CHAPTER - V
Several studies on xylem vessel development have been made by the use of light (Esau, 1965a,b; O’Brien, 1972) and Electron microscopy (Cronshaw, 1967; Cronshaw and Bouck, 1965; Esau et al., 1966a,b; O’Brien, 1970; O’Brien and Thimann, 1967b; Yata et al., 1970). These authors have documented the degenerative changes occurring during maturation of the conducting xylem elements. Wadzicki and Humphreys (1972, 1973) who examined cytodifferentiation of maturing pine trachids using scanning and transmission electron microscopy, stated that autolysis of the protoplast began with the formation of cytoplasmic spherules which contained cell organelles. The organelles were subsequently reported to be released into the central vacuole where they were digested. These authors favour the hypothesis that the digestion of individual cytoplasmic components occurs in the vacuolar sap containing hydrolases.

Gahan and Maple (1966), whose work was based on light microscopy, recorded localization of acid phosphatase in particulate structures, and subsequent release and diffusion of the enzyme throughout the differentiating xylem cell.

Matile (1969), however, suggested that Gahan and Maple (1966) were actually observing the development of vacuolar lysosomal apparatus instead of a release of enzymes from lysosomes.
The indication that acid phosphatase is localized in the dictyosomes of young xylem elements in bean, as observed by Charvat and Esau (1975), supports the view that acid phosphatase is packaged by the dictyosomes. These authors have also reported a strong acid phosphatase activity in the vessel elements during the last stages of differentiation. Hebant (1973), who made light-microscope studies on the localization of β-glycerophosphatase in different species of Bryophytes, observed a peak of strong acid phosphatase activity in water-conducting elements towards the end of their development. The author (Hebant, 1973) suggested that this intense activity was correlated with autolysis of the protoplast.

However, the most significant feature of acid phosphatase localization in the bean xylem is the consistency with which the enzyme was identifiable in the cell walls (Charvat and Esau, 1975). In the vessel element, specifically, the enzyme appears to be associated with the partial hydrolysis of the primary wall, characteristic of this cell in its later stages of differentiation (O'Brien, 1970).

**Microtubules and Cell-wall differentiation**

The differentiating tracheary elements of bean (Esau and Charvat, 1978) show the commonly observed correlation between distribution of microtubules and the stage of cell wall differentiation. Goosen-de-Roo (1973) found that, in the differentiating tracheary elements of Cucumber, the microtubules reappeared along the exposed primary wall in the
late stage of secondary wall growth. She explained this sequence by proposing that, the microtubules constitute a barrier to vesicles carrying wall material and that, in the early stages of secondary wall thickening, the vesicle content is incorporated between the 'gyres' of the helix where microtubules are absent at that time. Schnef (1974) opposes the view that the microtubules guide the vesicles toward the growing wall and, like Goosen-de Roo (1973), he thinks that release of vesicle content occurs along the wall surfaces, free of microtubules.

Investigators of xylem differentiation have emphasized the common phenomenon that secondary wall thickenings appear opposite one another in adjacent tracheary cells. Hopler and Gosset (1971) assume that the control of this correlation is exerted by the older cell upon the contiguous younger cell through factors residing in the wall. O'Brien (1972) has expressed a concern about proper interpretation of the wall thickening developing in a still elongating tracheary element.

The interpretation of the method of perforation of the end wall continues to be somewhat controversial. Sassen (1965) favours the notion that the end wall, as observed in the central vessel in the root of Hordeum vulgare, is detached in its entirety along the margin, and subsequently digested with the aid of enzymes introduced into the wall.
by means of dictyosome vesicles. According to the studies on vessel development in *Knightia excelsa* (Meylan and Butterfield, 1972) and *Laurelia novae-zealandiae* (Butterfield and Meylan, 1972) by the use of scanning electron microscopy, the breakdown of the endwall appears to be an enzymic process occurring in situ, with the wall parts to be removed, becoming granular. Furthermore, O'Brien (1974) suggested that the primary parts of both sides and the end walls are hydrolysed in a similar manner. However, in the side wall, the cellulose component of the wall persists, whereas in the end wall it is removed. But, in bean, the later stages of endwall modification was characterized by the appearance of numerous vesicles near both the surfaces of the end walls (Esau and Charvat, 1978). The presence of the vesicles may represent a stage of disintegration of the wall.

Other studies on formation, structure and breakdown of end walls in the vessels include those of Flash (1924); Esau (1936); Esau and Hewitt (1940); Buvat (1964); Esau et al., (1965); Niedermeyer (1974); Murmanis (1978) and Prestley et al., (1935). All these authors, except Prestley et al., (1935) have observed breakdown of endwalls occurring more or less simultaneously with the disintegration of vessel member protoplasm. Murmanis (1978), on the basis of his EM studies in *Quercus rubra*, suggested that with the protoplasmic contents (Mitochondria, Vesicles) being present at the site of breakdown, and considering the gradual steps in the
disintegration of the end wall, the process of breakdown of the wall must be an enzymatic process. Most workers have suggested that some wall materials could be preformed in the Golgi bodies, and transported to the wall, as the contents of vesicles and the fusion of vesicular membranes with the plasmalemma would then transfer the material into the cell wall (Hapler and Gowcomb, 1964; Wooding and Northcote, 1964; Cronshaw and Bouch, 1965; Cronshaw, 1965; Pickett-Heaps and Northcote, 1966a; Pickett-Heaps, 1966, 1967a). Autoradiographic investigations, using lignin precursors, have been carried out by Pickett-Heaps (1968). He used tritiated leucine, tyrosine, phenylalanine, methyl labelled methionine and cinnamic acid. His results indicated cinnamic acid incorporation in xylem wall thickening.

Nucleus and DNA in differentiating vessels:

The nucleus and the nucleolus in differentiating vessel members greatly enlarge and the nucleolus becomes lobate (Last, 1963; Esau and Charvat, 1978). The enlargement of the organelle is known to be correlated with the endoduplication of nuclear DNA (Lai and Srivastava, 1976). In a detailed analysis of differentiating metaxylem elements in onion, Innocenti and Avanzi (1971) and Avanzi found that the younger cells were engaged in chromosome endoduplication, whereas somewhat older cells showed extra replication of nucleolus-associated DNA. They interpreted this as due to amplification of cistrons (rDNA) coding for ribosomal RNA.
It appears that such nuclear activity associated with polyploidy makes the much elongated cell highly efficient in the production of the secondary wall during the limited interval of time that the proplast remains alive.

Innocenti and Avanzi (1971) observed the formation and release of multiple nucleoli in metaxylem of Allium, and concluded that this phenomenon is an additional expression of possible amplification of the genes coding for rRNA. Of late, nucleolar amplification during metaxylem differentiation has been studied by Langer and Koul (1984), and their observations clearly demonstrate the numerical increase in nucleolus up to 3 per nucleus. Another similarity between their work (Langer and Koul, 1984) and that of Avanzi et al., (1971) is the occurrence of nucleolar budding. These authors attribute nucleolar budding and consequent increase in nucleolar number to the extra replication of nucleolus associated DNA, within developing xylem cells.

Swift (1950) observed a geometric progression of nuclear DNA increase in the developing xylem cells of Zea roots. This was the first observation of its kind utilizing quantitative techniques to measure cell components in plant xylem cells. List (1963) attempted to further substantiate the hypothesis that in cells from developing primary xylem of roots, cell volume growth is highly correlated with DNA
duplication and nuclear volume doubling. He showed that one aspect of differentiation during cell enlargement, is the continued DNA doubling and that this may occur before intense vacuolation of cell sets in. He also concluded that DNA doubling is most likely a period of intense cytoplasmic synthesis in the metaxylem.

The final stage of nuclear breakdown seems to be an elusive phenomenon. Lai and Srivastava (1976) studied the nuclear transformations in the vessels of Zea. In their study, Liao and Charvat (1978) found the nuclear degradation associated with late secondary thickening in the side walls. Earlier, Walzicki and Humphreys (1972), who studied the protoplast breakdown in Pinus tracheids by means of scanning electron microscopy, found that the nucleus was among the last autolyzed components of protoplast.

The following 4 distinct ontogenetic stages of tracheary element formation has been recognized by Torrey (1953):

1. Cell origination associated with critical cell division — through gene activation.

2. Cell enlargement associated with DNA synthesis (endomitotic reduplication), protein synthesis, synthesis of primary cell wall materials, and determination of future secondary wall pattern.
3. Secondary wall deposition of cellulose and hemicellulose, and synthesis and deposition of lignin.

4. Wall lysis and cell autolysis associated with rupture of lysosomes, and lysis of portions of primary wall, cytoplasm and nucleus.
The central core of vascular tissue in many roots is separated from the cortex by the endodermal cells (Van Tieghem and Douloit, 1886) in which four developmental stages are usually recognized.

It was Williams (1947) who, for the first time recognized the meristematic phase of the endodermis in roots. The endodermal cells remain meristematic and often give rise to some or all the cells of the cortex, and the active meristematic phase of the endodermis described by Williams has been called by Hurst (1954) as "Proendodermis".

However, it was Van Fleet (1961) who showed that those cells recently initiated by the apical meristem, which are destined to become the endodermis, can be distinguished histochemically at a very early stage by their high phenol content. Furthermore, the end of the somatic division phase in the proendodermis of roots has been found to be marked by the appearance of phenol-quinone systems in the initials (Van Fleet, 1954) and that, quinones appear at the last stages of division of the mother cells. The proendodermis can often be detected at this stage, histochemically, by virtue of its high content of chemicals like dihydroxyphenols, hydroxynaphthols and hydroxyanthrols (Van Fleet, 1961). According to Van Fleet (1954), the quinone development in
the system (endodermal cells) is an internal indicator of redox phase and of a transition from division to the resting phase in differentiation. Such observations led him to conceptualize cellular differentiation in terms of histochemical characteristics established in the division phase, prior to elongation or 'differentiation' (Van Fleet, 1955; 1959).

A number of enzyme activities have been demonstrated by various workers. Esterases, particularly lipase, is a common enzyme in the endodermis (Van Fleet, 1950). Yin and Sun (1949) had earlier found a general coincidence of phosphorylase activity associated with sites of normal starch synthesis. This led Van Fleet (1961) to believe that phosphorylase activity to be most characteristic of the starch sheath endodermis.

The unpublished results of Cordes (1956), as described by Van Fleet (1961), indicate localization of phosphorylase in the starch sheath. Rhoades and Carvalho (1944) reported to have found starch deposited in the plastids of the bundle sheath. The starch sheath in aerial stems of *Smilax herbacea* seems to contain starch which is more resistant to hydrolysis than the starch grains in parenchyma cells of cortex or pith (Van Fleet, 1961). He further reports that when sections were heated in 12% HCl, hydrolysis to the erythro took place, starch in the phloem stained red with IKI, and starch
grains in the cortex and pith stained red. But, grains in the endodermis also stained red. This indicatively suggested that starch of the endodermis is resistant to hydrolysis by solutions of iodine and zinc chloride. It was concluded that some fatty or suberized pellicle is covering the starch grains in the starch sheath, this pellicle stained positively with Sudan III, Roseine and Sudan IV after hydrolysis with HCl. Van Fleet (1961) also found that the suberin-like pellicle, or plastid sheath encloses 5 to 15 grains, and that it may be responsible for isotropy in polarized light. He made similar observations on sections of sunflower, soybean and alfalfa stems (see Van Fleet, 1943–1950).

Presenting a definitive description of parenchymal sheath, plastids and their function in maize, Rhoades and Carvalho (1944) also demonstrated that starch accumulated during the day is transformed into soluble carbohydrates during the night. Discussing the nature of carbohydrates, Van Fleet (1961) logically states that 'Carbohydrates in the endodermis must be present in the form of hexose phosphate.' Otherwise, they would be lost from this tissue, and that the ester must be split by phosphatase if hexose is to leave the endodermal layer. Thus, he concludes that phosphatase apparently is inhibited in the endodermis and phosphorylase is active.

In a series of studies by Van Fleet (1947, 1952, 1959), it was conclusively observed that peroxidase activity
in the endodermis begins to develop prior to the occurrence of the casparian strip. This was the first enzyme (Peroxidase) to be localized in the endodermis (Wodzicka, 1916). Furthermore, it has been concluded by Lundegardh (1958) that peroxidase may conduct 10–15% of the total aerobic respiration in wheat roots; and that with a high concentration of this enzyme in the endodermis, it has been assumed (Van Fleet, 1961) that a respiratory carrier system may be responsible for stelar accumulation and selection of ions. Conclusively, Van Fleet (1961) has listed 10 enzyme systems characteristic of the endodermis including: 1. Lipase, 2. Phosphatase, 3. Polyphenolase, 4. \( \beta \)-glucosidase, 5. Amylase, 6. Phosphorylase, 7. Peroxidase, 8. Cytochrome oxidase 9. Lipoxidase and 10. Esterase.

During their investigation on the composition of the endodermis Molish (1921) and Tunman (1914) have evidenced for the presence mucoprotein like substances. However, definitive conclusions about the mucoids of the endodermis have still not been made.
1. **TOTAL INSOLUBLE POLYSACCHARIDES**:

   The cell walls of the prospective xylem vessel elements are PAS-positive and these cells show a few PAS-positive starch grains (PLATE 1; Fig.1). At this level of growth, the metaxylem initial cells are relatively larger than any of the surrounding pith cells. At a later stage of their differentiation, an increase in the number of PAS-positive starch grains was noticed in the prospective xylem vessel element cell (PLATE 1; Fig.2). A matter of significance here is a gradual increase in the total polysaccharide content of the prospective protoxylem elements that are adjacent to the pericycle layer (PLATE 1; Fig.2). The cells of the latter also show 6-7 hayline inclusions in the nucleoli.

   With further development of these xylem vessel elements, their secondary walls became more PAS-positive however the primary walls remain highly PAS-positive than the secondary walls (PLATE 1; Fig.3). An interesting feature of the mature metaxylem vessel elements is the presence of a small spherical inclusion-body which resembles the secondary walls in its PAS-positive stainability (PLATE 1; Fig.4).
the longitudinal sections of comparable growth levels, (PLATE I; Figs. 5 and 6) the linear sequence of prospective metaxylem vessel elements are recognized by their larger nucleoli, and these cells occasionally contained a binucleate nuclei (PLATE I; Fig.6). A gradual increase in the number and size of the PAS-positive starch grains was discernible in the developing metaxylem elements (PLATE I; Fig.7). The significant feature noticed is the association of few starch grains with the cell walls and the binucleate condition of maturing xylem vessel elements (PLATE I; Fig.7). On the whole, there is an overall increase in the PAS-positiveness of the cell walls of the differentiating row of metaxylem vessel elements (PLATE I; Figs. 5, 6 and 7). The mature metaxylem vessel elements with their secondary walls do not show any PAS-positive starch grains, and their secondary walls are relatively less PAS-positive than the primary walls. The degenerating nuclei in these cells stain positively with the PAS test (PLATE I; Fig. 8). The broken end walls between the successive mature xylem vessel elements are persistently PAS-positive.

2. POLARIZING LIGHT MICROSCOPY OF THE XYLEM VESSEL ELEMENTS:

All four primary xylem groups show the characteristic birefringence of their secondary lignified walls (PLATE II; Fig.9). It is interesting to note that the primary walls of these elements do not show any significant
birefringence. Intriguingly, with the differentiation of lateral roots the walls of certain cells opposite to the protoxylem elements show birefringence (PLATE II; Fig. 1). The protoxylem, opposite to the lateral root primordia shows distinct birefringence of its entire secondary walls (PLATE II; Figs. 14 and 15). The birefringence is also clear in a few xylem vessel elements at the base of the lateral root (PLATE II; Figs. 12 and 16).

The secondary xylem-groups which develop between the primary xylem groups at a very late stage, also show the birefringence in the walls (PLATE II; Fig. 10). The PAS-positive inclusion body of the metaxylem vessel elements is also birefringent under the polarized light (PLATE II; Fig. 11). At a level when the lateral roots develop opposite the protoxylem, even the endodermal cells show the birefringence in the lateral wall areas (PLATE II; Fig. 12). On the whole, the characteristic birefringence of the secondary lignified walls of all the xylem vessel elements is an invariable feature.

3. ASCORBIC ACID (AA):

There is a gradual increase in the AA content of both cytoplasm and nucleoli of the differentiating metaxylem vessel elements. The concentration increases towards their maturity in these areas (PLATE III; Fig. 17). This is in contrast to the cytoplasmic AA in the cells surrounding the
At a later stage of differentiation, AA-reduced silver grains are largely confined to the cell walls of the xylem elements (PLATE III; Fig. 19). However, small silver grains are also present along the walls of the neighbouring cells. The localization of AA shows different characteristics in the xylem cells at their maturity. In the maturing xylem vessel elements, AA deposition is thick and is largely confined to the lignified cell walls (PLATE III; Fig. 20). This is again in contrast to the very low AA content of the surrounding cells at this stage. The degenerating nucleus in these maturing xylem vessel elements has very little amount of AA associated with it (PLATE III; Fig. 20).

4. RNA AND DNA:

In the central stelar core the prospective xylem vessel elements in general and the prospective metaxylem vessel elements in particular are distinguishable by their high concentration of cytoplasmic and nucleolar RNA (PLATE IV; Figs. 21 and 22). With progressive maturation of the precursors of metaxylem vessel elements from near the apical initials (distal end) downwards (towards the proximal end) a significant increase in the size of the nucleus and nucleoli of these was noticed (PLATE IV; Fig. 21).
With the occurrence of mitotic division in the precursors of metaxylem vessel element, it was interesting to note a high amount of nucleolar and cytoplasmic RNA in that daughter cell which is towards the pericycle layer, while the other daughter cell towards the pith has low content (PLATE IV; Fig. 21). In general, the size of the nucleoli of the prospective metaxylem elements is relatively larger than any of the neighbouring parenchyma cells (PLATE IV; Figs. 21 and 22). The critical mitotic cell division occurring in one of the provascular cells is an intriguing feature because it is only the prospective metaxylem element derived from this mitotic division that contains higher cytoplasmic RNA in it. Furthermore, the size of the nucleolus also in the latter is relatively larger than the one in the other daughter cell (PLATE IV; Fig. 24). High RNA content of the prospective metaxylem vessel element persists even towards the maturity (PLATE IV; Fig. 25), and the substance (RNA) was richly confined to the wall areas. The degenerating nucleus in the maturing metaxytem vessel elements, invariably stains deeply with Azure B (PLATE IV; Fig. 25).

5. TOTAL PROTEINS:

The distributional pattern of total proteins is similar to that of RNA. Although the cytoplasmic and nucleolar protein content of 17-18 prospective metaxylem vessel elements beginning from their meristematic origin
(at the distal end) is moderately high, the subsequent prospective metaxylem vessel elements undergoing further differentiation (towards the proximal end) are prominently distinguished by their high protein content (PLATE V; Fig. 26).

An interesting feature of differentiation of these is the amplification of nucleolar size with the progressive maturation of the precursors of the metaxylem vessel elements. These cells invariably show a considerable increase in their size but, cytoplasmic proteins become low, while the nucleoli, at this stage are very large with high amount of proteins (PLATE V, Fig. 26; arrows adjacent to PLATE V, showing the course of differentiation and maturation from the distal towards the proximal end of the root apex).

6. ADENOSINE TRIPHOSPHATASE (ATPase) :

High ATPase activity was largely confined with the prospective four xylem groups and also with the central pith cells (PLATE VI; Fig. 27). In general, the enzyme activity was largely confined to the cell walls. The prospective xylem vessel elements invariably show characteristically high ATPase activity in their cytoplasm however, the particulate nature of enzyme activity was usually found near the walls of the elements (PLATE VI; Fig. 28).
7. CYTOCHROME OXIDASE:

In the prospective xylem vessel elements present near the apical initials (at the distal end) the activity of cytochrome oxidase is very low, unlike that in neighbouring parenchyma cells (PLATE VI; Fig. 29). However, in the relatively mature xylem vessels at the proximal end away from the apical initials its low activity is largely confined to the cell walls (PLATE VI; Fig. 30), whereas the neighbouring cells at this stage do not show any activity in the cytoplasm.

8. PEROXIDASE:

High activity of this enzyme is largely confined to the lignified secondary walls of the xylem vessel elements (PLATE VI; Figs. 31 and 32). The xylem vessel elements of the lateral root, which are in contiguity with the protoxylem group, also show high peroxidase activity greatly confined to their walls (PLATE VI; Fig. 32). However, feeble activity was also noticed in other surrounding cells. The cells at the base of the lateral root primordia also contain moderate peroxidase activity in them (PLATE VI; Figs. 31 and 32).

9. TOTAL LIPIDS:

A few sudan black B positive lipid bodies are associated with the secondary walls of the maturing metaxylem vessel elements whereas, the neighbouring pith
cells contain very high amount of lipids (PLATE VII, Fig.33). At the proximal end of the root tip in the fully mature xylem vessel elements, only the lignified secondary walls and the inclusion body in the metaxylem element give a positive reaction to Sudan black B test, while the surrounding pith and other parenchyma cells show a depletion of lipids (PLATE VII; Fig. 34).

10. ESTERASE:

The distributional pattern of esterase activity corresponds to that of lipids. In the xylem vessel elements at the distal end, the activity of esterase is relatively low as compared to its high activity in the neighbouring pith and other parenchyma cells (PLATE VII; Fig.35). With the maturation of the xylem elements at the proximal end of the roots the esterase activity was largely associated along with the secondary walls of these cells (PLATE VII; Fig.36). However, at this level the surrounding parenchyma cells do not show any enzyme activity.

11. LIGNIN:

Only the secondary walls of the proto and metaxylem vessel elements at the proximal end are positive to the phloroglucinol test (PLATE VII; Figs.37 and 38).
12. **ACID PHOSPHATASE**

Intense acid phosphatase activity was present along walls of developing xylem vessel elements (PLATE VII, Fig. 39) and a few neighbouring parenchyma cells show moderate to high acid phosphatase activity. The degenerating nucleus in the maturing metaxylem cells also show positive reaction indicating acid phosphatase activity (PLATE VII, Fig. 40). Occasionally the enzyme activity was noticed in the intercellular spaces. However, in general intense acid phosphatase activity is largely confined to the secondary walls of the mature xylem vessel elements.

II. **Endodermis in the 4 day old seedling root apices of Arachis hypogaea L. cv. BH 5-30**

The endodermis constitutes the inner row of cortical cells in the seedling roots of *A. hypogaea*. This layer, is invariably contiguous with the longitudinal file of the pericycle cells, and can be identified by the presence of a number of PAS-positive starch grains in its cells (PLATE I, Fig. 1).

1. **TOTAL INSOLUBLE POLYSACCHARIDES**

The walls of the pro-endodermal cells at the distal end near the apical initials are feebly PAS-positive, and the cytoplasm in them shows a faint PAS-positive tinge.
The maturing endodermal cells, farther at the proximal end away from the apical initials, possess small PAS-positive starch grains. At the level where the protophloem sieve elements begin to differentiate the mature endodermal cells invariably contain larger PAS-positive starch grain in them, contrary to the state in the neighbouring cells (PLATE 1; Fig.2). PAS-positive casparian strips (thickenings in the walls) also appear in these cells at the time of maturation of the primary xylem. The PAS-positive thickening of casparian strip however shows a characteristic birefringence under polarized light (See Fig.12 of PLATE II pertaining to xylem).

2. ASCORBIC ACID (AA):

The pro-endodermal cells at the distal end have relatively low cytoplasmic AA. However, at maturity, the endodermal cells show many AA-reduced silver grains unlike their neighbouring cortical cells (PLATE 1; Fig.3). The AA in the mature endodermal cells is largely confined to the cell walls.

3. TOTAL LIPIDS:

Although the distal pro-endodermal cells have quite low lipids in their cytoplasm, the mature ones at the proximal end contain high Sudan black B stainable wall areas (PLATE II; Fig.4) indicating the lipoidal nature of the casparian strips of these cells.
4. **ESTERASE**

The activity of esterase in the pro. and mature -
(endodermal cells) very high and is largely confined to
the walls (PLATE II; Fig. 5).

5. **CYTOCHROME OXIDASE**

The newly formed prospective endodermal cells,
derived from the apical initials at the distal end show
cytochrome oxidase activity in their cytoplasm and high
along the cell walls (PLATE II; Fig. 6). A few cells of
the central vascular cylinder and the pith are invariably
high in cytochrome oxidase activity. Moderate enzyme
activity often persists in the mature endodermal cells.

6. **PEROXIDASE**

Activity of peroxidase in the endodermis often
appears prior to the differentiation of caspian strips
(PLATE II; Fig. 7). The prospective xylem cells do not show
any significant peroxidase activity at this level.

The activity of most of the enzymes in the endodermal cells
seems to fluctuate at different levels.
PLATE I

Figs. 1-8. Tissue sections of seedling root apices tested for total insoluble polysaccharides with PAS method.

Fig. 1: Transverse section at a level with a row of 7-9 prospective xylem vessel elements. Note the relatively larger prospective metaxylem elements (blank darts) and the relatively smaller prospective protoxylem initials (black darts) opposite to pericycle layer (P). A few PAS-positive starch grains (open darts) are present in them. X 500.

Fig. 2: Transverse section at a level 7 µm higher than in Fig. 1. Note the increase in the number of PAS-positive starch grains (open darts) and the increase in the PAS stainability of the prospective protoxylem elements (black darts). X 500.

Fig. 3: Note the PAS-positiveness of the secondary walls of the xylem vessel elements. The stainability of the primary walls is relatively higher than the secondary walls. The spherical inclusion body (open dart) in one of the metaxylem vessel also reacts positively with PAS test and its stainability is similar to that of the secondary walls. X 500.

Fig. 4: Transverse section showing the fully mature xylem vessel elements at the proximal end of the seedling root. Note an increase in PAS-positiveness of the secondary walls (→). Also note the primary walls (→) have become more PAS-positive than the secondary walls. The spherical inclusion body (open dart) in the metaxylem vessel is intensely PAS-positive. X 500.

Fig. 5: Longitudinal section (L.S.) of prospective metaxylem vessel elements (blank darts). Note the larger size of their nuclei (arrows) and the few minute PAS-positive starch grains in these cells. X 500.

Fig. 6: L.S. of prospective metaxylem vessel elements (blank darts) at a higher state of differentiation than in Fig. 5. Note the increased stainability of the cell walls and also the increased number of PAS-positive starch grains (open darts). Also note the binucleate nature of one of the prospective metaxylem vessel (arrows). X 500.

Fig. 7: L.S. of maturing metaxylem vessel element (blank dart) at a level farther up than in Fig. 6. Note the significant increase in the number of PAS-positive starch grains (open darts) and the binucleate condition in the metaxylem cells. X 500.

Fig. 8: L.S. of metaxylem vessel elements nearing maturity (blank dart) with no PAS-positive starch grains in the elements. Note the PAS-positive nature of the degenerating nucleus (arrow). X 500.
PLATE II

Figs. 9 to 16: Unstained transverse sections of seedling root-apices as observed under the polarized light.

Fig. 9. Transverse section with mature primary xylem elements (open darts) in tetrarch pattern when only the secondary walls of the xylem vessels show characteristic birefringence. X 100.

Fig. 10. Transections of mature area of the primary root showing the beginning of secondary xylem (blank darts) differentiation alternating with the four primary xylem groups. X 500.

Fig. 11. Section showing the characteristic birefringence of the secondary walls of the xylem vessel elements. Note the birefringent nature of the inclusion body in one of the xylem vessel elements. X 500.

Fig. 12. Transection showing the birefringence of the protoxylem (PX)vessel element and in the walls of the flanking mature endodermal cells (En) at a level where the lateral root develops - Note the beginning of birefringent secondary wall formation in the central cells of the lateral root opposite the protoxylem vessels. X 500.

Fig. 13. Enlarged portion of one of the xylem group prior to the establishment of a connection between the xylem vessels of the primary and the lateral root. Note the birefringence developing in two cells opposite the protorylem (Px) of the primary root. X 500.

Fig. 14. A single primary xylem group of the primary root with metaxylen (Mx) vessels towards the pith and protoxylem towards the periphery. X 100.

Fig. 15. Enlarged area of protoxylem (blank dart) of fig.14 showing the birefringence of the entire secondary wall thickening which is typically scalariform. X 500.

Fig. 16. Showing the birefringence of the differentiating xylem vessel elements (open dart) in the lateral root opposite the protoxylem (Px) vessels of the primary root. X 200.
Figs. 17 to 20: Longitudinal (L.S) and transverse (T.S) section of seedling root—apices tested for ascorbic acid (AA) with acidified silver-nitrate method.

Fig. 17: L.S. of a row of prospective metaxylem vessel elements (blank darts). Note the increase in the size of the nucleoli (arrows) and also the increase in cytoplasmic AA in these prospective metaxylem vessel elements. X 500.

Fig. 18: T.S. of the root at a level in Fig. 17. Note the high AA concentration in the young metaxylem (Mx) and protoxylem (Px) vessel elements. X 500.

Fig. 19: T.S. at a higher level than in Fig. 18. Note the loss of cytoplasmic AA in the differentiating metaxylem (Mx) and protoxylem (Px) vessels. The primary walls give a positive reaction and small AA reduced silver grains (blank darts) are associated with the inner secondary walls of the xylem vessel elements. X 500.

Fig. 20: T.S. of metaxylem and protoxylem (Mx and Px) vessel elements towards maturity. Note the very high AA associated with the cell walls of only the xylem vessels (blank darts). The degenerating nucleus also in these cells is also positive to the test. The neighbouring cells contain very low AA unlike in their earlier stage of differentiation (See, fig. 18). X 500.
PLATE IV

Figs. 21 to 25: Longitudinal and transverse section of seedling root-spaces tested for RNA and DNA with Toluidine blue O and Azure B methods.

Fig. 21: Long section of seedling root tested for RNA and DNA with Toluidine blue O method.

Note: (a) the prominently high RNA content in the row of prospective metaxylem vessel elements (blank darts), than the neighbouring parenchyma cells.

(b) a considerable increase in the size of the nucleus (open dart) and the size of the nucleoli (while timed arrow).

(c) occasionally a few prospective metaxylem vessel elements undergo a vertical division (arrows). Of the two daughter cells, the one towards the pith has lower cytoplasmic and nucleolar RNA. X 500.

Fig. 22: Transsection of the prospective xylem vessel group showing relatively higher RNA in their cytoplasm and nucleoli than the neighbouring parenchyma cells. X 500.

Fig. 23: Transsection showing the later stages of maturing xylem vessel elements. The degenerating cytoplasmic components (blank darts) including the nucleus stain intensely with Azure B. X 500.

Fig. 24: Transverse section tested with Azure-B showing two asymmetric daughter cells (arrows) derived from the division of a metaxylem mother cell. Of these two, the cell with higher nucleolar and cytoplasmic RNA is the prospective metaxylem vessel element. Its sister cell towards the pith has relatively low RNA in its cytoplasm. X 500.

Fig. 25: Transsection at a higher level than in Fig. 24 showing high cytoplasmic RNA in the prospective metaxylem vessel (Mx) all along the inner wall. Note the low RNA in the neighbouring parenchyma cells in contrast to the earlier stage (Fig. 24) which is nearer the apical initials. X 500.
Fig. 26  Longitudinal section of a seedling root tested for total proteins with mercuric bromophenol blue method. Initially at the distal end the prospective metaxylem initials (4 black darts in the left row) near the apical initials have low proteins in them. In their linear sequence towards the proximal end leading to maturity as at higher levels, the prospective metaxylem elements, are distinguished by their high cytoplasmic proteins. These cells show an amplification of their nucleoli. At a later stage of maturity (black darts in the right row) of the metaxylem the cytoplasmic protein concentration declines whereas the nucleolar proteins remain very high. X 500.
Fig. 27 to 32: Fresh, freehand-sections of seedling root-apices tested for Adenosine triphosphatase (ATPase), Cytochrome oxidase and Peroxidase enzyme activities.

Fig. 27: ATPase enzyme activity is largely confined to the 4 distinct prospective xylem groups (black darts). Certain pith cells also show the enzyme activity. X 100.

Fig. 28: Note the ATPase activity in the cytoplasm of the prospective metaxylem (open dart) and protoxylem (blank dart). The enzyme activity is also particulate in nature. X 500.

Fig. 29: Cytochrome oxidase activity is very low in the prospective xylem elements (open darts), whereas the surrounding cells of the parenchyma show very high enzyme activity in their cytoplasm. X 500.

Fig. 30: Transaction showing groups of mature xylem vessel elements (black darts) with cytochrome oxidase activity, largely confined to the secondary walls of the elements. X 100.

Fig. 31: Section tested for peroxidase activity showing high enzyme activity in the secondary walls of proto- and metaxylem elements (darts). The cells, opposite to the protoxylem present in the center of the lateral root, also show high peroxidase activity. Moderate activity is also seen along the walls of those of cortex present around the base of the lateral roots (arrows). X 100.

Fig. 32: Note high peroxidase activity largely confined to the maturing xylem cells particularly associated with lignified secondary wall (darts). Feeble enzyme activity is also seen in other parenchyma cells also. X 500.
PLATE VII

Figs. 33 to 40: Fresh freehand sections tested for total lipids, lignin, Acid phosphatase (APase) and Esterase activities.

Fig. 33. Note the high amount of lipids in the parenchyma cells, surrounding the prospective metaxylem vessel elements. Very few lipid bodies (arrows) are present along the inner walls of the xylem elements. Note the absence of lipid stainability of the primary walls. X 600.

Fig. 34. Section showing Sudan black B positive secondary walls of mature xylem vessel elements (darts). Note the positive stainability of the inclusion body (arrow) associated with the wall in one of the metaxylem element. X 400.

Fig. 35. Note high esterase activity in the neighbouring parenchyma and low activity in the meta- and protoxylem cells along the walls. X 600.

Fig. 36. Note the absence of esterase activity in the parenchyma cells surrounding the fully mature xylem vessel elements, unlike in the earlier stage (Fig. 35). Note the high enzyme activity in the walls of the mature xylem vessel elements. X 600.

Fig. 37. Fresh free hand section, slightly dried at 37°C for 10 min. and tested for lignin using phloroglucinol test. Note the positive stainability of the secondary walls of both protoxylem (blank dart) and metaxylem (open dart) X 150.

Fig. 38. The longitudinal surface view of the phloroglucinol stained xylem vessels. X 300.

Fig. 39. Section showing slightly immature xylem vessel elements tested for acid phosphatase activity. X 150.

Fig. 40. Section at higher level than in fig. 39. Note the acid phosphatase activity associated the secondary wall thickening of the xylem vessels. High enzyme activity is seen with the degenerating nucleus in one of the metaxylem vessel (open dart). Also note the presence of APase activity in the intercellular spaces. X 300.
Fig. 1 to 3: Longitudinal and transverse sections of seedling root apices showing the endodermal cells at various levels of differentiation, tested for total insoluble polysaccharides and Ascorbic acid.

Fig. 1: Longissection tested for total insoluble polysaccharides, lower portion of the micrograph showing pro-endodermal cells (blank triangle) with faint cytoplasmic PAS-positive tinge. Farther away towards the proximal end, in the upper portion the maturing endodermal cells show many PAS-positive starch grains whereas the neighbouring pericycle cells (darts) do not contain any such grains. Note the gradual increase in the number and size of starch grains in the endodermal cells from the distal towards the proximal end. X 300.

Fig. 2: Transverse section of root briefly stained for total insoluble polysaccharides, at a stage where 2-3 protophloem sieve elements (blank darts) are present. Note the PAS-positiveness of the starch grains in the mature endodermal (En) cells. The neighbouring cells of the pericycle (P) lack starch grains. X 500.

Fig. 3: Transsection of the root showing distributional pattern of ascorbic acid. The mature endodermal cells (En) adjacent to the pericycle layer (P) showing relatively more number of AA-reduced silver grains. X 300.
PLATE II

Figs. 4 to 7: Fresh handcut sections tested for total lipids esterase, cytochrome oxidase and peroxidase activity.

Fig. 4. Transection showing the distributional pattern of lipids. Note the high lipid content in the endodermal cells (arrows), relatively low amount in the pericycle layer and very high in the inner provascular cells. X 300.

Fig. 5. Transection of the root at almost the same level as in Fig. 4, but tested for esterase enzyme activity. Note the high enzyme activity along the walls of the mature endodermal cells (blank darts). The esterase activity in the adjacent pericycle cells (P) is very feeble. X 300.

Fig. 6. Transection at a level near the apical initials, tested for cytochrome oxidase activity. Note the high enzyme activity confined to the proendodermal cells (arrows), and also in a few pith cells. X 300.

Fig. 7. Transection showing the distributional pattern of peroxidase activity. Note the appearance of activity in the pro-endodermal cells (blank darts), but not in the prospective metaxylem elements (Mx) at this stage. X 300.
III. Pith cells in the 4 day old seedling root apices of

*Arachis hypogaea* L. CV. DH3-10.

1. TOTAL INSOLUBLE POLYSACCHARIDES:

At the distal end near the apical initials the central pith cells do not contain any PAS-positive starch grains in them. However, the walls are PAS-positive. Small PAS-positive grains appear in the pith cells at a level where the endodermal cells begin to accumulate starch grains (PLATE I; Fig. 2). Further away from the apical initials where the first protophloem sieve elements differentiate, the pith cells have relatively thick PAS-positive walls and the number of PAS-positive starch grains in them increase (PLATE I; Fig. 3). In the dividing pith cells, the newly formed wall is relatively less PAS-positive.

At a level where the xylem vessel elements differentiate, the pith cells have many large PAS-positive starch grains and their walls are thickly PAS-positive. Most of the PAS-positive grains in them are present around the nuclei (PLATE I; Fig. 4). Furthermore, at the proximal end when the lateral root primordias begin to differentiate, the pith cells are characterized by their relatively less PAS-positive walls but these cells contain larger PAS-positive starch grains (PLATE I; Fig. 5).
On the whole from the distal to the proximal end a gradual increase in the size and number of PAS-positive starch grains is noticed in the central pith cells (PLATE I; Figs. 1 to 5).

2. TOTAL LIPIDS:

The pith cells at the distal end near the apical initials contain relatively low content of lipids in them (PLATE I; Fig. 7).

At the level where the first protoxylem sieve elements differentiate, the pith cells can be characterized by their rich cytoplasmic lipids (PLATE I; Fig. 8). Further at the proximal end away from the apical initials where the xylem vessels elements begin to differentiate, the pith cells have depleted their lipids and these cells have high lipid positive areas in their thick walls (PLATE I; Fig. 9).

3. ASCORBIC ACID (AA):

The nuclear stainability for AA in the pith cells at the distal end is significantly more and the intercellular spaces between the pith cells contain high AA (PLATE II; Fig. 10). At a higher level where the endodermal cells begin to accumulate starch, the intercellular AA between the pith cells begins to deplete and furthermore, the nuclear AA content also is low (PLATE II; Fig. 11). At the distal end wherein the xylem cells begin to differentiate, the
intercellular spaces between the pith cells do not contain any AA but these cells contain many AA reduced silver grains in their cytoplasm. These grains are mainly found at the periphery near the cell walls (PLATE II; Fig. 12).

4. TOTAL PROTEINS:

At a level where the first protophloem sieve elements differentiate, the pith cells contain moderately high cytoplasmic protein and high nucleolar proteins. The newly formed walls in these pith cells also stain positively for proteins (PLATE II; Fig. 13).

Further up, when the xylem vessel elements begin to differentiate the central pith cells contain very low cytoplasmic proteins and the nucleolar proteins also at this level are relatively less (PLATE II; Fig. 14).

At the proximal end where the lateral root primordias develop, the pith cells have very low cytoplasmic proteins. The intercellular spaces do not contain any protein positive material however, the nucleoli of the pith cells invariably contain high proteins (PLATE II; Fig. 15).
Serial transactions (T.S.) of fixed and fresh root apices of 4-day old seedlings, tested for total insoluble polysaccharides (Figs. 1 to 5) and total lipids (Figs. 6 to 9) showing the pith cells at different levels.

Fig. 1: T.S. at the level 'a' of fixed root tip at the distal and near the apical initials tested for total insoluble polysaccharides. Note the rich PAS-positive walls of central 4 to 5 pith cells, devoid of any PAS-positive starch grains. X 500.

Fig. 2: T.S. at the level 'b' where the endodermal cells begin to accumulate starch grains. At this level central pith cells show an increased PAS-positive walls and these cells contain small PAS-positive starch grains (darts). X 500.

Fig. 3: T.S. of the root showing pith cells at a level 'c' where the first protophloem sieve elements are completely differentiated. Note the further increase in size and starch content of the pith cells and cell divisions in some of them showing less PAS-positive newly formed walls (open darts). X 500.

Fig. 4: At a level 'd' where the xylem vessel elements differentiate, the pith cells contain larger PAS-positive starch grains (blank darts) associated with the nucleus. Note the increase in PAS-positiveness of the newly formed walls (open darts) and also the walls of old pith cells. X 500.

Fig. 5: At the level 'e' on appearance of lateral root primordia. Note a decrease in the PAS-positiveness of the cell walls of the pith cells. These cells contain many large PAS-positive starch grains (blank darts). X 500.

Fig. 6: T.S. at the level 'g' showing low concentration of total lipids in the pith cells. X 500.

Fig. 7: T.S. at the level 'h'. Note the significant increase in the lipid content of the pith cells greatly along the walls. X 500.

Fig. 8: Further away from the apical initials at level 'g'. The pith cells still have rich lipids in association with the walls. X 500.

Fig. 9: At the level 'd' where the xylem vessels differentiate. The pith cells are depleted of their cytoplasmic lipids parallelly with accumulation in the walls. Note; small lipid positive bodies (blank triangle) persist around the nucleus. X 500.
Transverse sections of fixed root apices showing the distributional pattern of ascorbic acid (AA) (Figs. 10 to 12) and total proteins (Figs. 13 to 15).

Fig. 10: At level 'a' the intercellular spaces (arrows) between the pith cells showing high AA in them. Note the similar high AA in the nucleus of these cells. X 300.

Fig. 11: Pith cells at level 'b' showing lesser AA in the intercellular spaces and also in the nucleus of these cells. X 300.

Fig. 12: At level 'd' the pith cells contain small AA-reduced silver grains along the inner periphery of cytoplasm (open darts) and also around the nucleus (blank triangles). Note the AA stainability of the nucleoli, its absence in the intercellular spaces and feeble stainability of cell walls X 500.

Fig. 13: At level 'c' showing the high protein content of pith cells in the nucleoli and the nucleoplasm, and also the cell walls including the newly formed ones (open darts). X 500.

Fig. 14: Note the low protein content of pith cells at level 'd' in the nucleoli and the cytoplasrn. X 500.

Fig. 15: At level 'e' cytoplasmic proteins in the pith cells is very low and absent in the intercellular spaces (open darts). Note the high protein positive cell walls and the nucleoli (arrows). X 500.
DISCUSSION

I. XYLEM VESSEL ELEMENTS IN THE 4 DAY OLD SEEDLING ROOT APICES

Torrey et al., 1971, have recognized four ontogenetic stages of xylem formation of which, they considered cell origination associated with critical cell division as the first stage of xylem differentiation. These authors have however, not presented any evidence to show the criticality of the cell division leading to origin of xylem. In the present investigation, the mitotic division occurring in the metaxylem mother cells could be considered as critical to the origin of xylem vessel element. For instance, of the two daughter cells generated by the mitotic division in the metaxylem mother cells, the larger daughter cell with high concentrations of RNA and proteins alone further differentiates into a metaxylem vessel element, whereas the other, relatively smaller cell with low RNA and proteins remains as a pith cell. The underlying metabolic differences brought about by the division is further reflected by the differences in the size of the nucleoli in these cells. Because it is known that nucleoli are larger in cells synthesizing proteins (List, 1963), the observed larger nucleoli of the prospective metaxylem cells than those of the surrounding pith cells indirectly evidence the probable cause for the enhanced protein synthesis in the cells of prospective metaxylem cells. It is, therefore,
conceivable that the mitotic division occurring in the metaxylem mother cells, present in the 4 prospective xylem areas, is critically decisive to ensure ontogenetic diversity and that the doctrine of product identity of mitosis is necessarily untenable in this case.

It has been suggested by Chinoy et al., 1971, that ascorbic acid combines with macromolecules and increases the formation of free radicals and charged transfer complexes and that this forms the basis of intensified energy transfer during the process of growth and morphogenesis. Based on the observed high concentrations of ascorbic acid, RNA, proteins and the high activity of ATPase in all the prospective xylem vessel elements of A. hypogaea it is reasonable to assume that charge transfer complex formation is enhanced in these cells during the process of their differentiation. A similar assumption has been made by Sethi and Malik (1974) for the morphogenesis and differentiation of different epidermal structures in Phaseolus mungo.

The general aspect of morphogenesis for the metaxylem cell appears to be one of a rather steep gradient of volume increase accompanied by enhanced synthesis of ascorbic acid, RNA and proteins, when compared with adjacent cells of the pith. The prospective metaxylem cells frequently grow to many times the volume of the surrounding cells within a few hundred microns from the initials. There is also a gradual increase in the size of the nucleoli of these cells. This observation is consistent with those of List (1963); Innocenti and Avanzi (1971) and Langer and Koul (1984).
Innocenti and Avanzi (1971) have also presented several lines of evidence that cells of immature metaxylem of the root of Allium cepa are active in the extrareplication of nucleolus associated DNA. Because, it is known from the studies on animals (Ritossa and Spiegelman, 1965; Gall et al., 1969; Lima De Faria et al., 1969) and also on plants (Avanzi et al., 1971) that certain nucleolus organizing regions contain cistrons coding for rRNA, they suspected that amplification of ribosomal cistrons is at work during metaxylem differentiation.

In A. hypogaea, the precursors of the metaxylem present at a greater distance from the apical initials contain characteristically larger nucleoli with high concentration of RNA. This observation can perhaps be considered as a positive evidence for amplification of cistrons coding for ribosomal RNA, more so because all these cells consistently show high RNA even in their cytoplasm. The observed amplification of the nucleoli in the prospective metaxylem cells of A. hypogaea is similar to that observed by Langer and Koul (1984) in Allium cepa. Langer and Koul (1984) and Avanzi et al., (1973) have reported the phenomenon of nucleolar budding in the developing metaxylem cells. However, in the present study no nucleolar budding was observed, at any stage of metaxylem differentiation.

The elongated and relatively older prospective metaxylem cells in A. hypogaea, particularly those at a greater distance from the apical initials, often contain two nuclei.
This perhaps reflects an increase in the DNA content of these cells. It appears that such a doubling of DNA is associated with enhanced synthesis of RNA and proteins thereby making the cell highly metabolic for synthesising the required biomolecules involved in the subsequent process of secondary wall formation. Increase in the number of nuclei in the cells of the metaxylem vessel elements of angiosperms has so far not been reported. However, in Marsilea tracheary elements, List (1963) has reported increase in nuclear number. His studies also show that in cells from developing primary xylem of roots, cell volume growth is correlated with DNA duplication and nuclear volume doubling.

The prospective xylem element in A. hypogaea shows a gradual increase in the PAS-positive starch grains from the distal towards the proximal (away from the apical initials) end. This increase can be correlated with the further increase in the PAS-positive walls of the prospective xylem elements. The prospective metaxylem elements at the proximal end invariably contain PAS-positive starch grains. Starch containing plastids have been reported to occur in the differentiating xylem elements of Beta and Cucurbita (Esau et al., 1966) and in Bean (Esau and Charvat, 1978). Esau et al., (1963) however, has not observed any plastids in the Cucurbita vessel members.

In A. hypogaea, the presence of starch grains in the prospective xylem vessel elements and the gradual
increase in the PAS-positive stainability of the secondary walls of the mature vessel elements followed by the general depletion of PAS-positive starch grains from these and surrounding parenchyma cells, indirectly suggests that the PAS-positive starch grains are probably utilized as the carbohydrate source for secondary wall formation in the xylem vessel elements.

It is also noticed that the PAS-positive secondary walls give a positive reaction to the Phloroglucinol -HCl staining. Because it is known that phloroglucinol-HCl staining provides a fairly reliable estimate of the extent of lignification in plant tissues (Hoelfsen, 1959), the positive stainability (with phloroglucinol-HCl) of the thick secondary walls of the mature xylem vessel elements suggests that these wall areas are lignin in nature. The observed positive stainability of the secondary walls of the xylem vessel element in A. hypogea with PAS-reaction for polysaccharides and the phloroglucinol-HCl test for lignin tends to support the view that the secondary walls are composed of lignin-carbohydrate complex. Lignin is believed to exist as a matrix with covalent bonds to the carbohydrate constituents of plant cell walls (Lai and Sarkanen, 1971). The studies of Esau (1966a) and Hopler et al., (1970) have also indicated that during the formation of the secondary wall the deposition of cellulose is accompanied by the deposition of lignin. The characteristic birefringence of the secondary walls of xylem under the polarized light has been known since long (see Torrey et al., 1971). The
birefringence of the mature xylem vessel elements, particularly of the secondary walls in *A. hypogaea*, is probably due to the cellulo-lignin compounds of their walls.

The studies of Freudenberg *et al.* (1952) and Siegel (1953) have suggested the involvement of peroxidase in biosynthesis of lignin, and the investigations of Lipetz and Garro (1965) further assume that peroxidase functions as a lignin polymerase. In the present study on *A. hypogaea*, the young prospective xylem vessel cells did not contain any appreciable amount of peroxidase activity but the enzyme reaction was strongest in the relatively mature xylem vessel elements. Peroxidase activity was largely found in association with the secondary lignified walls of the xylem vessels. This observation is in contrast to that of De Jong (1966) who did not observe any peroxidase activity in the lignifying xylem cells of onion roots. His studies further tried to suggest that peroxidase activity cannot be the determinative factor for lignification *in vivo*. In contrast to De Jong's (1966) study the investigations of Saunders *et al.* (1964) had earlier recorded that peroxidase is somehow involved in lignification. The later studies of Kidge and Osborne (1971) have shown that a form of peroxidase (EC 1.11.1.7) is attached to cell walls through ionic and possibly covalent bonds. It has also been suggested by Whitmore (1978) that wall-associated peroxidase is involved in dehydrogenative polymerization of phenolic monomers in the synthesis of lignin and also in establishment of covalent bonds between lignin and
carbohydrate in cell walls. It can, therefore, be speculated that the high peroxidase found in association with the lignified secondary walls of mature xylem vessel elements in A. hypogaea is involved in establishing covalent bonds between the lignin and carbohydrate components, and also in the polymerization of phenolic monomers. The activity of peroxidase in the differentiating xylem of A. hypogaea is similar to that reported by Hall and Sexton (1972), for seedling roots of Pisum sativum.

The lignified secondary walls of mature xylem vessel elements in A. hypogaea invariably stained positively with Sudan Black B indicating the presence of lipid components in it, and furthermore, the activity of esterase was also identical to that of the distributional pattern of lipids. Because, there are hardly any studies on the histochemical localization of lipids and also the activity of esterases in the developing xylem it is difficult to draw any conclusions on the role of these substances during the differentiation of the xylem vessel elements.

The high acid phosphatase activity along the walls of the maturing vessel elements in A. hypogaea indicates that this enzyme has some role in the formation of the secondary walls. Similar conclusions have been drawn by Charvat and Esau (1975) who studied the activity of acid phosphatase at ultrastructural level in xylem of Phaseolus vulgaris. The precise role of acid phosphatase in wall formation is not known, however the studies of Bartnick-Garcia (1973)
have shown that hydrolytic enzymes have an important function during wall formation in fungi. High concentrations of acid phosphatase has also been reported in the conducting tissues of bryophytes by Debant (1971). In *A. hypogaea* xylem, strong acid phosphatase activity is persistant along the secondary walls, it is also possible that this enzyme is associated with partial hydrolysis of the primary walls characteristic of these cells in their late stages of differentiation (O'Brien, 1970). The observed differences in the PAS-stainability of the primary walls in the mature xylem vessel element of *A. hypogaea*, indirectly supports the view that acid phosphatase present in association with the walls is probably involved in the hydrolysis of certain areas of the primary wall. Intense activity of acid phosphatase has been demonstrated in the differentiating tracheary elements by Shaykh and Roberts, (1974). The presence of acid phosphatase in cell walls of higher plants appears to be a common phenomenon (Diall and Sexton, 1974; Dalperu, 1969; Poux, 1970). In *A. hypogaea* acid phosphatase activity was frequently found in the intercellular spaces, the origin of which is obscure. The activity of this enzyme is often found at locations in the plant where massive short-distance solute transport occurs (See. Luttge and Schnepf, 1976 and references therein). Conceivably, in *A. hypogaea* acid-phosphatase plays a role in the short-distance transport of materials between the cortical cells and intercellular spaces, between the symplast and the apoplast of the root. Such a role for acid phosphatase has been
suggested by Uparko and Johnson (1982) for the roots of *Nymphoides peltata*.

The activity of cytochrome oxidase in the prospective xylem vessel elements of *A. hypogaea* is relatively lower than in the surrounding parenchyma cells. However, in the relatively mature xylem vessel elements, the reaction product seems to be associated with the secondary walls. Circumstantially, it can be inferred that this enzyme (cytochrome oxidase) has some role in the process of secondary wall formation, more so because this enzyme is known to be involved in oxidative processes (Zenchenko, 1964). It is highly probable that cytochrome oxidase is involved in the oxidative events during lignification of the xylem vessels, at later stages of differentiation.

...
A gradatory increase in the number of PAS-positive grains in the endodermal cells of *A. hypogaea* towards the proximal end which perhaps reflects the progressive naturation of these cells. These PAS-positive grains have been verified to be starch grains using the IKL test. The presence of starch grains in the endodermal cells has been reported by Van Fleet (1961); Scott and Peterson (1979) and Rhoades and Carvalho (1944). Van Fleet (1961) found that in *Smilax herbacea* the starch grains of the starch sheath are resistant to hydrolysis and furthermore he concluded that these starch grains are covered by a suberized pellicle. In the present study no such Sudan-positive pellicle could be found around the starch grains of the endodermis. Moreover, these starch grains were relatively more PAS-positive than those present in the neighbouring cortical cells.

Several workers, such as Huisinga and Kuiljff (1974); Frey-Wyssling (1959); Ziegenpeck (1921), and Warden (1935) doubt the occurrence of fatty acids in the endodermis owing to negative results obtained with Sudan dyes. However, the lipid (fatty acid) component of the wall areas of the mature endodermal cells in *A. hypogaea* has been demonstrated using Sudan black B. This observation is in agreement with the finding of Scott and Peterson (1979) in *Ranunculus acris* roots. Although the Sudan dyes have long been regarded as the standard histochemical staining reagents to confirm the
presence of lipid (fats), it is quite possible that sudan dyes may be less specific in their reaction properties than was previously suspected.

Lipid identification by means of Sudan black B staining has to be interpreted with caution as suggested by Frederiks (1977) and Fuller et al. (1977). Although the existence of lipid in the suberic layer has been substantiated by a number of researchers (Priestley and North, 1922; Priestley and Radcliffe, 1924; Tippett and O'Brien, 1976; Van Fleet, 1942; Wattendorf, 1974; and Sutherland, 1976), it has proved more difficult to show that suberin also contains an appreciable percentage of phenolic compounds.

The walls of the endodermal cells of *A. hypogaea* in an advanced stage of maturity invariably show predominantly localized acid phosphatase, esterase and peroxidase activity. High acid phosphatase activity has also been found in the endodermal cell of *Hordeum vulgare*, *Zea mays* and *Helianthus annuus* (Shaykh and Roberts, 1974). The increased level of acid phosphatase activity along the wall areas of the endodermal cells at their later stage of maturity, indirectly suggests the possible involvement of this enzyme in the process of suberization, a characteristic feature of these cells. The suberic nature of the wall areas is further reflected by the characteristic birefringence of the wall areas under polarized light. Suberin lamella is also known to be present on both radial and tangential walls of endodermis.
in *Hannouius* (Scott and Peterson, 1979). The Sudan black B positive stainability of the walls of the mature endodermal cells convincingly suggests suberinic nature of the wall areas. Similar stainability of the suberin in the endodermal cells has been found by Scott and Peterson (1979). An interesting feature of the endodermal cells *A. hypogaea* towards their maturity is the intense activity of esterase along the wall areas. It is highly probable that the enzyme is involved in events pertaining to the development of suberized walls. The observed high lipid positive areas in the wall and the activity of esterase perhaps suggests that the enzyme is involved in the process of splitting of fatty acids that may play a role in the synthesis of suberin precursors.

Unlike the activities of acid phosphatase and esterase, the peroxidase activity in the endodermis of *A. hypogaea* was found to occur prior to the development of the birefringent casparian strip or the suberinized wall areas. This finding is in agreement with that of Van Fleet (1947, 1959). In the mature endodermal cells, high peroxidase activity was observed in association with the cell walls. A similar observation has been made by De Jong (1967) in onion roots. The observed high concentration of ascorbic acid and intense peroxidase activity in the mature endodermal cells suggests that a respiratory carrier system may be involved for stelar accumulation and selection of ions as suggested by Van Fleet (1961). It has been proposed that a peroxidative mechanism is responsible for directing the flow of ions across permeability boundaries.
(De Jong, 1966). Because, a suberized wall areas of the mature endodermal cells can form potential permeability barriers, it is hypothesized that the peroxidative mechanism is operative to aid stelar accumulation of ions in the seedling roots of A. hypogaea.

III. THE PITH IN THE 4 DAY OLD SEEDLING ROOT APICES

A pith is present throughout the seedling root of A. hypogaea. Maturity of primary xylem leaves a considerable portion of the central cylinder as typical pith. Badami (1935) confirms this fact indirectly by reference to starch grains and tannin cells in the pith of the root. Prospective

Just above the apical initials, the central pith cells are devoid of PAS-positive starch grains, these cells however have PAS-positive walls. A gradual increase in the PAS-positiveness of the wall areas accompanied by accumulation of starch grains from the proximal towards the distal end perhaps indicates the changing carbohydrate metabolism underlying the process of maturation. The association of PAS-positive starch grains with the recently laid wall areas in the dividing pith cells is considered to be indicative of the utilization of starch grains in cell wall synthesis. At a level where the lateral root primordia begin to differentiate, the pith cells invariably contain large PAS-Positive starch grains. However, at this level, the PAS-positiveness of the
walls is slightly less the significance of which is unclear.
The intercellular spaces in the pith do not contain any PAS-positive material throughout the length of the root.

The young pith cells near the apical initials contain very low lipids whereas higher up where the endodermal cells begin to accumulate starch grains and also at a level where the differentiation of the xylem begins the pith cells invariably contain high concentrations of total lipids. An interesting feature of the pith cells at a level where lateral root primordia are formed, is the depletion of the lipids from the cytoplasm of the pith cells. This, perhaps, indicates the possible utilization of the lipid reserves for the process of lateral root differentiation.

The intercellular spaces in the pith tissue near the apical initials contain significantly high concentrations of ascorbic acid, a feature not observed in any other taxa studied to date. Furthermore, the ascorbic acid, associated with the nuclei of these cells is quite high. Because ascorbic acid is known to play a prominent role in intensified energy transfer mechanism during the process of growth and morphogenesis (Chinoy, et al., 1971), it can be suggested that the ascorbic acid in the intercellular spaces may be involved in mediating energy transfer events at the apical initial zone in the seedling root apices of A. hypogaea. This assumption as reasonable because, the meristematic apical initials (in the
vicinity of the young pith cells) generally are metabolically active.

Further away from the apical initials the ascorbic acid associated with the nuclei of the pith cells is relatively lower, but the ascorbic acid in the intercellular spaces is persistent. Intriguingly, at the level where the lateral root primordia begin to develop, the ascorbic acid in the intercellular spaces of the central pith cells is absent. However, small ascorbic acid reduced silver grains are present around the nuclei of these cells. It is highly probable that the ascorbic acid of the intercellular spaces is utilized for the process of lateral root differentiation.

The distributional pattern of total proteins is similar to that of RNA, in general. The young pith cells near the apical initials contain moderately high amount of proteins. The dividing cells of the pith clearly manifest the phenomenon of polarity and that these divisions are generally amitotic. The product cells are usually unequal in size and furthermore the nuclear stainability, for proteins is also differential. The newly laid wall areas of the recently divided pith cells consistently shows positive stainability for proteins. Further up at a level where the protophloem cells differentiate, the cytoplasmic protein concentration in the pith cells is significantly low. However, the nucleoli in these cells show moderately high protein content. At the proximal end away from the apical initials where the lateral root primordia develop, the pith cells contain very low
cytoplasmic proteins. In general, from the distal towards the proximal end a gradual decline in the ascorbic acid and proteins is noticed in the central pith cells which reflects the gradual decline in the metabolic activities in these cells.


Hurst, Fannie Mae. 1954. The vegetative anatomy of the genus Smilax with particular reference to the endodermis. Diss. Abst. 16(1); 14-15. Purdue Univ.


