THE FINE STRUCTURE OF DIFFERENTIATING XYLEM ELEMENTS

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ABSTRACT

Differentiating xylem elements of *Avena* coleoptiles have been examined by light and electron microscopy. Fixation in 2 per cent phosphate-buffered osmium tetroxide and in 6 per cent glutaraldehyde, followed by 2 per cent osmium tetroxide, revealed details of the cell wall and cytoplasmic fine structure. The localized secondary wall thickening identified the xylem elements and indicated their state of differentiation. These differentiating xylem elements have dense cytoplasmic contents in which the dictyosomes and elements of rough endoplasmic reticulum are especially numerous. Vesicles are associated with the dictyosomes and are found throughout the cytoplasm. In many cases, these vesicles have electron-opaque contents. "Microtubules" are abundant in the peripheral cytoplasm and are always associated with the secondary wall thickenings. These microtubules are oriented in a direction parallel to the microfibrillar direction of the thickenings. Other tubules are frequently found between the cell wall and the plasma membrane. Our results support the view that the morphological association of the "microtubules" with developing cell wall thickenings may have a functional significance, especially with respect to the orientation of the microfibrils. Dictyosomes and endoplasmic reticulum may have a function in some way connected with the synthetic mechanism of cell wall deposition.

INTRODUCTION

Since Crüger's (1855) original descriptions of cells of the velamen of orchid aerial roots, there have been several accounts of the relationship between the deposition of the secondary wall thickenings and cytoplasmic structure. Dippel in 1868 published descriptions and drawings showing that the direction of the thickenings in xylem vessels of *Impatiens* corresponded with that of protoplasmic streaming and was correlated with the larger refractive index of cellulose (*i.e.*, spirally). Schmitz (1880), working with orchid roots, and Strasburger (1882), using *Bryonia*, *Impatiens*, and *Sphagnum*, made similar observations. Barkley (1927) and Sinnott and Bloch (1945) using fixed material have described, in differentiating xylem elements, bands of dense cytoplasm in regions which later develop secondary wall thickenings. More recently, the system used by Sinnott and Bloch of redifferentiation of the parenchyma cells of wounded *Coleus* stems has been investigated at the fine structure level by Hepler and Newcomb (1963, 1964). They have described a concentration of organelles and vesicles in the cytoplasmic band within which the secondary wall thickenings are formed. Observations on *Cucurbita* xylem elements have been made by Esau, Cheadle, and Risley (1963), who described a change in the form of the endoplasmic reticulum from a cisternoid to a vesicular type and degenerative changes in the cytoplasmic components. Arrigoni and Rossi (1962) have described an abundance of protoplasmic organelles of every type and a large number of unidentified bodies and organelles in the differentiating elements from *Avena* coleop-

tiles. Porter and Machado (1960 *a*) and Porter (1961) described residual elements of the endoplasmic reticulum near the cell surface which were said to have a relation to the annular thickenings of the wall.

The arrangement of the cellulose microfibrils in the wall of differentiating xylem vessels is well established and it should, therefore, be possible with the use of modern techniques to relate the changes in cell wall structure during differentiation to the organization of the cytoplasm. The present study has been made on differentiating xylem cells of *Avena* coleoptiles which have been fixed and sectioned for observation by light and electron microscopy. The fine structural changes in the cytoplasm of these cells are described with respect to specialized differentiation of the xylem elements and the development of the thickened cell walls. Our observations suggest that, contrary to some of the above mentioned results, neither mitochondria, plastids, nor endoplasmic reticulum show any special orientation indicative of the pattern of cell wall deposition. Structural features of the cytoplasm do exist, however, which indicate that specific cytoplasmic components are functionally involved in cell wall deposition and orientation.

MATERIALS AND METHODS

Seeds of *Avena sativa* var. Victory were dehusked and germinated in 4-inch petri dishes on filter paper moistened with distilled water. They were grown at 25°C either in total darkness or in darkness after illumination for the first 12 hours with red light. Coleoptiles of different lengths were harvested at various times after germination. For light and electron microscopy segments of the coleoptiles were cut and fixed, either in phosphate-buffered 2 per cent osmium tetroxide for 24 hours, or for 2 hours in 6 per cent glutaraldehyde with a 3-hour wash in buffer, before a second fixation in phosphate-buffered osmium tetroxide for 12 hours at 4°C. The segments were dehydrated in a series of ethyl alcohol or acetone solutions and embedded in Epon or a 4:1 mixture of butyl and methyl methacrylate. For light microscopy sections were cut at 4 to 6 microns, using a Spencer microtome and a steel knife. For electron microscopy sections were cut on a Porter-Blum ultramicrotome with a diamond knife. Sections were stained either in Millonig's (1961) lead stain for 5 minutes or saturated uranyl acetate for several hours, and then viewed and photographed with a J.E.M. 6C electron microscope.

RESULTS

Cell Wall Development in the Differentiating Xylem Elements

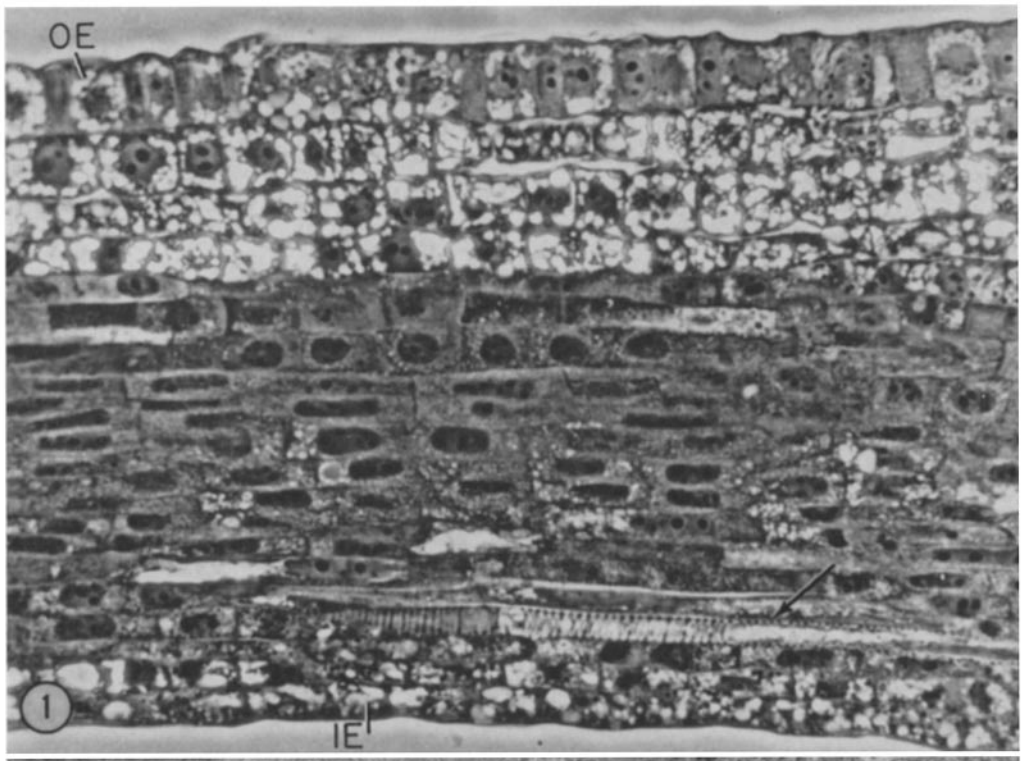
Observations in the light microscope have shown that fully differentiated xylem elements may be formed when coleoptiles are only 2 mm long (Fig. 1). In the electron microscope these xylem elements appear to be surrounded by a primary wall,

Key to Labeling

D, Dictyosome
ER, Endoplasmic reticulum
IE, Inner epidermis
M, Mitochondria
Mi, Microtubule
N, Nucleus
OE, Outer epidermis
P, Plastid
Pd, Plasmodesmata
PM, Plasma membrane
PW, Primary wall
T, Secondary wall thickening
TU, Tubules
V, Vacuole

FIGURE 1 Light micrograph of a longitudinal section of a 2-mm coleoptile. The section is through the differentiating vascular bundle, which consists of both mature and differentiating xylem and phloem elements. Elongate nuclei in the differentiating cells have several nucleoli. Adjacent parenchymal and epidermal cells have well developed vacuoles at this stage. Arrow, mature xylem element. × 430.

FIGURE 2 Electron micrograph of a longitudinal section through the differentiating region of a 1-cm coleoptile. A mature element with annular thickenings is adjacent to elongated cells which one would expect to differentiate into xylem elements. Note absence of any preferred orientation of organelles. Osmium tetroxide fixation. × 7,500.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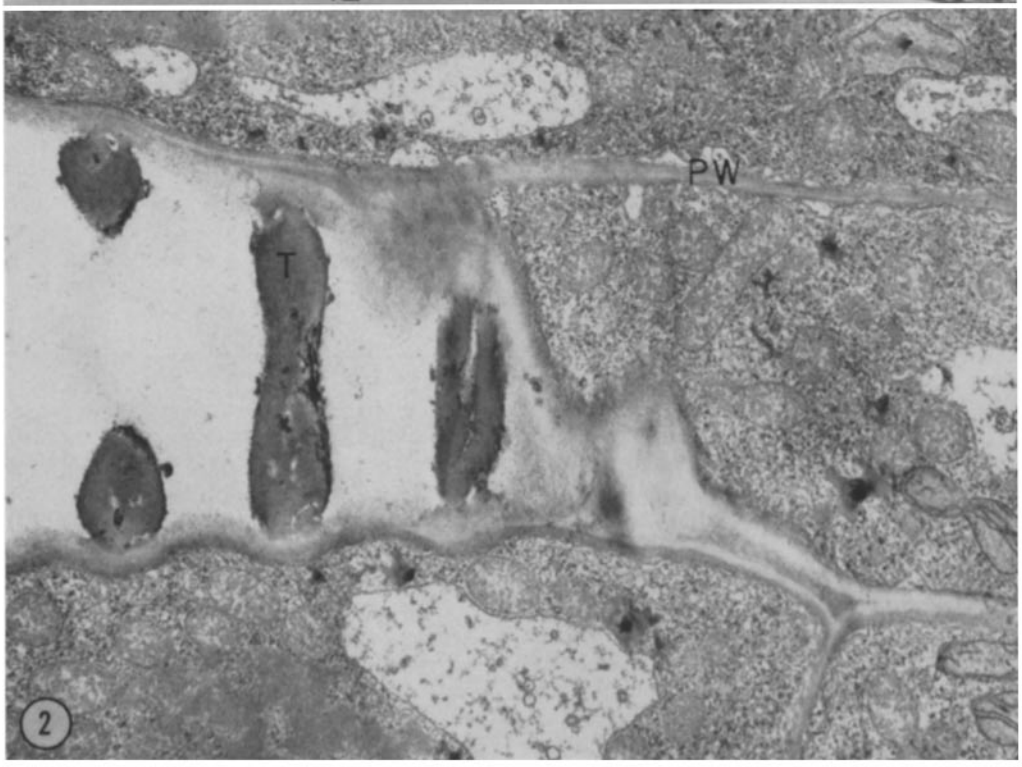
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with the microfibrillar direction clearly visible in most preparations (Figs. 3 to 5). The primary wall thins out as the cells elongate, until in extreme cases it is only a few microfibrils thick (Figs. 4 and 5). The massive secondary thickenings are deposited on the inside of the primary wall (Fig. 2). In the sections examined, cells showing stages of thickening development were less frequently encountered than either cells devoid of thickenings, or mature cells. Thus it may be concluded that the deposition is very rapid. The microfibrils run parallel to the direction of the thickenings (Figs. 3 and 9) and within the thickenings are arranged in concentric lamellae (Fig. 7) (Hepton *et al.*, 1956). In some cells there appears to be a groove along the edge of the thickenings. In cross-sections of the thickenings this groove gives a double-lobed appearance (Fig. 7), and in longitudinal sections the groove can be observed running along their innermost edge (Fig. 7). At their place of attachment to the primary wall the thickenings have a strip of wall material which appears to have the same electron density and packing of microfibrils as the thickenings themselves (Fig. 4). As the elements elongate and the primary wall thins, this basal strip remains attached to the thickenings (Fig. 4).

At an early stage of development, before secondary wall formation, both the side and end walls have numerous plasmodesmata which may occur in simple strands, or which may be grouped together in thinner areas of the primary wall. The secondary thickenings may be deposited over an area of plasmodesmata (Fig. 14). The cellulose thickenings appear to have reached their full dimension before any degeneration of the cytoplasm occurs (Fig. 13). In some cells, the thickenings tend to be electron opaque with a banded appearance (Fig. 3), and this appearance is usually most prominent when the cytoplasm is degenerating. This banded appearance is probably due to a fixation and staining of the enzyme system concerned with lignification, or to a staining of lignin itself which has already been deposited.

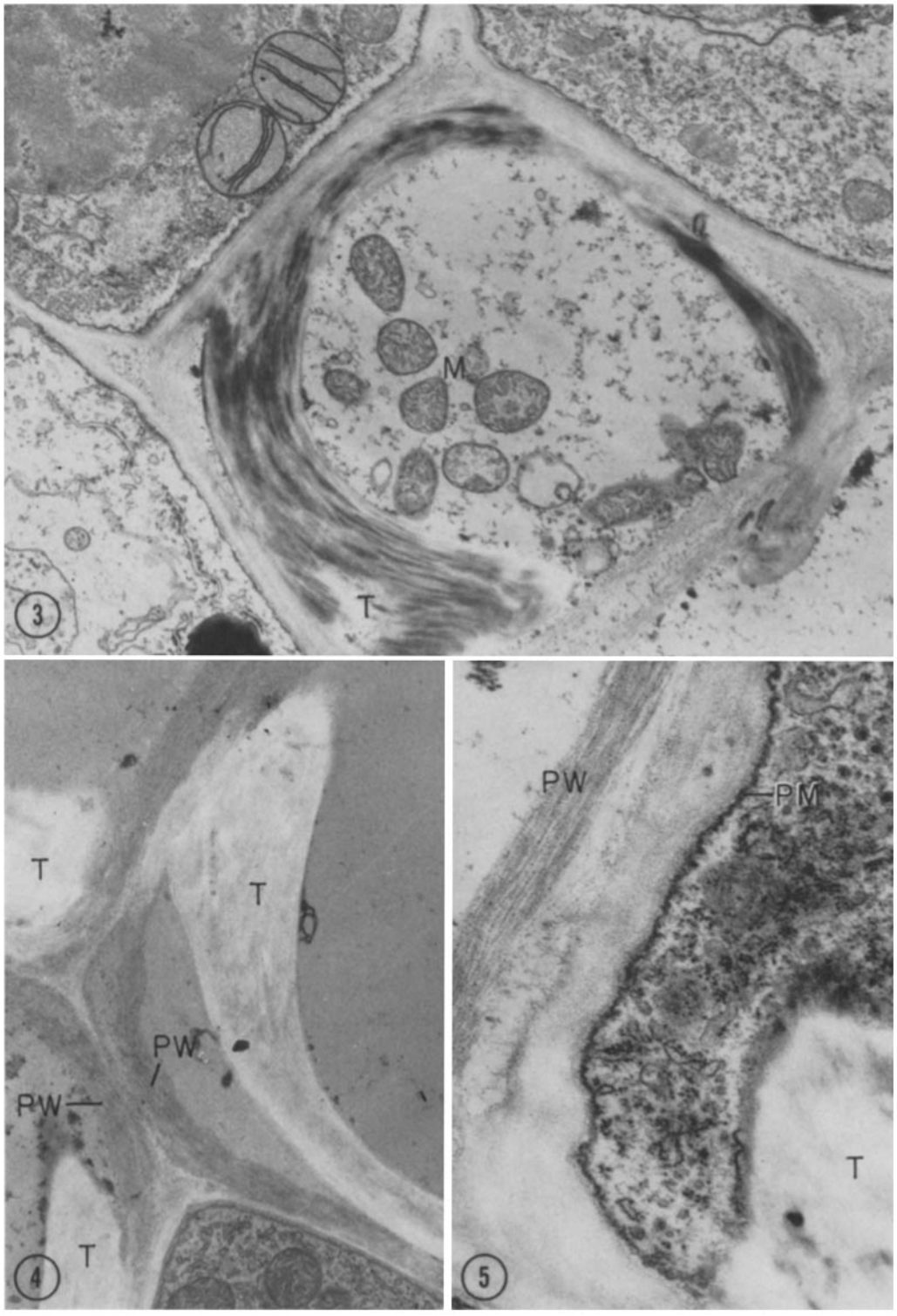
Organization of the Cytoplasm at the Time of Secondary Wall Deposition

The prominent wall thickenings mark the developing xylem elements. In a cross-section of the xylem region of a differentiating vascular strand, the majority of the cells are either surrounded by only a primary wall or have fully developed wall thickenings. Some cells, however, are in the process of depositing the localized wall thickenings, and the stage of cellular differentiation was judged by the degree of elaboration of the secondary wall thickenings. The vascular strands of the coleoptiles are very prominent following our fixation procedure, reflecting the dense cytoplasmic content of the cells which are more elongate and smaller in diameter than the cells of the surrounding parenchymatous tissue (Fig. 1). The prominent nuclei elongate as the cells enlarge (Fig. 1), and maintain this elongated form throughout the subsequent stages of development. In cross-section, nuclei often have a lobed appearance (Fig. 6). The nuclei are surrounded by the usual perforated double membrane (Fig. 6) which is continuous with portions of the endoplasmic reticulum. The nuclear pores seem to be less numerous in the differentiating xylem elements than in the surrounding parenchymal cells. The nucleoli appear to increase in number as the cells differentiate (Fig. 1).

FIGURE 3 Transverse section through an element with mature cell wall thickening from a 1-cm coleoptile. The thickening has a banded appearance consisting of electron-opaque and electron-transparent regions which are extended in the direction of the microfibrils. The cell lumen contains mitochondria. Osmium tetroxide fixation. × 12,000.

FIGURE 4 Transverse section through xylem elements of a 1-cm coleoptile. The primary wall is easily distinguished from the secondary thickenings. The direction of microfibrils is evident in the primary wall except at the more compact area where the thickening is attached. Osmium tetroxide fixation. × 12,000.

FIGURE 5 Transverse section through xylem cells of a 1-cm coleoptile which shows a mature element with its extended primary wall adjacent to a differentiating element. The plasma membrane of the differentiating element has a thickened appearance. Osmium tetroxide fixation. × 27,000.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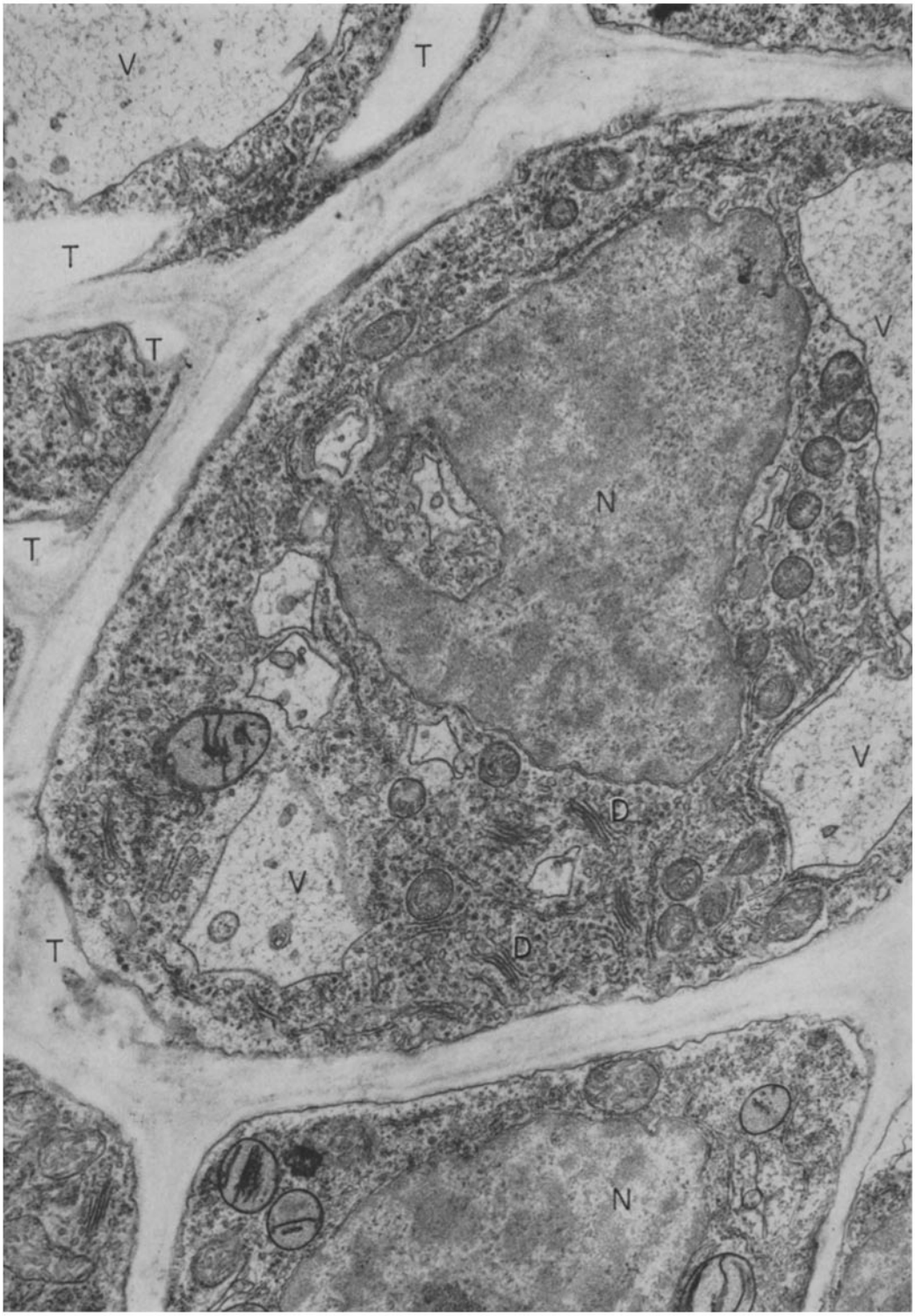


FIGURE 6 Transverse section through an element from a 1-cm coleoptile in which a band of wall thickenings is starting to form. Numerous dictyosomes, vesicles, and portions of ER are evident. At this stage, there are several small vacuoles. Osmium tetroxide fixation. × 11,000.

In some tissues, it has been suggested (Simon and Chapman, 1961) that mitochondria are structurally changed during cell differentiation and that the organization of the mitochondria depends upon the physiological state of the cells. However, at all stages of xylem development in *Avena* the mitochondria are similar in structure and even when the rest of the cytoplasm has disintegrated, intact mitochondria may be seen in the cell lumen (Fig. 3).

Plastids are present in most differentiating xylem elements and in the surrounding parenchymal cells (Figs. 6 and 9). The membrane system of the plastids stains more intensely than the membrane system of other organelles (see also Bouck, 1963). They are surrounded by the usual double membrane, and internally they show various substructures. A type of prolamellar body with an aggregation of tubules (Fig. 6) may be present, or there may be groups of three or four parallel lamellae (Fig. 9). In the parenchymal cells the plastids usually contain numerous starch grains, whereas in the xylem elements starch is usually absent. This may well reflect a different carbohydrate metabolism in the two cell types.

There are many long elements of rough ER in the differentiating xylem elements on which the ribosomes are usually arranged in groups (Figs. 6 and 9). Smooth ER is also well developed and consists of numerous anastomosing tubular elements which, at places, may be seen to be continuous with the long elements of the rough ER. At no stage does there seem to be a preferred orientation of the ER, either with respect to the primary wall structure or the secondary wall thickenings.

Dictyosomes are prominent in the differentiating xylem cells and in the parenchymal cells (Fig. 6). The dictyosomes consist of flattened discs, (usually about five) and associated vesicles. Two types of vesicles were observed, some with electron-opaque contents, and some with no apparent contents. The vesicles apparently free of contents are more numerous. Similar vesicles, not associated with the dictyosomes, are observed throughout the cytoplasm (Figs. 6 and 9), but the numerical ratio is changed. In the cytoplasm, vesicles with electron-opaque contents are at least as numerous as those without contents. These two kinds of vesicles appear to be a consistent and characteristic property of the differentiating xylem cells.

Ribosomes in the differentiating xylem elements are single, or are arranged in groups of five or more either along the elements of the rough endoplasmic reticulum or throughout the ground cytoplasm. The ribosomes in groups are often arranged as spirals of a fairly constant size (polysomes?). This arrangement in intact plant cells has also been demonstrated by Ledbetter (see Porter 1961) and by H. Falk (1962).

In the outer cortex of all the cells examined following glutaraldehyde fixation may be found microtubules similar to those described by Ledbetter and Porter (1963). These microtubules usually run parallel to the direction of the cellulose microfibrils in the most recently deposited region of the cell wall. A cross-section of a xylem element, before secondary wall deposition, with long microtubules parallel to the primary cell wall is shown in Fig. 11. The newly deposited microfibrils would be transversally oriented in this cell and hence parallel to the microtubules. Fig. 10 shows a cross-section of a differentiating xylem element in which thickenings are developing. In places where the section becomes a glancing section of the outer region of the thickening there can be seen a group of microtubules oriented in the same direction as the microfibrils in the developing wall thickening. Thus, it seems that the region of the cytoplasm adjacent to the thickenings contains a number of microtubules all oriented parallel to the thickenings. Long sections of microtubules are also seen parallel to the edge of the thickenings when they are cut in a direction parallel to the microfibrils. This association between the microtubules and the thickenings has been observed in all the differentiating xylem elements examined following glutaraldehyde fixation.

Early in development, the xylem cells may possess a component limited by a single membrane containing a structure with regularly spaced densely staining bands (Cronshaw, 1964). These bodies have an ordered three-dimensional structure, with spacing between the layers of approximately 160 A. They are abundant in the surrounding parenchymal cells and are similar to the structures described by Thornton and Thimann (1964). Lipid droplets, which are common in the surrounding parenchyma cells, are rarely seen in the differentiating xylem elements.

Young xylem cells have several small vacuoles which expand as the cell differentiates and eventually may fuse to form a large vacuole (Fig. 6).

This fusion usually occurs at a late stage in the development of the xylem elements. The vacuolar membrane may sometimes be resolved as a triple-layered structure, and in this case the two dense layers are of equal thickness (Fig. 11). Following the deposition of the secondary wall, degeneration of the cytoplasm starts with a breakdown of the vacuolar membrane. This is followed by a general degeneration of the cell components. The last components to persist are the cisternae of the rough endoplasmic reticulum and the mitochondria (Fig. 14). In mature elements, structurally intact mitochondria are often observed in an otherwise empty cell lumen (Fig. 3). Eventually these, too, disappear (Fig. 2).

The Cell Wall–Cytoplasm Interface

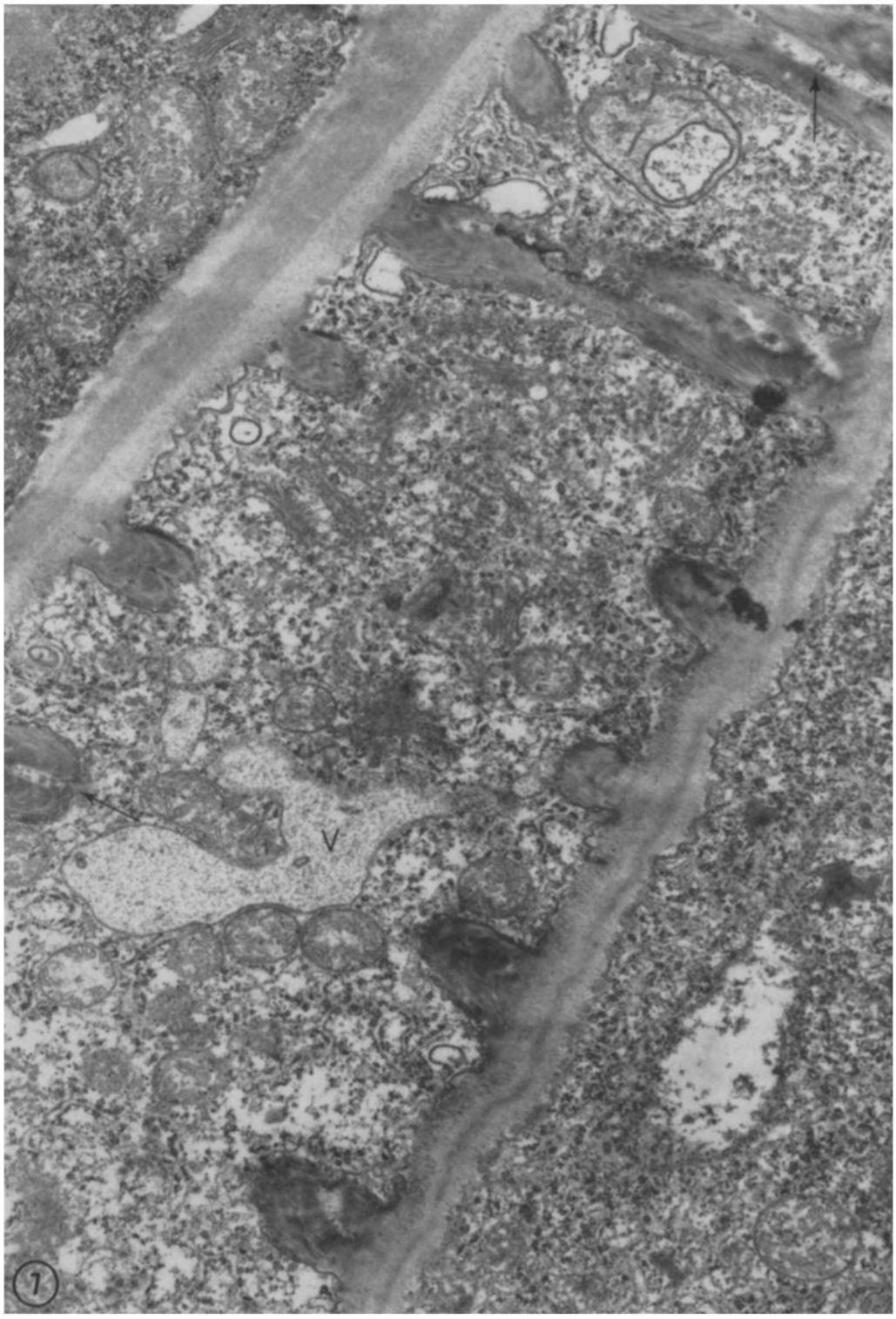
The plasma membrane may be resolved as the usual triple-layered structure, with two outer dense layers and a less dense central region. The dense layer adjacent to the cell wall is usually thicker than the inner dense layer (Fig. 8). The outer part of this membrane is very closely applied to the cell wall and is continuous from cell to cell through the wall at the region of the plasmodesmata. In cross-section the tubular nature of the plasmodesmata can be seen. The central area always contains an osmium-staining core with a less dense region between this core and the plasma membrane. In differentiating xylem elements, thicker areas of the plasma membrane may be found adjacent to the secondary thickenings or the primary wall (Fig. 5). A thickened structure of the plasma membrane has also been observed in differentiating phloem elements of pea (Bouck and Cronshaw 1965). However, at the present time it is not clear whether this structure is a real difference from the normal membrane or perhaps a fixation artifact. The cell wall–cytoplasmic interface at the area in which spiral or annular thickenings are being laid down shows no readily apparent difference from the cell wall–cytoplasmic interface at other regions of the wall.

There are numerous inclusions at the cell wall–cytoplasmic interface within any one cell. The most common of these is a group of tubules between the plasma membrane and the wall. These tubules may be seen in Fig. 12, in which the cell is slightly plasmolyzed. These tubules are of dimensions (*ca.* 200 A) similar to the microtubules seen in the peripheral cytoplasm. They often appear to branch, although it is difficult to distinguish true branching from a supposition of two tubules.

DISCUSSION

The present study attempts to show the organization of the cytoplasm in differentiating xylem elements of *Avena* coleoptiles at the time when the complex secondary wall is being deposited. The organization of the cytoplasm into localized bands, presumably rich in organelles (Crüger, 1855; Dippel, 1868; Schmitz, 1880; Strasburger, 1882; Sinnott and Bloch, 1945; Hepler and Newcomb, 1963), has not been verified in our material. The association of cytoplasmic bands and the site of secondary wall deposition have been illustrated best in fixed, dehydrated material (Sinnott and Bloch, 1945; Hepler and Newcomb, 1963), and in this respect our experimental procedures are certainly comparable. Yet in none of our preparations could an association of cytoplasmic accumulations or organelles with the developing thickenings be established. In files of differentiating cells, cells with well preserved cytoplasmic contents can be seen adjacent to mature xylem cells. The cells which presumably were destined to differentiate as xylem elements, as well as differentiating elements, showed no evidence of a spatial arrangement of organelles related to secondary wall deposition. Earlier workers have shown an association between cytoplasmic streams and secondary well deposition, and streaming has been cited as a possible mechanism for wall orientation. Our results suggest that there are no cytoplasmic accumulations at the regions of localized wall thickenings and that an organized streaming pattern which predetermines wall orientation is unlikely. In support of this view, many cases

FIGURE 7 Longitudinal section through a differentiating xylem element from a 1-cm coleoptile. In this oblique section, some of the secondary wall thickenings are cut in cross-section and some, where the section approaches a side wall, in longitudinal section. The groove along the thickenings is shown in nearly all cases (arrows). There is no apparent organization of the cytoplasmic components in the region of the developing thickenings. Osmium tetroxide fixation. × 18,000.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are known in which there is a marked difference between the direction of cytoplasmic streaming and orientation of the microfibrillar component of the walls as, for instance, in *Tradescantia* hairs and *Nitella* internode cells (Green, 1960; Probine and Preston, 1958). In *Nitella* and in other plant cells it is also now recognized that the outer layer of the cytoplasm is stationary, though the cells may exhibit marked cytoplasmic streaming. At the present time we have been unable to observe cytoplasmic streaming in the differentiating xylem elements of *Avena*.

In spite of the fact that there are apparently no cytoplasmic accumulations in which the secondary wall develops, it seems reasonable to assume that the development of the complex oriented wall system can be correlated with cytoplasmic components. There are several components which may be involved in this wall deposition. In the differentiating elements dictyosomes are very numerous. It has been suggested that dictyosomes play a role in secretion in other types of plant cells (Bouck, 1962; Bonneville and Voeller, 1963) and that they have a possible role in cell wall formation. Many observations have been made on the development of the cell plate during cell division in root meristems. Mollenhauer and Whaley (1962), Whaley *et al.* (1962), and Mollenhauer and Whaley (1963) have discussed the possibility of the dictyosomes having a secretory function in plant cells, and these authors have also described the fusion of secretory vesicles from the dictyosomes to form the cell plate (Whaley and Mollenhauer, 1963). In the light of these observations, it is possible that the role of the dictyosomes in the developing xylem elements is one of synthesis of substances concerned with wall formation. The matrix material that is deposited in the wall may, as has been suggested (Mollenhauer and Whaley, 1962), be formed by the dictyosomes in the form of vesicles which are then carried to the wall. Furthermore, in our material the vesicles containing electron-opaque bodies are found only in the differentiating xylem cells and arise apparently from the dictyosomes. It appears that the dictyosomes give rise to vesicles of both types, although the vesicles with contents are not so closely associated with the dictyosomes as those without contents. Another explanation is that the densely staining contents are formed after the vesicles leave the dictyosomes.

The ER is a further possible site of synthesis of precursors of wall polysaccharides or the enzyme system capable of producing them. It is well known that the ER may have a synthetic function (*e.g.*, Porter, 1961) and in the differentiating xylem elements there is a well developed system of ER. Porter and Machado (1960 *b*) and Whaley *et al.* (1960) have shown that the tubules of the ER are in close proximity to the developing cell plates of root meristems. Porter and Machado (1960) and Porter (1961) observed residual elements of ER near the wall of a developing xylem cell which they suggest are related to the developing annular thickenings. However, no association of ER with localized wall formation has been observed in the *Avena* xylem elements.

Two main factors that we have to consider are the synthesis of the enzyme system producing the cell wall constituents and the synthesis, deposition, and orientation of these constituents in the wall. Colvin *et al.* (1957) have shown that in *Acetobacter xylinum* the cells are capable of producing an enzyme system and precursors which move outside the cell and synthesize cellulose microfibrils. It seems unlikely that in differentiating xylem elements complete synthesis of the cell wall constituents takes place on the inner side of the plasma membrane. It is difficult to conceive how cellulose microfibrils could be formed inside the plasma membrane and then move out through it and become oriented in the developing wall. It seems more likely that the precursors of the wall substances are formed within the cytoplasm, carried to the plasma membrane, and transported through

FIGURE 8 Transverse section through a xylem element from a 1-cm coleoptile. The plasma membrane is triple-layered (arrow) with a more electron-opaque layer adjacent to the wall. Osmium tetroxide fixation. × 100,000.

FIGURE 9 Micrograph of a cross-section through a differentiating xylem element with a well developed wall thickening. Coleoptile length 1 cm. The primary wall and the wall thickening of an adjacent element are visible. There are numerous vesicles with electron-opaque contents (arrow). Osmium tetroxide fixation. × 48,000.


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it. Synthesis of the large macromolecules and their orientation would then be completed outside the plasma membrane. Thus, it is of interest that in some cells the plasma membrane has a thickened appearance, and this fact suggests that the enzymes associated with the final synthesis of the cell wall macromolecules and their orientation reside in its outer surface.

Ledbetter and Porter (1963) have described a system of microtubules in plant cells which they state could be either directly responsible for organization of the cell wall or responsible through through the mechanism of cytoplasmic streaming. The morphological association of these tubules with the developing cell walls provides us with circumstantial evidence that they may be functionally involved in wall deposition. Hepler and Newcomb (1964) have described, in redifferentiating parenchyma cells of *Coleus*, a system of microtubules and fibrils associated with thickened bands of wall material. The tubules are oriented in the same direction as the cellulose microfibrils of the developing thickenings. The fibrils are confined within cisternae of the endoplasmic reticulum and are oriented at right angles to the microfibrillar direction of the developing wall. In the *Avena* xylem elements, microtubules are very abundant in the outer cortex of cells fixed in glutaraldehyde, although fibrils have not been observed. The close association of these microtubules with developing wall thickenings and the general orientation of the microtubules in the same direction as that of the cellulose microfibrils of the developing thickenings lead us to the conclusion that they are functionally significant in wall deposition. Thus, we favor the opinion of Ledbetter and Porter that the system of microtubules in plant cells in some way contributes to the orientation of the cellulose framework of the cell wall. If the microtubules are functional in cell wall deposition or orientation, the fact that they are sometimes found outside the plasma membrane supports the view that the final elaboration of wall materials takes place in this region. An alternative site for wall deposition is in the outer cortex of the cytoplasm, with a consequent shedding of this layer into the wall and the formation of a new plasma membrane. This idea was put forward by Myers *et al.* (1956) on the evidence of electron-opaque layers in the cell wall which, they suggested, were regions of cytoplasm which had deposited wall materials, and had subsequently been incorporated into the wall. We have observed no evidence of such incorporation in the differentiating xylem elements of *Avena*. The clear area just inside the plasma membrane observed in our osmium tetroxide–fixed material, however, does leave open the possibility that a new plasma membrane is about to be deposited and a region of cytoplasm incorporated into the wall.

Frei and Preston (1961) have postulated that an oriented cytoplasmic component on the inner wall surface of *Chaetomorpha* may be responsible for the orientation of the deposited wall. This component may be similar in structure to the tubules we have described which occur between the plasma membrane and the cell wall, although in *Avena* these tubules are not well oriented. If this proves to be the case, these structures would have added significance in any consideration of cell wall deposition and orientation.

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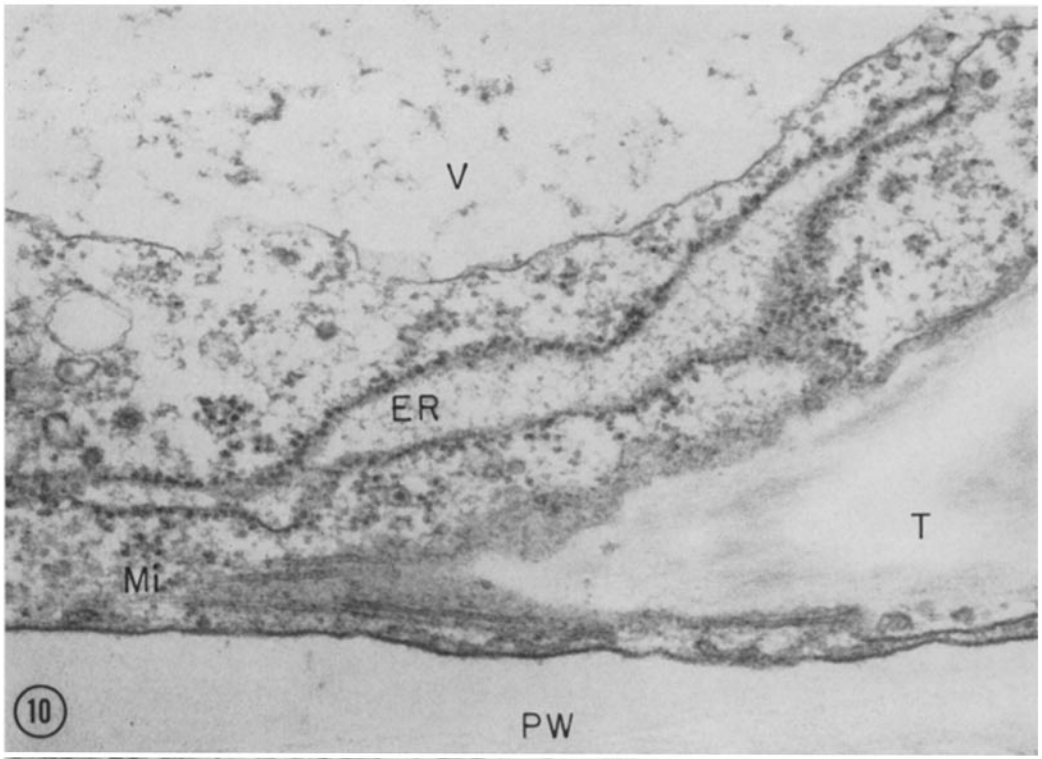
Note Added in Proof: In agreement with our results, Wooding and Northcote (1964) have recently published micrographs which show an association of

FIGURE 10 Transverse section of a developing xylem element from a 1-cm coleoptile after glutaraldehyde–osmium tetroxide fixation. The section is grazing with respect to the thickening. Numerous "microtubules" (*Mi*) are oriented in a direction parallel to that of the microfibrils in the developing wall thickening (*T*). × 45,000.

FIGURE 11 Transverse section of an extending xylem element (?) before deposition of secondary wall. Long microtubules are oriented parallel to the plasma membrane. Glutaraldehyde–osmium tetroxide fixation. × 65,000.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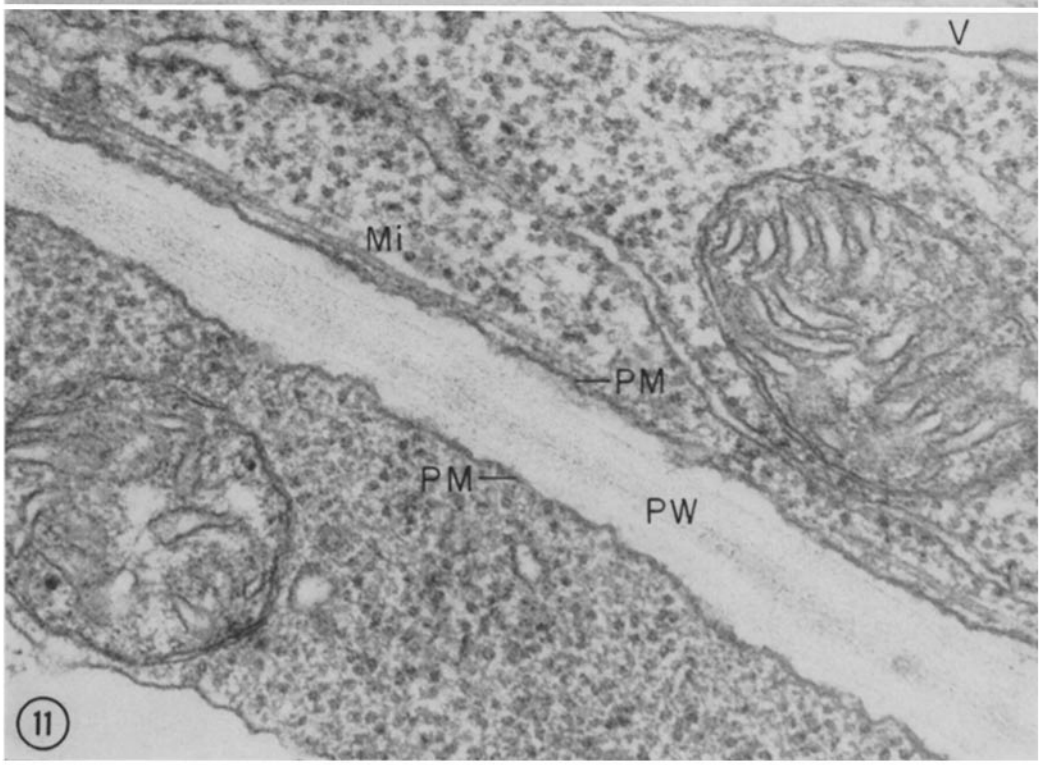
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microtubules and localized wall thickenings in differentiating xylem elements of *Acer pseudoplatanus*. A proliferation of Golgi bodies and vesicles during differentiation was also illustrated, and they suggest that the material of the developing thickenings stems from direct apposition of the material in the Golgi vesicles.

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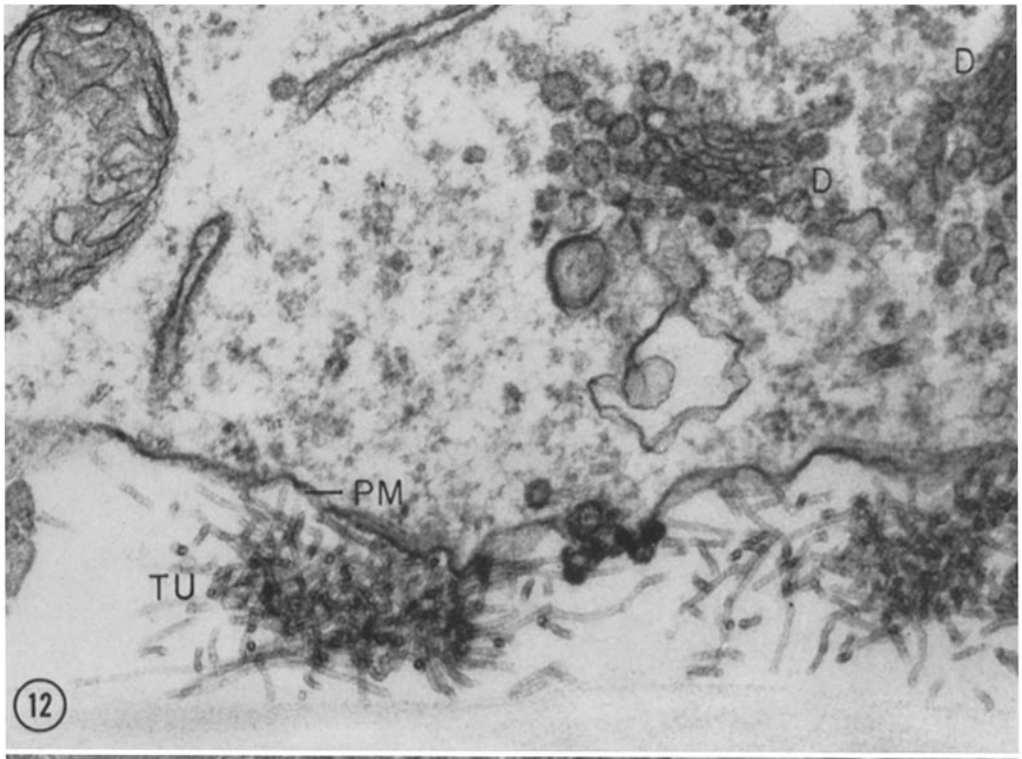
FIGURE 12 Transverse section through a differentiating xylem element from a 1-cm coleoptile. The plasma membrane has been pulled away from the cell wall, and the area between shows numerous "tubules." Glutaraldehyde–osmium tetroxide fixation. × 50,000.

FIGURE 13 Transverse section through differentiating xylem elements of a 1-cm coleoptile. The secondary wall thickening has probably reached its mature dimensions. The cytoplasmic components at this stage are still intact. Osmium tetroxide fixation. × 18,000.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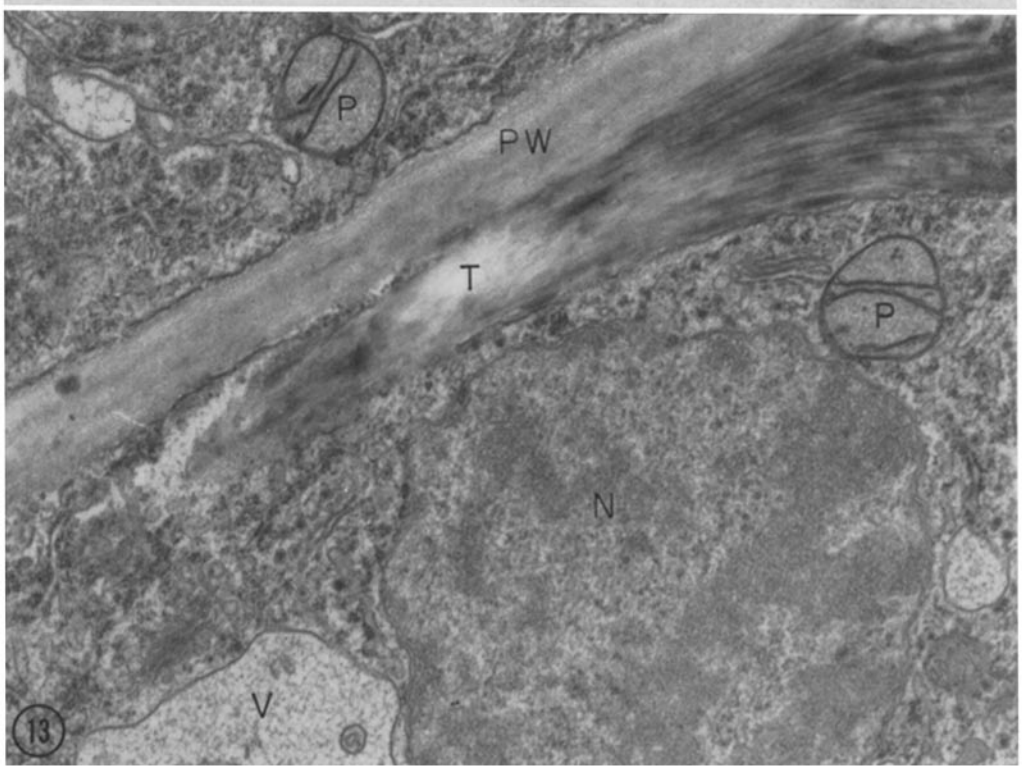
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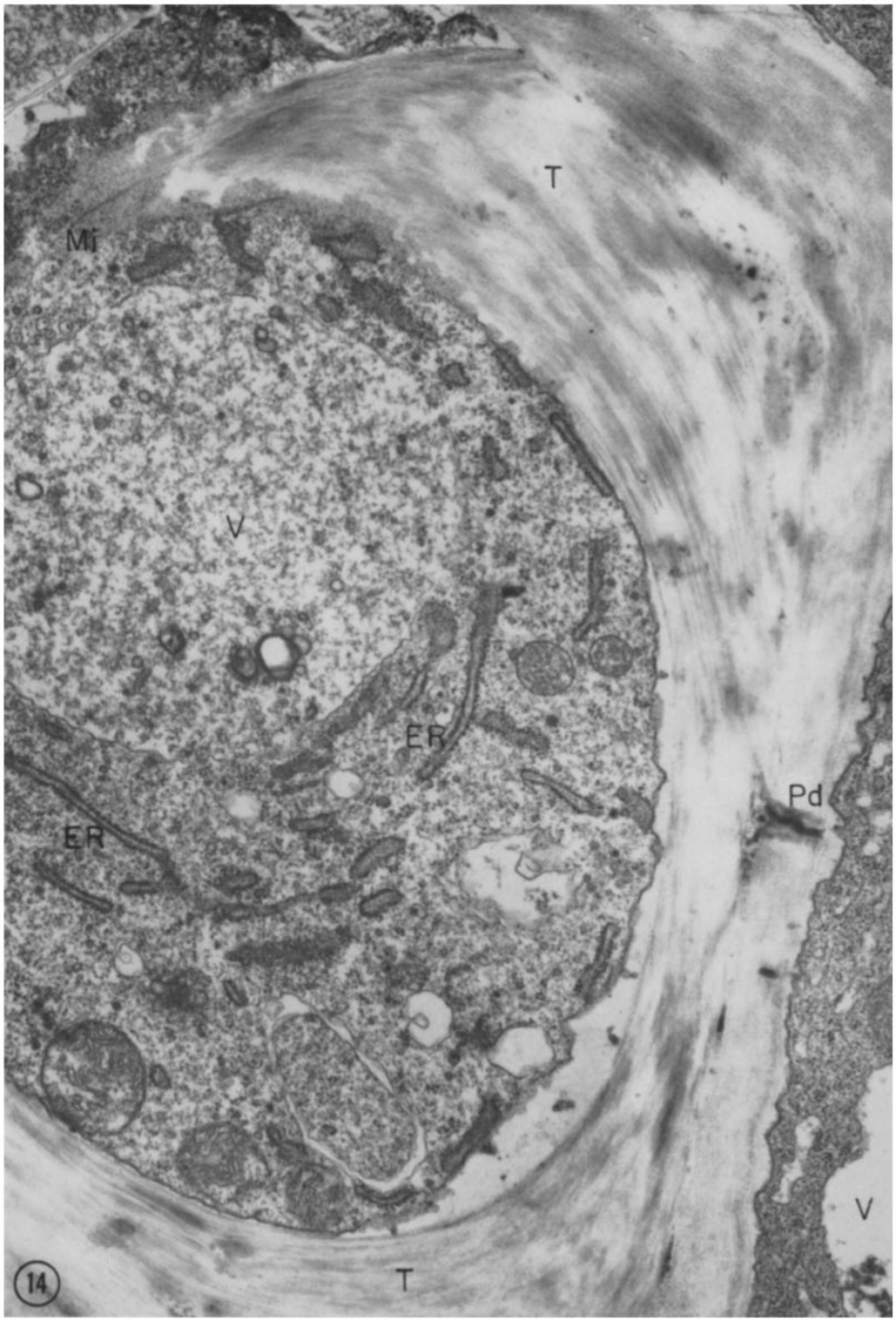

FIGURE 14 Transverse section of a xylem element from a 2-cm coleoptile which has a mature wall thickening. Cytoplasm at this stage shows signs of degeneration. Glutaraldehyde–osmium tetroxide fixation. × 28,000.

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