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The Development of the Endodermis and *Phi* Layer of Apple Roots

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Summary

Suberin lamellae and a tertiary cellulose wall in endodermal cells are deposited much closer to the tip of apple roots than of annual roots. Casparian strips and lignified thickenings differentiate in the anticlinal walls of all endodermal and *phi* layer cells respectively, 4–5 mm from the root tip. 16 mm from the root tip and only in the endodermis opposite the phloem poles, suberin lamellae are laid down on the inner surface of the cell walls, followed 35 mm from the root tip by an additional cellulosic layer. Coincidentally with this last development, the suberin and cellulose layers detach from the outer tangential walls and the cytoplasm fragments. 85 mm from the root tip the xylem pole endodermis (50%) of the endodermis) develops similarly, but does not collapse. 100–150 mm from the root tip, the surface colour of the root changes from white to brown, a phellogen develops from the pericycle and sloughing of the cortex begins. A few secondary xylem elements are visible at this stage.

Plasmodesmata traverse the suberin and cellulose layers of the endodermis, but their greater frequency in the outer tangential and radial walls of the *phi* layer when compared with the endodermis suggests that this layer may regulate the inflow of water and nutrients to the stele.

Keywords: Anatomy; Apple; Endodermis; Phellogen; *Phi* layer; Root.

1. Introduction

The structure and function of perennial roots is a neglected subject. The purpose of this paper is to describe the development of the endodermis and *phi* layer during the primary growth of apple roots and their senescence during the transition to secondary growth. This will be discussed in relation to the structural and experimental studies on water and nutrient uptake which

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have been done during the last decade (Clarkson and Sanderson 1979, Clarkson, Robards, and Sanderson 1971, Harrison-Murray and Clarkson 1973, Robards, Jackson, Clarkson, and Sanderson 1973, Ferguson and Clarkson 1975). In assessing the significance of primary apple roots, it should be emphasized that they form 1–85% of the total root surface area, depending on the time of year, the growth conditions in any year, and the age of the tree (Wilson and Atkinson 1979).

The primary development of *Malus* roots (Riedhart and Guard 1956–1957) and the closely related *Pyrus* roots (Esau 1943) is well documented. The present work, however, deals with the ultrastructural features of the endodermis and *phi* layer. This latter layer is common to *Rosaceous* species and is of some interest. It shares its origin with the endodermis. 550–800 μm from the root tip, the cells of the innermost layer of the cortex divide periclinally to form on the inside the future endodermis, and on the outside the cells destined to have their anticlinal walls thickened by lignin deposits—the so-called "*phi* (Φ) thickenings" (Russow 1875). These thickenings surround each *phi* layer cell similar to the way the Casparian strip surrounds each endodermal cell. Indeed the *phi* layer has been misinterpreted occasionally as the endodermis (Nightingale 1935, Stoutemeyer 1937), since the thickenings are much more apparent than the Casparian strips. The *phi* layer may be physiologically important and this possibility will be discussed.

2. Materials and Methods

Roots from M. 26, M. 27, and MM. 111 rootstocks were used. These were taken from rooted cuttings or stool shoots grown in pots in the glass house, or in open ground in the nursery for 1–2 years (M.A.F.F. 1969). Only minor differences between rootstocks were found, therefore reference to individual rootstocks will not be made.

Thick primary roots arising directly from the base of the cuttings were sampled and also thin secondary and tertiary roots arising from them. Portions of root up to 11 cm long and including the tip were cut into 2–4 mm pieces. These pieces were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 6.9 for 2 hours, rinsed in buffer and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hours. They were then dehydrated in a graded ethanol and epoxy propane series and embedded in either "Araldite" or "Emix" resin.

Ultrathin sections were stained in 2% aqueous uranyl acetate followed by lead citrate (Reynolds 1963) (15 minutes each). Sections 2–5 μm thick were cut from the same resin embedded material, heat stretched on water, mounted on glass slides and stained with periodic acid-Schiff reagent and toluidine blue. There was some variation in the distance behind the root tip at which stages of differentiation were reached, both between samples, and between primary, secondary, and tertiary roots. There was no pattern in this variation. Distances quoted here will, unless otherwise stated, refer to the maximum distance from the root tip found in this material.

Plasmodesmata frequencies quoted were obtained from transverse ultrathin sections 4–5 mm from the root tip. The average number per μm of cell wall was first found, and this figure converted, taking into account the section thickness and the diameter of the plasmodesmata, to a number per μm^{-2}.

3. Observations

3.1. Endodermal Development

The endodermis in apple roots differentiates 0.8 mm from the root tip and is characterized by small vacuoles containing tannins (Fig. 1). Vacuolation and tannin accumulation increase progressively farther back from the tip, the small vacuoles coalescing to form one large vacuole which restricts the cytoplasm to a thin peripherae layer (Fig. 2). Further development can be split into four successive stages; firstly, the differentiation of Casparian strips, secondly, the deposition of suberin lamellae, thirdly, the deposition of a cellulosic layer and fourthly, the collapse of the cells.

3.1.1. Stage I

The earliest stages in the development of the Casparian strip occur simultaneously opposite the xylem and phloem poles, and cannot be detected with the light microscope (Fig. 2). Electron microscope observations, however, show that the Casparian strip develops 4–5 mm from the root tip on the inner third of the anticlinal wall between endodermal cells (Fig. 3).
The wall surface is smooth in this region and the plasmalemma is closely appressed to it making the unit membrane more easily seen. The cell wall also appears more finely granular than in other areas.

3.1.2. Stage II

The second stage of endodermal development occurs initially opposite the phloem poles only. Opposite the xylem poles, the endodermis remains at Stage I for the present. The cytoplasm in both sets of endodermal cells darkens considerably and tannin accumulation continues.
Sixteen mm from the root tip suberin lamellae are laid down on the cell wall. They can be seen first adjacent to the Casparian strip, which they ultimately overlay after rapidly extending round the whole of the inner cell surface. The suberin is deposited in two forms; firstly as definite layers, and secondly as amorphous lobes (Fig. 4).
Suberin is thickest on the anticlinal walls just outside the Casparian strip, next thickest on the outer tangential wall, and thinnest on the inner tangential wall. The suberin on the inner tangential wall lacks the second amorphous layer (Fig. 5), and the suberin over the Casparian strip often lacks the initial lamellate layer (Fig. 6).
The relationship of the suberin layers with the primary cell wall also varies. Around most of the periphery the suberin appears appressed to the cell wall (Fig. 4), but in the region of the anticlinal wall outside the Casparian strips the two layers integrate (Fig. 7).

3.1.3. Stage III

The third stage in endodermal development first occcurs 30 mm from the root tip and at this level only opposite the phloem poles. This stage involves the deposition of a cellulosic layer inside the suberin, which is more coarsely granular than the primary cell wall (Fig. 4). At approximately this level, the first cambial divisions can be seen in the stele. Occasionally, cellulose deposition occurs before suberin deposition is complete, and therefore the tertiary cellulose wall comes into direct contact with the Casparian strip (Fig. 6). Fig. 4 shows that the new cellulosic layer fills the gaps between the amorphous lobes of the second suberin layer.

The cytoplasm at this stage stains very darkly and appears moribund, although the deposition of cellulose gives evidence to the contrary.

3.1.4. Stage IV

This stage occurs only opposite the phloem poles (Figs. 8 and 9) and rapidly follows the tertiary stage of endodermal development. The suberin and tertiary cellulose layers collapse inwards and the cytoplasm fragments. The suberin layer detaches from the outer tangential primary cell wall and from the anticlinal wall as far in as the area where the primary wall and suberin are in intimate contact—approximately one third of the distance out from the Casparian strip (Fig. 8). Over the rest of the inner surface of the cell the various wall components remain in place.

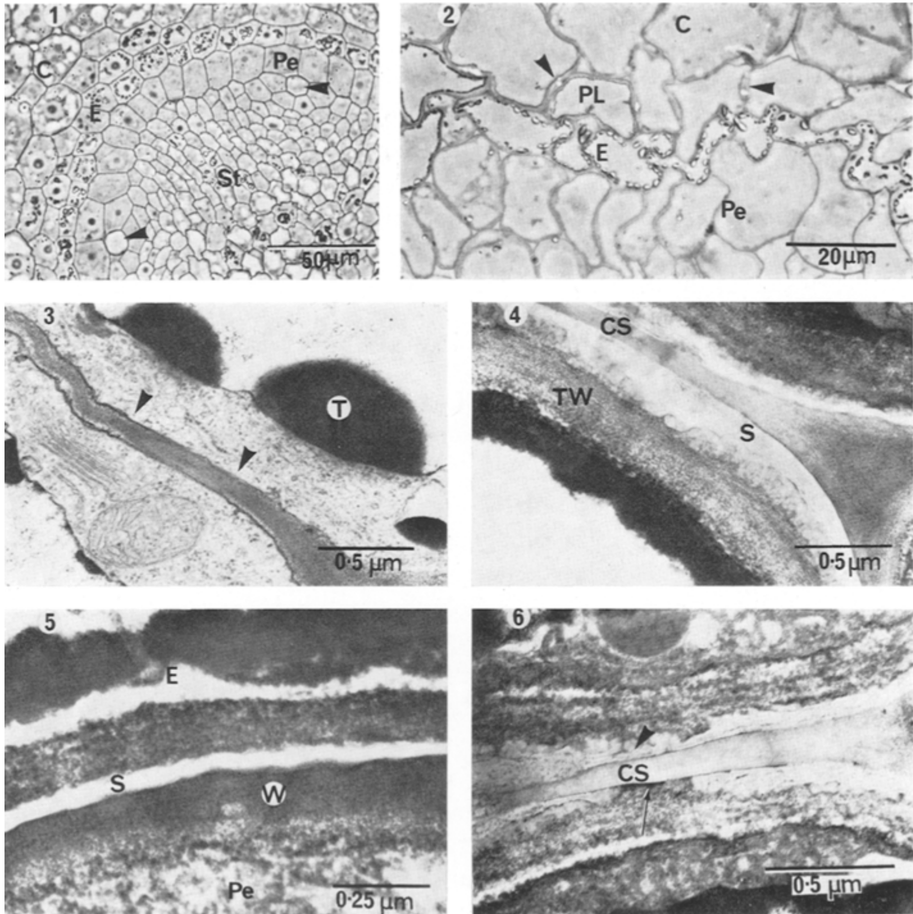
3.1.5. Developments in the Xylem Pole Endodermis

While the endodermal cells opposite the phloem poles are undergoing stage II and III development, those opposite the xylem poles remain at stage I (Figs. 9 and 10). Approximately 50 mm after the inward collapse of the secondary and tertiary wall components of the phloem pole cells, the xylem pole endodermis rapidly undergoes stage II and III development. The suberin lamellae become detached from the primary cell wall, but collapse is never as pronounced as in the phloem pole endodermis.

3.2. The Cork Cambium and Sloughing of the Cortex

The outward appearance of the roots changes from creamy white to light brown 100–150 mm from the root tip. This coincides internally with the early divisions of the phellogen which is derived from the pericycle (Figs. 11 and 12). Vascular cambial divisions are also well advanced at this level having given rise to several secondary xylem vessels (Fig. 11).

Here, the anticlinal walls between phloem pole endodermal cells split (Fig. 13). In places—usually opposite the xylem poles—the endodermis becomes bi- and tri-seriate (Fig. 12), although it is likely that the innermost cells



Abbreviations: *C* = cortex, *CS* = Casparian strip, *E* = endodermis, *Ep* = epidermis, *G* = Golgi bodies, *P* = phloem, *Pe* = pericycle, *Pg* = phellogen, *Pl* = *phi* layer, *PT* = *phi* thickening, *S* = suberin layers, *St* = stele, *T* = tannins, *TW* = tertiary cellulose wall, *W* = cell wall, *X* = xylem

Fig. 1. Light micrograph of TS root 0.9 mm from the root tip. Arrows indicate the first protophloem

Fig. 2. Light micrograph of TS root 5 mm from the root tip. Note the vacuolar tannins in the endodermis (*E*) and the rudimentary *phi* thickenings (arrows)

Fig. 3. Electron micrograph of the radial longitudinal wall between two endodermal cells from the same area as Fig. 2. Arrows indicate the limits of the Casparian strip

Fig. 4. Electron micrograph of radial wall between two stage III endodermal cells, 40 mm from the root tip. Note the two phases of suberin deposition—lamellae and lobes

Fig. 5. Electron micrograph of inner tangential wall of endodermis, 40 mm from the root tip. Note the lack of suberin lobes

Fig. 6. Electron micrograph of the Casparian strip 45 mm from the root tip. Note the lack of suberin over part of the Casparian strip on one side (small arrow) and the presence of lobed suberin on the other (large arrow)

in these regions are cells derived from the phellogen which have subsequently undergone secondary and tertiary development.

At this stage the cortex begins to break up throughout its depth, the cell walls having become increasingly thin and convoluted. Figs. 14 and 15 show the epidermis and underlying cortex of the same root at the level of transition from white to brown root. The two sections were taken a distance of only 4 mm apart; Fig. 14 shows the white root, Fig. 15 the brown root. The breaking and increasing convolution of cell walls can be seen in Fig. 15. Presumably the debris seen in this figure gives rise in part to the exterior brown colour.

3.3. The phi Layer

Lignification of the anticlinal walls of the *phi* layer occurs 4–5 mm from the root tip, at the same level as lignification of the protoxylem, and at about the same level as Stage I development of the endodermis (Fig. 2). The lignified portion of the cell wall eventually occupies up to one third of the cross-sectional area of the *phi* layer cells.

The cytoplasm of the *phi* layer contains a mass of organelles and membrane systems, the most notable being the Golgi bodies which are seen to be budding off vesicles with contents (Fig. 16). Microtubules can also be seen close to the lignifying cell walls (Fig. 17). The thickened walls are traversed by many plasmodesmata which, in a single section, do not appear to cross the wall completely. Serial sections, however, suggest that the plasmodesmata connect the cytoplasm of adjacent cells. At about the level in the root where the endodermis is close to Stage III development the thickenings in the anticlinal walls of the *phi* layer reach their maximum dimensions and the cytoplasm, while still retaining recognizable organelles, appears considerably less active and is restricted to a much thinner layer. Occasionally, further thickenings develop at the junctions between *phi* layer anticlinal walls and the inner

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Fig. 7. Electron micrograph of radial endodermal wall immediately outside the Casparian strip, showing the integration of the suberin layer with the original cell wall

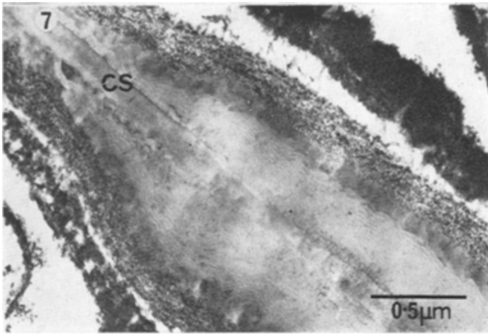
Fig. 8. Electron micrograph of radial endodermal wall 80 mm from the root tip, showing detachment of the suberin and cellulose layers from the original cell wall. The Casparian strip is arrowed

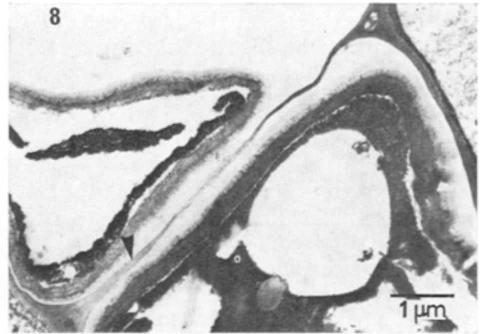
Fig. 9. Composite light micrograph of TS stele, 80 mm from the root tip. Note the intact and collapsed endodermal cells outside the xylem and phloem poles respectively, in the proportions 50 : 50

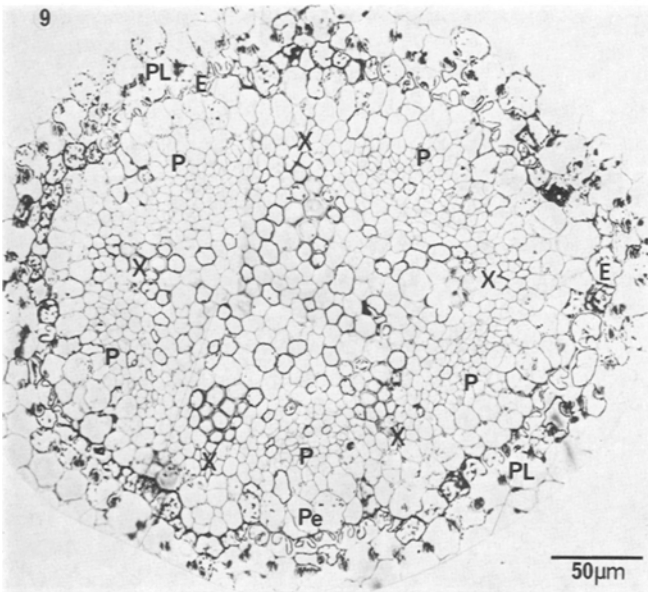
Fig. 10. Electron micrograph of radial wall between xylem pole (left) and phloem pole (right) endodermis 80 mm from the root tip. The phloem pole cell shows tertiary development

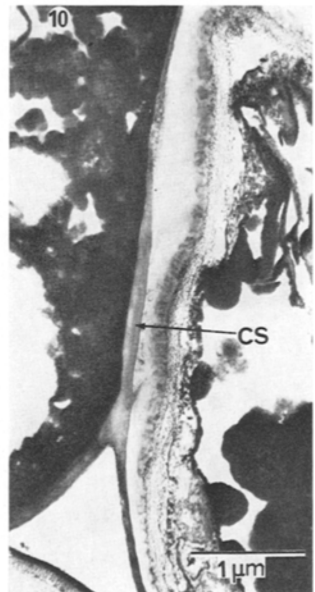
Fig. 11. Light micrograph of TS part of stele 105 mm from the root tip. Note the presence of a vascular cambium (small arrows) producing xylem vessels (large arrows)

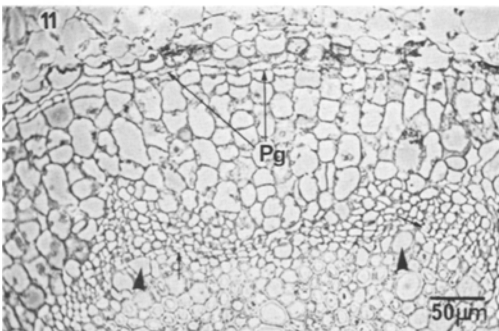
Fig. 12. Detail of part of Fig. 11 showing early divisions in the phellogen (*Pg*) and biseriate endodermis

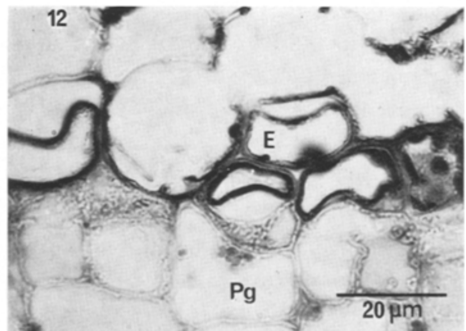












Figs. 7–12

tangential walls of the next layer of cortical cells. Eventually the *phi* layer is sloughed off with the cortex as described above.

3.4. *Plasmodesmata*

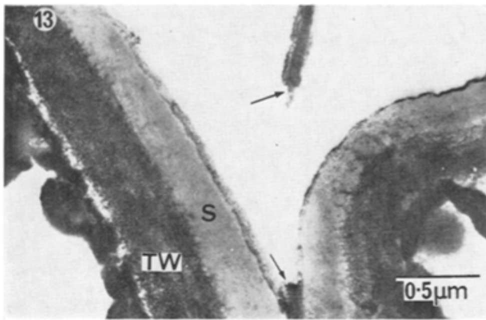
A large number of plasmodesmata (1.30 μm^{-2}) is found in the outer tangential wall of the *phi* layer, and a slightly higher number (1.57 μm^{-2}) is found in the radial walls. The frequency is considerably lower, however, in the common tangential wall between the endodermis and *phi* layer (0.67 μm^{-2}) and in the inner tangential wall (0.57 μm^{-2}) of the endodermis. Where a suberin layer is present, it is traversed by the plasmodesmata (Fig. 18).

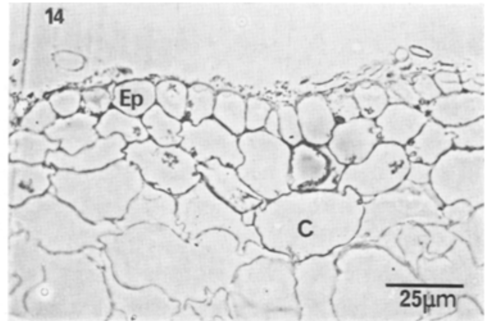
4. Discussion

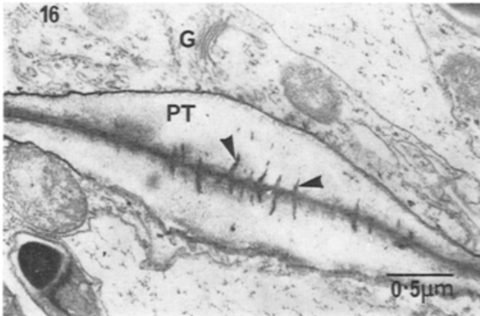
Although the distances from the root tip given here for the various developmental events vary from root to root, it is apparent that almost all these events take place considerably nearer the tip than in annual species. The exception is Stage I endodermal development; Casparian strips in apple endodermis are found 4–5 mm from the root tip, the same distance as that given by ROBARDS *et al.* (1973) for barley, and HARRISON-MURRAY and CLARKSON (1973) for marrow. Stage III in apples, however, is completed 100 mm from the root tip and the endodermal cells have collapsed, at least over the phloem poles. In contrast, it is 320 mm from the root tip before all barley endodermal cells have reached Stage III, with most wall thickening occurring 50–200 mm from the tip (ROBARDS *et al.* 1973), while in marrow endodermis, Stage II development occurs 100 mm or more from the root tip (HARRISON-MURRAY and CLARKSON 1973). In addition, the tertiary cellulosic layer in apple endodermis is considerably thinner than that laid down on the inner tangential walls of barley and marrow endodermis.

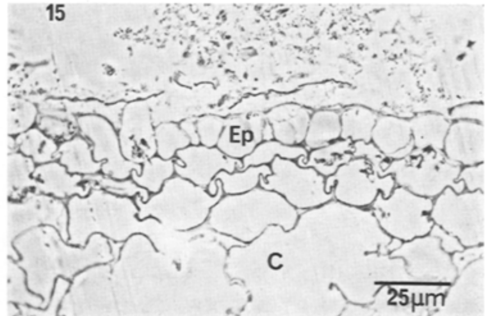
The present work shows that Casparian strips differentiate at the same time in all endodermal cells. This is in contrast to the situation reported by ESAU (1943) for *Pyrus*, where the Casparian strips were more conspicuous at the phloem poles. The thickened walls that she refers to at the phloem poles, however, can probably be accounted for by Stage II and III developments rather than Casparian strips.

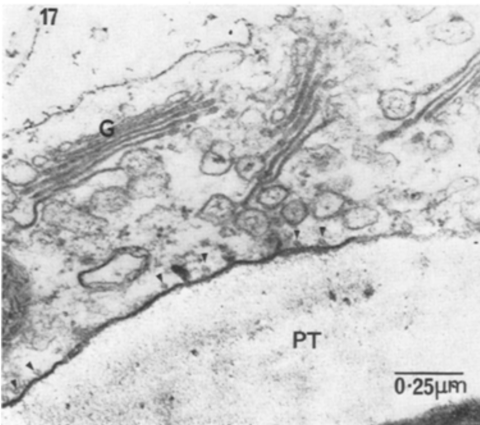
Figures presented here for apple roots show that although plasmodesmata are found in both inner and outer tangential walls of the endodermis—traversing the suberin lamellae, where present (Fig. 18)—they were at least twice as frequent in the outer tangential and radial walls of the *phi* layer cells. This may be of physiological importance. It is generally accepted that the Casparian strip prevents inward flow of water and nutrients in the apoplast (see ROBARDS and ROBB 1972). Substances in the apoplast must cross the plasmalemma of Stage I endodermal cells to gain access to the stele, while substances in the symplast may traverse the endodermis by means of the











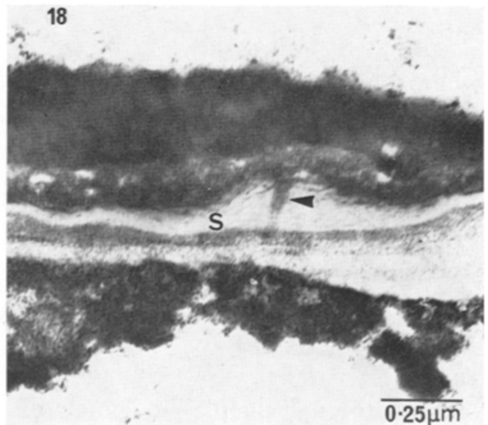


Fig. 13. Electron micrograph showing rupture of outer part of radial endodermal wall (arrows) in the same region as Fig. 11

Fig. 14. Light micrograph of TS outer cortex and epidermis from same region as Fig. 11. Cell walls are thin but intact

Fig. 15. Light micrograph 4 mm farther from tip than Fig. 14. Cell walls begin to rupture, with debris visible on outer surface

Fig. 16. Electron micrograph of TS *phi* layer radial wall, 5 mm from the root tip (see Fig. 2). Note plasmodesmata (arrows) on the median part of the lignified *phi* thickening (*PT*)

Fig. 17. Higher magnification electron micrograph of *phi* thickening, adjacent microtubules (arrows), and Golgi bodies (*G*)

Fig. 18. Electron micrograph of inner tangential wall of endodermis showing plasmodesm (arrow) traversing the suberin layer. The plasmodesm is not sectioned exactly longitudinally and therefore *appears* not to enter the pericycle (lower) cell

plasmodesmata (ROBARDS *et al.* 1973). It has been shown by CLARKSON, SANDERSON, and RUSSELL (1968) (see also HARRISON-MURRAY and CLARKSON 1973, CLARKSON and SANDERSON 1971, CLARKSON *et al.* 1971) that subsequent deposition of suberin lamellae in the endodermis presents no obstruction to nutrients which are known to move easily in the symplast (*e.g.*, potassium), the apparent pathway being plasmodesmata which they show traversing the suberin layer. On the other hand, transport of calcium, which moves with difficulty in the symplasm, ceases at this stage.

The existence of the *phi* layer presents something of a puzzle. Is its function purely one of mechanical strength (which seems unlikely), or does it have a definite physiological role? Lignified walls are reported to present approximately the same resistance to the transport of water and nutrients as does the plasmalemma (BRIGGS 1967, CLARKSON 1974); it may, therefore, be inferred that the lignified radial walls of the *phi* layer impede inward transport in the apoplast. This could account for the relatively high plasmodesmatal frequency in the outer tangential walls of the *phi* layer. Since there is about twice the number of plasmodesmata between the cortex and the *phi* layer than between the *phi* layer and endodermis, a build up of nutrients in the *phi* layer might be expected since the suberin lamellae of the endodermis must prevent transport across the plasmalemma. It has, however, been shown that secondary development in the xylem pole endodermis as compared to phloem pole cells is delayed relatively longer than in other plants, *e.g.*, marrow and barley, and so the plasma membranes of these cells would be available for transport purposes. Fig. 9 shows that about 50% of endodermal cells are in this unsuberized state. Obviously, not all *phi* layer cells are opposite the xylem poles, but the presence of a large number of plasmodesmata in the radial walls may well facilitate circumferential movement within the *phi* layer symplasm towards the unsuberized xylem poles where further penetration towards the stele could occur across the plasmalemma. In parallel with this pathway of movement, of course, would be the pathway through the plasmodesmata of the phloem pole endodermis, although this would presumably be at a slower rate due to lower plasmodesmata frequency. On collapse of the phloem pole endodermis, this latter pathway would cease due to the inevitable breakage of plasmodesmata in the outer tangential walls.

The cytoplasm of the *phi* layer cells appears highly active metabolically, containing a great number and variety of organelles and much endoplasmic reticulum; in this respect it contrasts with the endodermis. This apparent activity is also retained for much longer in the *phi* layer, and although much of the activity must be associated with the adding of material to the radial walls, it is possible that some is involved in the transfer of nutrients.

The onset of phellogen activity may be expected to have some considerable effect on nutrient flow to the stele. Quite apart from sloughing of the cortex,

including the *phi* layer, and the consequent breaking of plasmodesmatal connections, there is the addition of further layers of heavily suberized cells inside the endodermis. Traditionally, these would be seen as a barrier to water and nutrient flow, but evidence exists showing that water can enter secondarily thickened roots (KRAMER and BULLOCK 1966, ATKINSON and WILSON 1979, in press). In addition, CLARKSON, ROBARDS, SANDERSON, and PETERSON (1978) conclude from experiments on hypodermal-epidermal sleeves isolated from onions that suberin lamellae may not always present a "major barrier to diffusion in the apoplast". Their results may, however, have been affected by the enzyme digestion techniques they used to isolate the sleeves. Structural considerations here imply some similarities in the water and mineral uptake between apple roots, and barley and marrow roots. The extent of the structural differences between apple roots and those of barley and marrow, however, particularly the differences in relative length of unsuberized root and the presence of a *phi* layer in apple, indicate a need for detailed study of the physiology of uptake and transport through apple roots in relation to structure.

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