week old seedling roots and excised roots

These 4 week cultures were set up in 2 litre penicillin flasks as described in materials and methods (page 26). The growth and anatomy was recorded as in the previous experiment and the data are shown in Tables 1, 4A and 4B.

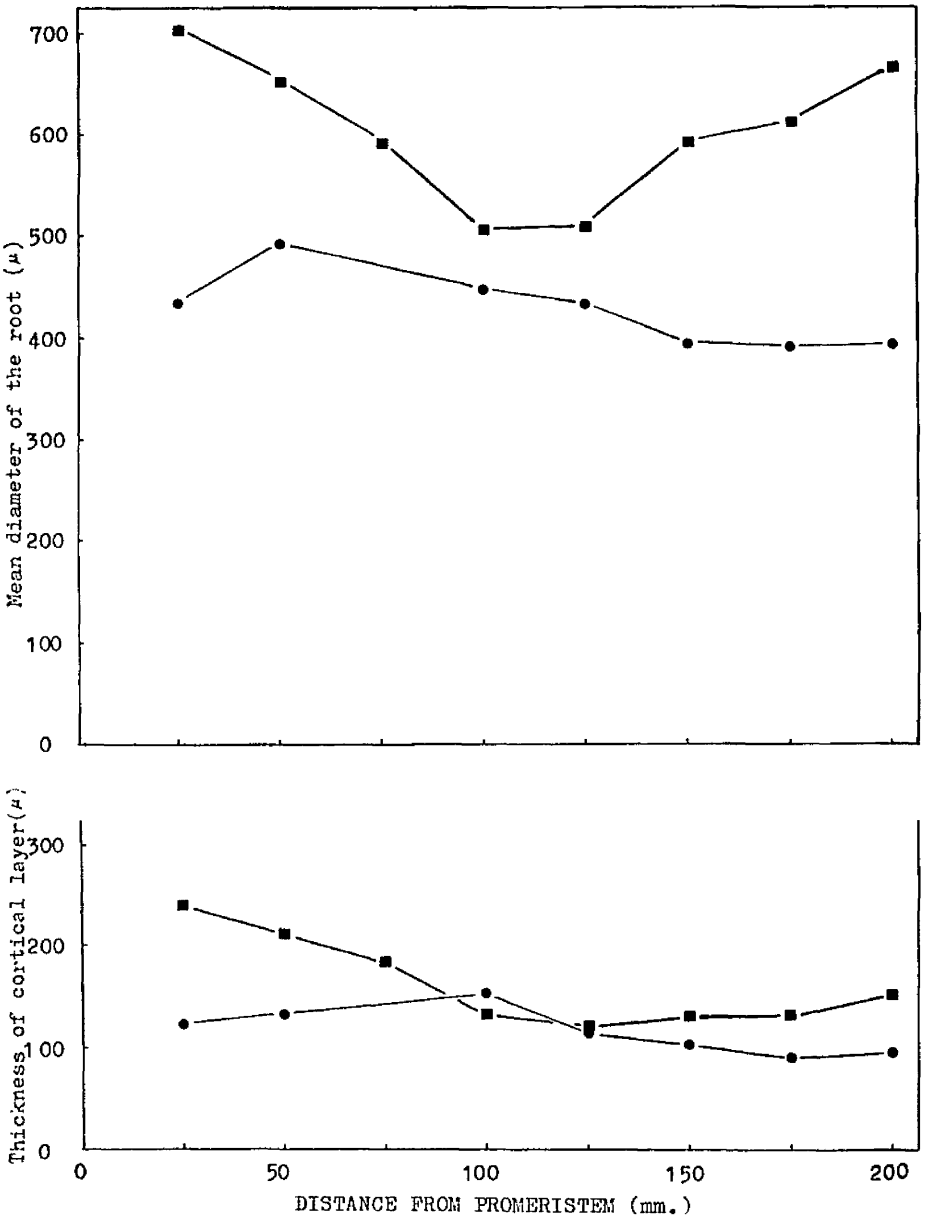


Fig. 7. A comparison of the root-diameter and the thickness of the cortical layer in four week old excised (●) and seedling (■) roots.

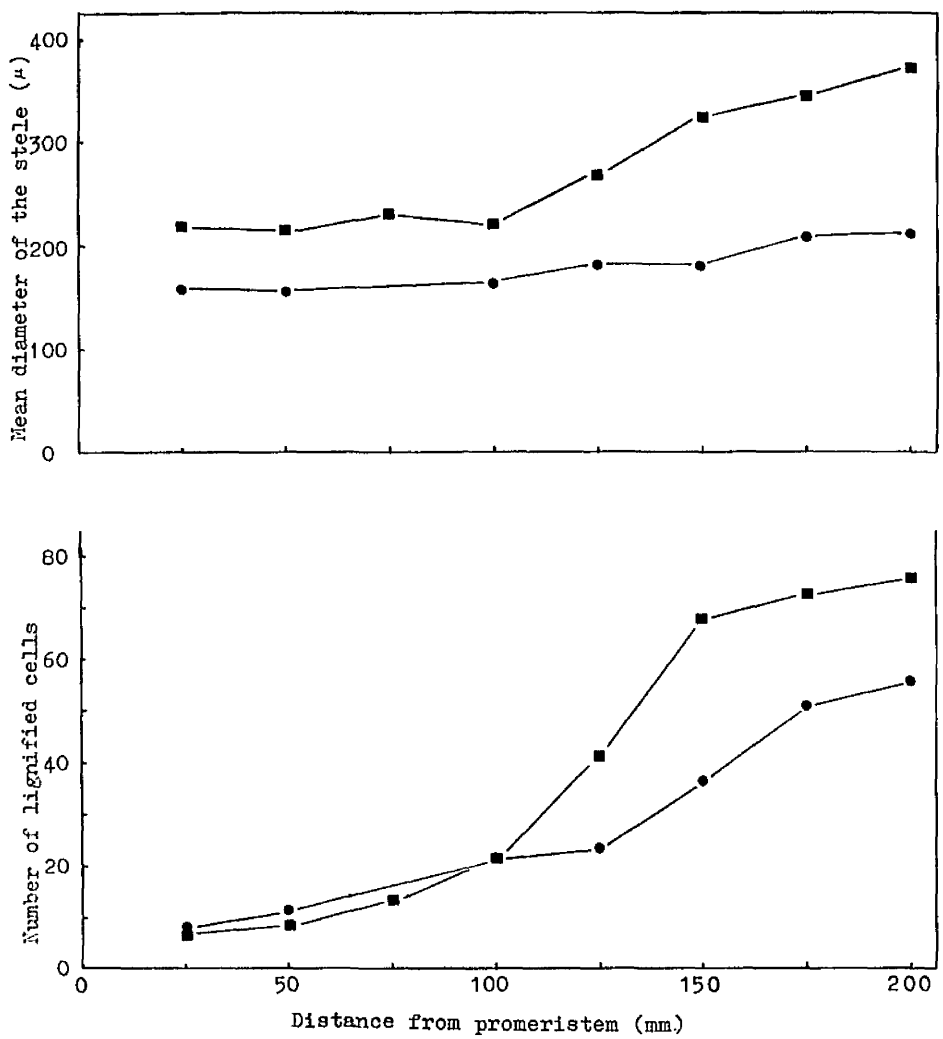
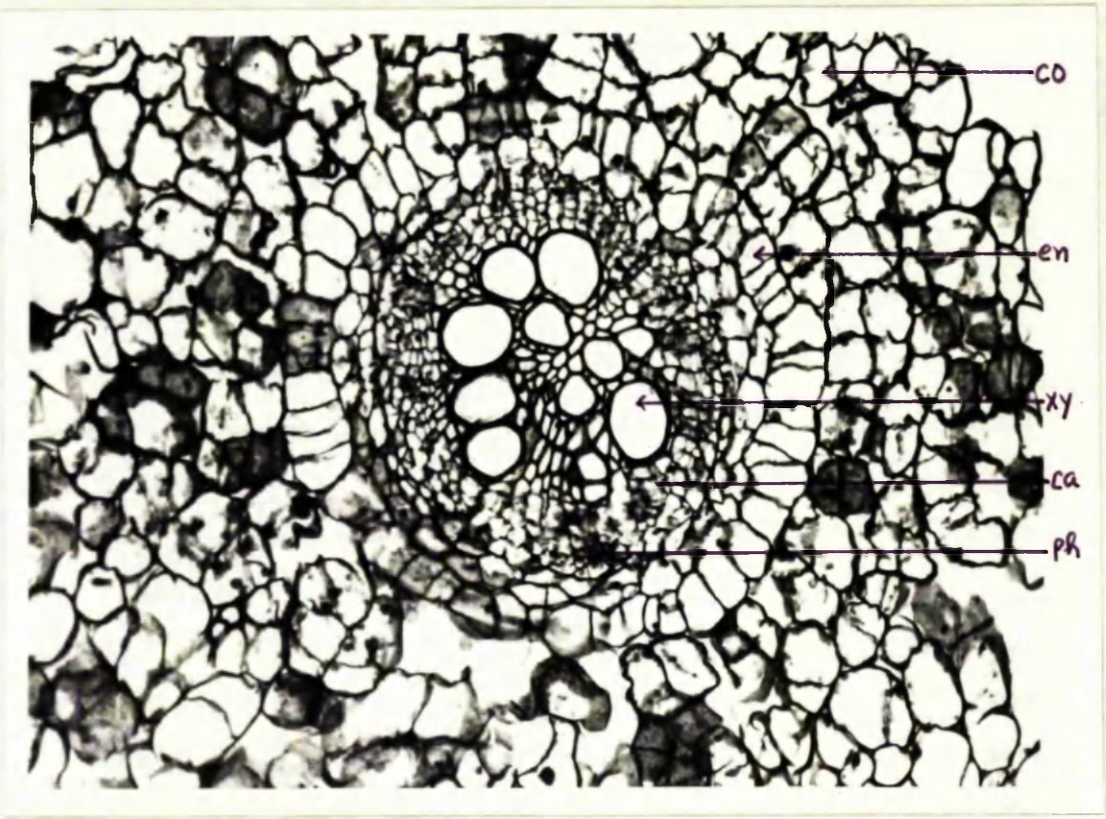
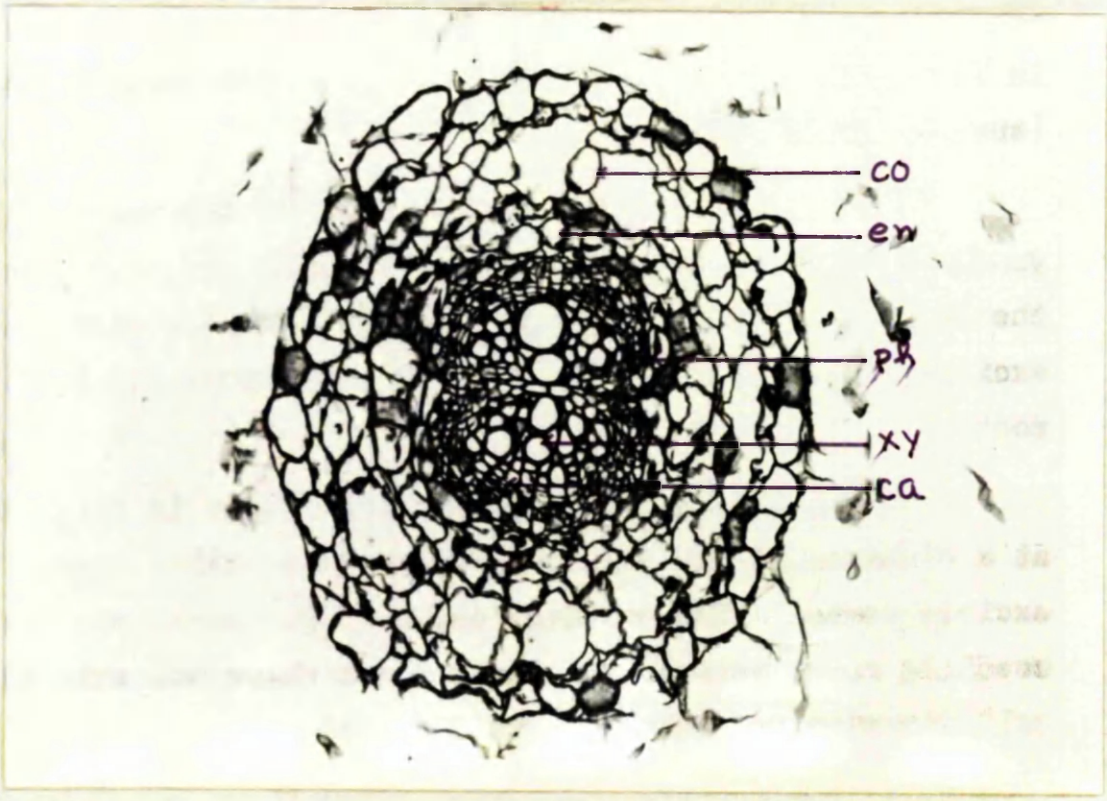


Fig. 8. A comparison of the stele-diameter and the number of lignified cells in four week old excised (●) and seedling (■) roots.



A



B

Fig. 9. Photomicrographs of transverse sections of four week old seedling (A)(X400) and excised (B)(X160) roots, 200 mm. from the apices.

As in the previous experiment the primary laterals of the excised roots were initiated in acropetal order whereas in the seedling root the arrangement was less regular.

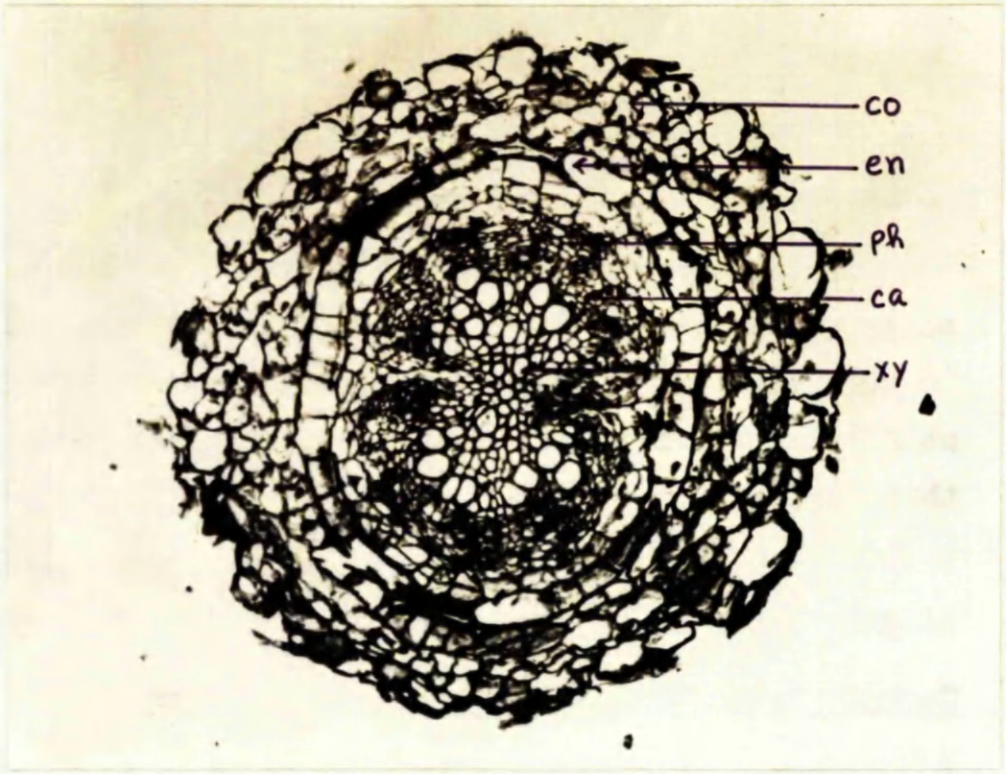
The results of the serial transverse sections of the apical region of excised and seedling roots are summarised in Figure 3B. There was little difference between four week old excised and seedling roots. However, in comparison with two week old seedling and excised roots, the derivatives of apical meristem differentiated closer to the apex. This may have been correlated with the decline in linear growth rate as previously reported for other dicotyledonous species (Heimsch, 1951; Clowes, 1961; Wilcox, 1962).

The measurements of the transverse sections of the segments taken from different distances from the apex are shown in Tables 4A and 4B. The observations made were as follows (summarised in Figures 7 and 8) :-

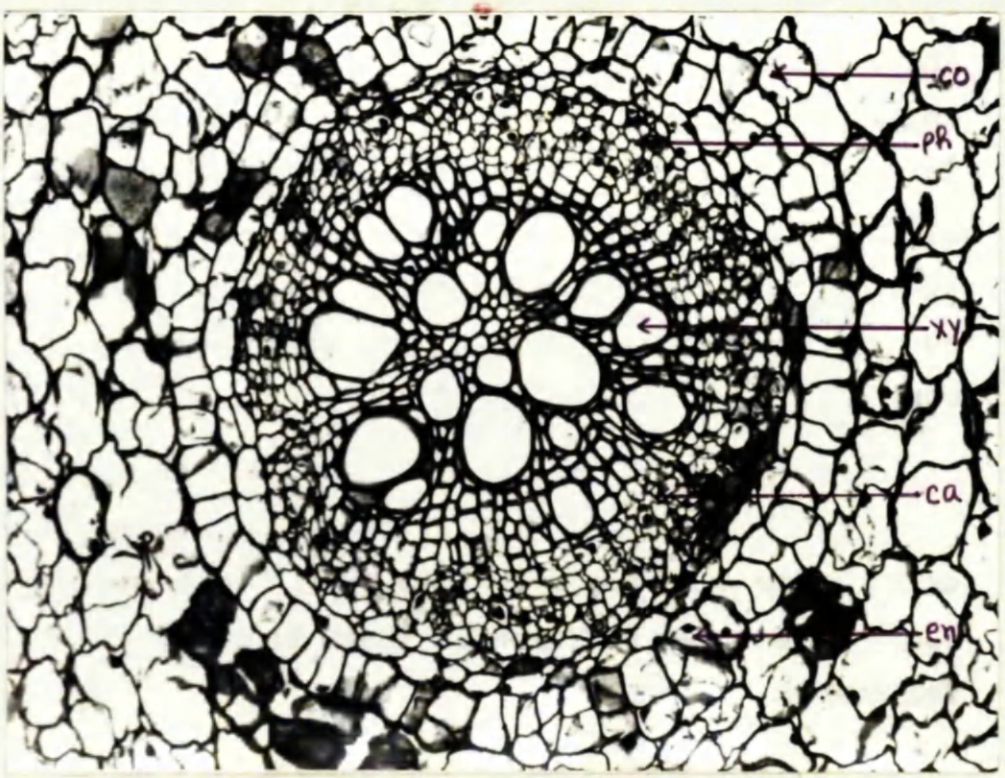
1. In the seedlings the diameter of the root decreases gradually from the promeristem to 100 mm. However, beyond this the diameter increases again. In contrast the diameter of the excised roots continues to decrease throughout the length of the root.

2. The thickness of the cortical layer is slightly increased at a distance of 100 mm. and greater in seedling roots, but not in excised roots. The cortical cells of the basal regions of seedling roots were closely packed and there was evidence that cell division had taken place (Fig. 9A).

3. The endodermis was very clear in seedling roots and in some cells transverse walls were observed, but in excised roots the endodermis was less obvious.



A



B

Fig. 10. Photomicrographs of transverse sections of basal parts of eight week old excised (A) (X160) and six week old seedling (B) (X400) roots.

4. The diameter of the stele was much greater in seedling roots than in the excised ones.

5. A very well organised vascular cambium was present in seedling roots at 175-200 mm. from the apex and further back (Figure 9A). In the case of excised roots a cambium was present at 200-220 mm. from the apex, but it was not better developed than that of two week old excised roots (Figure 9B).

6. There was much more xylem parenchyma present in the seedling roots than in the excised roots.

Cambial growth in cultures grown for longer periods in standard medium

The experiments described above showed that excised tomato roots when cultured for two weeks in standard medium initiated a vascular cambium. However this activity did not appear to be maintained as the four week old roots showed no further development. In contrast, although the cambium developed later in seedling roots, it appeared to be more active after four weeks. Cultures were set up and left for longer periods in order to confirm these findings. The roots were grown in 1 litre medium contained in 2 litre penicillin flasks.

The basal part of a 8 week old excised root is shown in transverse section in Figure 10A. The number of cambial derivatives were not increased from 4 week old excised roots but there were a few differences found in other tissues which are as follows :-

1. The cortex was more compact in 8 week old than in 4 week old excised roots grown in similar conditions. Transverse walls were present in some of the cortical cells of 8 week old roots indicating that cell division had occurred.

2. The endodermis was very clearly defined in comparison with two week and 4 week old excised roots.

The transverse section of the basal part of a six week old seedling root is shown in Figure 10B. The observations made were as follows :-

1. The cortical thickness was very much increased in comparison with four week old seedling roots. However, the cell size and the packing of the cells were not obviously different.

2. A large increase in the number of cambial derivatives indicated that the activity of the cambium had been maintained.

Conclusion

It is clear from the experiments described above that the excised tomato roots develop a vascular cambium if allowed to grow for two weeks or more. This cambium usually forms a complete ring but sometimes it is incomplete. Although the cambium is initiated, the duration of its activity seems to be limited since the number of derivatives in 4 week and 8 week old roots were only a little greater than in 2 week old roots. This suggests a difficiency in factors concerning cambial growth rather than cambium initiation. These findings are contrary to White's (1943) statement that secondary thickening does not occur in excised roottcultures. The reason for White's failure to demonstrate secondary thickening might have been the unsuitability of his original medium (the medium used in these studies was an 'improved' White's medium) or the short duration of each culture passage. Dormer and Street (1948) found that if excised tomato roots were left for 5 to 6 months in the same medium (either White's medium or White's medium supplemented with yeast extract) a certain amount of secondary tissue developed. However the cambium was often disorganised and it can be seen from their photographs that growth was much less than in the experiments described above. The earlier and better development of the cambium in these experiments might have been due to the 'improved' medium or possibly due to the different clonal material used. The excised roots of pea and radish fail to initiate a vascular cambium in a control medium (Torrey, 1963; Loomis and Torrey, 1964).

In comparison with the excised roots the cambium of seedling roots was initiated later but the growth was maintained throughout the duration of the experiments. The later initiation of the cambium might have been due to the growth delay due to germination, and this is supported by the observation that growth is similarly retarded.

THE EFFECT OF GROWTH REGULATORS ON THE SECONDARY GROWTH OF ISOLATED TOMATO ROOTS

In the following sections basically two types of experiments have been reported. In one type the roots were cultured totally immersed in a liquid medium supplemented with the appropriate growth factor(s). In the second type the roots were cultured with their bases inserted into an agar medium containing the growth factors and their tips were allowed to grow out into the standard liquid medium.

SECTION 2

THE EFFECT OF AUXINS ON GROWTH AND ANATOMY

The investigations reviewed in the introduction strongly implicate the auxins as substances important in determining cambial activity in both shoots and roots. Torrey and Shigemura (1957) showed that IAA induced vascular cambium in the 'initial tips' of pea, but failed to induce it in 'first transfer tips'.

In contrast to the work of Torrey and co-workers who used only initial and first transfer tips, the purpose of these investigations was to attempt to increase the cambial activity in continuously cultured clonal roots, i.e. roots which were originally derived from one seedling and maintained in culture for an indefinite time by subculturing every seven days into fresh medium.

Effect of IAA when the roots were totally immersed in liquid medium

Each of the root tips used for this experiment were taken from seven day old sector cultures and placed in 50 ml. medium containing the appropriate amount of IAA. Two concentrations of IAA were used - one (0.001 mg./l.) which inhibited linear growth of the main axis by approximately 50% and the other (0.00316 mg./l.) by approximately 75%. There were fifteen replicates for each treatment. After two weeks' incubation at 25°C, the growth of the roots was recorded as in the previous experiments.

For the anatomical investigations five replicates were taken for each treatment. Then each root was treated as follows: Three 10 mm. segments, starting 5 mm. from the cut end (so as to

Table 5A

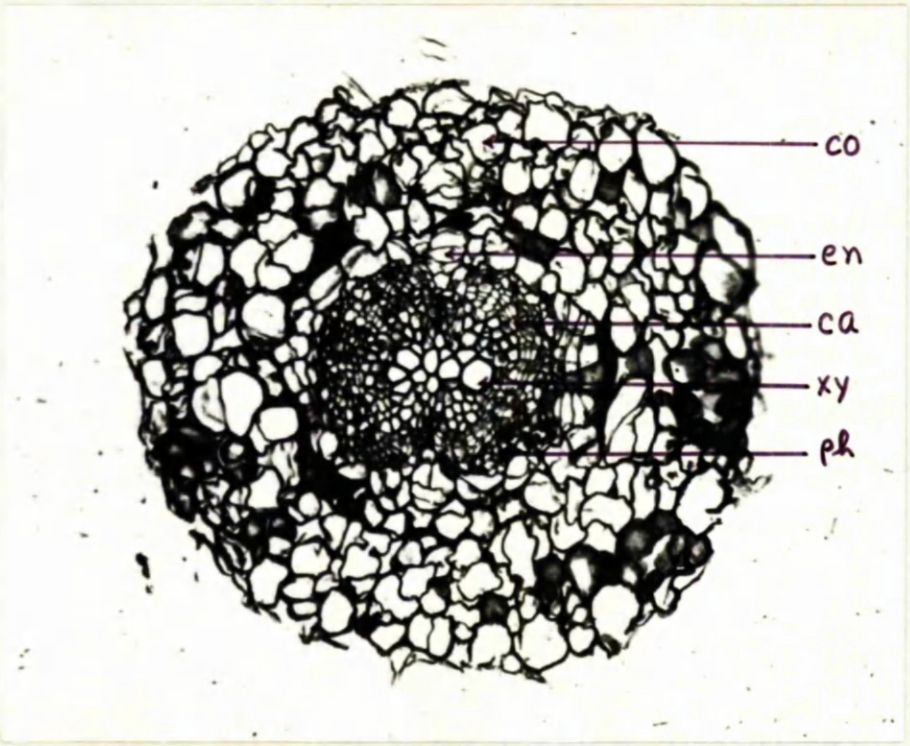
Effect of IAA on growth of the roots

Addition of IAA in mg/l.								
0			0.001			0.00316		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
144.5 ± 11	45 ± 9	385	85.5 ± 4	16 ± 1	182 ± 18	42.6 ± 1	5.4 ±0.7	57 ± 10

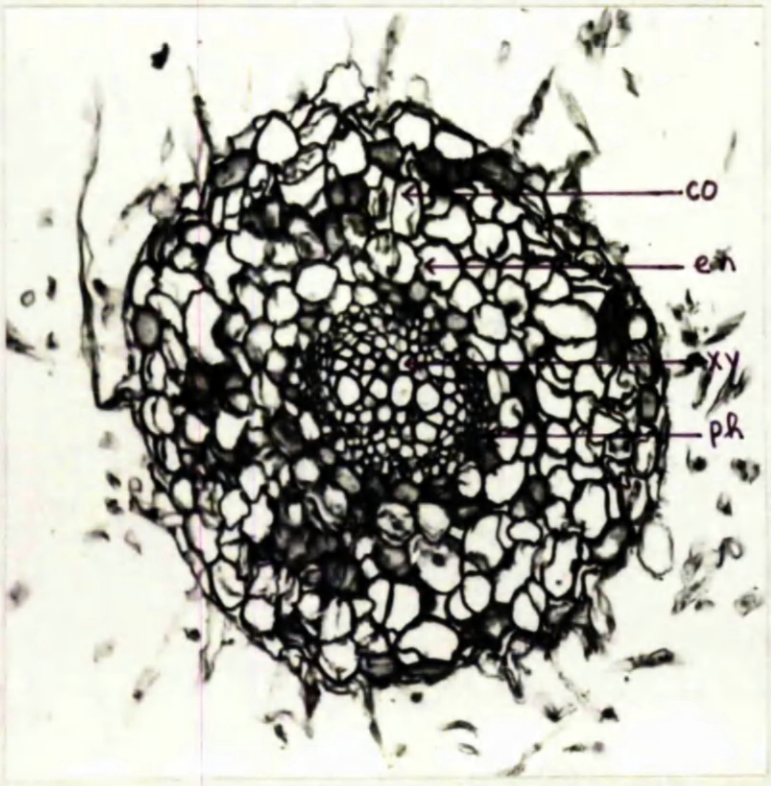
Table 5B

Effect of IAA on anatomy of roots

Addition of IAA in mg/l.	0			0.001			0.00316		
Distance from the base of the root (mm.)	10	20	30	10	20	30	10	20	30
Diameter of the root (μ)	411	405	406	429	395	421	394	392	395
Diameter of the stele (μ)	231 ± 12.7	226 ± 13.9	223 ± 10.4	211 ± 5.23	170 ± 10.4	169 ± 8.2	156 ± 6	165 ± 4.9	169 ± 10.7
Thickness of cortical layer (μ)	93	91	95	112	112	125	113	113	112
Number of xylem cells	37 ± 9.7	34 ± 6.6	25 ± 2.6	23 ± 4.4	20 ± 6.81	14 ± 1.46	14 ± 1.46	16 ± 8.3	11 ± 1.44
Cambium	2 rows	2 rows	-	-	-	-	-	-	-



A



B

C

Fig. 11. Photomicrographs of transverse sections of basal parts of roots grown in media containing 0.0 mg/l. (A), 0.001 mg/l. (B) and 0.00316 mg/l. (C) IAA (X160).

exclude wound tissue) were harvested and fixed. These segments were then embedded, cut at 12 μ and stained with Johansen's quadruple stain.

The growth measurements are shown in Table 5A. The auxin treatment, in addition to inhibiting linear growth, caused a marked swelling and browning of the tips and inhibited lateral development.

Examination of the transverse sections from the basal part of the root showed that the lower concentration of IAA had little or no effect on the diameter of the root whereas the higher concentration was slightly inhibitory (Table 5B). The diameter of the stele was very much decreased by the IAA treatment while the thickness of the cortical layer was significantly increased. The auxin treatment did not alter the basic primary distribution of vascular tissues (diarch) but it did markedly decrease the number of xylem cells in the stele (Figures 11A, 11B and 11C). There was no evidence that IAA enhanced vascular cambial activity. On the contrary, it appeared to inhibit the formation of a cambium.

Effect of NAA on growth and anatomy of the root

Previous investigations of the effects of auxins on the growth of excised tomato roots have indicated that NAA is more effective than IAA (Butcher, 1960)) and it has been tentatively suggested that the effect of NAA is similar to that of the hypothetical ageing hormone (Street, 1955).

NAA is also effective in initiating cambial activity in first transfer tips of pea (Torrey, 1963) and radish (Loomis and Torrey, 1964).

Table 6A

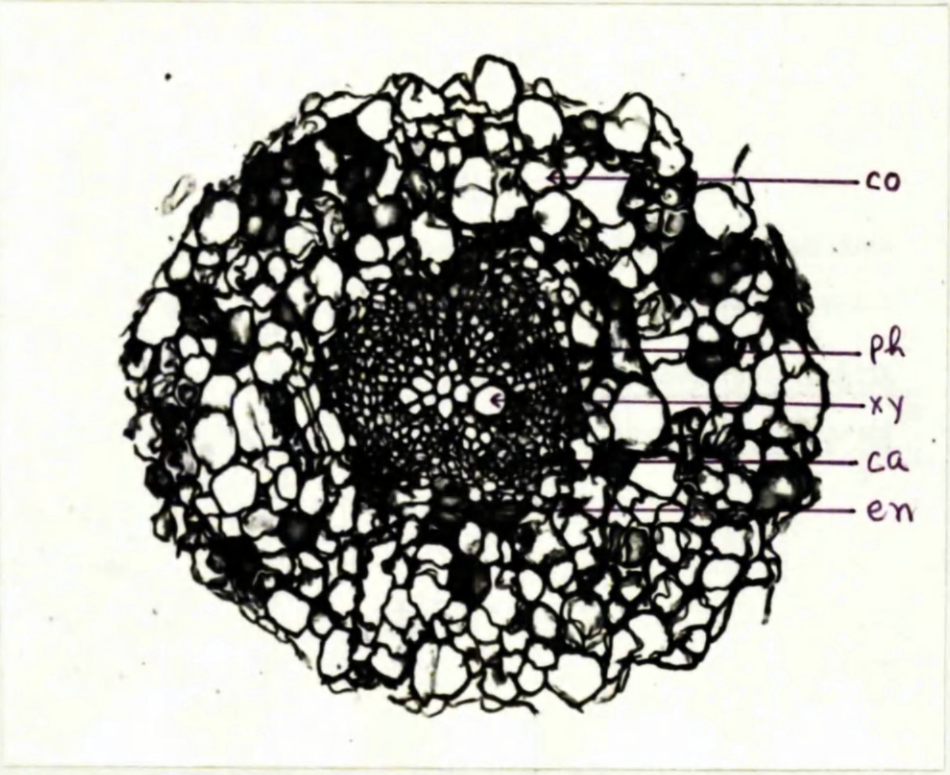
Effect of NAA on growth of roots

Addition of NAA in mg/l.								
	0		0.00075			0.0025		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
164 ± 5	34 ± 1	530	44 ± 1.9	9 ±0.8	120	43 ± 5	13 ± 2	119

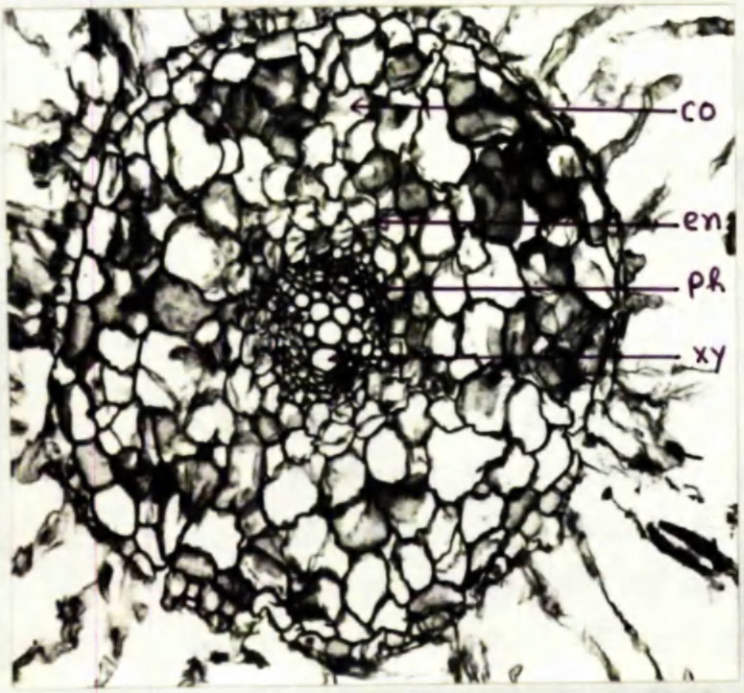
Table 6B

Effect of NAA on anatomy of roots

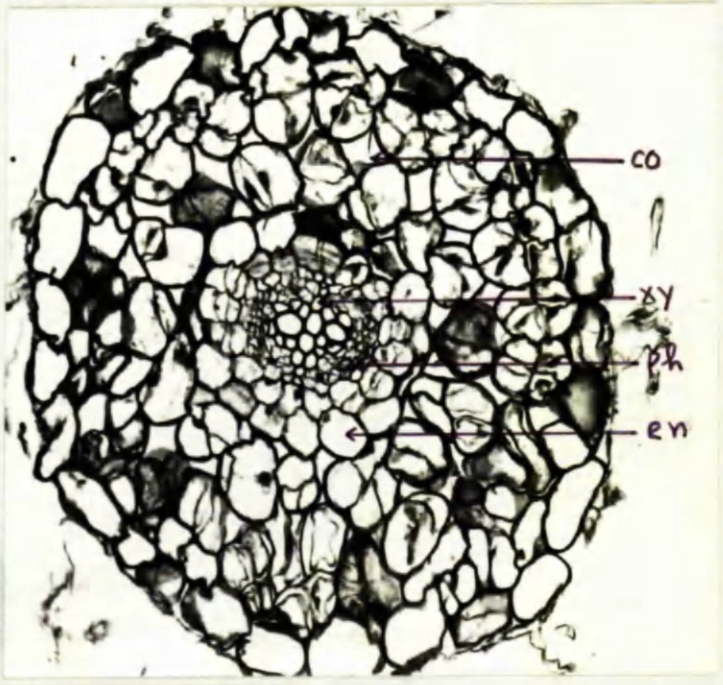
Addition of NAA in mg/l.	0			0.00075			0.0025		
Distance from the base of root (mm.)	10	20	30	10	20	30	10	20	30
Diameter of root (μ)	402	337	334	382	400	472	387	471	517
Diameter of stele (μ)	243 ± 4.9	184	168 ± 8.2	188.5 ± 9.3	175 ± 11.2	150 ± 10	190 ± 14	196 ± 13	160 ± 13
Thickness of cortical layer (μ)	79	76	87	117	117	139	98	148	117
Number of xylem cells	57 ± 10.2	48 ± 9.1	32 ± 3.8	16 ± 1.64	13 ± 0.6	12 ± 2.4	26 ± 8.5	22 ± 7.5	16 ± 1.64
Cambium	2 rows	2 rows	-	-	-	-	-	-	-



A



B



C

Fig. 12. Photomicrographs of transverse sections of basal parts of roots grown in media containing 0.0 mg/l (A), 0.00075 mg/l (B) and 0.0025 mg/l (C) NAA.

The experiments to be described here were carried out to see whether NAA was different from IAA in its effect on cambial growth.

Effect of NAA when the roots were totally immersed in liquid medium

The experiment was set up in the same way as for the previous experiment except that NAA was added to the medium at 0.00075 mg/l. and 0.0025 mg./l. instead of IAA. Fifteen replicates were used in each treatment and the cultures were kept in darkness at 25°C for two weeks. Growth was recorded and the anatomy examined as in the previous experiments.

The data for the growth measurements are shown in Table 6A. NAA, like IAA, inhibited the linear growth and the visible number of lateral roots and caused a swelling and browning at the main root apex.

Observations of the transverse sections of basal parts (Table 6B) revealed that NAA has similar effects on the anatomy of the root as IAA. The diameter of the root was markedly increased by NAA in 20 mm. and 30 mm. segments but the 10 mm. segments which included the tissues of the original inoculum were not significantly affected. The increase in diameter was entirely due to an increase in the width of the cortical cell layer and from the photographs (Figures 12A, 12B and 12C) it can be seen that this is largely due to an increase in cell size. This was more obvious with NAA than with IAA. In contrast to the control, the diameter of the stele in the basal part of the treated root was much less. As

Table 7A

Effect of IAA on growth when fed via the cut end

Addition of IAA in mg/l.								
0			0.001			0.00316		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
213.5 ± 4.7	55.6	887.7	196 ± 8.6	54	758	194 ± 13.8	49	755

with IAA, NAA did not influence the diarch vascular pattern and decreased the number of xylem elements. The transverse walls in endodermis and pericycle of treated roots were more noticeable in NAA treated roots (Figures 11B, 11C, 12B and 12C) than in IAA treated roots. There was no sign of vascular cambial activity in any of the NAA treated roots.

Effect of auxins on root growth and anatomy when fed via the cut end of the root

Experiments were set up in which the auxins (IAA and NAA) were fed via the cut end according to the procedure described in materials and methods (page 25). In these experiments complete White's standard medium was supplied in the flask whereas vitamins and sucrose were added to the vial. Root tips (20-25 mm.) were used and 15-20 replicates were set up for each treatment. After two weeks' incubation at 25°C in darkness, growth was recorded as usual.

For anatomical investigations five roots from each treatment were harvested and 10 mm. segments from the base cut by hand and stained temporarily by safranin O and glycerin.

Effect of IAA when supplied via the cut end of the root

The data for the growth measurements are shown in Table 7A. It can be seen that when auxin was supplied via the cut end, linear growth and lateral development was inhibited. In contrast to the previous experiments, where the roots were totally immersed in a liquid medium supplemented with auxin, there was no swelling or browning at the root apex.

Table 7B

Effect of IAA on anatomy when fed via the cut end

Addition of IAA in mg/l	0			0.001			0.00316		
Distance from the base of root (mm.)	10	20	30	10	20	30	10	20	30
Diameter of the root (μ)	351	-	-	445	-	-	450	-	-
Diameter of the stele (μ)	194 ± 5.5	-	-	241 ± 12	-	-	264 ± 11	-	-
Thickness of cortical layer (μ)	90	-	-	107	-	-	111	-	-
Number of xylem cells	34 ± 6.6	-	-	48 ± 3.9	-	-	53 ± 8.5	-	-
Cambium	-	-	-	-	-	-	-	-	-

Table 8A

Effect of NAA on growth when fed via the cut end

Addition of NAA in mg/l.								
0			0.00075			0.0025		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
213.5 ± 4.7	55.6	887	199.8 ± 17	51	556	193 ± 9.6	52	600

Table 8B

Effect of NAA on anatomy when fed via the cut end

Addition of NAA in mg/l.	0	0.00075	0.0025
Distance from the base of root (mm.)	10	10	10
Diameter of the root (μ).	345	425	405
Diameter of the stele (μ)	194 ± 5.5	296 ± 9.7	250 ± 7.2
Thickness of cortical layer (μ)	95.5	78	75
Number of xylem cells	34.8 ± 8.2	48.6 ± 3.9	54 ± 9.1
Cambium	-	-	-

An examination of the transverse sections of the basal parts of roots showed that IAA increased the diameter of the roots by approximately 25% (Table 7B). The diameter of the stele and the number of xylem cells inside the stele was increased. Unlike the roots bathed in auxin, the roots treated via the base had no transverse walls in the pericycle or endodermis. The treatment failed to initiate a vascular cambium.

Effect of NAA on growth and anatomy when supplied via the cut end of the root

In this experiment two concentrations of NAA (0.00075 and 0.0025 mg/l.) were tested and the results have been summarised in Tables 8A and 8B. The higher concentration of the auxin inhibited the linear growth of the main axis but did not cause browning or swelling at the root apex. The lower concentration had little effect on growth.

The effects of NAA on the anatomy of the basal part of the root were very similar to those of IAA. The diameter of the root and the number of xylem cells in the root were increased but there was no sign of any cambial activity.

Conclusions

The auxins at the concentrations tested did not enhance the activity of the cambium when added to the liquid medium. On the contrary they seemed to prevent the initiation of a vascular cambium in the basal regions of the excised roots. The diameter of the stele was decreased and there were fewer lignified cells.

Torrey and Shigemura (1957) working with isolated pea roots found that when the roots were allowed to grow on an agar medium containing auxins, the 'initial tips' developed a cambium whereas the 'first transfer tips' did not. They concluded that 'initial tips' having been freshly excised from a direct supply of materials stored in the cotyledons were rich in substances essential for vascular cambium initiation when stimulated by auxins, but that these tips cultured for a week on control media used up the materials.

Hughes and Street (1960) have demonstrated that similar inhibitory concentrations of IAA increased the diameter of regions near to the apices of excised tomato roots and the number of xylem cells within the stele, but no cambium was observed. These authors did not examine the basal regions of the roots.

In the experiments where the auxins were supplied via the cut end, no cambium was seen, however in the treated roots the diameters of the roots and the stele and the number of xylem cells were increased.

When Torrey (1963) similarly fed auxin via the root base, together with a high concentration of sugar (4%), first transfer pea roots developed a disorganised cambium. However, similar experiments with first transfer radish roots produced no cambium (Loomis and Torrey, 1964).

Table 9

Effect of kinetin on growth of roots.

Addition of kinetin in mg/l.												
0			0.003125			0.00625			0.0125			0.025		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
180±13	55±4	910	180±17	46±3	855	176±9	42±3	659	163±10	20±3	411	111±6	10±2	163

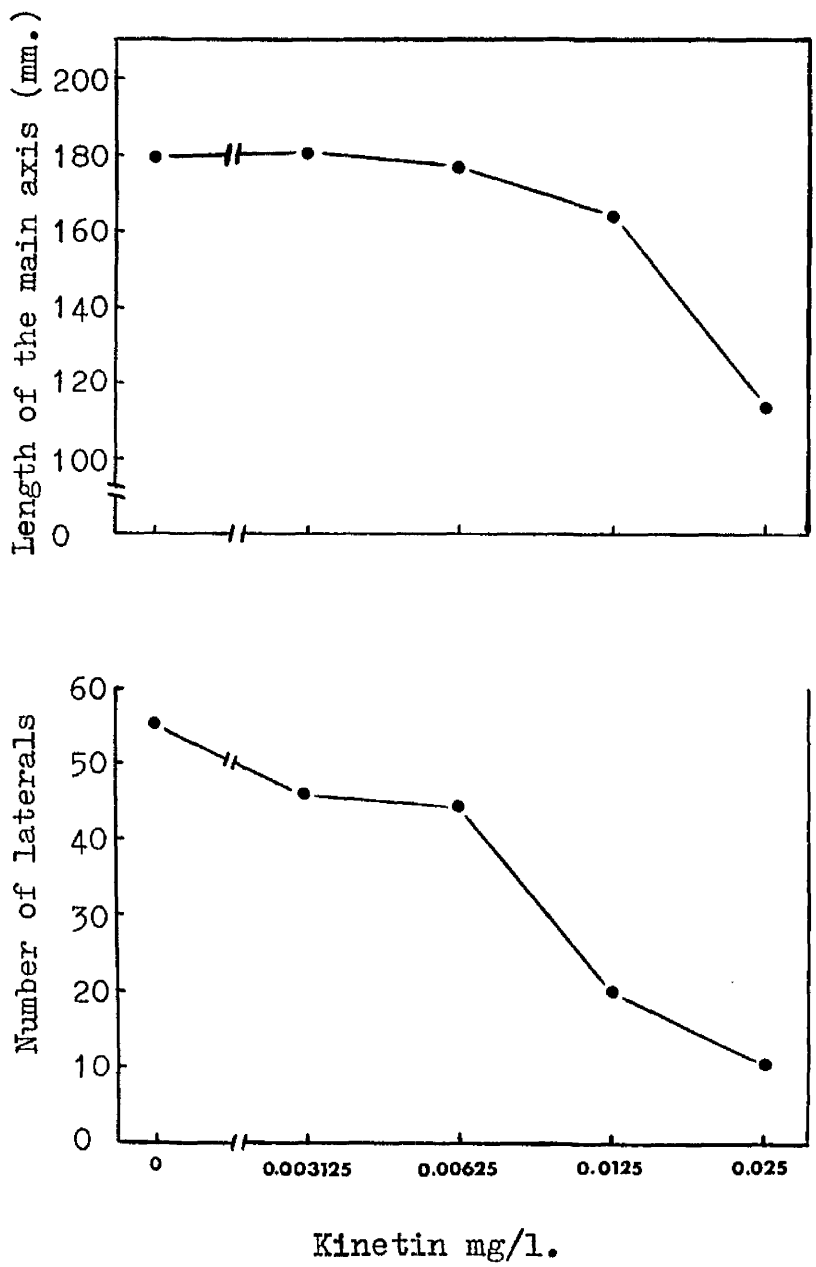


Fig. 13A. Effect of kinetin on the growth of roots.

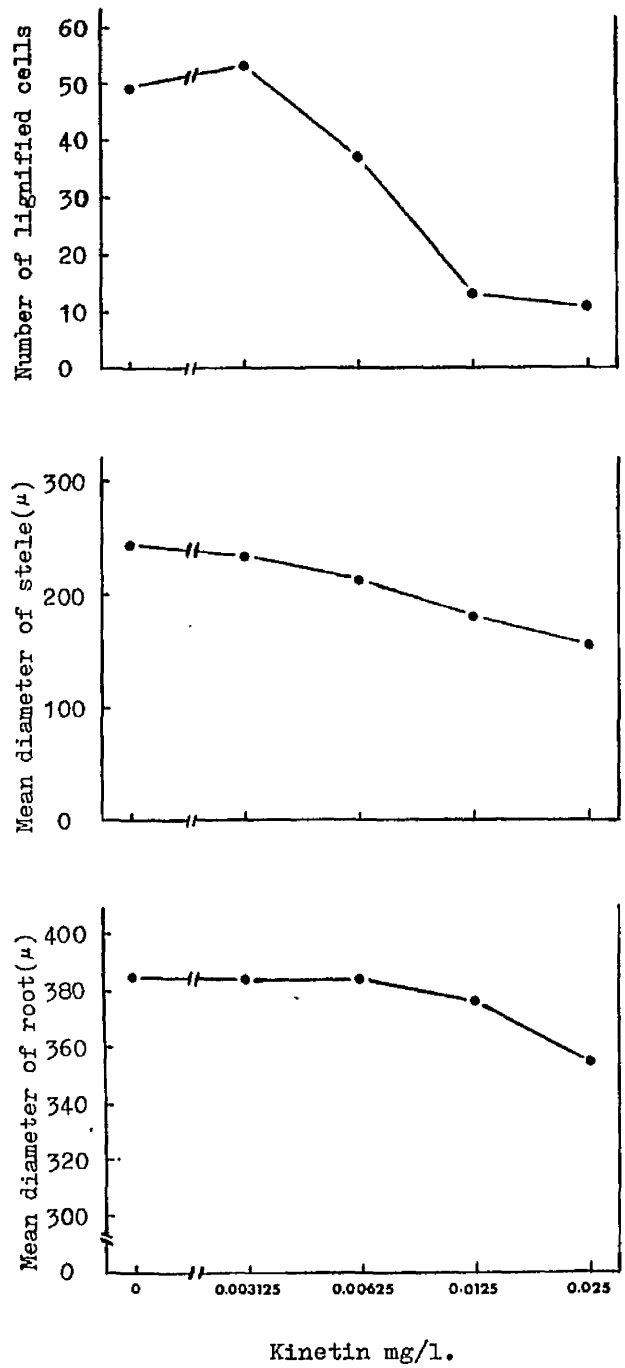


Fig. 13B. Effect of kinetin on the anatomy of roots.

SECTION 3

THE EFFECT OF 6-FURFURYLAMINOPURINE (KINETIN) AND 6-BENZYLAMINOPURINE (BA) ON GROWTH AND ANATOMY OF EXCISED ROOTS

Torrey (1963) found with 0.5 mm. decapitated and isolated pea roots that when IAA at 10^{-5}M and kinetin at 5×10^{-6}M were supplied to the medium the vascular pattern was changed from radial and alternate to a concentric arrangement with a vascular cambium. With isolated radish roots Loomis and Torrey (1964) reported that in addition to IAA and meso-inositol, cytokinins (kinetin and 6-benzylamino-purine) were essential for cambial activity.

The experiments in this section were carried out to see whether cytokinins are effective in promoting cambial activity in excised tomato roots. As in the previous section, growth substances were tested in two ways. In the first the roots were cultured in a cytokinin supplemented medium and in the second the roots were fed with cytokinin via the cut end of the roots.

Effect of kinetin when roots were totally immersed in the liquid medium

The experimental 'set-up' was the same as in the experiments with auxins except that kinetin was tested at four concentrations. The growth and anatomy was examined after two weeks.

The results (Table 9 and Figures 13A and 13B) show that kinetin, particularly at the higher concentrations, inhibited the growth of the main axis and the lateral roots. At the highest concentration of kinetin the roots were whiter as compared to the controls and the root hairs were more prominent. In 0.025 mg./l. the roots were distorted and some of them were coiled.

Table 10

Effect of kinetin on anatomy of basal part of root

Addition of kinetin in mg/l.	0	0.003125	0.00625	0.0125	0.025
Diameter of the root (μ)	385	383	384	377	352
Diameter of the stele (μ)	243 ± 4.9	232 ± 12.9	217 ± 6.9	176 ± 4	155 ± 19
Thickness of cortical layer (μ)	90	92	91	93	94
Number of xylem cells	48 ± 3.9	53 ± 5.2	37 ± 5.7	13 ± 1.11	11 ± 0.037
Cambium	2 rows	2 rows	-	-	-

Table 11

Effect of 6-benzylaminopurine on the growth of roots .

Addition of 6-benzylaminopurine in mg/l.														
0			0.003125			0.00625			0.0125			0.025		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
217±8	63	801	82±7.5	6	115	61±3.8	4	42	49±2.4	1	9	43±3.1	0.4	5

Table 12

Effect of 6-benzylaminopurine on anatomy of basal part of root

Addition of 6-benzylamino-purine in mg/l.	0	0.003125	0.00625	0.0125	0.025
Diameter of the root (μ)	386	352	345	321	300
Diameter of the stele (μ)	243 ± 4.9	212 ± 9.5	198 ± 10.5	172 ± 2.7	155 ± 19
Thickness of cortical layer (μ)	90	80	73	75	70
Number of xylem cells	53 ± 6.2	48 ± 3.9	24 ± 1.7	11 ± 0.031	7 ± 0.068
Cambium	2 rows	-	-	-	-

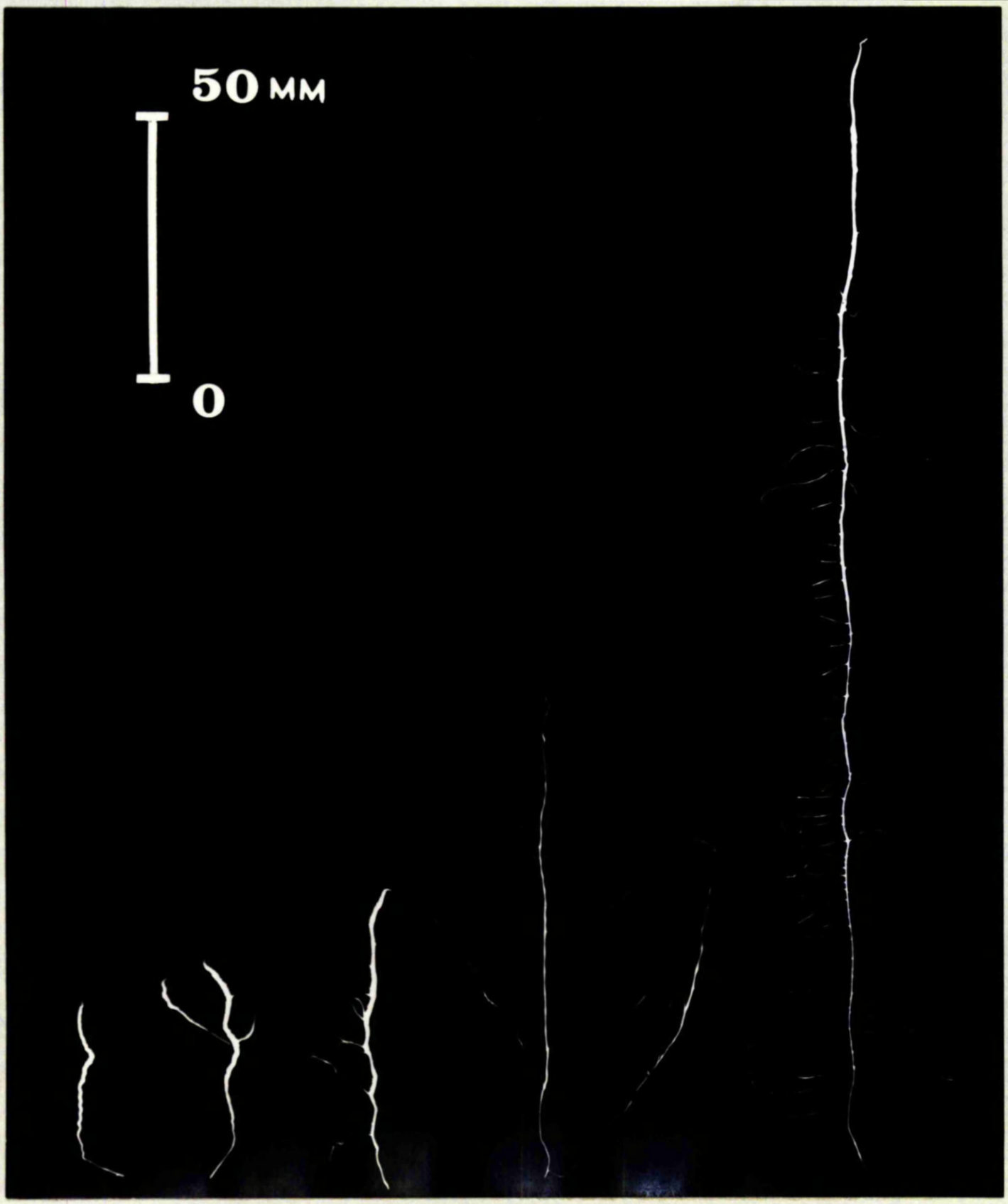


Fig. 14A. Shadowgraph of roots grown for 15 days in different concentrations of 6-benzylaminopurine.

A. 0.025 mg/l B. 0.0125 mg/l C. 0.00625 mg/l
D. 0.003125 mg/l and E. 0.0 mg/l.

Fig. 14B. Roots grown for 15 days in the presence of 0.025 mg/l. 6-benzylaminopurine showing coiling (X5).

Transverse sections of the basal parts of the roots revealed that the diameter of the root was slightly reduced in 0.0125 mg/l. and 0.025 mg/l. of kinetin (Table 10). These higher concentrations of kinetin markedly reduced the diameter of the stele and the number of xylem cells. Another obvious effect was that at high kinetin levels the endodermal cells lacked thickened lignified walls. No cambium was observed with 0.125 mg/l. and 0.025 mg/l. kinetin.

Effect of 6-benzylaminopurine on growth and anatomy of the root

6-benzylaminopurine was tested at the same concentrations and under similar conditions as kinetin. The growth measurements are shown in Table 11. The data show that all concentrations inhibited the length of the main axis and the number of laterals. At the concentrations of 0.0125 mg/l. and 0.025 mg/l. the roots were distorted and coiled (Figures 14A and 14B).

The measurements from the transverse sections of the basal part of the root, given in Table 12, show that the effects of 6-benzylaminopurine on the anatomy of the root were similar to those of kinetin. The diameter of the root was slightly reduced. The diameter of the stele and the number of xylem cells also were markedly decreased. No cambium was observed in any of the treated roots.

Effect of kinetin and 6-benzylaminopurine on growth and anatomy when fed via the cut end of the root.

The experiments were set up as described in the materials and methods (page 25). The kinins were supplied together with 2% sucrose and vitamins in the vial whereas the

Table 13

Effect of kinetin on growth when fed via the cut end

Addition of kinetin in mg/l.								
0			0.00625			0.025		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
172.7 ± 16	31	707	210 ± 5.4	37	1022	183 ± 5.7	33	844

Table 14

Effect of Kinetin on anatomy of basal part of root

Addition of kinetin in mg/l.	0	0.00625	0.025
Diameter of the root (μ)	448.5	352.5	348
Diameter of the stele (μ)	243 ± 4.9	198.5 ± 10.5	194.5 ± 5.5
Thickness of cortical layer (μ)	100.5	88.5	75.5
Number of xylem cells	37.6 ± 3.4	34.4 ± 2.9	32.6 ± 2.4
Cambium	-	-	-

Table 15

Effect of 6-benzylaminopurine on growth when fed via the cut end

Addition of 6-benzylaminopurine in mg/l.												
0			0.00625			0.0125			0.025		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
172.7±16	38	792	202.7±10	44	871	191.6±9.2	43	891	186±4.7	45	939

Table 16

Effect of 6-benzylaminopurine on anatomy of basal part of root

Addition of 6-benzylamino-purine in mg/l.	0	0.00625	0.0125	0.025
Diameter of the root (μ)	448	360	351.5	349.2
Diameter of the stele (μ)	243 ± 4.9	198 ± 10	194 ± 5	186 ± 7.1
Thickness of cortical layer (μ)	105	102	89	73.5
Number of xylem cells	40 ± 5	39 ± 5.7	32 ± 2.4	26 ± 8.4
Cambium	-	-	-	-

flask contained the standard medium. There were 15-20 replicates for each treatment and the cultures were incubated for two weeks in darkness. Growth measurements and anatomical examinations were done as before.

Effect of kinetin

As shown by the growth measurements (Table 13), kinetin stimulated the linear growth of the main axis and even at the highest concentration used it was not inhibitory. It did not markedly influence lateral development. The kinetin treated roots had prominent root hairs along their whole length.

Kinetin reduced the diameter of the root and the diameter of the stele (Table 14). Theeffect on the number of xylem cells within the stele was less marked than in the immersed roots. No cambium was observed in any of the roots.

Effect of 6-benzylaminopurine

In this experiment three concentrations (0.00625, 0.0125 and 0.025 mg/1.) were tested and the results are shown in Tables 15 and 16. The effect of 6-benzylaminopurine was similar to that of kinetin. The length of the main axis was increased particularly in the lowest concentration (0.00625 mg/1.) but the growth of the laterals was only slightly increased.

BA decreased the overall diameter of the root, the diameter of the stele, the width of the cortical layer and the number of xylem cells within the stele. No cambium was seen in any of the treatments.

Conclusions

The cytokinins (kinetin and 6-benzylaminopurine) at the concentrations tested did not enchance the activity of the cambium. It was shown that they markedly reduced the diameter of the stele and the number of lignified xylem cells within the stele. The cytokinins had little effect on the the anatomy when supplied via the base. It has been reported (Torrey, 1963; Loomis and Torrey, 1964) that cytokinins when supplied alone do not stimulate cambium activity in first transfer pea and radish roots.

Excised tomato roots growing totally immersed in the cytokinin supplemented medium were markedly inhibited and distorted whereas roots treated with similar concentrations via the cut end were stimulated. This difference could be explained by arguing that cytokinin absorption through the base and its translocation was so slow that it reached a stimulatory and not an inhibitory concentration in the tip region. There have been several reports that cytokinins have a low mobility in plants (Miller, 1961). Butcher and Street (1960b) found that kinetin prolonged the period of high growth rate in excised tomato roots when cultured in media having high levels of sucrose (3%). However, with media containing lower levels of sucrose, only inhibition of linear growth was observed.

Table 17 A

Effect of gibberellic acid on the growth of roots

Addition of gibberellic acid in mg/l												
0			1			5			50			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
176.6±10	48	776	149.6±10	46	374	115.6±5.2	40	229	59±3.6	12	26

Table 17B

Effect of gibberellic acid on anatomy of basal part of root

Addition of gibberellic acid in mg/l.	0	1	5	50
Diameter of the root (μ)	411	390	300	270
Diameter of the stele (μ)	232 ± 12	212.5 ± 3.3	155 ± 19	155 ± 19
Thickness of cortical layer (μ)	93	90	75	60
Number of xylem cells	53 ± 8.5	44 ± 2.5	23 ± 1.9	16 ± 0.894
Cambium	2 rows	-	-	-

SECTION 4

EFFECT OF GIBBERELLIC ACID ON GROWTH AND ANATOMY OF EXCISED TOMATO ROOTS

Bradley and Crane (1957) found that gibberellic acid (GA_3), when sprayed on the shoot, stimulated the cambial activity in the stems of apricot spur shoots. Wareing (1958) reported that gibberellic acid had little effect when added alone but when added together with an auxin it gave a pronounced increase in cambial activity in disbudded woody shoots. In the case of excised roots of pea (Torrey, 1963) and radish (Loomis and Torrey, 1964) it was found that gibberellin had no effect, when supplied via the base, on cambial activity whether it was added alone or together with other growth substances.

The effect of gibberellic acid on growth and anatomy of excised tomato roots was tested and has been reported in this section.

Effect of gibberellic acid when roots were totally immersed in the liquid medium

Three concentrations of gibberellic acid were tested. The results (after two weeks) are summarised in Tables 17A and 17B. The growth of the main axis and the number of laterals were inhibited by gibberellic acid, particularly at higher concentrations (5 mg/l. and 50 mg/l.).

Gibberellic acid reduced the diameter of the root, the diameter of the stele, the thickness of the cortical layer and the number of xylem cells inside the stele at the concentrations of 5 and 50 mg/l. However, roots grown in 1 mg/l. were not very much different from controls. No cambium was observed in any of the treated roots.

Table 18A

Effect of GA_3 on growth of the root when fed via the cut end

Addition of GA_3 in mg/l.								
0			5			50		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
206 ± 7.7	39	737	190 ± 12	45	861	48 ± 4.1	12	97

Table 18B

Effect of GA_3 on anatomy of basal part of root

Addition of GA_3 in mg/l.	0	5	50
Diameter of the root (μ)	448	386	335
Diameter of the stele (μ)	243 ± 4.9	220 ± 9.2	186 ± 7.1
Thickness of cortical layer (μ)	100	88	75
Number of xylem cells	53 ± 8.5	48 ± 4.1	37 ± 9
Cambium	-	-	-

Effect of gibberellic acid on growth and anatomy when supplied via the cut end of the root

The growth measurements taken after two weeks of incubation are given in Table 18A. Gibberellic acid at 50 mg/l. inhibited both the length of the main axis and the number of laterals per root. However 5 mg/l. had little effect on the growth of the root.

Gibberellic acid reduced the overall diameter of the root and the stele and the number of xylem cells inside the stele. No cambium was observed in any of the treated roots (Table 18B).

Conclusions

Gibberellic acid at the concentrations tested did not stimulate the cambial activity in excised tomato roots. At a high concentration (50 mg/l.) it reduced the diameter of the root and the stele and the number of xylem cells. The effects on root anatomy were very similar to those of the auxins.

The observations made here were similar to those of Torrey (Torrey, 1963; Loomis and Torrey, 1964) who found that GA_3 was inactive in initiating a cambium in first transfer tips of pea and radish.

Table 19 A

Effect of meso-inositol on the growth of the roots

Addition of meso-inositol in mg/l.												
0			50			100			200			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
198.6±7	51.5±3	955.5	215±13	60±2	1092	215±13	68±6	1201	217±7	62±3	1133

Table 19B

Effect of meso-inositol on anatomy of basal part of root

Addition of meso-inositol in mg/l.	0	50	100	200
Diameter of the root (μ)	420	425	388	355
Diameter of the stele (μ)	232 $\pm$ 12.9	235 $\pm$ 6.1	217 $\pm$ 6.9	212 $\pm$ 9.5
Thickness of cortical layer (μ)	87	78	76	86
Number of xylem cells	63 $\pm$ 4.9	70.7 $\pm$ 3.9	57 $\pm$ 10.2	53 $\pm$ 8.5
Cambium	2 rows	2 rows	Incomplete ring	Incomplete ring

SECTION 5

EFFECT OF MESO-INOSITOL ON GROWTH AND ANATOMY OF EXCISED ROOTS

Loomis and Torrey (1964) reported that meso-inositol when added with an auxin and a cytokinin enhanced the cambial activity of isolated radish roots, although it had little effect when added alone. The following experiments were carried out as a preliminary to the study of interactions between meso-inositol, auxins and cytokinins.

Effect of meso-inositol in liquid medium

Meso-inositol was added at concentrations of 50, 100 and 200 mg/l. The growth measurements and the observations on the anatomy of basal parts were recorded after two weeks.

The final growth measurements show (Table 19A) that meso-inositol slightly enhanced the growth of the main axis and the number of laterals. However, observations made during the growth period indicated that during the first week inositol markedly increased the growth but in the second week the control roots caught up to the inositol treated roots.

Meso-inositol had little effect on the basal part of the root although it did appear to give a small increase in the number of xylem cells in the stele at 50 mg/l. A vascular cambium was seen in all treatments including the controls, but the number of derivatives was reduced at 100 and 200 mg/l. (Table 19B).

Table 20 A

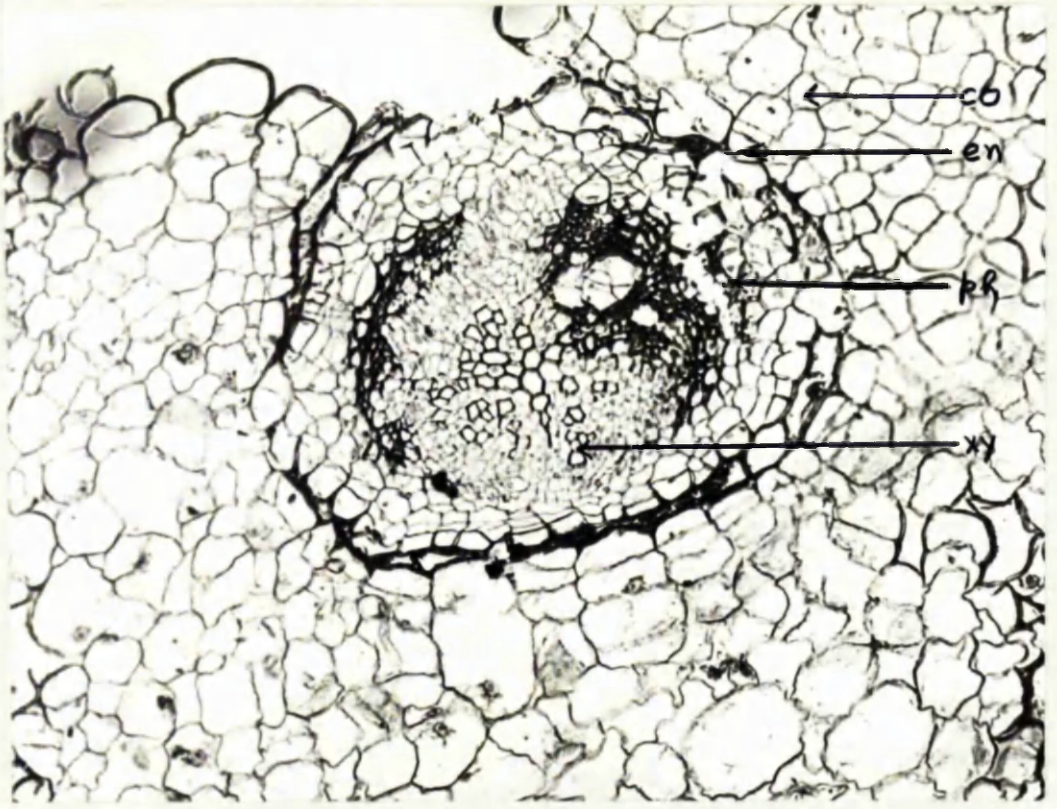
Effect of meso-inositol on growth when fed via the cut end

Addition of meso-inositol in mg/l.												
0			50			100			200			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	
172.7±16	64	698	208±21	76	995	188±13.2	66	748	179±17.7	59	857	

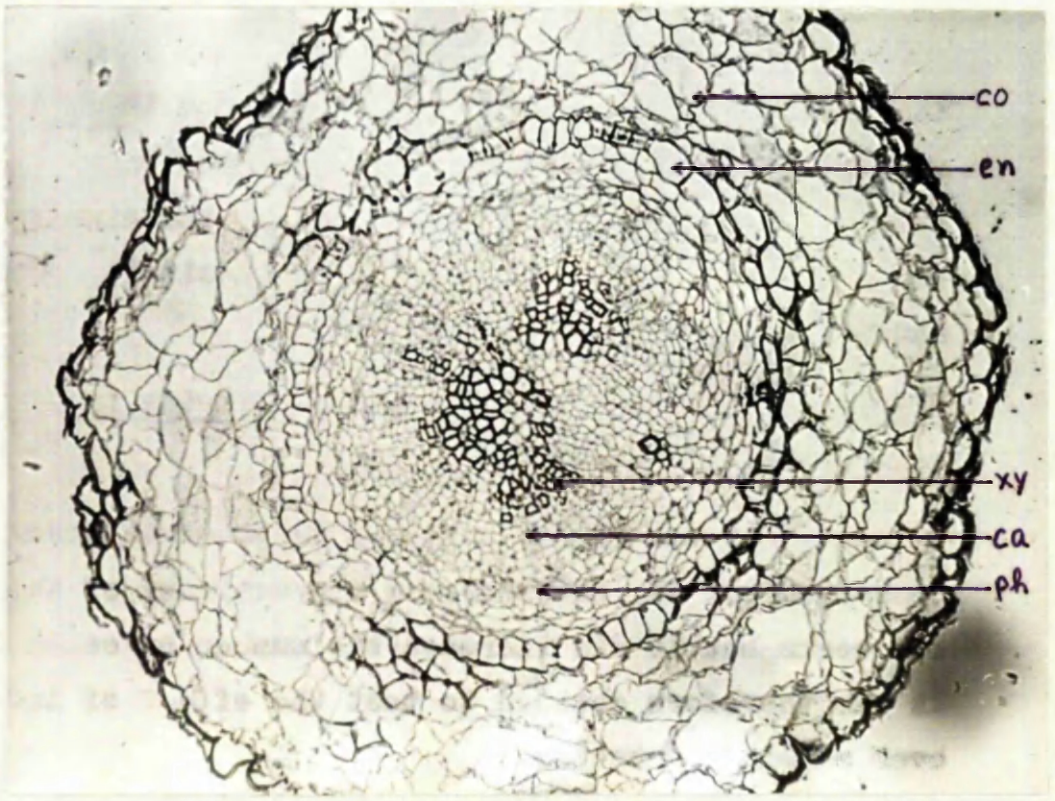
Table 20B

Effect of meso-inositol on anatomy of basal part of root

Meso-inositol in mg/l.	0	50	100	200
Diameter of the root (μ)	385	425	367	364
Diameter of the stele (μ)	223 ± 4.7	226 ± 7.2	199 ± 4.5	195 ± 5.4
Thickness of cortical layer (μ)	75.5	100.5	82	89.5
Number of xylem cells	51 ± 7.2	56 ± 6.8	47 ± 7.1	49 ± 5.1
Cambium	-	-	-	-



A



B

Fig. 15. Photomicrographs of transverse sections of basal parts of roots grown in media containing 0.0 mg/l (A) (X400) and 50 mg/l meso-inositol (B) (X400).

Effect of meso-inositol when fed via the cut end

Meso-inositol was supplied in the vial with 2% sucrose and vitamins whereas the flask contained the complete liquid medium. Twenty replicates of each treatment were incubated in darkness, 25°C, and harvested for growth measurements and anatomical investigations.

The data for growth measurements are shown in Table 20A. The growth of the main axis was stimulated by 50 and 100 mg/l. of inositol treatments. The lateral growth was similarly enhanced although the number of visible laterals was not greatly influenced.

Meso-inositol at 50 mg/l. increased the diameter of the root and the diameter of the stele (Table 20B). The increase in the diameter of the stele was chiefly due to an increase in the number of non-lignified cells rather than lignified cells. However, the number of xylem cells and the diameter of the stele was reduced in 100 and 200 mg/l of inositol. No cambium was observed in any of the roots.

Effect of meso-inositol on cambial activity in cultures grown for longer periods

It was observed in the experiments described above that meso-inositol did not increase the activity of the cambium after two weeks but it did increase the number of cells in the stele. It was therefore decided to test the effect of inositol in cultures over a longer duration.

The results proved to be interesting in that after six week the meso-inositol treated roots had well-developed secondary tissues (Figures 15A and 15B) compared with the control roots. The number of lignified cells was increased over those

of the controls, but the most striking effect was the large increase in the number of unlignified cells derived from the cambium. The cortex and endodermis were also well developed unlike in the control roots where the cortex in particular was split and disorganised. The control roots resembled those described by Dormer and Street (1948). Their roots which were cultured for 5-6 months exhibited disorganised secondary growth. On the other hand the roots grown on a meso-inositol supplemented medium had a more normal structure.

When these meso-inositol treated roots were compared with six week old seedling roots (Figure 10B) some obvious differences were apparent. There were no large xylem elements in the stele and the cortex was much less extensive than the seedling roots.

Table 21

The effect of meso-inositol on the growth rate during a 15-day incubation period

Harvesting Days	Addition of meso-inositol in mg/l.					
	0			50		
	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
3rd day	25 ± 1.02	0.6	0.6	27 ± 1.48	0.4	0.2
6th day	80 ± 4.17	14	51	93 ± 4.25	15	55
9th day	127 ± 6.9	38	333	159 ± 5.9	49	380
12th day	165 ± 5.7	48	548	196 ± 9.7	61	706
15th day	204 ± 10.5	49	988	210 ± 10.3	61	1098

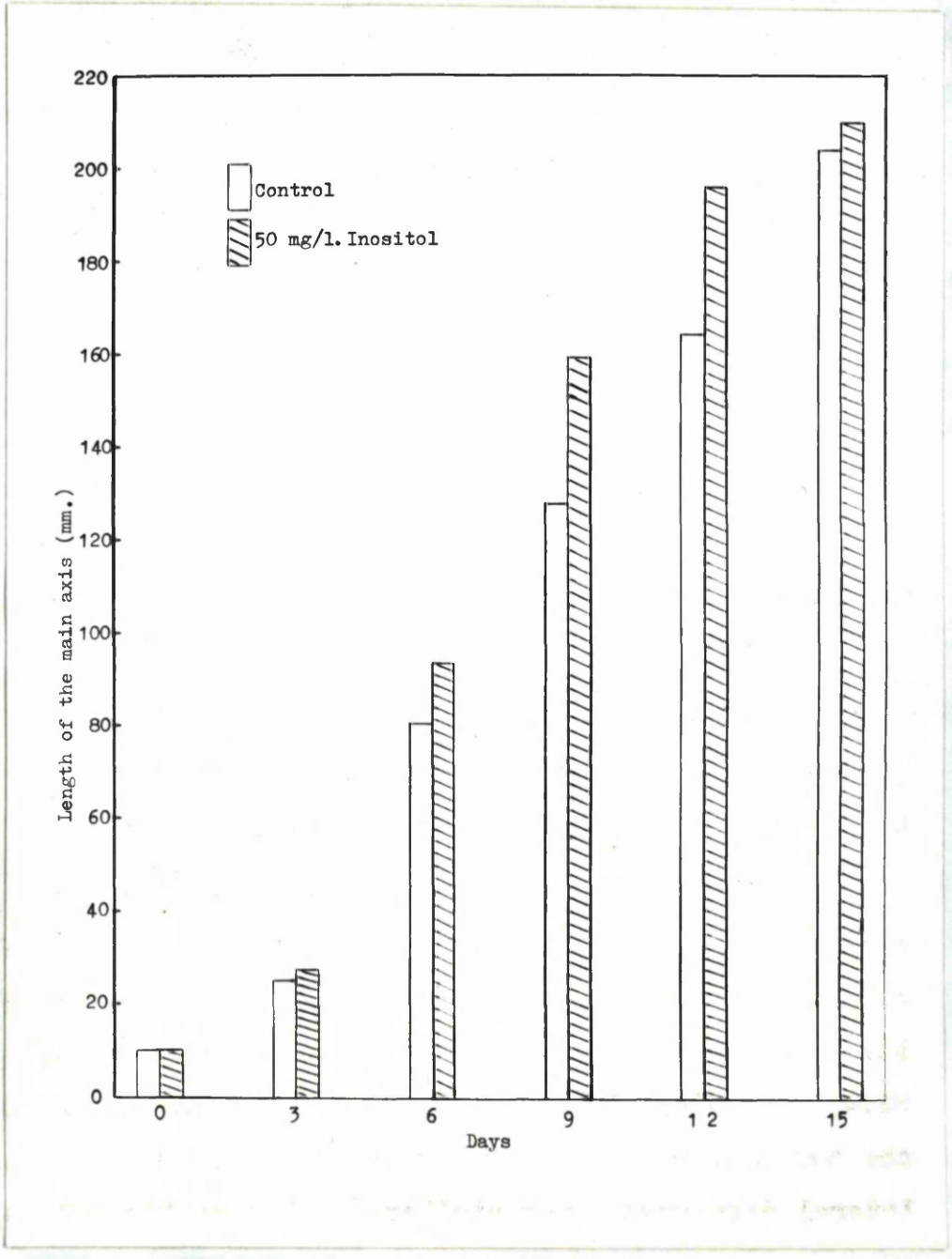


Fig. 16A. Effect of meso-inositol on the rate of growth during a 15-day incubation period.

FURTHER EXPERIMENTS ON THE GROWTH PROMOTING ACTIVITY OF MESO-INOSITOL

The influence of meso-inositol (50 mg/l.) on the growth rate during a 15 day incubation period

The previous experiments indicated that in certain conditions of culture the addition of meso-inositol enhanced the growth of isolated tomato roots. Since this had not previously been reported, it was decided to investigate this effect further.

In the first inositol experiment it was suggested from observations made during the incubation period that inositol enhanced growth initially but that later the growth of the controls caught up to the treated roots. The experiment to be described here was designed to confirm these observations.

Initially 50 replicates were set up for the controls and 50 for the meso-inositol treatment (50 mg/l.). Ten replicates of each treatment were then harvested and measured at three day intervals during the 15 day growth period.

The results presented in Table 21 and Figure 16A confirmed that meso-inositol markedly increased the linear growth of the roots during the first nine days. However, from 9-12 days the growth rates of the controls and the meso-inositol treated roots were very similar and from 12-15 days the growth rates of the controls exceeded that of the treated roots. The effect on lateral development was similar to that on the main axis growth except that most of the stimulation was between 9-12 days instead of 6-9 days.

Table 22B

Effect of meso-inositol on growth of roots in media containing different concentrations of sucrose after 15 day culture period

Meso-inositol mg/l.	1 per cent			2 per cent			3 per cent		
	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
0	180 ± 19	56	545	198 ± 7	51	955	100 ± 8.4	26	193
50	198 ± 24	67	885	215 ± 13	60	1092	106 ± 9.2	24	175
100	249 ± 33	55	911	215 ± 13	69	1201	90 ± 6.3	21	138
200	219 ± 23	50	872	217 ± 7	62	1133	85 ± 7.9	19	99

Table 22A

Effect of meso-inositol on growth of roots in media containing different concentrations of sucrose after 7 day culture period

Meso-inositol mg/l.	1 per cent			2 per cent			3 per cent		
	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
0	108 ± 10.9	23	116	100 ± 6	29 ± 2	172	99 ± 3.9	18	110
50	116 ± 12.9	26	120	158 ± 4	45 ± 3	233	97 ± 10.7	17	119
100	108 ± 10.9	21	102	150 ± 7	43 ± 2	223	93 ± 9	19	96
200	106 ± 6.03	24	124	153 ± 3	48 ± 2	325	91 ± 9.8	16	79

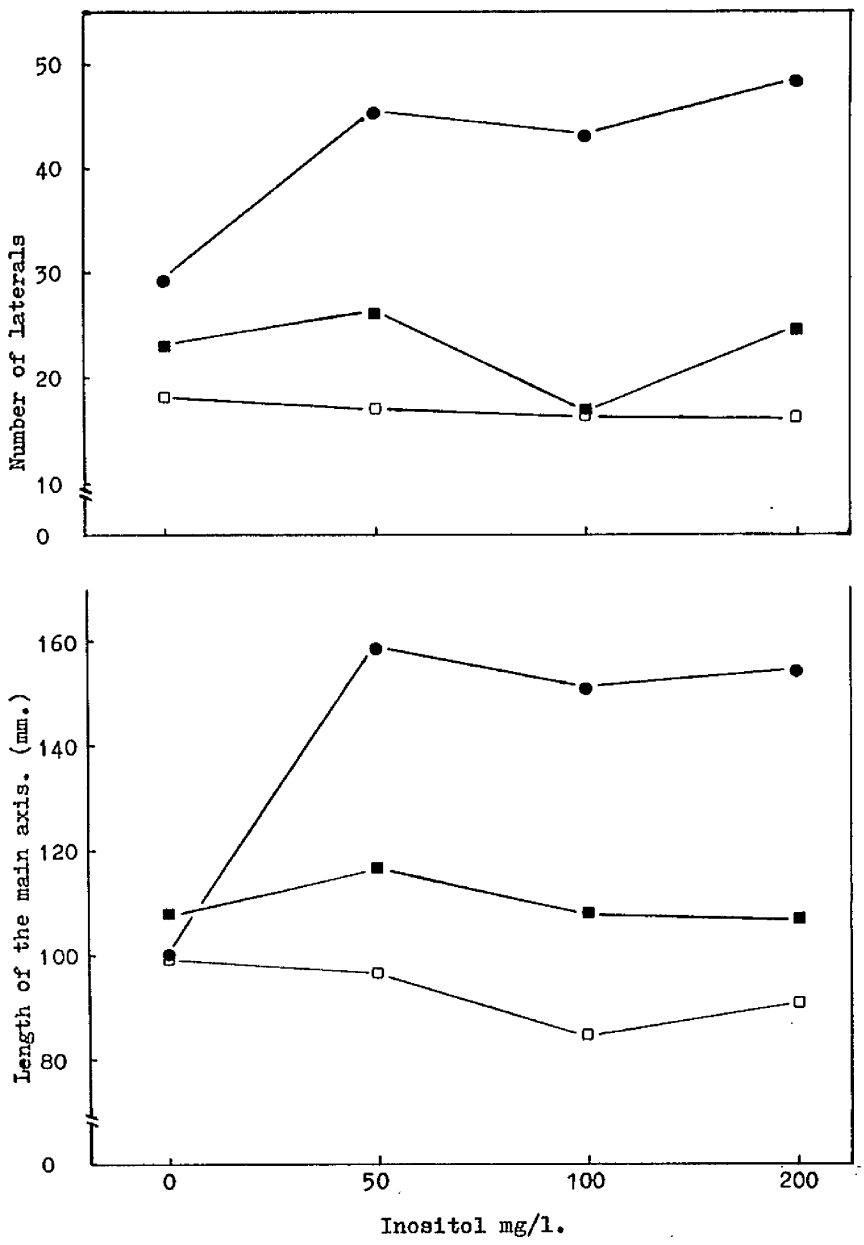


Fig. 16B. Effect of meso-inositol on growth in media containing 1% (■), 2% (●) and 3% (□) sucrose.

Effect of meso-inositol on the growth of roots in media containing different concentrations of sucrose

(a) 7-day passage: Previous work indicated that the responses of excised tomato roots to added growth factors such as auxins, kinins and gibberellins are often determined by the sucrose concentration in the culture medium (Butcher and Street, 1960 a; and 1960b).

The results shown in Table 22A and Figure 16B confirmed that in 2% sucrose the addition of meso-inositol at 50, 100 and 200 mg/l. produced a marked increased in growth (approximately 50%). However meso-inositol had very little effect in media containing 1% or 3% sucrose.

(b) 15-day passage: When the above experiment was repeated with a 15-day growth passage, it was found that meso-inositol greatly stimulated growth in 1% sucrose media, gave a small stimulation in 2% and a small inhibition in 3% (Table 22B).

Thus these experiments have indicated that the response of excised tomato roots to meso-inositol depends both on the age of the root apex (number of days after inoculation) and the sucrose level in the medium.

Effect of meso-inositol on the growth of the roots in light

It has been observed by Steinhart, Anderson and Skoog (1962) in spruce callus that the response to meso-inositol was more marked in dark than in light as measured by an increase in the dry weight of the callus.

The effect of inositol on root growth in continuous culture in light was investigated. Although inositol

Table 23

Effect of meso-inositol on growth in light in media containing different concentrations of sucrose

Meso-inositol mg/l.	1 per cent			2 per cent			3 per cent		
	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
0	108 ± 8.7	10	77	127 ± 7	26	113	85 ± 7.9	17	124
50	100 ± 8.4	15	65	113 ± 4	26	123	-	-	-
100	106 ± 9.2	9	62	142 ± 7	27	247	90 ± 6.3	20	172
200	102 ± 5.4	9	68	125 ± 4	22	156	104 ± 3.6	22	207
400	-	-	-	-	-	-	85 ± 5.2	16	143

Table 24

Effect of sorbitol on growth in media containing two per cent sucrose when the roots are grown in dark

Sorbitol in mg/l.											
0			50			800			1600		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
192±20.6	54	796	191±11.2	50	770	166±19.2	43	563	165±18.	47	584

Table 25

Effect of meso-inositol on seedling roots in dark and light

Meso-inositol mg/l	Dark			Light		
	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
0	233 ± 3.9	51	1103	222 ± 10.9	42	1000
50	233 ± 7.8	54	1261	238 ± 9.8	49	1080
100	215 ± 6.7	57	1181	233 ± 3.9	46	1069
200	227 ± 8.2	48	1168	237 ± 7.6	48	1124

Table 26A

Effect of meso-inositol on the anatomy of seedling roots in dark.

Meso-inositol mg/l.	0	50	100	200
Diameter of the root (μ)	721	985	671	630
Diameter of the stele (μ)	350 ± 14	522 ± 17.8	308 ± 14.3	283 ± 11.4
Thickness of cortical layer (μ)	172	192	183	147
Number of xylem cells	90.6	225*	108*	112*
Cambium	Three complete rows	Five complete rows	Two complete rows	Two complete rows

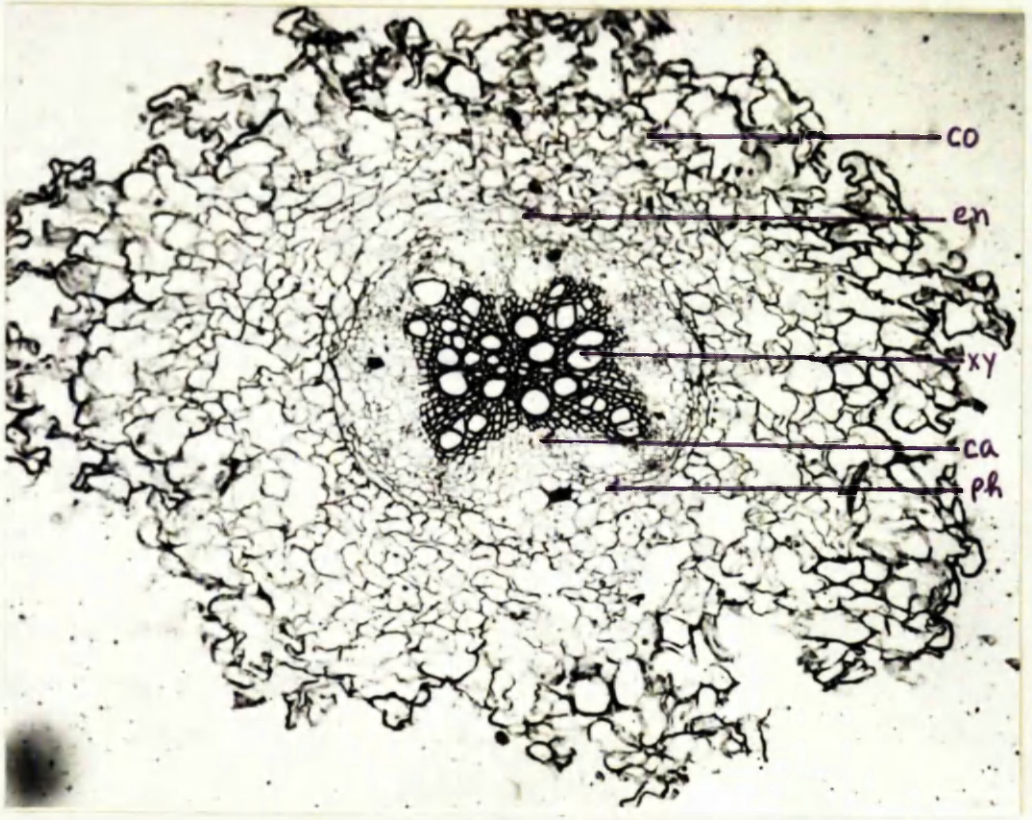
* This value is an estimate since the cells were too numerous to be counted accurately.

Table 26B

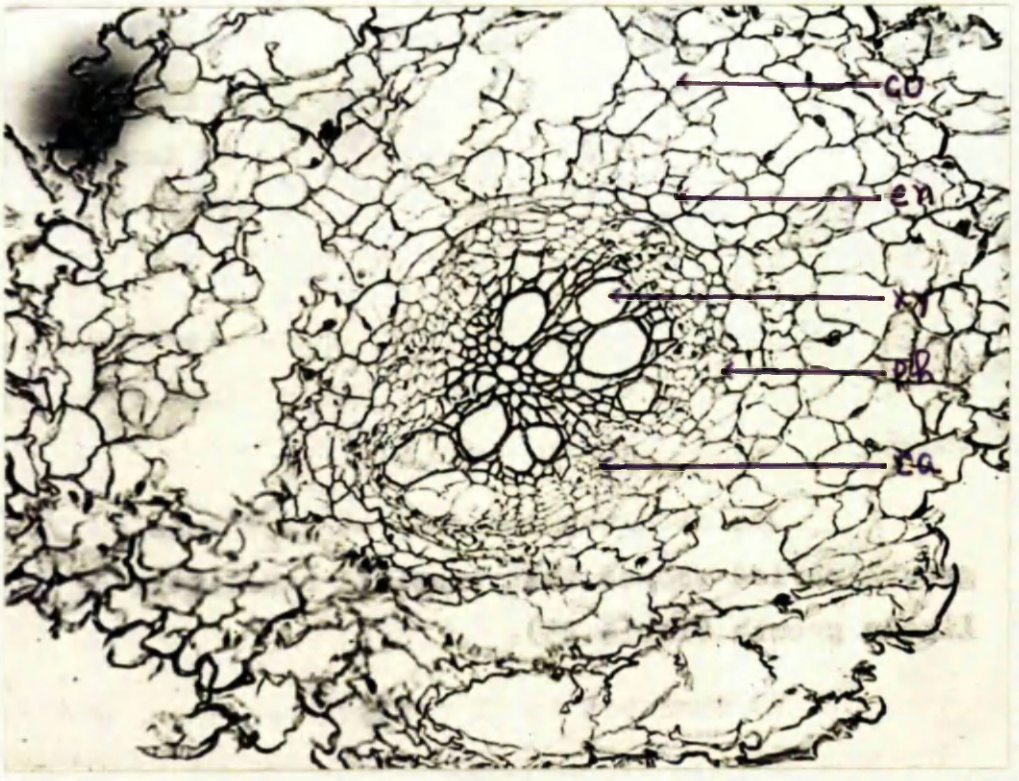
Effect of meso-inositol on the anatomy of seedling roots in light

Meso-inositol mg/l.	0	50	100	200
Diameter of the root (μ)	590	671	1016	917
Diameter of the stele (μ)	286 ± 12.9	299 ± 16.8	596 ± 6.8	477 ± 9.4
Thickness of cortical layer (μ)	172	175	215	208
Number of xylem cells	79	97*	196*	134*
Cambium	Two rows	Three complete rows	Five complete rows	Four complete rows

* This value is an estimate since the cells were too numerous to be counted accurately.



A



B

Fig. 17. Photomicrographs of transverse sections of basal parts of roots grown in 100 mg/l meso-inositol in light (A)(X160) and in dark (B)(X400).

significantly increased the growth in the one week growth period, the effect was less marked than in the dark. It was also found that the optimum stimulation occurred at higher meso-inositol levels i.e. 100 mg/l in 2% sucrose and 200 mg/l. in 3% sucrose. As in the roots grown in dark, meso-inositol did not enhance root growth at 1% sucrose level (Table 23).

Effect of sorbitol in dark

In addition to meso-inositol, another hexitol, sorbitol was found to be effective in promoting the growth of carrot tissue explants (Pollard, Shantz and Steward, 1961). However sorbitol when tested alone had either no effect or was slightly inhibitory at the concentrations tested (Table 24) with tomato roots in 2% sucrose.

Effect of meso-inositol on the seedling roots of tomato

Since the previous experiments had shown that inositol had a marked effect on the growth rate of isolated roots, it was decided to see whether it also had an effect on the intact seedling root system.

Seedling cultures were set up (as described on page 26) and a range of meso-inositol concentrations were tested both in continuous light and in dark.

The observations made at intervals during the four week growth period showed that inositol had little or no effect on linear growth (Table 25).

In contrast to the effect on linear growth rate meso-inositol had a dramatic effect on the activity of the cambium particularly at 50 mg/l. in the dark and at 100 mg/l. in the light (Tables 26A and 26B and Figures 17A and 17B). Both the width of

the lignified ~~cambial~~ zone and the number of lignified xylem derivatives were markedly increased. In addition to this the cells of the endodermis of the treated roots were more prominent than the controls and had a better developed periderm. Due to the growth of the vascular cambium and the periderm the cortex was ruptured as shown in Figures 17A and 17B.

Conclusions

Meso-inositol did not appear to enhance the activity of the vascular cambium in two week old roots, however at the appropriate concentrations it clearly increased its activity in 6 week old roots as compared to the controls. Although the cambial activity was less than in comparable seedling roots, it was more extensive than those of the excised roots grown in the previous experiments. Experiments with seedling roots similarly showed that meso-inositol enhanced the activity of the vascular cambium.

Meso-inositol had little effect on the anatomy of the excised tomato roots when fed via the cut end. Similar results were obtained by Loomis and Torrey (1964) who found that meso-inositol increased cambial activity in the presence of cyto kinin and an auxin in first transfer radish roots but it had no effect when added alone.

In the experiment following up the stimulating effects of meso-inositol on elongation it was found that it strongly promoted linear growth in the first, but not in the second week. It was also shown that the response of the roots depended on the sucrose level in the medium. In the first week it was most effective in 2% but had little effect in 1% or 3%. In contrast in the second week meso-inositol was most effective in 1 sucrose. It is interesting to note that Butcher and Street (1960a and 1960b) have shown that the response of excised roots tomato roots to other growth factors such as auxins, cytokinins and gibberellins are often determined by the sucrose level in the culture medium. For example auxins and gibberellins stimulated the growth of roots when the sucrose level was less

than 1% but had little effect or were inhibitory at higher levels. In contrast, cytokinins stimulated growth at high levels of sucrose, i.e. 3%, but only inhibited growth at lower levels. These authors also found that the age of the root influences the response to the growth substances.

As far as the author is aware there have been no previous reports indicating that additions of meso-inositol to White's root culture medium enhances the growth of excised tomato roots, although Loomis and Torrey (1964) briefly reported that inositol fed via the base of radish roots stimulated growth. However there have been many reports that it is essential or beneficial to the growth of various callus tissues (Pollard et al, 1961; Steinhart et al, 1962; Linsmaier and Skoog, 1965).

The chromatographic examination of plant extracts has revealed the presence of meso-inositol in many species (Plouvier, 1963). Its widespread occurrence and its likelihood of being an essential growth factor has been discussed by Anderson and Wolter (1966). They have pointed out that plant cells must have inositol to build into phospholipid molecules, and perhaps to use in the synthesis of wall polysaccharides. Inositol also serves as a phosphate acceptor in phytate synthesis. Braun and Wood (1962) and Wood and Braun (1961) have suggested that inositol facilitates the uptake of inorganic ions by plant cells.

These observations that meso-inositol increases the growth of excised tomato roots by up to 60% in the standard medium suggests that with this clone at least 50 mg/l. meso-inositol should be added as a standard supplement.

SECTION 6

INTERACTION BETWEEN THE EFFECTS OF NAA, IAA, CYTOKININS AND MESO-INOSITOL

Digby and Wareing (1964) have suggested from their work with disbudded woody shoots that there is a very marked synergism between IAA and GA_3 in both cambial cell division and xylem differentiation indicating that both are important in regulating cambial activity. Sorokin, Mathur and Thimann (1962) showed that auxins (IAA or 2,4-D) together with kinetin, produced more xylem than auxin alone in the second internode of etiolated 'Alaska' pea epicotyls. In the case of first transfer root tips of radish, Loomis and Torrey (1964) found that the vascular cambium was most active when meso-inositol, auxin (IAA or NAA) and a cytokinin (6-benzylaminopurine) were present. Gibberellin had no effect on this system.

In the previous sections, when growth substances were tested, only meso-inositol enhanced the activity of the vascular cambium of isolated tomato roots. The following interaction experiments were carried out to see whether or not combinations of the growth factors were more effective.

As in the previous experiments the substances were supplied either in the liquid medium in which the roots were totally immersed or via the cut end of the root using the modified Raggio and Raggio technique.

Effect of high concentrations of NAA, kinetin and meso-inositol on growth and anatomy of excised roots

First of all, inhibitory concentrations of kinetin and auxin were used since Loomis and Torrey (1964) found that

Table 27

Interaction between NAA, kinetin and meso-inositol on growth of the roots

Growth substances in mg/l.	Growth Measurements		
	L.M.A.	N.L.	T.L.L.
Controls	172 ± 16.6	50	567
Kinetin 0.025	102 ± 13.3	7.7	75
Inositol 200.0	190 ± 12	58	610
NAA 0.00075	75 ± 13.9	23	184
Kinetin 0.025 + Inositol 200.0	116 ± 9.9	11	162
NAA0.00075 + Inositol 200.0	88 ± 14.8	29	289
NAA 0.00075 + Kinetin 0.025	62 ± 3.8	10	99
NAA 0.00075 + Kinetin 0.025 + Inositol 200.0	53 ± 2.3	9	119

STUDIES ON THE CONTROL OF SECONDARY THICKENING IN EXCISI ROOTS OF *LYCOPERSICUM ESCULENTUM* MILL.

(Summary)

by

SUNITA SINHA.

The anatomy of excised tomato roots was compared a contrasted with that of cultured seedling roots and the have been presented in the first section of this thesis. remaining experimental work comprises a study of the eff of various growth substances on the initiation and growt of the cambium in excised tomato roots. Each substance w tested in two ways :-(a) The roots were cultured immerse a liquid medium supplemented with the compound(s). (b) T growth substances were fed via the cut end using a modi Raggio and Raggio technique. At the end of each experime the roots were measured and the anatomy of the basal reg of the roots was examined. The substances tested include auxins, cytokinins, a gibberellin, meso-inositol and var complex mixtures such as coconut milk, yeast extract and acid hydrolysed casein. The auxins, cytokinins and meso- inositol were also tested in various combinations.

It has been shown that a well developed vascular c is initiated in two week old excised roots in the standa medium but that the activity of the cambium was not main In contrast the cambium initiated in seedling roots was up to six weeks. Furthermore, IAA and NAA, at the concen tions tested, prevented the formation of a cambium and caused some reduction in the number of xylem cells in th stele. However when auxins were supplied via the cut end the root no marked effect was observed.

Kinetin and 6-benzylaminopurine, added to the liqu medium, inhibited the formation of the cambium and grea

reduced the diameter of the stele and the number of xyle cells within the stele. When the cytokinins were supplie via the cut end, they increased the linear growth of the roots but had little effect on the anatomy of the base. effects of gibberellic acid were similar to those of the auxins in that it prevented the formation of a cambium a reduced the number of xylem cells in the stele.

Although meso-inositol did not have a stimulatory on cambial activity after two weeks it did markedly prol its activity and the roots grown in a meso-inositol supp mented medium for six weeks showed extensive secondary thickening. No effect on cambial activity was observed w the meso-inositol was fed via the cut end. In experiment with intact seedling roots meso-inositol similarly stimu the activity of the vascular cambium. After one week's g meso-inositol increased the growth of excised tomato roo by up to 60% when added to the standard medium containi 2% sucrose. However, it was much less effective in medi containing 1% or 3% sucrose. After two weeks it was most effective in the medium containing 1% sucrose. Sorbitol, another cyclitol, had little effect on the growth of roo

When the various combinations of IAA or NAA, cytol and meso-inositol were tested for effects on cambial gro no positive interactions were observed. It was found tha the lower concentrations combinations of NAA, kinetin an meso-inositol produced thick roots, but this reflected a effect on the thickness of the cortex rather than on the

At the concentrations tested coconut milk, yeast e and acid hydrolysed casein had no detectable effect on c activity when added to the liquid medium or when fed via cut end of the root.

Excised roots cultured for six weeks in media supplemented with meso-inositol developed a considerable amount of secondary tissues which were derived from a

vascular cambium. It is suggested that meso-inositol may be a factor limiting the cambial growth in excised tomat roots in a standard medium. However meso-inositol did n produce roots with secondary tissues as extensive as in comparable seedling roots and it was considered that oth unidentified factors are also involved in cambial growth

Table 28

Effect of interaction between NAA, kinetin and inositol on the anatomy of the roots

Growth substances mg/l.	0	Kinetin 0.025	Inositol 200	NAA 0.00075	Kinetin + inositol	NAA + inositol	NAA + kinetin	NAA + kinetin + inositol
Diameter of the root (μ)	410	328	385	494	358	495	388	364
Diameter of the stele (μ)	239 ± 14	121 ± 15	223 ± 104	210 ± 5.4	168 ± 8.2	246 ± 19	185 ± 4.7	168 ± 8.2
Thickness of cortical layer (μ)	90	109	87	142	103	120	109	111
Number of xylem cells	53 ± 8.5	9.6 ± 1.08	54 ± 9.1	34.8 ± 8.2	11 ± 1.4	49 ± 6.8	16 ± 0.89	7 ± 1.4
Cambium	Two rows	-	Incomplete ring	-	-	-	-	-

concentrations inhibitory to linear growth were the most effective in stimulating the activity of first transfer tips of radish.

The growth and anatomy was recorded after two weeks (Tables 27 and 28). The results show that :-

1. Inositol partially overcomes the inhibition of linear growth caused by either kinetin or auxin.

2. The effects of kinetin and auxin, when used together, were greater than either of the two used alone.

3. The least growth was obtained when NAA, kinetin and meso-inositol were present together.

In the treatments where the substances were added alone the previous results were confirmed, i.e. 0.025 mg/l. kinetin reduced the diameter of the root and the stele and markedly decreased the number of xylem elements (Table 28). NAA at 0.00075 mg/l., on the other hand, increased the diameter of the root and the thickness of the cortical layer but decreased the diameter of the stele. Meso-inositol at 200 mg/l. caused a slight decrease in root and stele diameters. Kinetin and NAA prevented the formation of a cambium. The combination of growth substances used in this experiment did not cause any increase in the thickness of the roots or the diameter of the stele as compared with the controls and in the treatments where the growth substances were added singly cambial activity was not enhanced. On the contrary, cambial growth was prevented.

Effect of lower concentrations of NAA, kinetin and meso-inositol on growth and anatomy of the root

The high concentrations of the growth factors used in the experiment described above inhibited rather than

Table 29

Interaction between NAA, kinetin and meso-inositol on growth of the roots

Growth substances in mg/l.	Growth Measurements		
	L.M.A.	N.L.	T.L.L.
Control	182.6 ± 16	57	541
Kinetin 0.00625	180 ± 9.8	57	685
Inositol 50.0	215.7 ± 9.2	61	1029
NAA 0.00025	178 ± 7.5	64	686
Kinetin 0.00625 + Inositol 50.0	216 ± 7.8	63	1038
NAA 0.00025 + Inositol 50.0	153 ± 15.6	59	771
NAA 0.00025 + Kinetin 0.00625	168 ± 1.2	52	753
NAA 0.00025 + Kinetin 0.00625 + Inositol 50.0	163.6 ± 13.1	58	814

Table 30

Effect of interaction between NAA, kinetin and meso-inositol on the anatomy of the roots

Growth substances in mg/l	Control	Kinetin 0.00625	Inositol 50	NAA 0.00025	Kinetin + Inositol	NAA + Inositol	NAA + Kinetin	NAA + Kinetin + Inositol
Diameter of the root (μ)	385.5	376.5	361.5	421.5	346.5	373.5	390	445.5
Diameter of the stele (μ)	212.5±3.3	208±8.3	205.5±7.7	214.5±6.7	186±7.1	220.5±9.2	206±7.7	212±3.3
Thickness of cortical layer (μ)	82.5	82.5	84	96	85	82.5	96	112.5
Number of xylem cells	63.4±4.9	40.4±5.4	54±9.1	31±5.1	40±5.4	48±3.9	32±3.8	16.6±1.6
Cambium	Two rows	-	One row	-	-	-	-	-

Table 31

Effect of interaction between IAA, NAA and meso-inositol on growth when supplied via the cut end

Growth substances in mg/l.	L.M.A.	N.L.	T.L.L.
Control	212.5 ± 3.3	47	881
NAA0.0005	212 ± 3	52	908
IAA 0.001	206 ± 7.7	45	826
Inositol 50.0	232 ± 12.9	53	832
Inositol 50 + NAA 0.0005	217 ± 6.9	52	1048
Inositol 50 + IAA 0.001	194 ± 5.5	38	603

increased cambial activity. In this experiment the same substances were tested at lower concentrations.

At the concentrations used in this experiment kinetin (0.00625 mg/l.) and NAA (0.00025 mg/l.) had little effect on linear growth whereas meso-inositol at 50 mg/l. promoted the growth of the main axis and increased the number of laterals and their total length. Again the growth was not enhanced by adding all the growth substances together (Table 29).

Although the treatment where kinetin, NAA and meso-inositol were added together produced roots of the greatest thickness, this was due entirely to the increase in thickness of the cortex. The effect of interactions on the diameter of the stele was either inhibitory or negligible. The number of xylem cells were greatly reduced (Table 30).

Interactions between NAA, IAA and 6-benzylaminopurine on growth and anatomy of the root when supplied via the cut end

Loomis and Torrey (1964) observed in excised radish roots that when IAA or NAA was supplied via the cut end of the root together with cytokinin and inositol, a very well organised vascular cambium was induced. On the other hand only limited rows of cambium could be induced by auxin and inositol.

The first experiment was set up to see the effect of IAA or NAA with inositol on growth and anatomy of the root when supplied via the cut end.

The growth measurements presented in Table 31 show that auxins were ineffective in stimulating the length of the main axis when they were provided in the vial either singly or together with inositol.

Table 32

Effect of interaction between IAA, NAA and meso-inositol on anatomy when fed via the cut end

Growth substances (mg/l)	Control	NAA 0.0005	IAA 0.001	Inositol 50	NAA + Inositol	IAA + Inositol
Diameter of the root (μ)	385	457.5	445	415.5	367.5	358
Diameter of the stele (μ)	213.5±5	264±12	250±7.2	213.5±5	241±12.2	213±4.7
Thickness of cortical layer (μ)	85.5	100.5	107	91.5	77	90.5
Number of xylem cells	48±3.9	52.6±8.5	48±3.9	54.6±9.11	48±3.9	48±4.1
Cambium	-	-	-	-	-	-

Table 33

Effect of interaction between NAA, inositol and 6-benzylaminopurine on growth when fed via the cut end

Growth substances in mg/l	L.M.A.	N.L.	T.L.L.
Control	196 ± 8.6	44	678
BA 0.00625	206 ± 7.7	56	888
Inositol 50	208 ± 21	76	995
NAA 0.0005	212 ± 3	52	908
Inositol 50 + BA 0.00625	204 ± 10.5	40	857
NAA 0.0005 + Inositol 50	215 ± 6.7	45	918
NAA 0.0005 + BA 0.00625	210 ± 10.3	44	787
NAA 0.0005 + BA 0.00625 + Inositol 50	203 ± 10	41	891

Table 34

Effect of interaction between inositol, 6-benzylaminopurine and N.A.A. on the anatomy when fed via the cut end of the roots

Growth substances mg/l	Control	B.A. 0.00625	Inositol 50	N.A.A. 0.0005	B.A. + Inositol	N.A.A. + Inositol	B.A. + N.A.A.	B.A. + N.A.A. + Inositol
Diameter of root (μ)	441	390	422	457.5	375	380.5	420	388
Diameter of stele (μ)	243±4.9	200	226	264	211	241	210	198
Thickness of cortex (μ)	95	88.5	100.5	100.5	117	85	105.5	120
No. of xylem cells	52	48	53	52	41	48.5	42	38
Cambium	-	-	-	-	-	-	-	-

The data of the anatomical investigations (Table 32) show that the diameter of the root was increased when IAA, NAA or inositol was added alone. But when IAA or NAA was supplied with inositol, the diameter of the root as well as the diameter of the stele was reduced. No marked effect was observed on the number of xylem cells and the initiation of cambial activity.

Effect of interation between NAA, meso-inositol and 6-benzylaminopurine on growth and anatomy of the root when supplied via the cut end

The data for the growth measurements after two weeks are shown in Table 33. They indicate that growth substances had a small stimulating effect on the length of the main axis irrespective of whether they were added singly or together. Meso-inositol (50 mg/l.) stimulated the number of laterals but the others had very little effect.

The observations from the transverse sections of the basal parts of the roots are summarised in Table 34. The data show that the combinations of the growth substances had no effect on the initiation of vascular cambium. The diameter of the root was reduced in all treatments except the NAA treatment where it was slightly increased. When NAA, BA and inositol were added together the diameter of the stele was much reduced and the thickness of the cortical layer was increased.

Conclusions

In the experiments described in this section there was no evidence that auxins, cytokinins and meso-inositol added in various combinations enhanced the activity of the vascular cambium either when added to the liquid medium or when fed via the cut end of the root. On the contrary it was found that they often prevented cambium formation as compared with controls. It was found that at the lower concentrations combinations of NAA, kinetin and meso-inositol produced thickened roots. However, this reflected an effect on the thickness of the cortex and not the stele. Thus it may be concluded that either these growth factors were not tested at suitable concentrations or that other factors were limiting the development of the cambium. There was insufficient time to carry out detailed interaction experiments with gibberellins, however preliminary experiments indicated that gibberellic acid in combination with other growth factors had no stimulatory effects on cambial development.

These results were very different from those of Loomis and Torrey (1964) since they found in radish roots that auxins, cytokinins and meso-inositol, when added together (via the base), produced a more active cambium. Even in their experiments the diameter and extent of cambial activity was limited.

Table 35

Effect of acid hydrolysed casein on growth

Addition of acid hydrolysed casein in mg/l															
0			25			50			100			200			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
180	55	910	111	34	230	106	31	207	92	23	143	81.5	18	103

Table 36

Effect of acid hydrolised casein on anatomy of basal part of root

Acid hydrolysed casein in mg/l.	0	25	50	100	200
Diameter of the root (μ)	425	382	377	366	350
Diameter of the stele (μ)	245	192	186	156	155
Thickness of cortical layer (μ)	90	90	93	105	110
Number of xylem cells	49	41	37	25	22
Cambium	2 rows	-	-	-	-

Table 37

Effect of acid hydrolysed casein on growth when fed via the cut end

Addition of acid hydrolysed casein in mg/l.												
0			25			50			100			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
223	44	799	207	35	429	213	43	734	183	43	431

SECTION 7

EFFECT OF ACID HYDROLYSED CASEIN, YEAST EXTRACT AND COCONUT MILK ON GROWTH AND ANATOMY OF TOMATO ROOTS

Earlier experiments have shown that specific growth substances (i.e. auxins, kinins and gibberellic acid) were inactive in stimulating the cambial activity in excised roots of tomato. Meso-inositol had little effect in two weeks although in experiments of longer duration it caused some enhancement of cambial growth. Therefore, in this section the complex mixtures were tested. The concentrations of complex mixtures selected were similar to those used by other workers for organ and tissue cultures (Robbins, 1922; White, 1934; Overbeek, Conklin and Blakeslee, 1941; Steward and Caplin, 1951; Roberts and Street, 1955; Steward, Mapes and Smith, 1958; Loomis and Torrey, 1964).

Effect of acid hydrolysed casein when supplied in the liquid medium

Four concentrations of acid hydrolysed casein (25, 50, 100 and 200 mg/l.) were tested. The data presented in Table 35 show that all concentrations used were inhibitory to the length of the main axis and the number of laterals.

Acid hydrolysed casein reduced the diameter of the root, the diameter of the stele and the number of xylem cells (Table 36). No cambium was observed in any of the treated roots.

Effect of acid hydrolysed casein when fed via the cut end

The results given in Table 37 show that lower concentrations of acid hydrolysed casein (25 and 50 mg/l.) had

TABLE 38

Effect of acid hydrolised casein on anatomy when fed via the cut end

Acid hydrolised casein in mg/l.	0	50	100	200
Diameter of the root (μ)	385	340	363	369
Diameter of the stele (μ)	195	182	183	189
Thickness of cortical layer (μ)	95.5	85	90	90.5
Number of xylem cells	41	40	39	40
Cambium	-	-	-	-

Table 39

Effect of yeast extract on growth

Yeast extract in mg/l.												
0			25			50			100			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
182	54	910	91	25	95	95	15	75	67	15	85

Table 40

Effect of yeast extract on anatomy of basal part of root

Yeast extract in mg/l.	0	25	50	100
Diameter of the root (μ)	430	390	386	376
Diameter of the stele (μ)	241	200	192	186
Thickness of cortical layer (μ)	92	90	97	105
Number of xylem cells	50	38	32	27
Cambium	2 rows	-	-	-

Table 41

Effect of yeast extract on growth when fed via the cut end

Yeast extract in mg/l.												
0			25			50			100			
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
190	34	723	158	31	492	161	32	497	185	38	799

Table 42

Effect of yeast extract on anatomy of basal part when fed via the cut end

Yeast extract mg/l.	0	25	50	100
Diameter of the root (μ)	385	364	367	380
Diameter of the stele (μ)	213.5	195	199	210
Thickness of cortical layer (μ)	75.5	89	82	79
Number of xylem cells	42	39	41	42
Cambium	-	-	-	-

Table 43

Effect of coconut milk on growth

Coconut milk in mg/l.								
0			50			100		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
180	56	910	64	11	63	38	6	35

little effect on the growth of the roots. However 100 mg/l. was slightly inhibitory.

The results of anatomical investigations (Table 38) indicate that acid hydrolysed casein, when supplied via the cut end, slightly reduced the diameter of the root but otherwise it had little effect.

Effect of yeast extract when supplied in the liquid medium

Three concentrations of yeast extract (25, 50 and 100 mg/l.) were tested. All concentrations were found to be inhibitory to the growth of the main axis and the laterals (Table 39).

Anatomical examinations of the basal parts of the roots revealed that yeast extract did not enhance the cambial activity. However, the diameter of the root and the stele and the number of xylem cells were slightly reduced (Table 40).

Effect of yeast extract when fed via the cut end

The growth measurements after two weeks showed that lower concentrations of yeast extract (25 and 50 mg/l.) were inhibitory to the length of the main axis. The number of laterals was not affected by the treatment (Table 41).

No significant increase was observed in the diameter of the root, diameter of the stele, thickness of cortical layer and the number of xylem cells. No cambium was observed in any of the treated or control roots (Table 42).

Effect of coconut milk when supplied in the liquid medium

The growth measurements are shown in Table 43. The results indicate that both the concentrations were inhibitory to growth.

Table 44

Effect of coconut milk on anatomy of basal part of root

Coconut milk in mg/l.	0	50	100
Diameter of root (μ)	402	382	372
Diameter of stele (μ)	244	158	142
Thickness of cortical layer (μ)	79	117	120
Number of xylem cells	57	38	31
Cambium	2 rows	-	-

Table 45

Effect of coconut milk on growth when fed via the cut end

Coconut milk in mg/l								
0			50			100		
L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.	L.M.A.	N.L.	T.L.L.
231.5	55	787	217	45	701	203	42	847

Table 46

Effect of coconut milk on anatomy of basal part when fed via the cut end

Coconut milk in mg/l.	0	50	100
Diameter of the root (μ)	370	350	360
Diameter of the stele (μ)	190	185.5	183
Thickness of cortical layer (μ)	90	90	92
Number of xylem cells	39	41	40
Cambium	-	-	-

The results summarised in Table 44 show that coconut milk reduced the diameter of the stele but increased the thickness of the cortical layer. No cambium was observed in any of the treated roots.

Effect of coconut milk when fed via the cut end

The results of growth measurements and anatomical observations are given in Tables 45 and 46. No stimulatory effect was found after two weeks. No difference was observed from controls in the transverse sections of the basal parts of treated roots. There was no sign of a vascular cambium in any of the treated roots.

Conclusions

None of the complex mixtures tested (as reported in this section) were found to be active in promoting the cambial activity of excised tomato roots when they were added in the liquid medium or when they were given via the cut end of the roots. It was therefore concluded that either these mixtures did not contain substances capable of stimulating cambial growth or that their effects were masked by the presence of inhibitory compounds.

Loomis and Torrey (1964) reported that the cambial activity of radish roots was initiated by 10% coconut milk plus auxin (10^{-5} M). They suggested that coconut milk was supplying the natural cytokinins.

GENERAL DISCUSSION

In these investigations it was found that a vascular cambium developed in excised tomato roots if allowed to grow in modified White's standard medium for two weeks. However the activity of this cambium was limited and even after eight weeks the number of xylem derivatives was not increased over those of the two week old roots. Attempts to prolong or increase the activity of the cambium were for the most part unsuccessful. Additions of auxins (IAA and NAA), cytokinins (kinetin or 6-benzylamino purine) and gibberellic acid, when supplied to the liquid medium or via the cut end of the roots, failed to increase the cambial activity and often these hormones appeared to inhibit the formation of the cambium. Of all the growth factors tested only meso-inositol enhanced the growth of the cambium and after six weeks produced roots with a considerable amount of secondary tissue. But even with this substance there was less growth than in comparable seedling roots. When the auxins, cytokinins and meso-inositol were added in various combinations no increase of cambial activity was observed.

The experiments using the Raggio and Raggio technique were unsuccessful in that they did not enhance the activity of the cambium, in contrast no cambium even in the controls was seen in the roots. The lack of cambium in the control roots is not easily explained since the only difference between these roots and those in normal culture was that the cut end was inserted into an agar medium. One possible explanation is that an inhibitor of cambium initiation diffuses from the agar. This possibility was not tested, but the report that tomato roots do not grow well on agar media (Day, 1943) does add support to this idea.

These results in many respects are different from those of Torrey (1963) and Loomis and Torrey (1964) who worked with pea and radish roots. These authors were not very successful in initiating active cambia in excised roots growing on their normal ~~cultures containing~~ standard media with or without the addition of growth substances, but found the Raggio and Raggio technique very effective. Their best cambial development in first transfer pea roots was obtained when IAA, adenosine sulphate, L(+)-aspartic acid, L(+)arginine HCL, L(+)-glutamic acid, glycine, asparagine, urea (all at 10^{-3} M) and 8% sucrose were fed via the cut end of the roots.

With the first transfer tips of radish a mixture of auxin, kinin and meso-inositol proved to be the most effective. Torrey (1963) suggested that this might be due to the concentration gradients of growth substances being set up along the length of the root and that this is important in cambial development.

Although in most of the experiments with tomato roots the growth substances appeared to be transported in the root (They influenced the growth of the apical meristem) there was no evidence that concentration gradients of substances tested were important in cambial growth. However it is possible that the agar effect could have masked any such stimulation.

Cambial activity was not increased by the addition of coconut milk, casein hydrolysate or yeast extract. Thus there was no evidence that these substances contained factors which were limiting the growth. Loomis and Torrey (1964) found that 10% coconut milk plus auxin inhibited cambium growth in radish roots but they attributed this to the natural cytokinins present in the coconut milk. However they did not mention that coconut milk also contains meso-inositol (Pollard et al, 1961) and gibberellins (Radley and Dear, 1958).

Although the growth hormones used have been tested over a wide range of conditions it is still possible that the conditions necessary to demonstrate their role have been missed. For example gibberellic acid was not tested in combination with the other growth factors. This should have been done since Wareing (1958) found that gibberellic acid and IAA were synergistic in their effects on cambial growth in disbudded shoots of Populus robusta. Further experiments involving the use of the growth factors in media containing different concentrations of sucrose might also be fruitful, particularly in the light of the findings of Wetmore and Rier (1963) working with callus cultures. They found that the ratio of auxin to sucrose determined the initiation, location and growth of cambium-like layers.

The results from these studies indicate that the factors limiting the growth of the cambium in excised tomato roots are different from those limiting its activity in pea and radish roots.

The observations that auxins, kinins and gibberellic acid do not prolong the activity of the cambium suggest that there may be other factors yet to be discovered which are limiting the growth of the secondary tissues in tomato roots. On the other hand the findings that meso-inositol to some extent enhances the growth suggest that it may have a role in cambial activity.

GENERAL SUMMARY

1. In the first section of this thesis the anatomy of excised tomato roots was compared and contrasted with that of cultured seedling roots. The remaining experimental work comprises a study of the effects of various growth substances on the initiation and growth of the cambium in excised tomato roots. Each substance was tested in two ways :-

(a) The roots were cultured immersed in a liquid medium supplemented with the compound(s).

(b) The growth substances were fed via the cut end using a modified Raggio and Raggio technique (page 25).

At the end of each experiment the roots were measured and the anatomy of the basal region of the roots was examined. The substances tested included auxins, cytokinins, a gibberellin, meso-inositol and various complex mixtures such as coconut milk, yeast extract and acid hydrolysed casein. The auxins, cytokinins and meso-inositol were also tested in various combinations.

2. It has been shown that a well developed vascular cambium is initiated in two week old excised roots in the standard medium but that the activity of this cambium was not maintained. In contrast the cambium initiated in seedling roots was active up to six weeks.

3. It has been shown that IAA and NAA, at the concentrations tested, prevented the formation of a cambium and caused some reduction in the number of xylem cells in the stele. However when auxins were supplied via the cut end of the root no marked effect was observed.

4. Kinetin and 6-benzylaminopurine, added to the liquid medium, inhibited the formation of the cambium and greatly reduced the diameter of the stele and the number of xylem cells within the stele. When the cytokinins were supplied via the cut end, they increased the linear growth of the roots but had little effect on the anatomy at the base.

5. The effects of gibberellic acid were similar to those of the auxins in that it prevented the formation of a cambium and reduced the number of xylem cells in the stele.

6. Although meso-inositol did not have a stimulatory effect on cambial activity after twoweeks it did markedly prolong its activity and roots grown in a meso-inositol supplemented medium for six weeks showed extensive secondary thickening. No effect on cambial activity was observed when the meso-inositol was fed via the cut end. In experiments with intact seedling roots meso-inositol similarly stimulated the activity of the vascular cambium.

7. After one week's growth meso-inositol increased the growth of excised tomato roots by up to 60%. when added to the standard medium containing 2% sucrose. However, it was much less effective in media containing 1% or 3% sucrose. After two weeks it was most effective in the medium containing 1% sucrose. Sorbitol, another cyclitol, had little effect on the growth of tomato roots.

7. When the various combinations of IAA or NAA, cytokinins and meso-inositol were tested for effects on cambial growth, no positive interactions were observed. It was found that at the lower concentrations combinations of NAA, kinetin and meso-inosito produced thick roots, but this reflected an effect on the thickness of the cortex rather than on the stele.

8. At the concentrations tested coconut milk, yeast extract and acid hydrolysed casein had no detectable effect on cambial activity when added to the liquid medium or when fed via the cut end of the root.

9. Excised roots cultured for six weeks in media supplemented with meso-inositol developed a considerable amount of secondary tissues which were derived from a vascular cambium. It is suggested that meso-inositol may be a factor limiting the cambial growth in excised tomato roots in standard medium. However meso-inositol did not produce roots with secondary tissues as extensive as in comparable seedling roots and it was considered that other unidentified factors are also involved in cambium growth.

BIBLIOGRAPHY

ANDERSON, L. and WOLTER, K.E., 1966. Cyclitols in plants : Biochemistry and physiology. Ann. Rev. Plant Physiol., 17, 209-222.

AVERY, G.S., BURKHOLDER, P.R. and CREIGHTON, H.B., 1937. Production and distribution of growth hormones in shoots of Aesculus and Malus, and its probable role in stimulating cambial activity. Amer. J. Bot., 24, 51-58.

BAILEY, I.W., 1943. Some misleading terminologies in the literature of 'Plant tissue culture'. Science, 98, 539.

BOLL, W.G., and STREET, H.E., 1951. Studies on the growth of excised roots. I. The stimulatory effect of molybdenum and copper on the growth of excised tomato roots. New Phytol., 50, 52-75.

BONNER, J., 1940. On the growth factor requirements of isolated roots. Amer. J. Bot., 27, 692-701.

BONNER, J. and DEVIRIAN, P.S., 1939. Growth factor requirements of four species of isolated roots. Amer. J. Bot., 26, 661-665.

BRADLEY, M.V. and CRANE, J.C., 1957. Gibberellin stimulated cambial activity in stems of apricot spur shoot. Science, 126, 972-973.

BRAUN, A.C. and WOOD, H.N., 1962. On the activation of certain essential biosynthetic systems in cells of Vinca rosea, L. Proc. Natl. Acad. Sci. U.S., 48, 1776-1782.

BUTCHER, D.N., 1960. Ph.D. Thesis, University of Wales.

BUTCHER, D.N. and STREET, H.E., 1960a. The effects of gibberellins on the growth of excised tomato roots. J. Expt. Bot., 11, 206-216.

BUTCHER,D.N. and STREET,H.E., 1960b. Effects of kinetin on the growth of excised tomato roots. Physiol.Plant., 13, 46-55.

CAMUS,G., 1949. Recherches sur le role des bourgons dans les phenomenes de morphogenese. Rev.Cytol.Biol.Veg., 9, 1-199.

CLOWES,F.A.L., 1961. Apical meristems. Blackwell Scientific Publications, Ltd., Oxford.

COSTER,C., 1927. Zur anatomie und physiologie der zuwachszonen und jahresringbildung in den tropen. Ann.Jard.Bot.Buitenzorg., 37, 49-160.

COSTER,C., 1928. Zur anatomie und physiologie der zuwachszonen und jahresbildung in den tropen. Ann.Jard.Bot.Buitenzorg., 38, 1-114.

DAY,D., 1943. Growth of excised tomato roots in agar with thiamin plus pyridoxine, nicotinamide or glycine. Amer.J.Bot., 30, 150-156.

DIGBY,J. and WANGERMANN,E., 1965. A note on the effect of the shoot and root apex on secondary thickening in pea radicles. New Phytol., 64, 168-170.

DIGBY,J. and WARDING,P.F., 1964. The role of growth hormones in cambial activity (MORPH). Proc.10th.Int.Bot.Cong.Edinburgh. 357-358 (abstract).

DORMER,K.J. and STREET,H.E., 1948. Secondary thickening in excised tomato roots. Nature, 161, 483.

GARNER,W.W. and ALLARD,H.A., 1920. Effect of relative length of day and night and other factors of the environment in growth and reproduction in plants. J.Agric.Res., 18,553-605

GARNER,W.W. and ALLARD,H.A., 1923. Further studies in photoperiodism, the response of the plant to relative length of day and night. J.Agric.Res., 23, 871-920.

GAUTHERET,R.J., 1957. Histogenesis in plant tissue cultures. J.Natl.Cancer Inst., 19, 555-590.

GILL,N., 1933. The relation of flowering and cambial activity. New Phytol., 32, 1-12.

GOUWENTAK,C.A. and HELLINGA,G., 1935. Beobachtungen uber wurzelbildung. Med.Landbouwhoogeschool Wageningen., 39, 1-6.

HANNAY,J.W. and STREET,H.E., 1954. Studies on the growth of excised roots. III. The molybdenum and manganese requirement of excised tomato roots. New Phytol., 53, 68-80.

HEIMSCH,C., 1951. Development of vascular tissues in barley roots. Amer.J.Bot., 38, 523-537.

HUGHES,E.W.D. and STREET,H.E., 1960. Effects of inhibitory concentrations of 3-indolylacetic acid and 3-indolyl-acetonitrile on cell division and tissue differentiation in excised tomato roots. J.Expt.Bot., 11, 198-205.

JOHANSEN,D.A., 1940. Plant microtechnique. McGraw-Hill Book Company, Inc., London-New York.

JOST,L., 1891. Uber dickenwachsthum und jahresringbildung. Bot.Zeit., 49, 485-630.

JOST,L., 1893. Uber beziehungen zwischen der blattentwickelung und der gefassbildung in den pflanzen. Bot.Zeit., 51,89-138.

JOST,L., 1935. Wuchsstoff und zellteilung. Ber.d.Bot.Ges., 53, 733-750.

KASTENS,E., 1924. Beitrage zur kenntnis der funktion der siebrohren. Mitt.Inst.Allg.Bot. Hamburg., 6, 33-70.

KERBLE,F., 1910. Plant-animals. Camb.Univ.Press.

KUNNING,H., 1950. Untersuchungen uber die wirkstoffregulation der kambiumtatigkeit. Planta, 38, 36-64.

LEVINE,M., 1951. The effect of growth substances and chemical carcinogens on fibrous roots of carrot tissue grown in vitro. Amer.J.Bot., 38, 132-138.

LINSMAIER,E.M. and SKOOG,F., 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol.Plant., 18, 100-127.

LOOMIS,R.S. and TORREY,J.G., 1964. Chemical control of vascular cambium initiation in isolated radish roots. Proc.Natl. Acad.Sci. U.S., 52, 3-11.

MILLER,C.O., 1961. Kinetin and related compounds in plant growth. Ann.Rev.Plant.Physiol., 12, 395-408.

OVERBEEK,J.van., CONKLIN,M.E. and BLAKESLEE,A.F., 1941. Factors in coconut milk essential for growth and development of very young Datura embryos. Science, 94, 350-351.

PLOUVIER,V., 1963. Sur la recherche des itols à chaine droite et des cyclitols chez les vegetaux. Relations entre leur repartition et la classification systematique. Bull.Soc. Chim.Biol., 45, 1079-1118.

POLLARD,J.K., SHANTZ,E.M. and STEWARD,F.C., 1961. Hexitols in coconut milk : their role in nurture of dividing cells. Plant.Physiol., 36, 492-501.

PRIESTLEY,J.H., 1930. Studies in the physiology of cambial activity. III. The seasonal activity of the cambium. New Phytol., 29, 316-354.

RADLEY,M. and DEAR,E., 1958. Occurence of gibberellin-like substances in the coconut. Nature, 182, 1098.

RAGGIO,M. and RAGGIO,N., 1956. A new method for the cultivation of isolated roots. Physiol.Plant., 9, 466-469.

RIER,J.P. and BESLOW,D.T., 1967. Sucrose concentration and the differentiation of xylem in callus. Bot.Gaz., 128, 73-77.

RISSER,P.G. and WHITE,P.R., 1964. Nutritional requirements of spruce tumor cells in vitro. Physiol.Plant., 17, 620-635.

ROBBINS,W.J., 1922. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. Bot.Gaz., 74, 59-79.

ROBERTS,E.H. and STREET,H.E., 1955. The continuous culture of excised rye roots. Physiol.Plant., 8, 238-262.

SASS,J.E., 1959. Botanical microtechnique. Constable and Company Ltd. London.

SHEAT,D.E.G., FLETCHER,B.H. and STREET,H.E., 1959. Studies on the growth of excised roots. VIII. The growth of excised tomato roots supplied with various inorganic sources of nitrogen. New Phytol., 58, 128-141.

SNOW,R., 1932. Growth regulators in plants. New Phytol.,31, 336-354.

SNOW,R., 1933. The nature of the cambial stimulus. New Phytol., 32, 288-296.

SNOW,R. and LE FANU,B., 1935. Activation of cambial growth. Nature, London, 135, 149.

SODING,H., 1936. Uber den einfluss von wuchsstoff auf das dickenwachstum der baume. Ber.Deutsch Bot.Ges., 54, 291-304

SOROKIN,H.P., MATHUR,S.N. and THIMANN,K.V., 1962. The effect of auxins and kinetin on xylem differentiation in the pea epicotyl. Amer.J.Bot., 49, 444-454.

STEINHART,C.E., ANDERSON,L. and SKOOG,F., 1962. Growth promoting effect of cyclitols on spruce tissues. Plant Physiol., 37, 60-66.

STEWARD,F.C. and CAPLIN,S.M., 1951. A tissue culture from potato tuber : The synergistic action of 2,4-D and coconut milk. Science, 113, 518-520.

STEWARD,F.C., MAPES,M.O. and SMITH,J., 1958. Growth and organised development of cultured cells. I. Growth and divisions of freely suspended cells. Amer.J.Bot., 45, 693-703.

STREET,H.E., 1955. Factors controlling meristematic activity in excised roots. VI. Effects of various 'anti-auxins' on the growth and survival of excised roots of Lycopersicum esculentum Mill. Physiol.Plant., 8, 48-62.

STREET,H.E., 1966. The physiology of root growth. Ann.Rev.Plant Physiol., 17, 315-344.

STREET,H.E., McGONAGLE,M.P. and LOWE,J.S., 1951. Observations on the 'staling' of White's medium by excised tomato roots. Physiol.Plant., 4, 592-616.

THIMANN,K.V., 1936. Auxins and the growth of roots. Amer.J.Bot., 23, 561-569.

TORREY,J.G., 1951. Cambial formation in isolated pea roots following decapitation. Amer.J.Bot., 38, 596-604.

TORREY,J.G., 1959. Experimental modification of development in the root, 189-222. In D.Rudnick (ed.), Cell,organism and milieu. Ronald Press, New York.

TORREY,J.G., 1963. Cellular patterns in developing roots. Symp XVII. Soc.Expt.Biol., 17, 285-314. Cambridge Univ. Press.

TORREY,J.G. and SHIGENURA,Y., 1957. Growth and controlled morphogenesis in pea-root callus tissue grown in liquid media. Amer.J.Bot., 44, 334-344.

WARMING,P.F., 1949. Photoperiodic control of leaf growth and cambial activity in P.sylvestris. Nature, 163, 770-771.

WARMING,P.F., 1958. Interaction between indoleacetic acid and gibberellic acid in cambial activity. Nature,181,1744-1745.

WARDING,P.F. and ROBERTS,D.L., 1956. Photoperiodic control of cambial activity in Robinia pseudacacia L.New Phytol., 55, 356-366.

WEINTRAUB,R.L., 1940. Plant tissue cultures. Ann.Rep.Smithsonian Inst., 357-368.

WENT,F.W. and THIMANN,K.V., 1937. Phytohormones. Experimental Biology Monographs. Macmillan and Co., New York.

WETMORE,R.H. and SOROKIN,S., 1955. On the differentiation of xylem. J.Arnold Arbor., 36, 305-317.

WETMORE,R.H. and RIER,J.P., 1963. Experimental induction of vascular tissues in callus of angiosperms. Amer.J.Bot.,50, 418-430.

WHITE,P.R., 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol., 9, 585-600.

WHITE,P.R., 1938. Accessory salts in the nutrition of excised tomato roots. Plant Physiol., 13, 391-399.

WHITE,P.R., 1943. A hand-book of plant tissue culture. Tempe,Ariz : The Jacques Cattell Press Inc.

WHITE,P.R., 1963. The cultivation of animal and plant cells. 2nd Ed. Ronald Press, New York.

WILCOX,H., 1962. Growth studies of the root of incense cedar, Libocedrus decurrens. I. The origin and development of primary tissues. Amer.J.Bot., 49, 221-236.

WINTER,H., 1954. Der einfluss von wirkstoffen, von rontgen-und electronenstrahlen auf die cambiumtatigkeit von Beta vulgaris. Planta, 44, 636-668.

WOOD,H.N. and BRAUN,A.C., 1961. Studies on the regulation of certain essential biosynthetic systems in normal and crown-gall tumor cells. Proc.Natl.Acad.Sci. U.S.,47, 1907-1913.