The Effects of Auxins and Kinetin on Xylem Differentiation in the Pea Epicotyl
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THE EFFECTS OF AUXINS AND KINETIN ON XYLEM DIFFERENTIATION IN THE PEA EPICOTYL[1]

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ABSTRACT

SOROKIN, HELEN P., S. N. MATHUR, and KENNETH V. THIMANN. (Harvard U., Cambridge, Mass.) The effects of auxins and kinetin on xylem differentiation in the pea epicotyl. Amer. Jour. Bot. 49(5): 444–454. Illus. 1962.—Treatment of isolated segments from the second internode of etiolated 'Alaska' pea epicotyls with indoleacetic acid or 2,4-D results in: (1) activation of fascicular cambium, and initiation of some interfascicular cambium, resulting in abundant production of secondary xylem, and in formation of hyperplastic tissue; (2) partial or even total occlusion of proto- and metaxylem. The secondary xylem formed consists of short vessel members with scalariformly reticulate or pitted walls, which often lack vertical connection with each other, being interrupted by unlignified cells. When IAA is used, the hyperplastic growth mainly takes the form of root primordia, whereas 2,4-D initiates the formation of callus, but not of root primordia. The growth of this callus causes a characteristic split at the base of the internode. Treatment with kinetin, alone or in combination with the auxin, changes the above structure markedly. It leads to the initiation, over the entire circumference of the core of the internode, of a still more active cambium, which forms several layers of secondary xylem; this consists mainly of long vessel members with pitted walls. Hyperplastic growth is completely absent, and the xylem does not become occluded. Thus the effect of kinetin is to make the xylem more normal and to alter the epicotyl structure from herbaceous to more-or-less woody.

THE INITIAL purpose of this investigation was to study the effects of auxins and kinetin on the development of the transport system in the stem and bud of the pea plant during the inhibition of lateral bud development. As the work developed, however, it became clear that the changes in the internode are much more extensive than had been expected, and that they required detailed study before changes in the other parts could be interpreted. This paper, therefore, describes some of the histological changes which occur in excised, etiolated sections of the *Pisum* epicotyl after treatment with 2,4-dichlorophenoxyacetic acid or indoleacetic acid,[2] with and without kinetin, and also after growing decapitated plants on intact roots in 2,4-D solutions. Attention is devoted here to the internode, and a description of the changes in the node and bud will be given in another paper.

There are many earlier studies of the anatomy of stems in relation to treatment with auxins, particularly with the bean, *Phaseolus vulgaris* L., as the experimental plant, and 2,4-D and IAA as the auxins. The majority of these have been made on whole green plants. Regardless of the mode of application of auxin, e.g., laterally round the stem in lanolin (Hamner and Kraus, 1937), at the base of the primary leaves (Beal, 1945), in aqueous spray (Swanson, 1946), to the ends of decapitated internodes (Whiting and Murray, 1946), to the roots (Allard, De Rose, and Swanson, 1946), or as a spray in carbowax (Eames, 1950)—in all cases the effects are similar. There is a general proliferation of the tissues between the cortex and the primary xylem, "destruction" of the phloem and often the production of root initials. With the exception of the occlusion of some vessels, and the occasional occurrence of isolated wound tracheids, changes in the xylem of *Phaseolus* have not been particularly noted. However, in other plants, from the earliest days of work with applied auxins, increase in the amount of xylem either through activation of cambium or under the additional influence of wounding, have been reported by Snow (1935), Söding (1936), Avery, Burkholder, and Creighton (1937), Gouwentak (1936), and Jacobs (1952).

Although the epicotyl of *Pisum sativum* L. is one of the most frequently used materials for studies of auxin action, its anatomical responses to auxins have been comparatively little studied. Swelling of the bases of lateral buds into little tubers following the direct application of IAA has been reported (Thimann, 1937) and in *Vicia faba* L.—the stem structure of which is somewhat similar to that of the pea—treatment of a decapitated stem with IAA (1.5% in lanolin) gave rise to a tumor-like swelling (Palser, 1942). In this tissue the parenchymatous cells were found to proliferate, but no change in the phloem or xylem was described, with the exception of the appearance of wound tracheids in the callus tissue. Notable

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This work is a part of a continuing program of anatomical research on the living cells and tissues of the pea epicotyl, studied in parallel with physiological experiments. It was supported by grants to Helen Sorokin (8 Cliff Street, Winchester, Mass.) from the American Philosophical Society and the American Academy of Arts and Sciences, and to K. V. Thimann from the National Science Foundation, grant No. G9084.

[2] Abbreviated 2,4-D and IAA respectively.

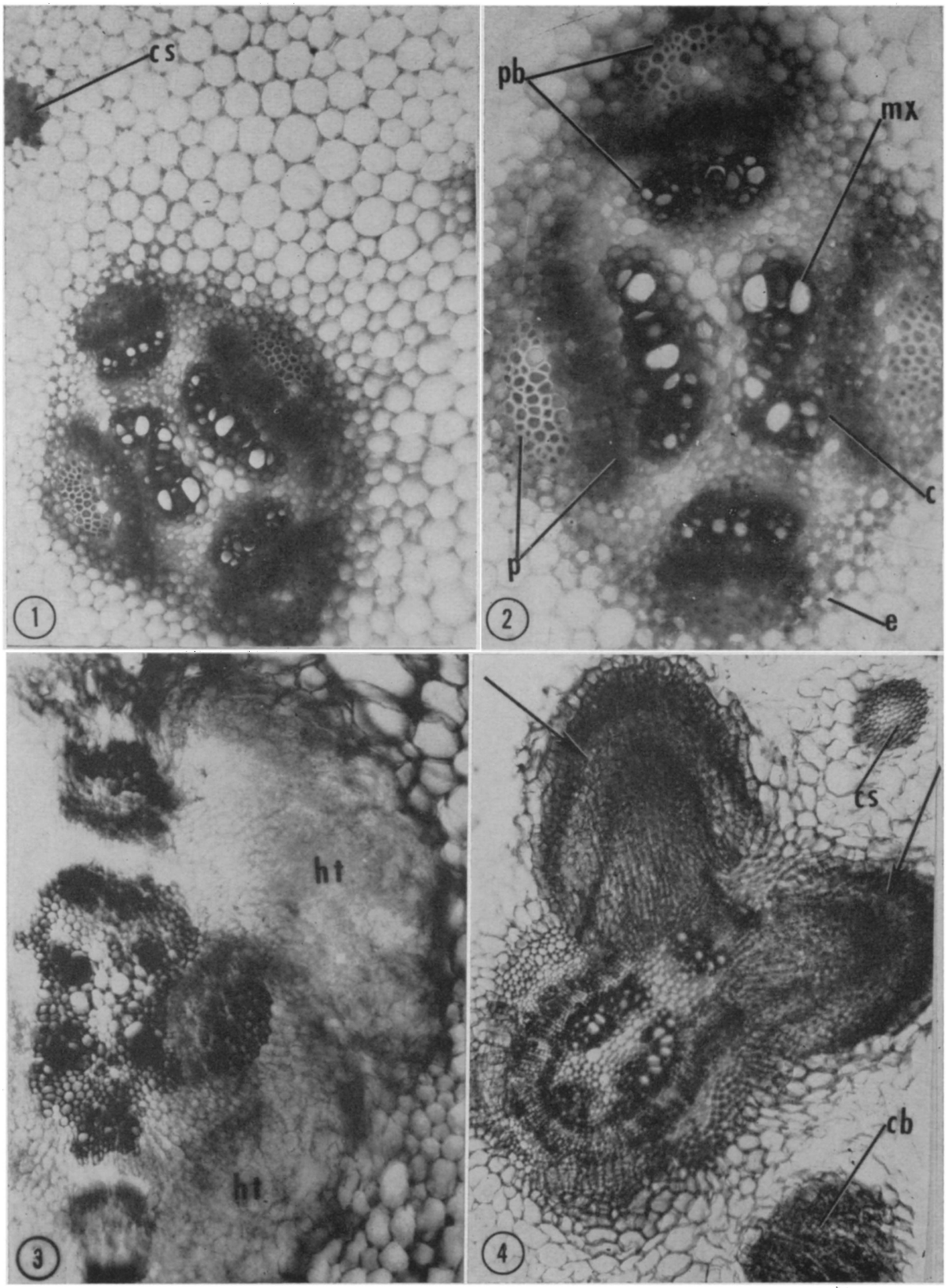
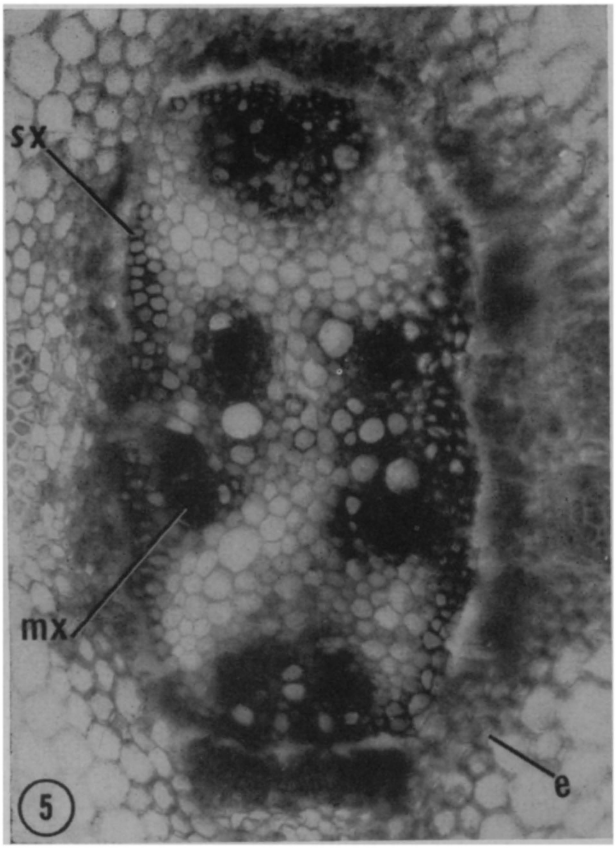


Fig. 1–4. Transections through the second internodes of pea epicotyls. c, cambium; p, phloem; mx, metaxylem; e, endodermis; pb, polar bundle; cs, cortical strand; cb, cortical bundle; ht, hyperplastic tissue.—Fig. 1. Control, fresh stem in I_2 in KI showing the stele, part of the cortex and of the cortical strand. ×50.—Fig. 2. Control, typical distribution of tissues in the part of the second internode under investigation. ×79.—Fig. 3. Excised, grown in 0.15 mg/liter of 2,4-D. Hyperplasia spreads into the cortex and forms symmetrical growth. ×50.—Fig. 4. Grown on intact roots in 4 mg/liter of 2,4-D. Acetocarmine. Hyperplasia is in the form of wedges (arrows) which extend the whole length of the internode. Cortical strand unchanged, cortical bundle shows hyperplasia near and about the xylem. ×50.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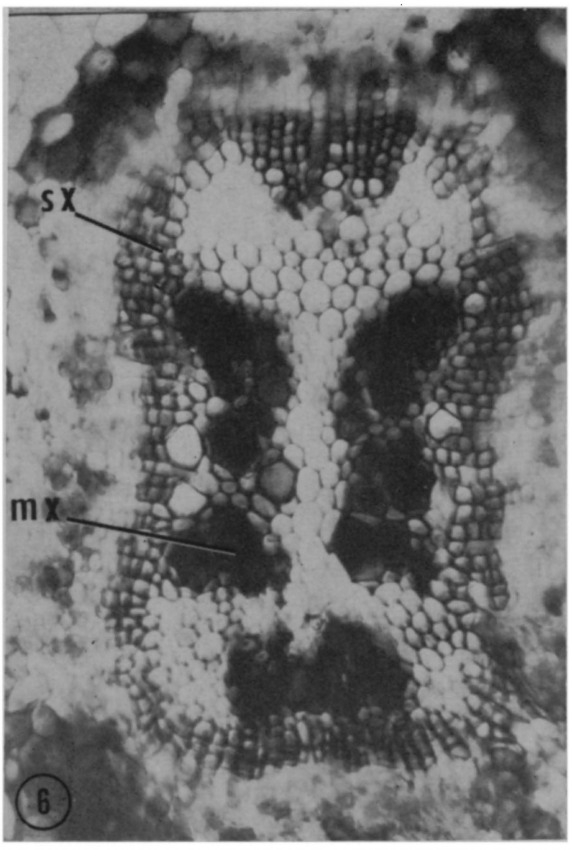
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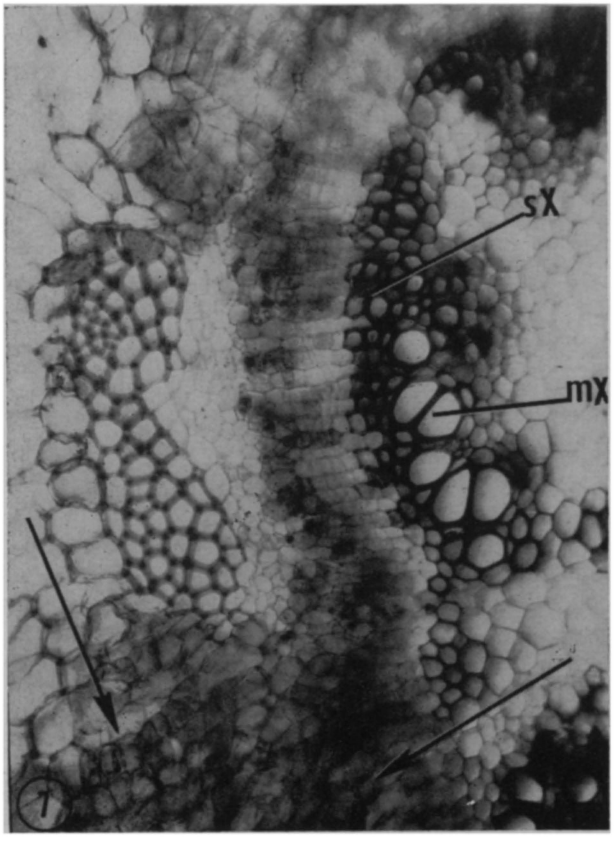
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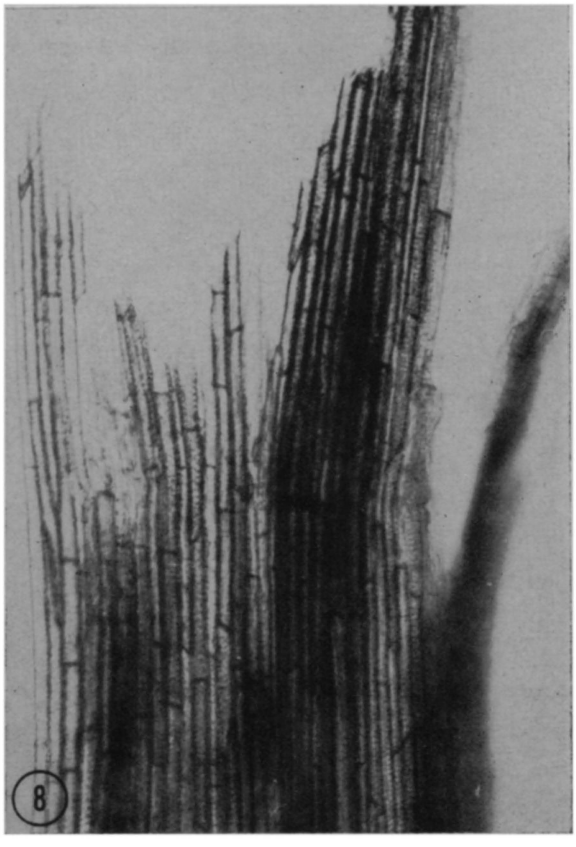
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also was the absence of root primordia in the treated material. In a histological study of excised stem segments of 'Alaska' peas grown in IAA (1–100 ppm), Solberg and Higinbotham (1957) found neither hyperplastic tissue nor differentiation of xylem, but IAA plus sucrose increased the number of thick-walled xylem elements.

The effects of IAA on the roots of *Pisum sativum* have been studied more thoroughly. Pea roots grown in a semifluid agar medium were treated by Bond (1948) with 5 different substances dissolved in lanolin. Among these, 1% 2,3,5-T caused proliferation of the pericycle with consequent tearing of the cortex, while tryptophane increased the stelar tissue mainly by proliferation of xylem parenchyma, accompanied by some cambial activity. Numerous cells of the stele differentiated as scalariform tracheids, especially those derived from the xylem parenchyma. The protoxylem elements, however, became plugged, and there was very little proliferation of the phloem. In roots treated with IAA, there was a moderate hypertrophy of cortical cells in the region of elongation, and some initiation of lateral roots from the pericycle.

In tissue cultures of decapitated roots, a regenerated meristem with a symmetrical hexarch vascular pattern was produced by 5×10^{-6} M IAA, and the roots reverted back to their original triarch pattern when transferred to auxin-free medium (Torrey, 1957). Subsequent treatment with intermediate auxin concentrations produced pentarch and tetrarch patterns. Torrey proposed that the auxin influences the radial dimensions of the new meristem, resulting in a larger procambial cylinder at the level where the vascular tissue pattern is first formed, thus increasing the number of vascular strands.

MATERIALS AND METHODS—The stem segments were prepared exactly as those used for bud inhibition experiments (Wickson and Thimann, 1958, 1960; Thimann and Laloraya, 1960). 'Alaska' peas were grown in water in the 25 C dark room with occasional weak red light. After 7 days, when the third internode[3] was approaching its maximum length, the plants were cut 5 mm above the 2nd node and 30 mm below it to give a 35-mm segment, the basal end of which was at least 0.5 mm, and usually several mm, above the first node. Ten of these segments were placed radially in 10-cm Petri dishes containing 6 ml of solution, bud upwards, exposed to 1500 ft-c white light at 20 C. The solution contained 1.5% sucrose plus 10^{-4} M pure penicillin G, together with the auxin or kinetin. Usually the solutions were changed on the 3rd, 5th and 8th day, and the sections examined on the 6th–10th day. For the experiments with application via the roots, the plants were decapitated 5 mm above the base of the 3rd internode on the 7th day and placed upright with their roots in the test solutions.

The transections were cut free-hand in the living condition between slit pieces of pith marked beforehand into 2-mm segments. All 10 plants of each experiment, as well as the 10 corresponding controls, were sectioned every time. After having been returned to distilled water, the sections were treated on the slides with I_2 in KI for starch, 5% phloroglucinol in 95% alcohol and in HCl, or Mäule reagent (Schneider and Zimmermann, 1922) for lignified cell walls. Since the phloroglucinol preparations slowly faded, they were examined immediately in glycerine jelly. The preparations treated with Mäule reagent were mounted in resin and are permanent. Longitudinal sections were made after the pieces of epicotyl were attached to a paraffin-coated block with low-melting-point paraffin (53–55 C) in such a way that only about one-third of the material touched the paraffin. If the whole block were then cooled rapidly, the upper two-thirds of the tissue remained in living condition. In addition to the above treatment, the longitudinal sections were stained with 0.005% Janus green B. The callus tissue was also stained in acetocarmine.

Photographs were made with a 35-mm camera attachment to a Zeiss microscope with neofluar objectives 6.3/0.20; 16/0.72; 40/0.75; and K oculars 8× with magnification changer for factors 1×,1.6×, and 2.5×. Kodak tri-X pan film was used throughout. Negatives are enlarged as specified.

ANATOMY OF CONTROL PLANTS—Before the histological effects of the treatments are considered, a brief review of the structure of control plants is essential. The earlier studies of the seedling anatomy of *Viciae* have established the existence of a zone of transition between root and stem which is not restricted to the cotyledonary node only, but extends to the upper end of the second internode (Gérard, 1881; Van Tieghem, 1884; Hérail, 1885; Tourneaux, 1910; Compton, 1912;

[3] The internodes of the seedling are counted beginning from the bottom, the first being the oldest, directly above the cotyledonary node, and the next node above is called the first node. This is the system used by anatomists (cited below) and is also commonly employed by physiologists.

Fig. 5–8. Sections of the second internode grown with 2,4-D. sx, secondary xylem; mx, metaxylem.—Fig. 5. Excised, grown in 0.15 mg/liter. Middle of the internode. Secondary xylem forms almost complete cylinder surrounding pith and primary xylem. ×79.—Fig. 6. Grown on intact roots in 4 mg/liter. Occlusion of primary xylem. Activation of fascicular and interfascicular cambium resulting in formation of several layers of secondary xylem. ×79.—Fig. 7. Same culture as in Fig. 6. Acetocarmine. Fascicular and interfascicular cambium activated, secondary xylem formed, hyperplastic growth extends into the cortex (lower part of photograph). ×126.—Fig. 8. Excised, grown in 1.5 mg/liter. Stele dissected out, mostly secondary xylem. ×79.

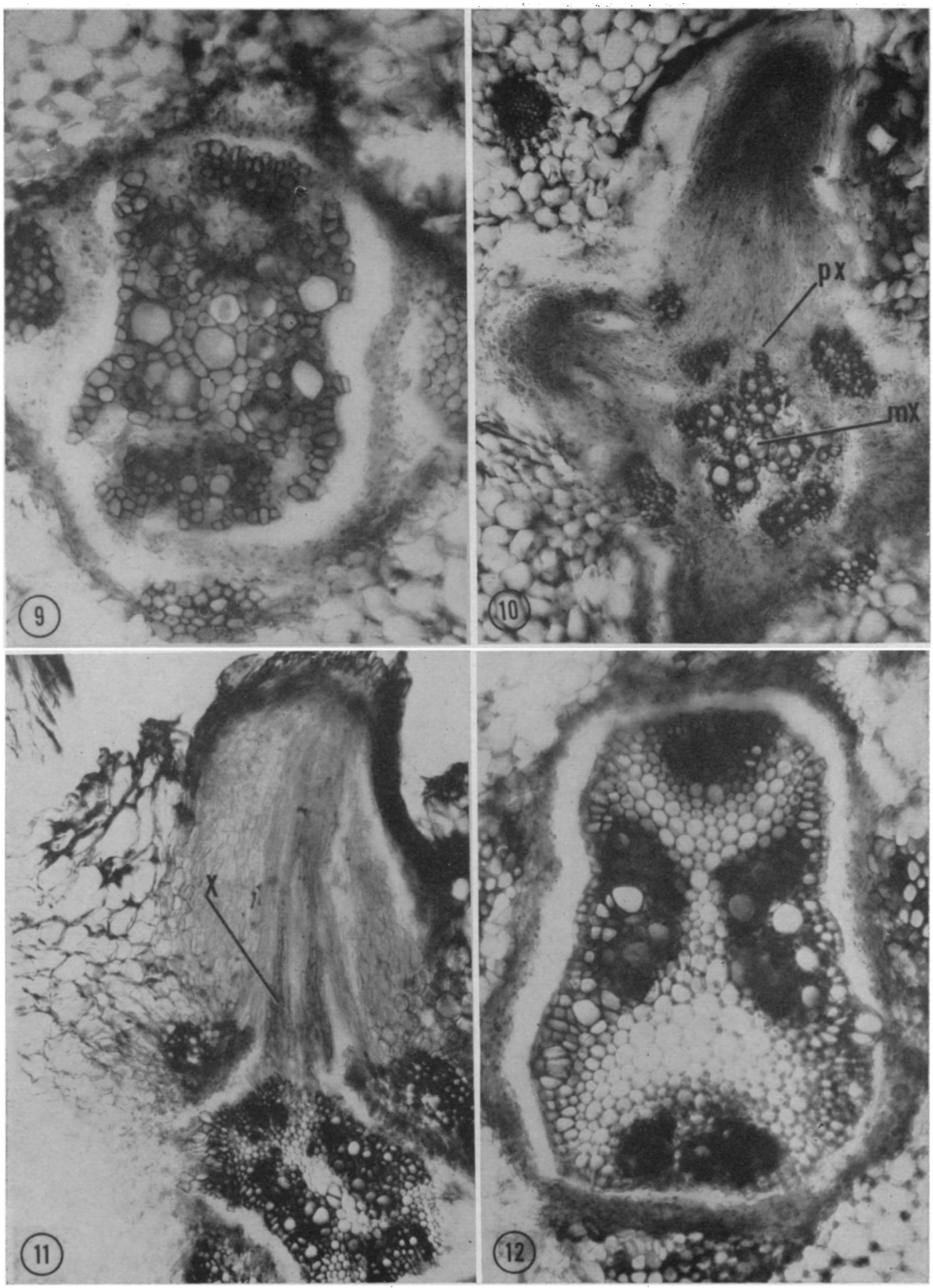


Fig. 9–12. Excised epicotyls grown with IAA. px, protoxylem; mx, metaxylem; x, tracheary xylem elements.—Fig. 9. Grown in 3 mg/liter. Metaxylem in the center, some secondary xylem, hyperplastic growth surrounds the inner tissues. ×79.—Fig. 10. Grown in 3 mg/liter. Section of the internode at ca 4 cm above the root. The central part of the stem acquires structure characteristic of tetrarch roots. Metaxylem in the center, lateral root primordia are emerging opposite the protoxylem points. ×50.—Fig. 11. Grown in 0.3 mg/liter. Scalariformly thickened tracheids appear in the root primordium. Central part of stem does not show the exarch xylem plate seen in Fig. 10. ×50.—Fig. 12. Grown in 0.3 mg/liter. Hyperplastic growth, occlusion of primary xylem, development of secondary xylem. ×79.

Gourley, 1931; Hayward, 1938). This "anomaly" consists of reorganization of the mixed exarch-endarch type of bundles to the purely endarch structure which is characteristic of the stem. As previously summarized by Hayward (1938), a transection made through the middle part of the second internode thus shows the central stele occupying about one-third of the stem diameter (Fig. 1), and surrounded by a cortex of about the same width containing 2 fibrous strands (Fig. 1, cs) and 2 fibrovascular bundles. The central stele is bounded by an endodermis (Fig. 2, e) which contains a small amount of starch. Two polar bundles are present in the stele (Fig. 2, pb), and the lateral groups of metaxylem (Fig. 2, mx) are separated by pith parenchyma from each other and from the xylem of the polar bundles (Hayward, 1938). No interfascicular cambium is present normally, but the fascicular cambium (Fig. 2, c) forms 4 separate and distinctly outlined regions, 2 of which are abaxial and parallel to the metaxylem, the other 2 parallel to the xylem of the polar bundles. Correspondingly, the phloem of the stele (Fig. 2, p) forms 4 groups, each adjacent to the cambial region, and containing phloem fibers, sieve tubes, companion cells, and parenchyma cells. This tissue arrangement is characteristic for the whole length of the second internode in untreated plants. Up towards the second node some changes occur in the xylem, but these are not considered in the present study. The tissue distribution also changes slightly in approaching the first node. In none of the control plants investigated were any root primordia observed at the first or second nodes.

HISTOLOGICAL CHANGES INDUCED BY AUXINS—Four distinct effects were observed in the treated internodes: (1) activation of fascicular and initiation of interfascicular cambium; (2) hyperplastic growth; (3) formation of secondary xylem; and (4) occlusion of primary xylem. Because the responses of the tissues to different auxins, and to different concentrations of the same auxin, differed in several important respects, each treatment will be discussed separately.

Effects of 2,4-D—The excised internodes treated with 0.15 mg/liter ($= 7 \times 10^{-7}$ M) 2,4-D for 6 days or longer show very characteristic enlargement at the base. This enlarged portion, 4–6 mm in length, exhibits a 1-sided longitudinal slit which extends upward about 4 mm from the lower cut surface. In depth this slit reaches through the cortex to the central stele. A cross-section 2–4 mm above the lower cut surface shows considerable development of hyperplastic tissue. The origin of this tissue has not been followed from the time of its inception, but the later stages suggest that it may be derived from undifferentiated cambial derivatives, rather than from the endodermal cells, to which the similar tissue caused by 2,4-D in *Phaseolus* stems was ascribed (Eames, 1950).

By the sixth or seventh day from the beginning of the treatment the hyperplastic tissue may be 2 or 3 times the diameter of the stele (Fig. 3, ht). This tissue resembles callus, i.e., it is a parenchyma undergoing an active uncoordinated growth. The cells are of varied size and shape, and the nuclei often elongated or irregular. The outer layers of this proliferating tissue appear to be more actively dividing than the inner ones. In some cases the outgrowth is more or less equal in all directions (Fig. 3), while in others the growing tissue pushes out the cortex on one side and ruptures it. This is the cause of the characteristic basal slit referred to above.

In excised epicotyls, the hyperplastic tissue extends only 2–4 mm upwards from the base, but in the decapitated stems with their roots in 4 mg/liter of 2,4-D the hyperplastic growth may extend the whole length of the second internode. It decreases somewhat in the middle portion, but increases again near the second node, and is well-developed in the basal part of the third internode. Serial transverse sections (Fig. 4) indicate that the more or less radially seriated extensions of the callus tissue into the cortex, although they appear root-like, are actually in the shape of wedges (Fig. 4, arrows). There is no xylem development in these wedges in the direction perpendicular to the main axis of the internode. Superficially these wedges resemble the extensions of proliferating tissue into the cortex of bean roots treated with 2,4-D (Wilde, 1951), with the difference, however, that in the latter case they are actually lateral roots with a webbing of thin tissue between. Neither mature roots nor real root primordia were seen in the 2,4-D-treated internodes of our material.

Within the callus tissue, vertically oriented xylem elements occur occasionally in the form of scalariformly thickened wound tracheids. A ring of isolated "nests" of such tracheids was observed in the callus at the base of the third internode in decapitated stems grown on intact roots. They were similar to the nodules of xylem reported to occur in the callus of *Syringa* grown with IAA (Wetmore and S. Sorokin, 1955).

Occlusion of the xylem is a very characteristic result of 2,4-D treatment (Fig. 3, 5, 6). Between the second and fourth millimeter from the bottom of the excised internodes, where the amount of callus-like tissue is greatest, the central stele shows a pronounced occlusion of both proto- and metaxylem (Fig. 3). This occlusion probably extends the whole length of the metaxylem, since it is still very marked at 8–10 mm above the cut surface (Fig. 5). Occlusion of the primary xylem is also very prominent in the internodes grown with 4 mg/liter of 2,4-D on intact roots (Fig. 6).

Although in *Phaseolus* treatment with 2,4-D causes no marked development of xylem (Eames, 1950), in *Pisum* the extraordinary development of xylem is the outstanding result of most of the vigorous activity of both fascicular and inter-

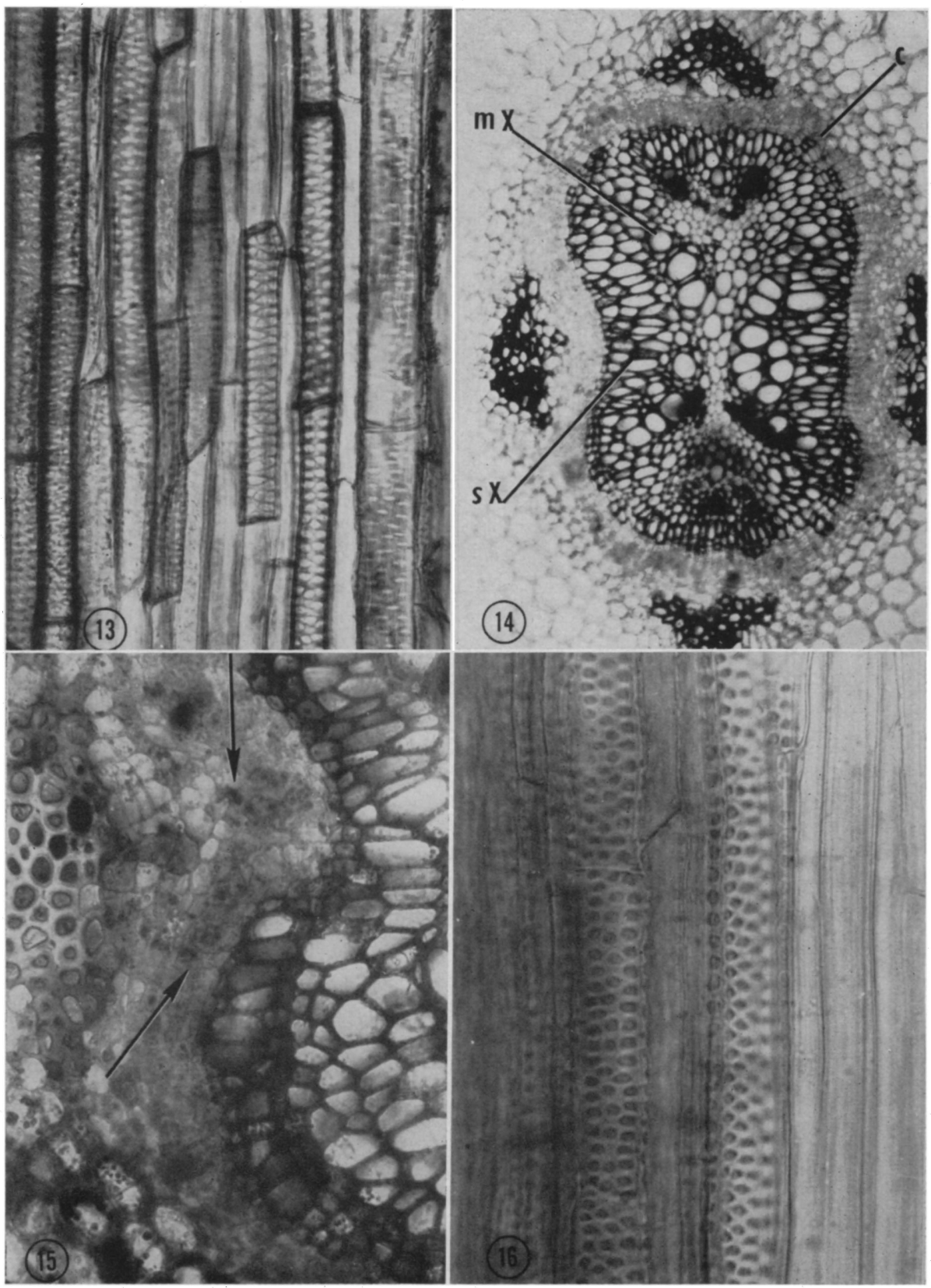


Fig. 13–16. Fig. 13. Excised, grown in 0.3 mg/liter of IAA. Typical elements of the secondary xylem have a variety of wall patterns and do not always form good vertical connections. ×320.—Fig. 14–16. Excised, grown with 4 mg/liter of kinetin and 2 mg/liter of IAA. mx, metaxylem; sx, secondary xylem; c, cambium.—Fig. 14. Very active fascicular and interfascicular cambium produce large amount of secondary xylem. Hyperplastic growth is absent. Metaxylem is not occluded. ×79.—Fig. 15. Mitotic figures are often seen in the active cambial region (between arrowheads). ×200.—Fig. 16. Pitted vessel members with alternate pit arrangement. ×126.

fascicular cambium. The activated cambium often shows the formation of secondary xylem on its adaxial side, and at the same time initiation of hyperplastic growth on the abaxial side (Fig. 7, sx and arrows). In preparations stained with acetocarmine the cells showing hyperplasia appear red, and are therefore darker in the photomicrograph. At 8–10 mm above the basal end the secondary xylem forms an almost complete oval, cylindrical sheath (Fig. 5, sx) which encloses the pith and the primary xylem. At this level the secondary xylem has a few small discontinuities around its circumference (Fig. 5). As the distance from the basal end increases, the amount of secondary xylem becomes very variable. In some sections it diminishes gradually in amount until finally good contact between the pith and the endodermis becomes apparent. In others, including those grown on intact roots, the secondary xylem ring, which appears quite complete in transection (Fig. 6, sx), extends the whole length of the internode. As a result the central part of these internodes can be easily dissected by needles from the rest of the cortex and teased apart for microscopical examination (Fig. 8). It contains tracheary elements whose wall thickenings occur in a variety of patterns—scalariform, reticulate, scalariform-reticulate and pitted.

The elements of the secondary xylem vary in length from 40 to several hundred microns, the most frequently observed length being 150–200 μ. Often in a vertical row of precursor cells only a few had developed into vessel members, the others still not showing the characteristic wall thickenings. The tissue varies in thickness from 1 to 3 layers. The cells adhere to each other closely, though quite a few parenchyma cells are seen among them. The tracheary elements in the secondary xylem produced after treatment with IAA are exactly of the same type and are shown at a greater magnification in Fig. 13.

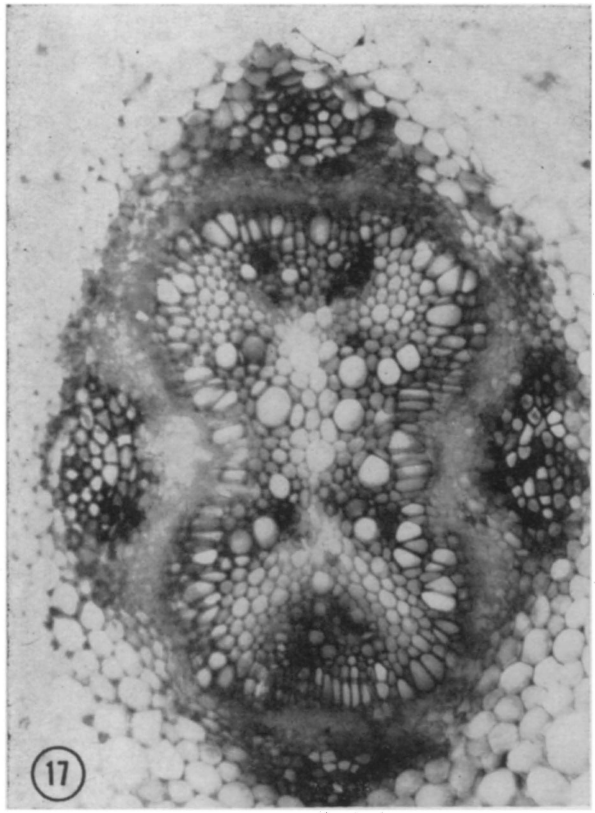
Effects of IAA—The internodes grown in 3 mg/liter ($= 1.7 \times 10^{-5}$ M) of IAA show no external evidence of enlargement except for occasional slight splitting at the base. In the transection through the lower third of the internode (Fig. 9, 10) the most conspicuous changes are (1) formation of hyperplastic tissue; (2) reversion of the siphonostele to a condition reminiscent of the protostele of the roots; (3) activation of fascicular and interfascicular cambium and its conversion into secondary xylem; (4) frequent occlusion of the primary xylem.

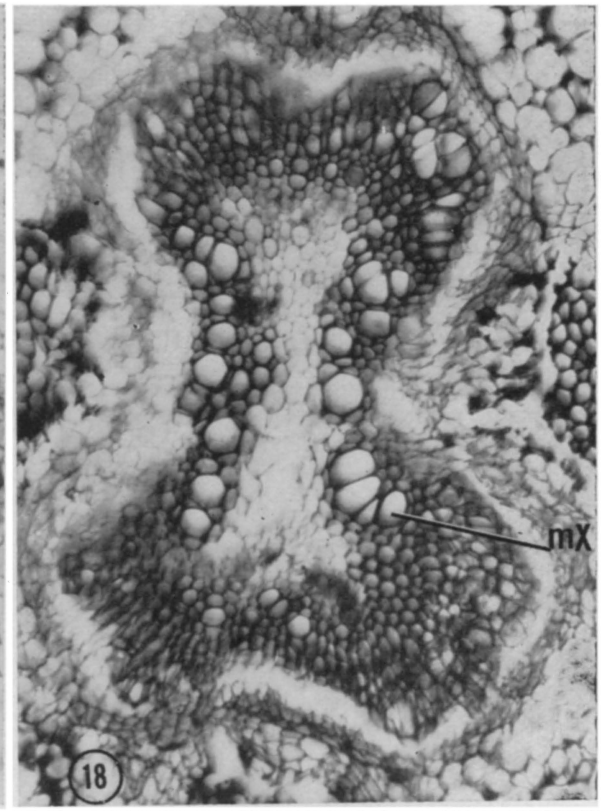
The hyperplastic growth induced by the IAA at this concentration gives rise to what appear to be root primordia (Fig. 10). It is important to emphasize that the portion of the second internode discussed here is removed from the root by the length of the first internode, the first node, and by the lowermost portion of the second internode—a total distance of about 4 cm. Under no conditions were roots formed at the corresponding distance in control plants. When root primordia are initiated the internode anatomy becomes reminiscent of that of the root, i.e., the central core contains an exarch xylem plate with four protoxylem points and metaxylem in the center (Fig. 10). The lateral root primordia emerge opposite the protoxylem points. Not all of the primordia reach the surface of the stem, and the differentiation of xylem in the direction perpendicular to the stem axis is only slight. Better organized xylary elements are observed in root primordia on internodes treated with 0.3 mg/liter of IAA (Fig. 11); here scalariformly thickened tracheids are beginning to be arranged in rows, the axis of which is perpendicular to the xylem of the stele (Fig. 11, x). The central core in such internodes does not show the exarch xylem plate seen at 3 mg/liter of IAA. It is interesting to note that the pea root normally has a triarch pattern of vascular tissue (Hayward, 1938), which is changed under the influence of IAA (Torrey, 1957). Thus, a hexarch pattern is produced in auxin media at a concentration of 5×10^{-6} M ($= 0.9$ mg/liter), and it reverts back to triarch when transferred to auxin-free medium. Furthermore, when such roots are again transferred to intermediary auxin concentrations, pentarch and tetrarch roots occur (Torrey, 1957). Whether the anatomical structure of the treated internode, which is reminiscent of the tetrarch root (Fig. 10), is produced accidentally by about the same concentrations of IAA as used in the experiments with roots, or whether there is a casual relationship between the 2 phenomena, remains to be investigated.

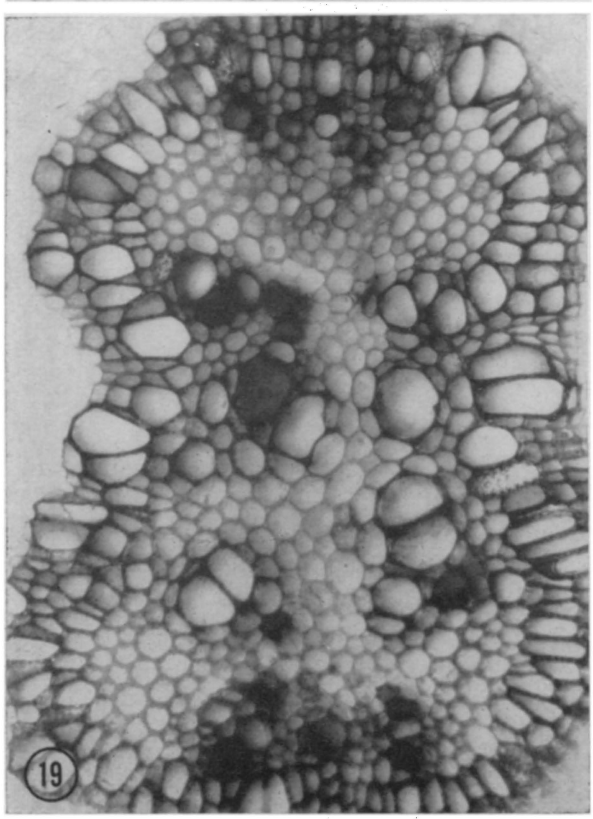
At a concentration of 2 mg/liter, longer roots (up to 5.5 mm) are occasionally observed, whereas if the internode is debudded beforehand, most sections develop several short roots (1–10 mm long) at a distance of about 2 mm above the basal cut surface.

Fascicular and interfascicular cambium become activated in all the concentrations of IAA employed in these experiments, and secondary xylem is always formed. The structure of this xylem is very similar to that induced by 2,4-D and described above (Fig. 13), although the amount varies from plant to plant. In some cases (Fig. 12) the new xylem forms a 1- or 2-layered cylinder which surrounds the pith with the primary xylem; in other cases (not illustrated) several layers of secondary xylem remind one of the structure of the internode grown in the presence of kinetin (see below). The primary xylem usually becomes either completely or partially occluded.

HISTOLOGICAL CHANGES INDUCED BY KINETIN—Previous anatomical studies of the effects of kinetin allow only a few generalized conclusions, namely: (1) kinetin considerably increases mitotic activity, as was shown first in excised tobacco pith tissue treated with kinetin in combination with IAA (Das, Patau, and Skoog, 1956); (2) it inhibits root elongation (see lit. in Laetsch and







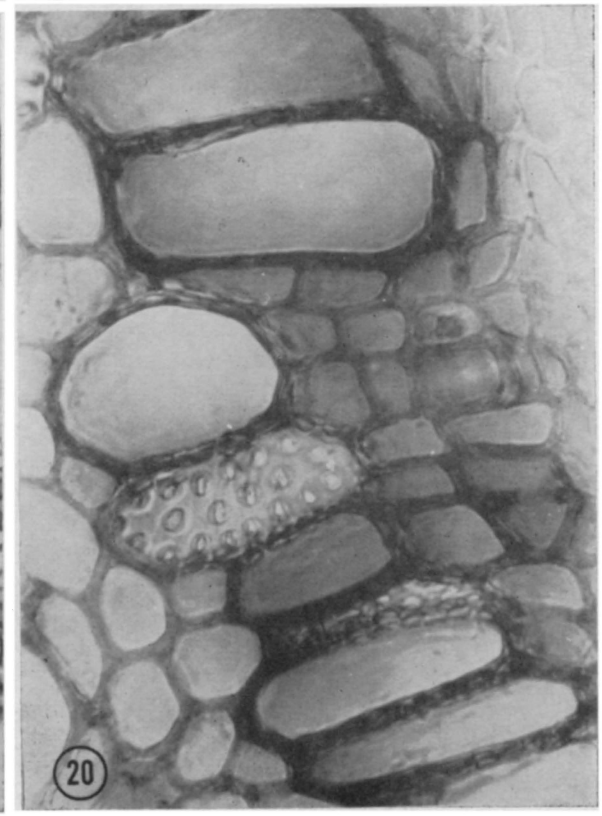


Fig. 17–20. Excised second internodes after treatment with kinetin.—Fig. 17. Grown with 1.5 mg/liter. Interfascicular and fascicular cambium form secondary xylem which surrounds pith and primary xylem. ×79.—Fig. 18. Grown with 0.15 mg/liter of 2,4-D plus 3 mg/liter of kinetin. Very active cambium forms abundant secondary xylem. Metaxylem not occluded. ×79.—Fig. 19. Grown with 4.5 mg/liter of kinetin; phloroglucinol and HCl. Several layers of narrow cells of secondary xylem completely surround pith and primary xylem. ×126.—Fig. 20. Detail of Fig. 19 to show the pits of the vessel members. ×500.

Briggs, 1961); and (3) it promotes or initiates callus formation. Specific conditions are apparently required for callus formation, since, e.g., in *Phaseolus* only the petioles produced callus, while the hypocotyl did not (Humphries, 1960); epicotyls of *Vicia faba* formed callus strongly, while those of *Pisum* did not (Wickson and Thimann, 1958).

In pea internodes treated with kinetin (4.5 and 1.5 mg/liter, $= 2 \times 10^{-5}$ and 7×10^{-6} M) alone or in combination with auxins, mitotic divisions were found abundantly in the cambial region (Fig. 15, between arrowheads), resulting in the formation of well-organized zones of activated fascicular and interfascicular cambium (Fig. 14, 17, 18). Hyperplastic growth, such as was seen after treatment with 2,4-D or IAA, did not occur, whether the kinetin was applied alone or in combination with the auxins. The development of the xylem was accelerated and regularized. It consisted of a compact tissue of narrow tracheary elements of pitted vessels with alternate pits (Fig. 16, 20). These elements did not become occluded. The newly formed secondary xylem, of one to several layers, was in the shape of a solid irregular cylinder which surrounded the pith and the primary xylem in the lowermost third of the internode, but was interrupted in the middle and upper part. Regardless of the condition of growth, concentration of kinetin, or presence or absence of either of the added auxins, the general pattern of this xylem development remained more or less similar. The presence of endogenous auxins may perhaps account for the differentiation of secondary xylem in plants treated with kinetin alone. In Fig. 17 the internode was grown with 1.5 mg/liter of kinetin, in Fig. 19 with 4.5 mg/liter, in Fig. 18 with kinetin at 3 mg/liter in combination with 0.15 mg/liter of 2,4-D, and in Fig. 14 with kinetin at 4 mg/liter plus 2 mg/liter of IAA. The results of these treatments were all very similar, though there were slight differences in the number of layers of secondary xylem produced, and there were slightly more periclinal divisions after 2,4-D treatment (Fig. 18). The metaxylem, which is readily recognizable after these treatments, was not occluded (Fig. 18, mx).

DISCUSSION—Considering the results as a whole, it may be concluded that treatment of the stem sections with auxins causes cambium activation and *abnormal* growth; hyperplastic tissue and imperfectly formed xylem are the principal evidences for this. When kinetin is added the growth becomes more *normal*, callus does not develop and the xylem elements become regular and apparently functional. In a rather over-simplified way it might be said that the stem has now become essentially converted from herbaceous to woody. It may be suggested, therefore, that perhaps the formation of woody stems normally takes place under the combined influence of an auxin and a kinetin-like compound.

It is suggestive that in several woody species the combination of indoleacetic acid with gibberellin has a greater effect on the formation of normal xylem than either one alone (Wareing, 1958). It is true that the effects of gibberellin and kinetin are, in other respects, not comparable, but at least the evidence that more than one factor is concerned supports the above conclusion.

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