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A Histological Study of Lateral Root Initiation and Development in *Zea mays*

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With 32 Figures

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Summary

A light microscopic study has been made of the origin and development of lateral roots in *Zea mays*.

The initiation of a lateral occurs adjacent to a xylem pole and involves an increase in cytoplasmic basophilia and subsequent divisions of cells of the pericycle and the parenchyma of the stele of the mother root.

Cells derived from the parent pericycle form most of the young lateral but its epidermis and root cap are composed of cells of endodermal origin.

Two different types of polysaccharides are secreted by cells of the young lateral root. One type which is PAS-positive and non-metachromatic, is produced by the epidermal cells, while the other type, metachromatic and only slightly PAS-positive, is secreted by the root cap cells.

1. Introduction

Lateral roots arise endogenously and must traverse living cortical and epidermal tissues in the course of their growth to the outside of the parent root. The means by which this passage is effected has been the subject of controversy in the literature since the classic investigations of lateral root development by van Tieghem and Douliot in 1888.

The mechanisms which have been postulated involve either a mechanical tearing of the parent tissues by the lateral, or, what would seem much more efficient, the enzymatic removal of parent tissue from the path of the advancing primordium. Although the theory of mechanical breakthrough has had plenty of supporters (*e.g.* Pond 1908, von Guttenberg 1940) more investigators have favoured enzymatic hydrolysis, either as the only mechanism of cell breakdown, or in conjunction with mechanical passage (*e.g.* van Tieghem and Douliot 1888, Eames and MacDaniels 1947, Bonnett and

Torrey 1966). However, there seem to have been only three investigations of lateral root development which provide direct evidence for either mechanism.

Pond (1908) used the ingenious approach of forcing root tips to grow through tissues of other roots. The histological appearance of the damaged roots was then compared with that of similar tissue pierced by appropriately-sized glass rods and with that of tissue around lateral roots. Pond found no significant differences in the appearance of the tissues in each of these cases and concluded that only mechanical pressure is involved in the emergence of lateral roots in *Vicia faba* and *Lupinus albus.* A similar approach was used earlier by Pierce in 1894 (quoted in Torrey 1965) who concluded also that passage through the cortex is purely mechanical. Pond's conclusions that enzymes are not involved in lateral passage were based on the observations that in the species he examined neither the walls nor the starch content of adjacent cortical cells seem to be eroded during the passage of lateral primordia.

Recently, Sutcliffe and Sexton (1968) have demonstrated histochemically that there is β-glycerophosphatase activity in disintegrating cortical cells around developing lateral roots of *Pisum.* These authors conclude that in this case the tissues of the lateral are not secreting these hydrolyzing enzymes but rather that the pressure of the emerging primordium damages the cortical cells and induces them to produce hydrolytic enzymes and thus bring about their self-destruction.

One of the reasons for the lack of studies of enzyme involvement in lateral root emergence is the difficulty in finding a good system in which it is possible to study separately the enzymology of the lateral primordia and the surrounding cortical tissues. However, roots of *Zea mays* seem to provide just such a system.

It was first shown by Laties and Budd (1964) that the cortex can be stripped easily from corn roots and we have found that the cortex separates from the stele by the breaking of either the outer periclinal walls of the endodermis or the walls of the innermost cortical parenchyma cells. In either case the lateral primordia remain attached to the stele (see Fig. 47, O'Brien and McCully 1969). The laterals can subsequently be removed easily since they break cleanly at their base when a small pressure is applied. Thus lateral primordia and cortical tissue can be studied separately.

The present paper reports a study of lateral root initiation and development in *Zea mays* which was undertaken to provide a knowledge of the histology of this system for use in conjunction with a biochemical and histochemical study of the enzymes involved in the penetration of the cortex by the lateral roots.

This histological study is of interest in its own right since there have been relatively few investigations of lateral root development in Monocotyledons.

Furthermore, there have been few reports of studies with any species, which include photomicrographs of the complete sequence of stages from initiation of a lateral root primordium to its emergence from the parent root and these studies have been done prior to the introduction of new histological methods which facilitate high resolution light microscopic observations of both cell walls and cytoplasm.

2. Materials and Methods

Seeds of *Zea mays* var. Seneca Chief were surface sterilized in commercial Javex (5.25% sodium hypochlorite) for 1 minute, rinsed 5 times in sterile distilled water and germinated on 1% agar plates at 27° C for 70 to 100 hours (method of Dr. A. OAKES, personal communication). Under these conditions intact roots readily form laterals.

By the use of toluidine blue stained hand sections (see O'BRIEN and MCCULLY 1969) it had been determined that a linear developmental series of lateral root primordia occurs within approximately the first 24 mm of the main root, distal to the last emerged lateral. Such regions of the roots were fixed at 0° C in 10% acrolein in tap water, dehydrated (also at 0° C) through a methyl cellosolve, ethanol, propanol, and butanol series, and embedded in glycol methacrylate. Details of this fixation and embedding procedure are available elsewhere (FEDER and O'BRIEN 1968).

Serial sections, 1 to 2 μ thick, were cut with glass knives on a Porter Blum MT 1 ultramicrotome. Sections were placed in drops of sterile, filtered, distilled water on new glass slides and air dried.

Sections were stained with toluidine blue (O'BRIEN, FEDER, and MCCULLY 1964), acidic acid fuchsin, by the periodic acid-Schiff's (PAS) reaction (see JENSEN 1962), or by the periodic acid-acriflavine reaction (KASTEN 1959). Two different aldehyde blocking agents were used with the latter methods; a saturated solution of 2,4-dinitrophenylhydrazine in 15% aqueous acetic acid for 15 minutes at room temperature (modified from DANIELLI 1949), or a solution of chlorous acid for 20 minutes (modified from RAPPAY and VAN DUIJN 1965).

Photomicrographs were taken on Kodak Panatomic-X or Contrast Process Pan 4″ × 5″ sheet film.

3. Results

Under the growth conditions of the present study, lateral roots of *Zea mays* var. Seneca Chief are initiated in an area approximately 70–90 mm from the apex of the main root. While no specific pattern of distribution is evident, lateral primordia frequently occur in groups of up to 3 or 4 along the main root and it is not uncommon to have three lateral primordia in one cross-section of the main root.

3.1. Initiation and Development of the Lateral Root Primordium

The first indication of lateral root initiation is a marked increase in the cytoplasmic basophilia in two pericycle cells and a few underlying parenchyma cells at the tip of a xylem pole (Fig. 1). Changes in the walls of these same tissues also occur at an early stage in lateral root development. Normally in this region of the root the walls of the cells of the pericycle and the

parenchyma of the stele are thick and they stain bright green with toluidine blue [1], suggesting that they are lignified (see O'Brien, Feder, and McCully 1964). However, this staining reaction disappears and these cell walls become thinner (Fig. 2) in the area where a lateral root is initiated. The loss of wall material is accompanied by a loss in birefringence and is clearly demonstrated by viewing unstained sections with polarized light (cp. Figs. 3 and 4).

The two pericycle cells which first show the increased basophilia lie end to end along the long axis of the root so that only one of them appears in a cross section (Fig. 1). It is in these cells that the first divisions which can be associated with lateral root outgrowth are observed. Fig. 5 shows a longitudinal section in which it can be seen that these divisions occur simultaneously in both cells. Fig. 5 also shows that at this stage a whole group of parenchyma cells in the stele adjacent to the dividing pericycle are intensely basophilic and are also dividing. Such cells are not seen elsewhere, suggesting that they are also in some way involved in the very early development of the lateral primordium.

Soon after mitotic activity has been initiated in the two pericycle cells, one observes that further divisions follow rapidly, and that there has been considerable enlargement of the pericycle in this area so that it bulges outwards in both directions, and compresses both the overlying endodermis and the underlying xylem parenchyma (Figs. 6 and 8). Thus at this stage several

[1] These walls also give a positive reaction to the chlorine-sulphite test for lignin (see Siegel 1953).

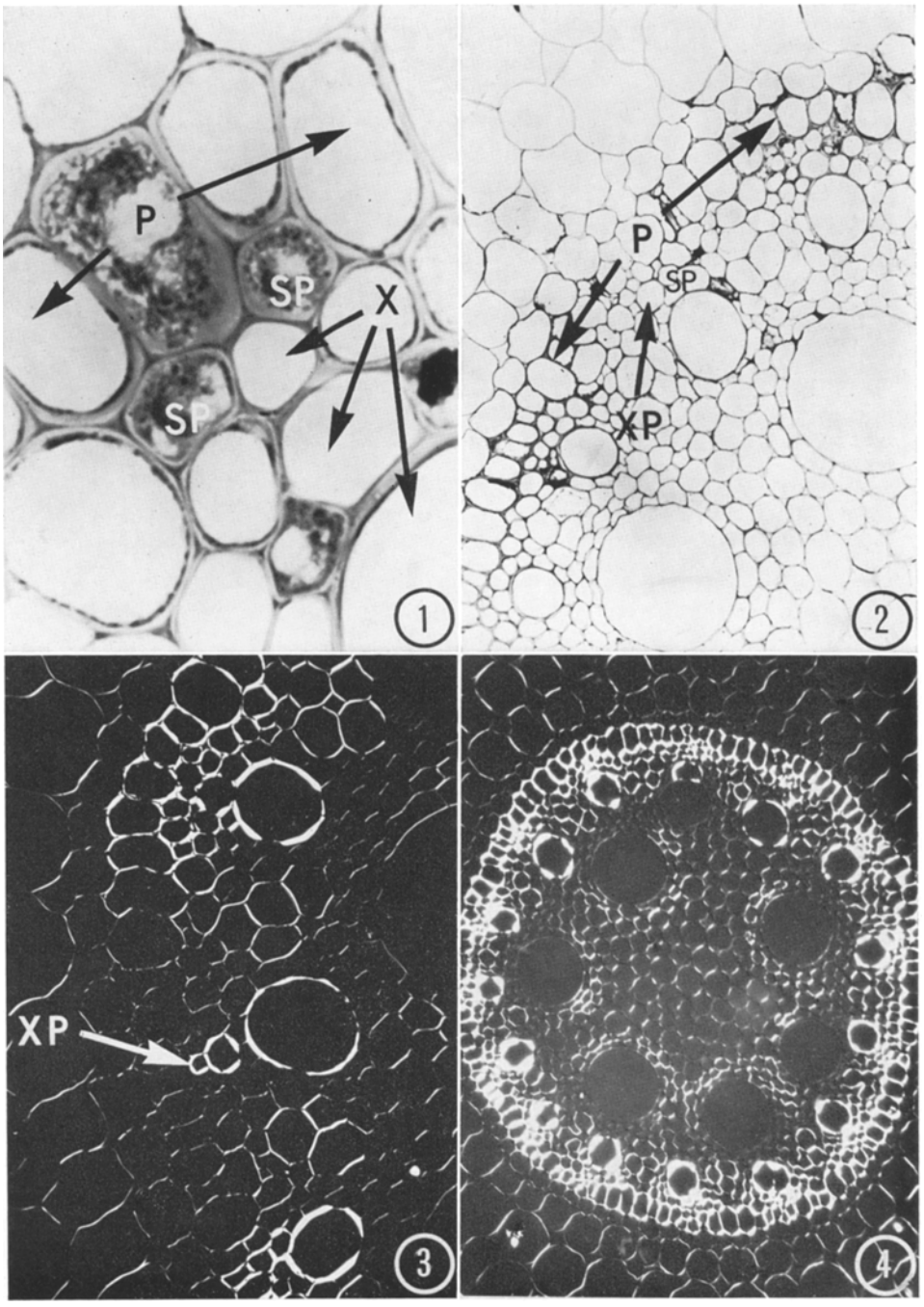
All sections shown are of root tissue of *Zea mays*, fixed with acrolein and embedded in glycol methacrylate. Unless otherwise indicated, sections are stained with toluidine blue. The solid black arrow which appears in the upper right corner of many of the figures indicates the direction of growth of the lateral root primordium.

Fig. 1. Transverse section (*TS*) of a main root in the region of initiation of a lateral primordium, showing intense basophilia in the cytoplasm of one cell in the pericycle (*P*) adjacent to a xylem point and in parenchyma cells (*SP*) of the stele around the xylem (*X*). ×1,380

Fig. 2. *TS* of main root in the region of initiation of a lateral primordium showing thinning of the walls of cells in the pericycle layer (*P*) and in the stelar parenchyma (*SP*) around one of the xylem points (*XP*). The wall thinning is accompanied by loss of the characteristic greenish stain of these walls with toluidine blue. This staining is evident in the walls of corresponding cells around the other xylem points not involved in lateral root initiation (the greenish colour appears light grey in the photomicrograph). ×310

Fig. 3. A *TS* similar to that shown in Fig. 2 but with the section unstained and photographed with polarized light. Note the loss of birefringence and the thinning of the cell walls of the pericycle and stelar parenchyma cells around a xylem point (*XP*) involved in lateral initiation. ×450

Fig. 4. *TS* of main root distal to the section shown in Fig. 3. Lateral initiation has not started in this region and the thickened walls of the pericycle and stelar parenchyma cells are strongly birefringent. Polarized light. ×107



Figs. 1–4

pericycle derivatives may be observed, and it should also be noted that some divisions have now occurred in the endodermis.

It is difficult to obtain sections showing stages of lateral development intermediate to those shown in Figs. 5 and 6. However, after the initial pericycle divisions, pericycle cells on both sides of the dividing cells and in the same tangential plane also begin to divide. Thus by looking at pericycle tissue in longitudinal sections adjacent to one such as that shown in Fig. 6 what may be an earlier stage in the development in the pericycle derivatives can be demonstrated (Fig. 7). Here, a single new cell wall has been laid down in each pericycle cell, subsequent to the nuclear divisions such as those shown in Fig. 5. It can be seen that the new cell divisions have been asymmetric, and that only one end of each pericycle cell is involved. Thus, the 4 daughter cells produced differ in size, those closer to the stele being larger than those adjacent to the endodermis.

At this stage it becomes clear that the asymmetric divisions of the pericycle cells have produced two types of daughter cells of quite different developmental potential. The larger, more vacuolated cells formed adjacent to the stele of the main root (Fig. 6) divide only infrequently and appear to play but a small part in the subsequent development of the main body of the lateral primordium (Figs. 12, 13, 14, and 19). However, when such cells lie over the mother root vascular supply they frequently show heavy basophilia and some cell division (especially clear in Fig. 13 but also seen in Figs. 9 and 11) suggesting that they will be involved in the formation of vascular

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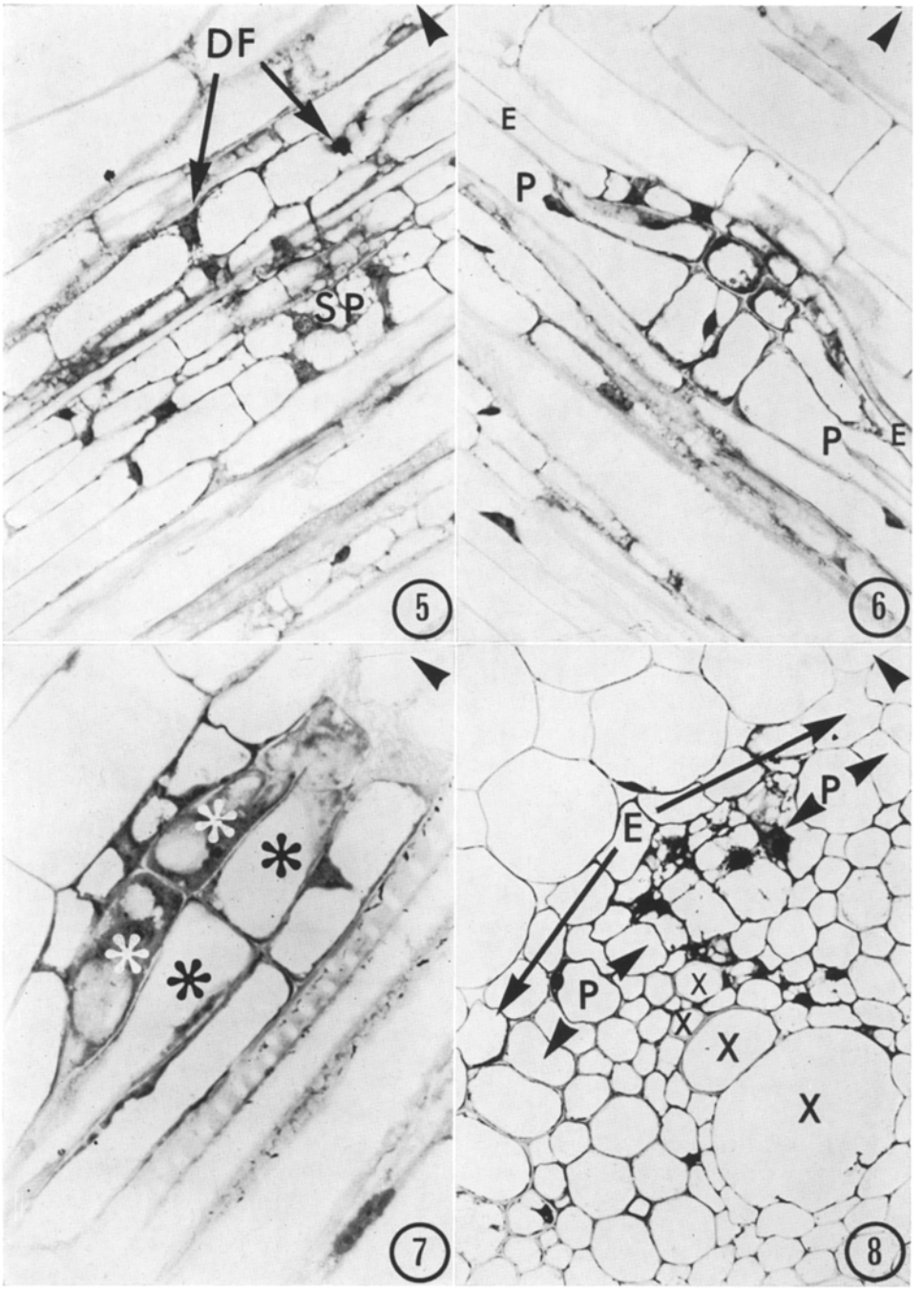
Fig. 5. Longitudinal section (*LS*) of main root in the region where a lateral has been initiated showing the first 2 division figures (*DF*) in the pericycle layer. Note the increased basophilia and evidence of recent cell divisions in the stelar parenchyma cells (*SP*) immediately under the dividing pericycle cells. ×415

Fig. 6. *LS* of main root showing a median section of a lateral primordium at a somewhat later stage in development than shown in Fig. 5. The pericycle (*P*) has expanded in the area where the original 2 pericycle cells involved in lateral initiation adjoined. At this stage one periclinal and anticlinal division have occurred in each of these 2 original pericycle cells (see Fig. 7) and cell division has started in the endodermis (*E*) just above the expanding pericycle. ×415

Fig. 7. A section of the same primordium shown in Fig. 6 but removed from the latter section by ca. 20 μ. This section is tangential to the young primordium and shows what is probably an earlier stage in the development of pericycle derivative cells than is shown in Fig. 6. The area where the two pericycle cells adjoin is greatly expanded and the first periclinal division in each of these cells has produced two very different cell types, a small cell (white asterisks) with small vacuoles and strongly basophilic cytoplasm and a large, highly vacuolated cell (black asterisks). ×670

Fig. 8. *TS* of main root passing through the centre of a young lateral primordium at a stage of development between those shown in Figs. 5 and 6. Cell division has occurred in the pericycle (*P*) but not in the endodermis (*E*). Increased basophilia and evidence of recent divisions can be seen in some of the parenchyma cells of the stele adjacent to the xylem (*X*). ×430



Figs. 5–8

connections with the main root. The daughter cells which are produced closest to the cortex are smaller and more basophilic. These cells divide rapidly both in anticlinical and periclinal planes and give rise to most of the body of the primordium.

The configuration shown in Fig. 7 could be arrived at in two ways. The adjoining pericycle cells could each divide asymmetrically in a semi-periclinal plane, and these divisions could then be followed by radial expansion of the area. Alternatively, the portions of the two pericycle cells close to their abutting walls could enlarge first and then be cut off by asymmetric division. In either case a further anticlinal division in each of the four derivatives would lead to the cell arrangement shown in Fig. 6.

It is worth noting that if the second possibility occurs, the configuration of the pericycle cells before the division would be remarkably similar to that of pericycle cells involved in the formation of lateral primordia in wheat roots in which cell division has been blocked by colchicine (cp. Fig. 11, Foard *et al.* 1965, with our Fig. 7).

The initial divisions in the endodermis are confined to cells immediately adjacent to pericycle cells which are dividing (Figs. 6 and 7). However, a wave of division spreads rapidly to adjacent endodermal cells and for a short time endodermal cells over a wider radius than that of the dividing pericycle cells show increased basophilia (Fig. 10) and subsequently divide. All the cell divisions of the endodermis which occur during the early development of the lateral primordium are anticlinal and form a single layer of cuboidal epidermal cells over the entire primordium. As development continues, a few of these epidermal cells at the tip of the lateral divide almost

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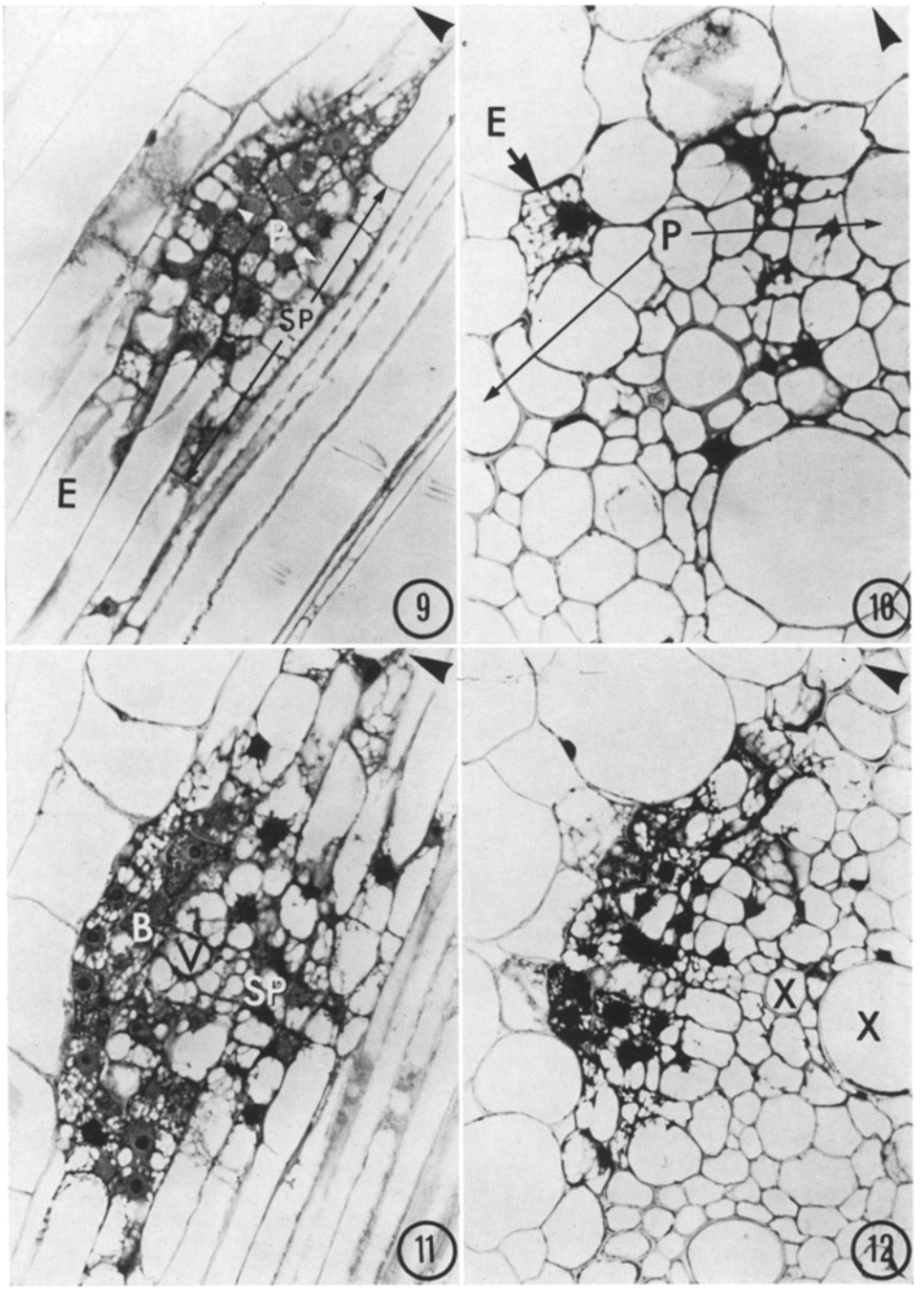
Fig. 9. *LS* of a main root through the centre of a young lateral primordium in which cell divisions have occurred in both pericycle (*P*) and endodermis (*E*). Note that the now multicellular layer of pericycle derivatives is little thicker than the original layer. Recently divided cells are evident in the file of stelar parenchyma cells (*SP*) adjacent to the primordium. ×400

Fig. 10. *TS* of a main root passing through a lateral primordium at the same stage of development as that shown in Fig. 9. This section is not through the centre of the lateral primordium but passes through it at the outer limit of pericycle activity. At this level there is increased cytoplasmic basophilia associated with primordium development in an endodermal cell (*E*) in contact with cells of the pericycle (*P*) which are apparently not yet stimulated. ×600

Fig. 11. *LS* of main root through centre of a lateral primordium at a later stage of development than shown in Figs. 9 and 10. The cells (*B*) derived from the pericycle, which lie toward the outside of the mother root are densely cytoplasmic and basophilic while those (*V*) lying toward the stele are larger and more vacuolated. Note the cell division which has occurred in the underlying parenchyma (*SP*) of the stele. ×400

Fig. 12. *TS* of main root through centre of a lateral primordium at a stage similar to that shown in Fig. 11. The differences between the outer and inner pericycle derivatives (legend, Fig. 11) is clearly shown. Note that at this stage the primordium is centred directly over a xylem point (*X*). ×425



Figs. 9–12

synchronously in a periclinal plane to produce two layers of cells, an inner layer of initials and the first layer of root cap cells (Figs. 15, 16, and 17). The epidermal cells not involved in root cap formation continue to divide anticlinically and elongate in an anticlinal direction so that they become distinctly columnar in shape, but they do not divide periclinally.

By the time a stage comparable to that shown in Fig. 15 is reached, the number of basophilic and mitotically active parenchyma cells in the stele has greatly increased, especially in the areas surrounding the directly underlying protoxylem elements of the mother root (see also Fig. 19).

During its early development (stages up to that shown in Fig. 15) the lateral primordium remains approximately centred over the original xylem point but a subsequent asymmetric distribution of cell division around the xylem point results in a shift of the centre of the primordium to a position directly over a phloem bundle of the main root thus placing the primordium under the influence of two xylem points, one on each side of the phloem bundle, rather than the original single xylem point (cp. Figs. 14 and 18). By this stage small basophilic cells completely surround the phloem bundle (Fig. 15). Later the area containing such cells extends circumferentially under the largest protoxylem elements and may, by the time the lateral reaches the surface, be as long as 1/4 the circumference of the stele.

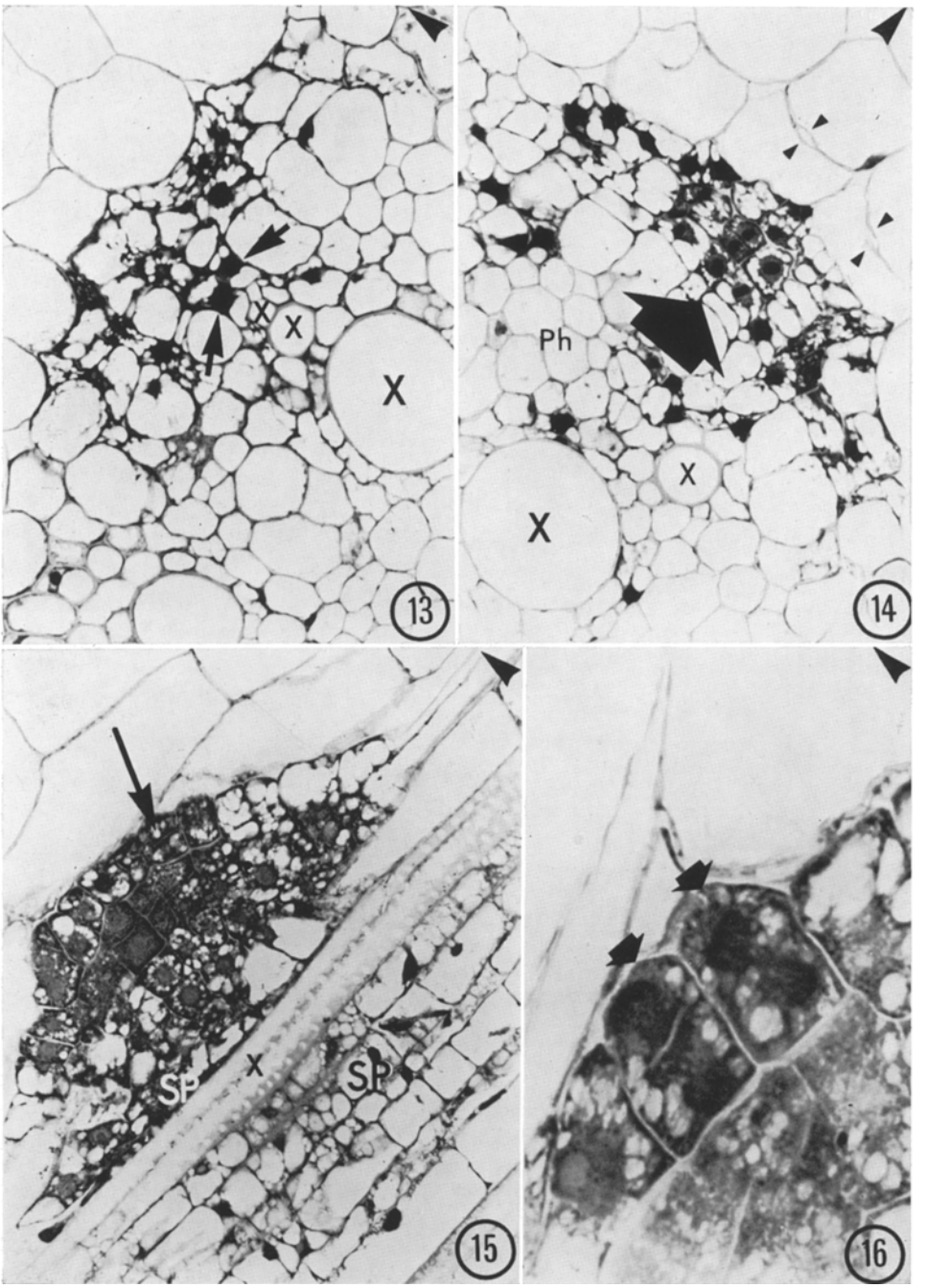
By the time the lateral is half way through the cortex (Fig. 20) the various tissues characteristic of the mature root apex are clearly differentiated. A small but morphologically distinct root cap has been formed by further periclinal divisions of the root cap initials. This cap is now similar to the cap of main roots (unpublished observations) and of older laterals (Figs. 29, 31, and 32)

Fig. 13. *TS* of main root through the centre of a lateral primordium at a similar stage as that shown in Fig. 12. In this section it can be seen that not all the inner pericycle derivatives are large and highly vacuoled, exceptions being those (arrows) directly over the xylem point (*X*) which are small with strongly basophilic cytoplasm. It is in derivatives of these latter cells that vascularization of the lateral eventually proceeds. ×425

Fig. 14. *TS* of main root through centre of a primordium at a stage slightly older than shown in Figs. 12 and 13. The primordium is no longer centred (arrow designates centre) directly over a xylem element but now lies over the mid-point between the underlying xylem (*X*) and phloem (*Ph*) points. Dissolution of the middle lamella is apparent (small arrows) between cortical cells in the path of the primordium. ×425

Fig. 15. *LS* of main root through centre of a lateral primordium somewhat further developed than those shown in earlier figures. Root cap initials are being formed by periclinal divisions in cells at the tip of the primordium, which are of endodermal origin. A new cell wall from such a periclinal division can be seen (arrow). By this stage there has been considerable cell division in the primordium and in the stelar parenchyma (*SP*) surrounding the underlying xylem (*X*). ×425

Fig. 16. A higher magnification of a section similar to that of Fig. 15 showing periclinal division in two cells (arrows) forming root cap initials at the tip of a lateral primordium. ×1,400



Figs. 13–16

in two ways. Firstly, the peripheral portions of the cap which do not lie directly over the initials are separated from the underlying epidermal cells by a layer of PAS-positive, non-metachromatic polysaccharide (Figs. 18 and 20), and secondly, the root cap cells contain abundant starch grains (Fig. 20). However, the young root cap differs from the mature cap of either lateral or main roots in several ways. It appears to fit more tightly over the meristem (possibly the result of pressure against the parent root and the fact that the polysaccharide layer separating it from the epidermal cells is thinner than in mature apices), it does not extend as far down the flanks of the primordium (cp. Fig. 20 with Figs. 29, 31, and 32) and there is no sign of erosion of root cap cells. Polysaccharide secreted by the cap cells occupies a much smaller volume around the tip of the lateral at this stage (Fig. 20) than it does in the mature root cap. As the primordium matures further, often prior to emergence, the cap does extend down the flanks, but massive secretion of polysaccharide, and sloughing of cells does not occur until the lateral has emerged.

Immediately behind the root cap the meristematic area may be distinguished by mitotic figures and cells which have not undergone much vacuolation, although mitotic figures are observed throughout the rest of the lateral, especially in the root cap and epidermis. The region where the stele will develop can be distinguished easily from the cortical area in which the cells are larger and more vacuolated (Figs. 20 and 23). Some cells of the stele are slightly elongated suggesting that provascular tissue is differentiating but

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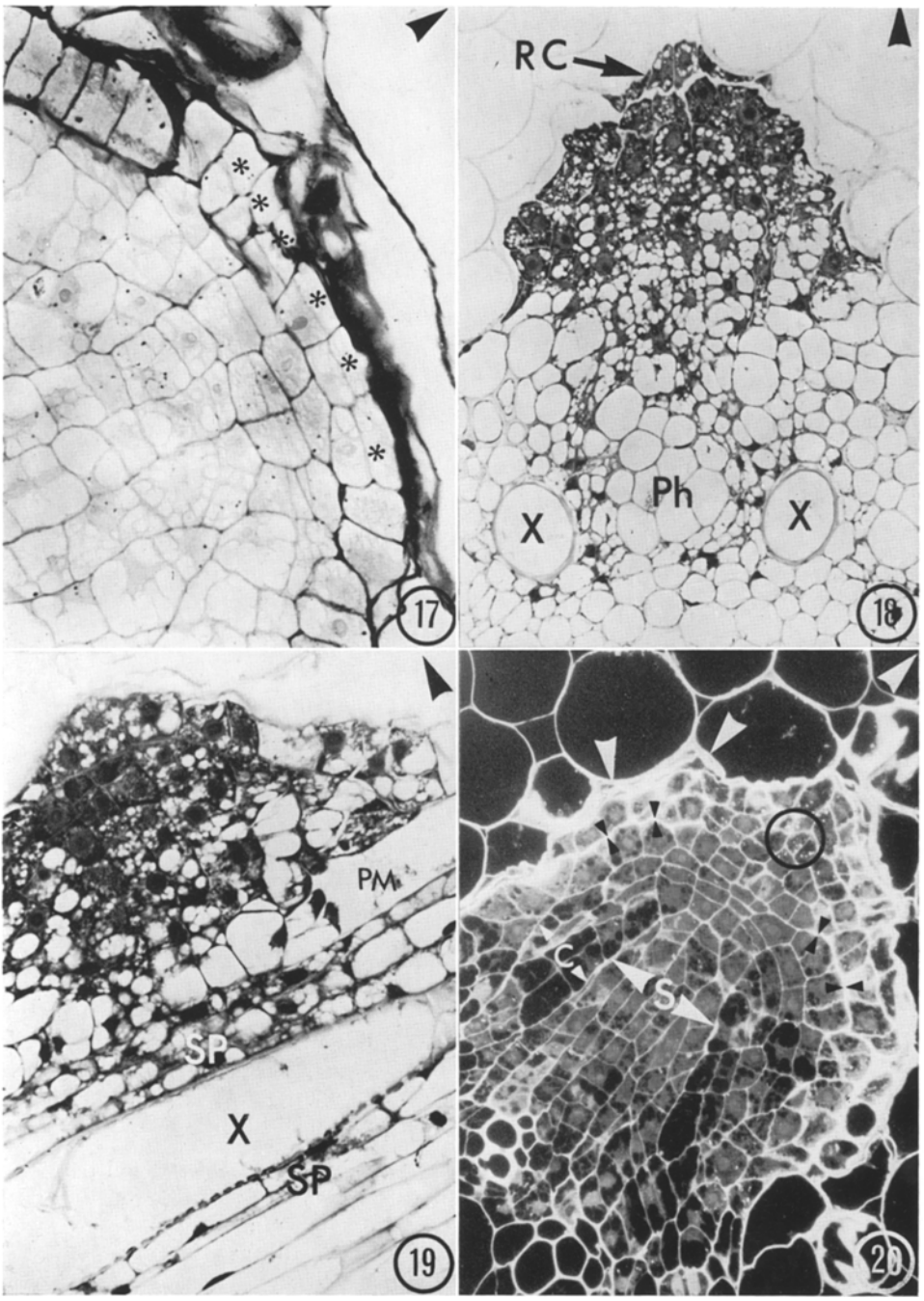
Fig. 17. *LS* of main root through centre of lateral primordium which has already differentiated a row of root cap cells (black asterisks) at its tip. The outer surface of the young lateral is surrounded by a thick layer of strongly PAS-positive material. PAS reaction. ×550

Fig. 18. *TS* of main root through a lateral root primordium at a stage somewhat later than shown in Fig. 17. By this stage the primordium is centred over a phloem pole (*Ph*) between *two* xylem points (*X*). Small basophilic derivatives of stelar parenchyma now surround the phloem bundle and partially surround the xylem bundles. The root cap (*RC*) appears smaller than it actually is at this stage as the section is not median in the primordium. The thick layer of polysaccharide (unstained) between the flanks of the root cap and the epidermis is apparent. ×375

Fig. 19. *LS* of main root through centre of a lateral primordium at a stage of development slightly earlier than shown in Fig. 18. This section shows the activity in the stelar parenchyma (*SP*) around the xylem (*X*) associated with lateral outgrowth. An original undivided pericycle cell (*PM*) of the main root can be seen. ×400

Fig. 20. Median *LS* of a young lateral root which has traversed approximately ½ the width of the main root cortex. The stele (*S*) and cortex (*C*) are blocked out, the root cap has enlarged and starch grains (white dots in circled area) have developed in the root cap cells. The epidermal and root cap cells are each surrounded by a considerable amount of periodic acid-acriflavine positive material and this material has accumulated in the space formed where the root cap overlaps the columnar epidermal cells (small black arrows). Accumulated walls of cortical cells (white arrows) can be distinguished. Periodic acid-acriflavine. Fluorescence microscopy. ×450



Figs. 17–20

no true vascular tissue forms until the primordium has reached the outside of the main root.

While the primordium is growing through the cortex of the parent root a large number of small cells are produced since cell division of the primordium keeps pace with cell enlargement. However, as soon as the lateral breaks through the surface of the mother root there is a rapid expansion of cells particularly in a direction parallel to the longitudinal axis of the lateral and this elongation occurs mainly in the basal regions of the primordium so that the root tip is carried rapidly away from the parent root (Fig. 31).

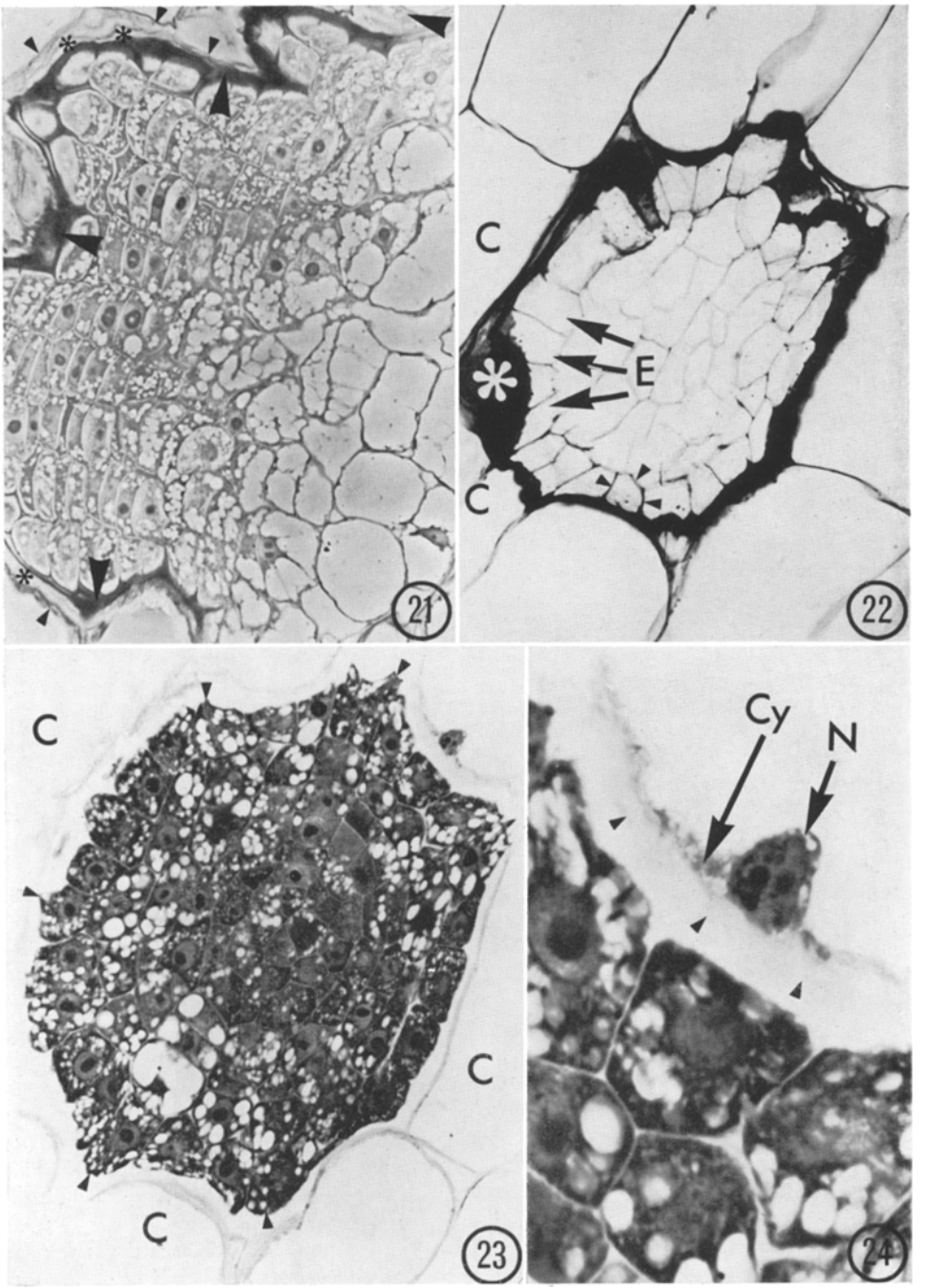
Up to the time of emergence, anticlinal divisions and expansion of the columnar epidermal cells keep pace with the increase in surface area of the primordium, and except for the area occupied by the root cap they form a uniform epidermis which can be followed back easily almost to the endodermis of the parent root. However, cells of the transition region between epidermis and endodermis are not distinctly columnar (Fig. 25). When the rapid expansion of the basal cells of the lateral occurs, the division of the epidermal cells does not keep pace, their continuity with the parent endodermis is broken and the surface around the base of the lateral becomes delimited by large highly vacuolated cells (Fig. 31). These cells are continuous with the outer cortical cells underlying the columnar epidermis in the distal part of the lateral and these cells unlike the other epidermal cells are not derived from the parent endodermis but have their origin in pericyclic derivatives.

Fig. 21. *LS* of a lateral at a similar developmental stage to that shown in Fig. 20. This section is considerably off centre so that no root cap cells are seen. The section shows clearly the heavy accumulation of strongly PAS-positive material (large black arrows) around the root. Toward the tip this layer is surrounded by a thinning slightly PAS-positive layer (asterisks). The accumulated cortical cell walls (small black arrows) lie outside these layers. PAS reaction. Phase contrast optics. ×220

Fig. 22. Median *TS* near tip of a lateral root primordium at a stage of development comparable to that shown in Figs. 20 and 21. The area between the epidermal cells of the lateral (*E*) and the main root cortex (*C*) is filled with a layer of strongly PAS-positive material (asterisk). Smaller amounts of PAS-positive material accumulate at the sides and around the base of the columnar epidermal cells (arrows). PAS reaction. ×400

Fig. 23. Section similar (but closer to base of primordium) to that shown in Fig. 22 except that it is stained with toluidine blue. The PAS-positive material which surrounds the lateral (Fig. 22) is unstained by this method and the convex apices of the epidermal cells are seen projecting into this layer especially opposite gaps (small arrows) between cortical cells (*C*). Note lack of distortion in the surrounding cortical cells. ×450

Fig. 24. Higher magnification of a portion of the section shown in Fig. 23. Two layers can be distinguished in the extracellular material around the lateral, an inner unstained layer and an outer slightly metachromatic layer (arrows denote interface). The irregular appearance of the cytoplasm (*Cy*) and nucleus (*N*) of the adjacent cortical cell suggests that the contents of this cell are disintegrating. No cell wall can be detected between the contents of cortical cell and the extracellular material. ×1,590



Figs. 21–24

The root cap and meristematic region of the lateral is carried approximately 1.5 mm away from the parent root by rapid elongation of the basal cells. Then growth slows down again. Approximately the distal 0.5 mm of the lateral primordium remains covered by the columnar epidermal cells (Fig. 31) throughout the life of the lateral. The remaining emerged part of the lateral is covered by vacuolated cells which are much elongated in a direction parallel to the longitudinal axis, and are derived from the columnar epidermal cells at the lateral tip. It is interesting to note that the columnar epidermal cells do not undergo this marked change in shape while the lateral is still within the parent cortex (Fig. 31).

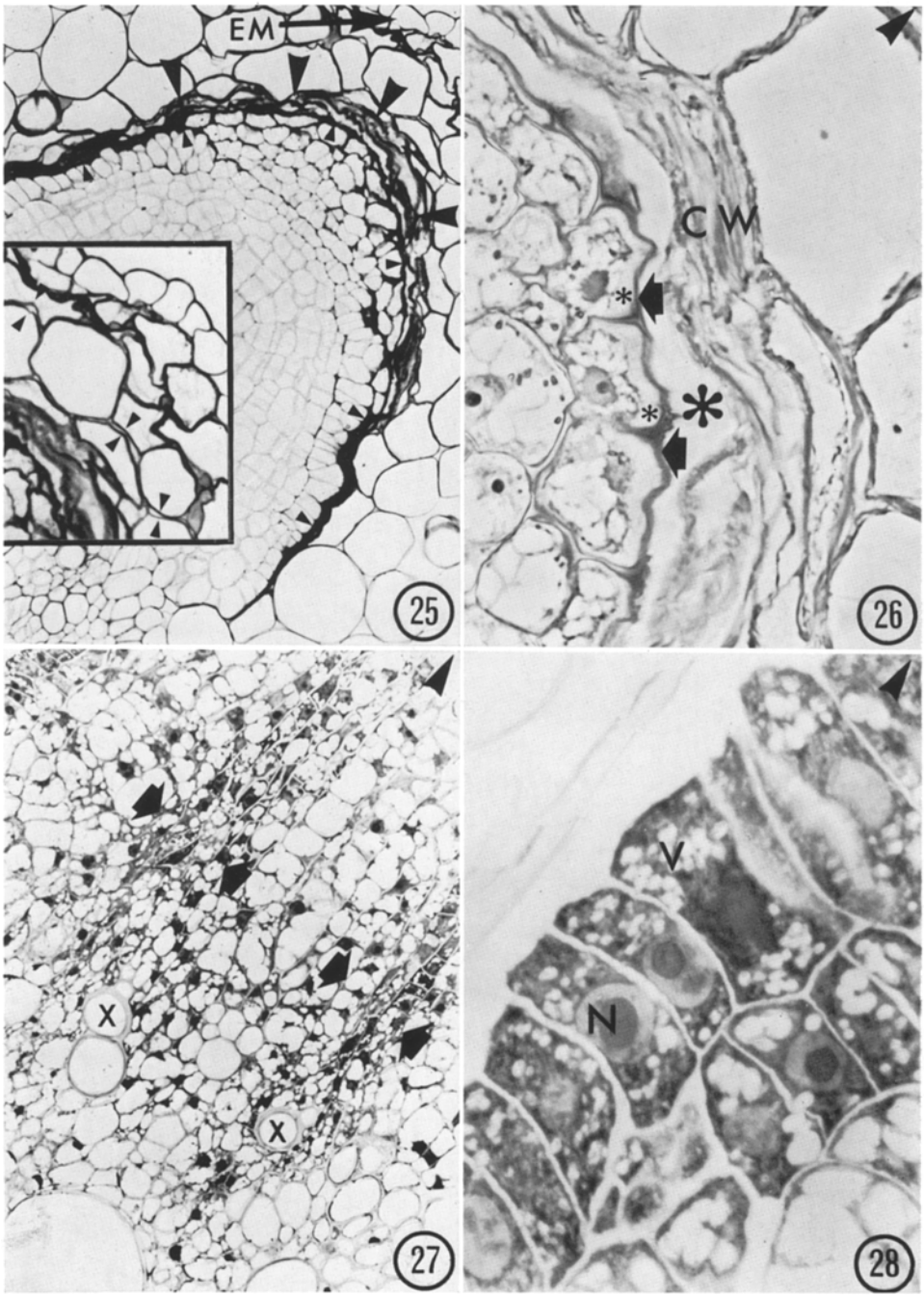
Xylem tissue cannot be distinguished until shortly after the lateral has emerged when it first appears as short and irregular elements extending from the mother root xylem a short way into the base of the primordium. These newly formed elements only occur in tissue of stelar origin, or that which originated from the layer of cells produced by the original asymmetric divisions of pericycle cells. However, by the time the lateral has reached a length approximately three times the radius of the mother root, files of metaxylem elements can frequently be observed in the lateral, differentiating in an acropetal direction (Fig. 32).

Fig. 25. Median *LS* of a lateral root primordium which has extended to within two cells of the epidermis (*DM*) of the main root. The layer of PAS-positive extracellular material (small black arrows) still surrounds the primordium but is thicker over the columnar epidermal cells. This material also occurs between the root cap cells and around the sides and base of the epidermal cells of the lateral. By this stage of lateral development there is an obvious accumulation of cortical cell walls (large black arrows) at the tip of the primordium. Inset: Enlarged view of region at the tip of the primordium of the same section showing the dissolution of the middle lamella (small arrows) between cortical cells. ×275. Inset ×430

Fig. 26. A higher magnification of a similar section to that of Fig. 25 showing the accumulation of cortical cell walls (*CW*) at the tip of the primordium. A thin layer of strongly PAS-positive material (arrows) covers the outer surface of the outer root cap cells and a very lightly PAS-positive material (small asterisk) lies between the plasma membrane and the outer wall of these same cells. Similar lightly PAS-positive extracellular material (large asterisk) lies between the strongly PAS-positive layer and the accumulated cortical cell walls. PAS reaction. Phase contrast optics. ×900

Fig. 27. *LS* showing part of the underlying stele and the basal portion of a lateral primordium at a similar stage of development to the one shown in Fig. 25. A line of small basophilic cells (arrows), probably provascular tissue, runs into the primordium from one of the underlying xylem points (*X*). ×325

Fig. 28. High magnification of a portion of an *LS* of a lateral primordium at the same stage as those shown in Figs. 25 to 27 showing details of the columnar epidermal cells. Each cell has a large nucleus (*N*) with prominent nucleolus and the cytoplasm is strongly basophilic and contains numerous small vacuoles (*V*). The walls of these cells are unstained by toluidine blue. ×2,050



Figs. 25–28

3.2. Secretion of Extracellular Polysaccharides by Epidermal and Root Cap Cells of the Developing Lateral

The columnar epidermal cells (Figs. 17, 21, 25, 28–30), whose origin has already been described are distinctive not only because they are elongated but also because they have large nuclei, strongly basophilic cytoplasm, and numerous small vacuoles (Fig. 28). Early in the development of the lateral these cells become covered by a thin layer of non-metachromatic, PAS-positive material, which is apparently polysaccharide. The material can first be clearly demonstrated at the time of periclinal divisions which initiate the root cap (Figs. 16 and 17). At this stage polysaccharide covers the surface of the primordium including the root cap and also occupies the space between the root cap and the outer part of the meristem proper except in the region of the cap initials where there is cell continuity between the cap and the rest of the root tip (Figs. 17 and 18, and Fig. 20, small black arrows). As the lateral develops further more material accumulates along the flanks (Figs. 21–25 and 28), so that the epidermal cells are covered by a thick layer of polysaccharide into which they project. This is particularly clear in Figs. 22 and 23 which show transverse sections of the young lateral on its way out of the parent cortex. The strongly PAS-positive, non-metachromatic material accumulates not only on the outside of the primordium, but also to a lesser extent between the lateral and basal walls of the epidermal cells (Fig. 22, but see also Fig. 28 where this is especially clear).

Prior to the development of the root cap, as already stated, the epidermal cells at the tip of the primordium secrete a polysaccharide with similar staining

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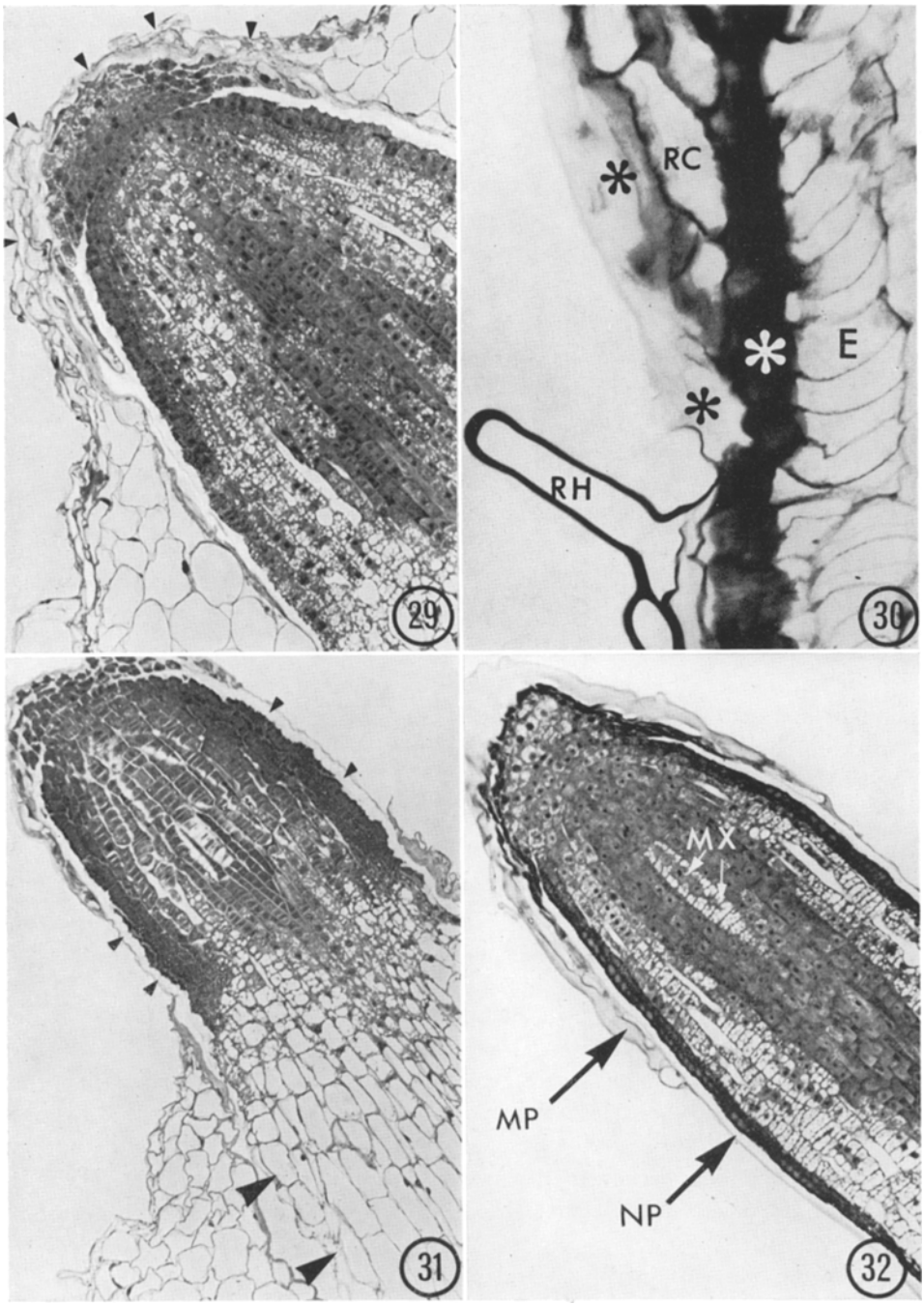
Fig. 29. Median *LS* through a lateral root which has not yet emerged but is distending the partially lignified epidermis and sub-epidermal layers (arrows) of the main root. Metachromatic extracellular material has accumulated around the outside of the root cap. This is sharply contrasted with the non-metachromatic extracellular material lying over the epidermal cells of the lateral (see also Figs. 30, 31, and 32). ×200

Fig. 30. *LS* through a lateral which has just emerged showing details of the breakthrough point which happens to be adjacent to a root hair (*RH*) on the mother root. The two types of extracellular polysaccharides secreted by the lateral are evident. A layer of lightly PAS-positive material (black asterisk) lies on top of the root cap cells (*RC*) and a layer of strongly PAS-positive material (white asterisk) lies on the surface of the lateral root epidermal cells (*E*). PAS reaction. ×850

Fig. 31. *LS* of a recently emerged lateral root. The layer of non-metachromatic extracellular material (small black arrows) still lies over the columnar epidermal cells of the lateral but it does not extend over the large highly vacuolated cells which now cover the basal portions of the primordium (large black arrows). ×150

Fig. 32. Near median *LS* through lateral root which has extended ca. 4.5 mm from the epidermis of the main root. By this stage the root cap has increased in size and is developing back along the flanks of the root. Metaxylem (*MX*) is differentiating. The metachromatic (*MP*) and non-metachromatic (*NP*) extracellular polysaccharides are clearly distinguished. ×150



Figs. 29–32

properties to that produced along the flanks of the primordium (Figs. 17 and 20). The first formed root cap cells (cells just derived from the epidermis) continue to secrete this polysaccharide for a limited time, but subsequently all the root cap cells produce an extracellular polysaccharide differing from that of the epidermal cells in that it is only slightly PAS-positive and stains metachromatically with toluidine blue (Fig. 26). A considerable amount of this second type of extracellular polysaccharide builds up between the cell membrane and the walls of the root cap cells (Fig. 26) before it moves to the exterior and accumulates around the outside of the root cap and between the walls of the inner root cap cells. During this process it is obvious that the second type of polysaccharide must move through deposits of the non-metachromatic, PAS-positive material (Fig. 26).

Secretion of the metachromatic extracellular material increases greatly once the lateral emerges and the deposit becomes much thicker on the outside of the root cap (Figs. 29, 30, and 32). This polysaccharide is never associated with the columnar epidermal cells and is always separated from them by the thick layer of PAS-positive polysaccharide which they secrete (Figs. 29 to 32).

3.3. Passage of the Lateral through the Tissues of the Main Root

A dissolution of the middle lamella is observed between the cortical cells immediately surrounding the primordium almost as soon as the lateral begins to grow outward (arrows, Fig. 14) and this is particularly apparent in front of the root cap (inset, Fig. 25). In the initial stages of lateral growth some accumulation of walls of collapsed cortical cells is observed all around the primordium (Figs. 20 and 21) but further accumulation of cell walls is mainly at the tip of the developing primordium (Figs. 22–26). The lack of accumulated wall material along the sides of the young lateral is evident in cross-sections of primordia (Figs. 22 and 23), where frequently it is not possible to distinguish any cell wall enclosing the portions of the cortical cells adjacent to the lateral (Figs. 23 and 24), even when the sections are examined with a polarizing microscope.

Nuclei and cytoplasm of cortical cells adjacent to the primordium appear abnormal in that basophilic staining of the nuclei is blotchy compared to that of other cortical cells while the cytoplasm stains very lightly and appears dispersed with no definite cytoplasm-vacuole interfaces (Fig. 24). There is little compression of the cortical cells along the sides of the primordium (Figs. 22 and 23).

Unlike the walls of intact cortical parenchyma cells, the accumulated walls of the collapsed cells do not stain metachromatically with toluidine blue. This suggests that the polyuronide component of both the walls, and the middle lamellae between them has been removed. Furthermore, there are

only occasional scattered remnants of basophilic and acid fuchsin-positive material between the layers of walls indicating that most of the cytoplasm of the collapsed cells has been broken down.

While the developing lateral is traversing the cortex, there is almost no distortion of the surrounding tissue (Figs. 15, 20, and 22). However, the walls of the epidermal and subepidermal cells of the mother root are partially lignified and these present an obstacle to passage of the lateral quite unlike that of the cortical cells. Growth through these layers appears to be dependent much more on mechanical pressure since for the first time distortion of the mother root is noticeable (Fig. 29) and the lateral may bulge out from the main root as much as 200 μ with the epidermal and subepidermal layers stretched but remaining intact around them. When the epidermal layer finally breaks, the fragments remain along the flanks of the primordium (Figs. 30 and 31) and possibly serve a protective function during the rapid growth phase of the lateral.

4. Discussion

4.1. Origin of Laterals

It is apparent that in corn the early stages of lateral root initiation involve changes not only in the pericycle tissues but also in a considerable number of parenchyma cells in the adjacent portion of the stele.

Although RYWOSCH (1909) describes meristematic tissue forming what he refers to as a cambium in the stele under a young lateral root in *Zea mays*, he does not suggest that there are changes in the stelar cells at as early a stage of lateral development as is seen in the present study. Such early involvement of stelar tissues is not reported in general accounts of lateral root initiation (*e.g.* CLOWES 1961, ESAU 1965, FAHN 1967, STOCKING 1956) and it may be that this phenomenon does not occur in dicotyledons, the group in which most morphological studies of lateral roots have been made. Alternatively, most studies of this phenomenon have been done using thick (5–12 μ) sections of tissue in which the cell contents have been disrupted by destructive coagulating fixatives. Under these conditions the intense basophilia developed by the stelar cells may not be apparent. Two reports of studies of lateral root formation in wheat (FOARD *et al.* 1965) and barley (HACKETT and STEWART 1969) include photomicrographs (Figs. 1 and 2, FOARD *et al.*; Figs. F and D, HACKETT and STEWART) which suggest the early participation of stelar parenchyma tissue.

The involvement of the stele tissues at the very early stages of lateral development in corn is also emphasized by the changes in the cell walls, not only of the activated pericycle cells, but also of the underlying stelar parenchyma. The reduction of birefringence and the thinning of the walls of these cells in the absence of much apparent cell expansion suggests some breakdown

of cellulose. The green staining reaction of these cell walls with toluidine blue is almost gone by the time that the first pericycle divisions are seen and disappears completely shortly after.

The loss of lignin or lignin-like components is only observed in the area where a lateral will form and it seems reasonable to assume that once an area is stimulated to form a primordium, the lateral precursor cells are induced to form a lignin-degrading enzyme. The question of whether in this case the lignin itself is broken down or whether the wall component to which it is bound is affected so that the lignin is lost during preparation is easily answered by the appearance of toluidine blue-stained hand sections of fresh material in which the green staining component is also absent from the walls of pericycle and stelar parenchyma cells involved in lateral formation.

The early literature (*e.g.* Schellenberg 1896 and Warburg 1893) suggests that lignified cells can neither expand nor divide. However, the occurrence of delignification, though not common, has been reported both in wounded and healthy plants (Block 1941, Roelofsen 1959) so that the possibility of the presence of a lignin degrading enzyme in a higher plant is not without precedent.

4.2. Development

The periclinal divisions of pericycle cells associated with early stages of lateral root initiation have been described in a wide variety of Angiosperms (see, for example, Clowes 1961, Esau 1965). However, it has not been shown as in the present case that these divisions are not only physically asymmetric but that the daughter cells have quite different potentialities in the further development of the lateral.

It is clear that in *Zea* the innermost cells produced by the asymmetric pericycle divisions, together with some of the proliferated parenchyma cells of the stele form the basal tissues of the lateral and the bridge between it and the vascular supply of the main root. The main body of the lateral originates from proliferation of cells of pericyclic origin and the columnar epidermal cells and the cells of the root cap are of endodermal origin.

Interpretations of the origin of the different tissues of young laterals vary. In particular the endodermis and pericycle of the mother root have been assigned a variety of roles. For example, Janczewski (1874) considers that the proliferated pericycle cells give rise to the central cylinder of the lateral while the cortex, epidermis and root cap originate from a combination of cells originating from the endodermis and one or two adjacent cortical layers. Popham (1955) reiterates Janczewski's concept of the role of endodermis and pericycle, but considers that the former tissue may also contribute to the stelar tissue of the lateral. A quite different, and more common interpretation (van Tieghem and Douliot 1888, von Guttenberg 1940) is that all cells of the lateral originate from the pericycle while the cells of the endodermis

together with a few derivatives of the mother root cortex proliferate to form a cap-like structure, variously called a "digestive pouch" (VAN TIEGHEM) or "Tasche" (VON GUTTENBERG). This temporary cap is considered to be retained only while the lateral is within the mother root and to be shed upon emergence and replaced by a normal root cap.

BONNETT and TORREY (1966), describe the origin from the parent root endodermis of the epidermis and root cap of the lateral roots of *Convolvulus*. Although these authors did not look at intermediate morphological stages between the root cap of a young lateral and the root cap of a mature apex they felt that differences in these root caps suggest that the earlier one of endodermal origin is shed when the lateral emerges.

Much of the variation in interpretation of the origin of lateral root components obviously stems from species variation, however, the function of a temporary root cap of endodermal origin has been ascribed specifically to the lateral roots of *Zea mays* (SCHADE and VON GUTTENBERG 1951). These authors describe the formation under the temporary cap, of a second root cap formed from tissue of pericyclic origin at the tip of the lateral primordium. Both caps are carried out of the mother root and the outer one is shed along with its initials so that all root cap tissue formed subsequently is of pericyclic origin.

We find no evidence from the present study that a root cap of endodermal origin is temporary. There is no doubt that the initial cells of the cap are derived from the epidermis of the lateral (Fig. 17) and this tissue in turn is clearly of endodermal origin. True, the original root cap cells must eventually be shed in the normal course of maturation and erosion of cap cells but there is no evidence that the original initials do not function throughout the life of the new root. Certainly the columnar epidermal cells which are clearly of endodermal origin persist on the tip of the longest laterals which we examined (emerged approximately 1.5 cm) and such cells are also found on the lower 3–5 mm of main root tip.

4.3. Polysaccharide Secretion

It is apparent that cells of the lateral roots of corn produce two different types of polysaccharides which accumulate outside the cell walls. The histochemical results suggest that the polysaccharide secreted by the columnar epidermal cells and the very young root cap cells because it is strongly PAS-positive, contains a fair proportion of residues of sugars with free hydroxyl groups on vicinal carbon atoms, *e.g.* glucose (see PEARSE 1968). On the other hand this material is not metachromatic even after sections are demethylated by saponification (see LILLIE 1965) suggesting that it contains few if any residues bearing carboxyl groups or methyl esthers (see PEARSE 1968). The PAS and the metachromatic staining reactions of the polysaccharide secreted by the cells of the outer portions of the root cap are the reverse of those of

the first type suggesting the presence of few if any residues with hydroxyl groups on vicinal carbon atoms, but rather of residues with carboxyl groups (*e.g.* galacturonic acid). There seem also to be distinct physical differences between the two types of polysaccharides. The one associated with the epidermal cells and the root cap initials appears to be viscous and firm, since in both fresh material and in the sections, the layer covering the epidermal cells is of uniform thickness with a clear-cut outer margin, whereas the polysaccharide associated with the outer root cap cells appears much more fluid, having indefinite limits and smearing easily in fresh section. Preliminary work in our laboratory suggests that two types of polysaccharides with the same histochemical characteristics as those of the lateral are secreted by the cells of the epidermis and root cap of main root apices.

The secretion of large amounts of extracellular polysaccharides by cells of the root caps of the main roots of *Zea* has been described by a number of workers (see Clowes and Juniper 1968). This material has been analyzed chemically (Jones and Morré 1967) and shown to contain glucose, galactose, some pentoses and galacturonic acid. It is not exactly clear how our histochemical data fits the results of gross chemical analysis and at this stage two points should be kept in mind. Firstly, the material analyzed chemically may have been a mixture of the two polysaccharides associated with the root cap, and secondly, since we took no special precautions (see Pearse 1968) to retain polysaccharides during fixation some components may have been lost. This particular problem is currently being investigated.

If enzymatic activity is involved in the passage of the lateral root through the parent cortex we are faced with two problems. Firstly, where are the enzymes produced, and secondly, how is self-destruction of the lateral prevented? At this time answers to either of these questions can only be speculative. The appearance of the columnar epidermal cells is unusual in higher plants although such highly polarized cells are frequent in animal tissue where they are invariably associated with a secretory and/or an absorptive role (see, for example, Bloom and Fawcett 1968). A cell type of similar appearance does occur in the scutellar epithelium of germinating seeds of grasses and in this case the appearance of this distinct morphology is correlated directly with the secretion of starch hydrolyzing enzymes by these cells and the development of their capacity to absorb products of this hydrolysis (see O'Brien and McCully 1969). The polarized lateral root epidermal cells with their intense basophilia are obvious candidates for enzyme synthesizing and secreting cells. It should be pointed out, however, that cells of quite similar appearance form the epidermis of main root tips of *Zea*. In both situations these cells may be involved only with the synthesis and secretion of polysaccharides.

The layer of polysaccharides which coat the emerging lateral from the very early stages of its development may well play a protective role by preventing

self hydrolysis, possibly by the formation of inactive enzyme complexes which are only activated once the enzyme has moved through the polysaccharide. Protection against autohydrolysis has been postulated as a role for extracellular polysaccharides in such animal tissues as small intestine (SPICER and SUN 1967) but such a function in higher plants has not been considered.

4.4. Mechanism of Passage through the Tissue of the Mother Root

The present observations suggest that in the case of *Zea mays,* both enzymatic and mechanical mechanisms are involved in passage of the lateral through the mother root but mechanical pressure alone is not of much importance until the lateral reaches the lignified epidermal and subepidermal layers of the main root. The cortical cells in the path of the extending lateral are definitely separated by breakdown of the middle lamella region, the cells are collapsed and the protein and nucleic acid contents disappear, all observations which decidedly implicate pectinases, proteases and nucleic acid hydrolyzing enzymes. Whether these enzymes are released by the tissues of the lateral or are induced in the cortical cells is not clear.

The involvement of a cellulose degrading enzyme in the corn system is less certain. If there was no cellulose breakdown the amount of cortical cell wall accumulation ahead of the lateral seems somewhat less than one would expect from the number of cell layers traversed. Furthermore, the accumulated walls when viewed with polarized light are not uniformly birefringent suggesting wall breakdown. The absence of walls of collapsed cells along the flanks of the primordium could result from them being caught and carried out of the root by the broad tip of the lateral. However, situations such as that shown in Fig. 24, where even when the section is viewed with polarized light no cortical cell wall can be detected adjacent to the non-birefringent extracellular polysaccharide coating the sides of the primordium, certainly suggest that there is some cellulose breakdown. In this case the enzymatic hydrolysis may be too slow to keep up with the accumulation of walls at the tip of the rapidly advancing lateral.

BONNETT (1969), in a light and electron microscope study of lateral root emergence in *Convolvulus* concludes on the basis of the large accumulation around the lateral of walls of collapsed cortical cells that there is little evidence for the action of a cellulose-degrading enzyme in that system. However, in BONNETT's study as in the present one, the disappearance of the contents of the collapsed cortical cells definitely suggests the activity of other hydrolytic enzymes.

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References

Block, R., 1941: Wound healing in higher plants. Bot. Rev. **7**, 110—146.

Bloom, W., and D. W. Fawcett, 1968: A textbook of histology. Philadelphia: W. B. Saunders Co.

Bonnett, H. T., 1969: Cortical cell death during lateral root formation. J. Cell Biol. **40**, 144—159.

— and J. G. Torrey, 1966: Comparative anatomy of endogenous bud and lateral root formation in *Convolvulus arvensis* roots cultured *in vitro*. Amer. J. Bot. **53**, 496—507.

Clowes, F. A. L., 1961: Apical meristems. Oxford: Blackwell's.

— and B. E. Juniper, 1968: Plant cells. Oxford: Blackwell's.

Danielli, J. F., 1949: A critical study of techniques for the cytochemical demonstration of aldehydes. Quart. J. Micro. Sci. **90**, 67—74.

Eames, A. J., and L. H. MacDaniels, 1947: An introduction to plant anatomy. 2nd ed. New York: McGraw Hill Co.

Esau, K., 1965: Plant anatomy. New York: J. Wiley and Sons.

Fahn, A., 1967: Plant anatomy. Oxford: Pergamon Press.

Feder, N., and T. P. O'Brien, 1968: Plant microtechnique: some principles and new methods. Amer. J. Bot. **55**, 123—142.

Foard, D. E., A. H. Haber, and T. N. Fishman, 1965: Initiation of lateral root primordia without completion of mitosis and without cytokinesis in uniseriate pericycle. Amer. J. Bot. **52**, 580—590.

Guttenberg, H. von, 1940: Der primäre Bau der Angiospermenwurzel. In: Handbuch der Pflanzenanatomie. K. Linsbauer (ed.), Berlin: Gebrüder Borntraeger.

Hackett, C., and H. E. Stewart, 1969: A method for determining the position and size of lateral primordia in the axes of roots without sectioning. Ann. Bot. **33**, 679—682.

Janczewski, E. de, 1874: Recherches sur l'accroissement terminal des racines dans les Phanerogames. Ann. Sci. Nat. **20**, 208—233.

Jensen, W. A., 1962: Botanical histochemistry. San Francisco: W. H. Freeman Co.

Jones, D. D., and D. J. Morré, 1967: Golgi apparatus mediated polysaccharide secretion by outer root cap cells of *Zea mays*. II. Isolation and characterization of the secretory product. Z. Pflanzenphysiol. **56**, 166—169.

Kasten, F. H., 1959: Schiff-type reagents in cytochemistry. I. Theoretical and practical considerations. Histochemie **1**, 466—509.

Laties, G. G., and K. Budd, 1964: The development of differential permeability in isolated steles of corn roots. Proc. nat. Acad. Sci. (U. S. A.) **52**, 462—469.

Lillie, R. D., 1965: Histopathologic technique and practical histochemistry. New York: McGraw Hill Co.

O'Brien, T. P., N. Feder, and M. E. McCully, 1964: Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma **59**, 367—373.

— and M. E. McCully, 1969: Plant structure and development. New York: The Macmillan Co.

Pearse, A. G. E., 1968: Histochemistry. Theoretical and applied. London: J. & A. Churchill Ltd.

Pond, R. H., 1908: Emergence of lateral roots. Bot. Gaz. **46**, 410—421.

Popham, R. A., 1955: Zonation of primary and lateral root apices of *Pisum sativum*. Amer. J. Bot. **42**, 267—273.

Rappay, Gy., and P. van Duijn, 1965: Chlorous acid as an agent for blocking tissue aldehydes. Stain Technol. **40**, 275—277.

Roelofsen, P. A., 1959: Encyclopedia of plant anatomy, Pt. 4, The plant cell wall, 3. Berlin: Gebrüder Borntraeger.

RYWOSCH, S., 1909: Untersuchungen über die Entwicklungsgeschichte der Seitenwurzeln der Monocotylen. Z. Bot. **1**, 253—283.

SCHADE, C., und VON GUTTENBERG, 1951: Über die Entwicklung des Wurzelvegetationspunktes der Monokotyledonen. Planta **40**, 170—198.

SCHELLENBERG, H., 1896: Beiträge zur Kenntnis der verholzten Membranen. Jahrb. Wiss. Bot. **29**, 237—266.

SIEGEL, S. M., 1953: On the biosynthesis of lignin. Physiol. Plant. **6**, 134—139.

SPICER, S. S., and D. C. H. SUN, 1967: Carbohydrate histochemistry of gastric epithelial secretions in dog. Ann. N.Y. Acad. Sci. **140**, 762—783.

STOCKING, C. R., 1956: Histology and development of the root. In: Handbuch der Pflanzenphysiologie. W. RUHLAND (ed.), Berlin-Göttingen-Heidelberg: Springer-Verlag.

SUTCLIFFE, J. F., and R. SEXTON, 1968: β-glycerophosphatase and lateral root development. Nature **217**, 1285.

TIEGHEM, P. VAN, et H. DOULIOT, 1888: Recherches comparatives sur l'origine des membres endogènes dans les plantes vasculaires. Ann. Sci. Nat. Bot. **8**, 1—660.

TORREY, J. G., 1965: Physiological basis of organization and development in the root. In: Handbuch der Pflanzenphysiologie. W. RUHLAND (ed.), Berlin-Heidelberg-New York: Springer-Verlag.

WARBURG, O., 1893: Über den Einfluß der Verholzung auf die Lebensvorgänge des Zellinhaltes. Ber. dtsch. bot. Ges. **11**, 425—441.

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