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THE RELATIONSHIP BETWEEN CELL ORGANELLES AND CELL WALL THICKENINGS IN PRIMARY TRACHEARY ELEMENTS OF THE CUCUMBER I. MORPHOLOGICAL ASPECTS*

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SUMMARY

During the formation of cell wall thickenings in primary tracheary elements of the hypocotyl of cucumber, the morphology or topography of cell organelles was studied in relation to the size of the developing thickenings.

In the initial stage, endoplasmic reticulum is smooth and vesicular, whereas afterwards it is rough and cisternal. The diameter and the electron density of the contents of Golgi vesicles in the successive stages differ. In the initial stage, cortical microtubules are present along the whole plasmalemma; in the following stages they occur only around the thickenings; and, in the final stage, they are again present along the whole plasmalemma. Lomasomes and cytoplasmic fibrils are present only in the beginning of thickening formation.

The changing morphology of the endoplasmic reticulum in the different stages is related to different functions in thickening formation. The smooth vesicular form buds off vesicles which are probably added to the lomasomes. The rough cisternal form synthesizes proteins (enzymes). In addition, it seems that it transports substances and conducts vesicles. The changing morphology of Golgi vesicles is probably related to varying amounts and nature of substances in the vesicles in various stages. The orientation of microtubules and the distance between them indicate that microtubules form a barrier against Golgi vesicles. Lomasomes probably deposit substances during the initial stage in the developing thickenings. There are no indications that cytoplasmic fibrils are involved in thickening formation.

1. INTRODUCTION

During the ontogeny of a primary tracheary element, cell wall material is deposited by the protoplast only at certain places against the primary cell wall. These depositions form the cell wall thickenings characteristic of the mature xylem cells. In the xylem of the cucumber (*Cucumis* 'Sporu-Origineel') hypocotyl these thickenings have a ring- or spiral-like shape. Thickenings in adjacent tracheary elements are deposited directly opposite to one another across the common primary cell wall.

These cell wall thickenings are nearly always referred to as secondary. In some cases this is based on the structure of the thickenings; in other instances it is based on their ontogeny. Neither criterion, however, is fully satisfactory. The first, a structural criterion, refers to the orientation of the cellulose micro-

* Dedicated to Professor Karstens on the occasion of his retirement.

fibrils which are parallel to each other and at a right angle to the cell axis. Using this criterion, however, the thickening itself gradually changes from primary to secondary. During the formation of the thickening, various cell wall substances and cellulose precursors must be deposited before cellulose microfibrils are formed. The second criterion is not satisfactory because the thickenings are formed before the surface growth of the primary cell wall is completed. In the present paper, therefore, the term "cell wall thickening" is used without further qualification.

The difficulties in classifying the nature of the thickenings in xylem tracheary elements have also been discussed by O'BRIEN (1972). He assumed that, in tracheary elements, the thickenings are secondary walls formed in zones of non-extension within the elements.

According to FREY-WYSSLING & MÜHLETHALER (1965) it is not possible to make a distinction between a primary or a secondary cell wall on morphological characteristics. The distinction is to be based on the ontogenetic development of the cell wall.

The cell wall thickenings contain various substances: pectic substances, hemicelluloses, cellulose, lignin, and protein (ROELOFSEN 1959). The pectic substances and the hemicelluloses form the matrix on which a framework of cellulose is deposited simultaneously or in sequence. Lignin incrusts the developing thickenings from a certain stage onwards.

The site of deposition in the thickening may differ for the various cell wall substances. Pectin seems to be deposited, especially at the beginning of thickening formation, as a kind of substratum for further deposition of cell wall material in the thickenings as described by MAJUMDAR (1940). For cellulose an appositional manner of deposition in the thickenings is assumed (ROELOFSEN 1959). Lignification seems to start from the site of attachment of the thickening to the primary cell wall (HEPLER, FOSKET & NEWCOMB 1970).

Little is known about the sequence of deposition of the various cell wall substances or whether the substances are deposited in their final state. They may be deposited as precursors and synthesis is only completed after passage through the plasmalemma. For instance, in the case of lignin, it is possible that some of the precursors and enzymes are already present in the thickenings and have been deposited at various places in the thickenings. Perhaps only one special factor was to be deposited at this special site of attachment of the thickenings to start the lignification from this point.

Therefore, the relationship between cell organelles and cell wall thickenings should be investigated for each cell wall substance separately.

The process of the formation of the thickenings has been the subject of many electron microscopical studies in which the possible role of the various cell organelles is discussed (reviews by CRONSHAW 1965; ESAU, CHEADLE & GILL 1966a, b; ROBERTS 1969 and TORREY, FOSKET & HEPLER 1971). In these studies the arguments for assuming a relationship between a particular cell organelle and the cell wall thickenings differ in nature. In morphologically oriented studies the evidence is usually topographical (ESAU, CHEADLE & RISLEY 1963 and BUVAT

1964a, b, c). In other studies the arguments are based on the detection of cell wall precursors by radioactive labeling techniques (PICKETT-HEAPS 1968 and WOODING 1968) or on studying the effects of the removal of a particular cell organelle by a destructive agent (PICKETT-HEAPS 1967 and HEPLER & FOSKET 1971). None of these papers, however, presents a complete picture of the sequence of events in the thickening formation as it occurs in one plant species.

The present paper concentrates on the formation of the thickenings in tracheary elements of the hypocotyl of *Cucumis* 'Sporu-Origineel' in the course of time. The morphology and the topography of cell organelles are studied during the process of thickening formation. Particular attention is paid to changes in the morphology, or in the localization of cell organelles, in relation to the size of the thickenings during their development. The possible function of the various cell organelles in the formation of the thickenings is also discussed.

2. MATERIAL AND METHODS

2.1. Culture of the plants

Seeds of cucumber (*Cucumis* 'Sporu-Origineel' from de Ruyter and Son, Bleiswijk, Holland) were germinated on moistened filter paper in petri dishes measuring 22 cm in diameter and 5 cm deep. The petri dishes were placed in a controlled culture room. The illumination regime was 16 hours light (Philips TLF 40 W., 10 tubes per metre; 1.50 m above the dishes) and 8 hours darkness; the temperature varied from 25°C during the light period to 20°C during the dark period. Germinated seeds and young seedlings were collected after 24, 48, 72, or 96 hours.

2.2. Electron microscopy

Slices of the hypocotyl about 2 mm thick, cut from the part below the insertion of the cotyledons to a distance of 6 mm, were prepared for electron microscopy. In this part of the hypocotyl all stages of cell wall thickening formation in xylem tracheary elements can be found. These range from the initial stages found in procambial cells to fully developed cell wall thickenings in mature tracheary elements. The extension growth of cell walls in this part of the hypocotyl has not been completed.

Each slice was cut into four wedges of about equal size, each containing a large vascular bundle. These parts were fixed by two different methods:

1. Placed for four hours at room temperature in a solution of glutaraldehyde diluted to a concentration of 6.5% in a 0.2 M sodium cacodylate buffer, pH 7.6. This was followed by washing in water for 10 minutes before a post-fixation in a 1% osmium tetroxide solution in a 0.28 M sodium veronal acetate buffer, pH 7.4, for two hours at 4°C (1a). Sometimes a 2% osmium tetroxide solution, in the same buffer, but now with a pH of 7.6 was used (1b). The only difference seen with the post-fixation with 1% osmium tetroxide is a slightly increased contrast in the sections for electron microscopic photography.

2. Left for four hours at room temperature in a 6.5% solution of glutaraldehyde in a 0.1 M sodium cacodylate buffer, pH 7.3, followed by washing in the same buffer for 10 minutes. Thereafter a post-fixation in a 1% solution of osmium tetroxide in the same buffer for two hours at room temperature.

We chose fixation method 2 because, after using fixation method 1, plasmolysis sometimes occurred in the tracheary elements. Using fixation method 2, no plasmolysis in the tracheary elements was observed.

After dehydration in alcohol the pieces were embedded in Epon 812. Thin sections were cut with a glass knife on an LKB-ultramicrotome. The sections were stained with a saturated uranyl acetate solution (U) in water for 45 minutes. This was followed by staining with lead citrate. The lead citrate staining lasted for 8 minutes when REYNOLDS' (1963) technique was used (R) or for 5 minutes when using the technique of VENABLE & COGGESHALL (V) (1965).

The stained sections were then examined with a Philips EM 300 electron microscope operated at 60 or 80 Kv.

2.3. Stages in the process of the formation of the cell wall thickenings

Only radial, or approximately radial, sections of tracheary elements were used for further study (*plate I*). In such sections, the surface area of the transversely cut cell wall thickenings were measured and four classes were distinguished in relation to the size of the thickenings. The reason why more tangentially sectioned tracheary elements could not be used was that the size of the thickenings in those sections varies and is always larger than in a radial section of the same tracheary element.

In addition, the morphology or the topography of cell organelles in the vicinity (that is, within 3 microns distance from the plasmalemma) of the thickenings was studied. The diameters of the tracheary elements studied varied from 10 to 20 microns. In the wider cells a large central vacuole was present, whereas the cytoplasm was found only along the cell wall in a small zone about 3 microns wide at the most.

In order to establish a possible relationship between organelle morphology or topography and the formation of the thickenings, the thickenings and their surrounding cytoplasm were grouped into four classes according to the size of the thickenings as mentioned above. These four size classes (1 to 4), which are given in *fig. 1*, can be considered to represent arbitrarily delineated successive stages in the formation of the thickenings. To complete this ontogenetical progression, we also added classes 0 and 5. In class 0 thickenings have not yet developed; in class 5 they are fully developed and there is no protoplasm left in the cell. The size of the thickenings in the cells studied is about 3.25 square microns at the most.

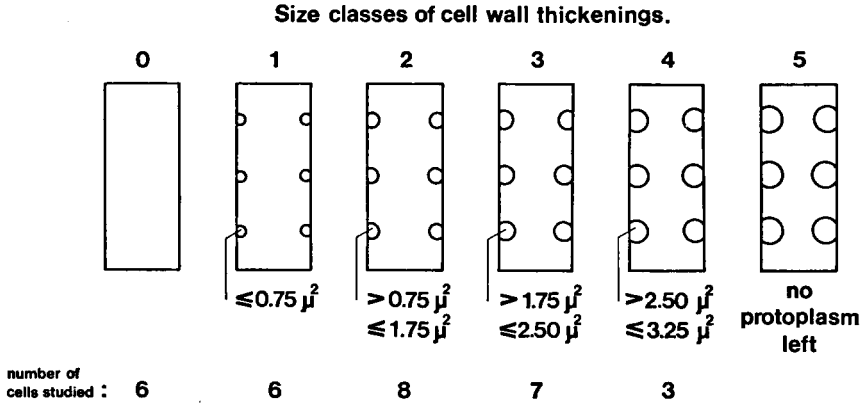


Fig. 1. Representation of size classes of cell wall thickenings in radial sections of tracheary elements.

3. RESULTS

3.1. General remarks

In this section the morphology or topography of the various cell organelles and its possible correlation to the various size classes of the thickenings (i.e., stages in the formation of the thickenings) is described.

In the tracheary elements studied, the following cell organelles or structures were observed: endoplasmic reticulum, ribosomes, Golgi bodies, plastids, cortical microtubules, lomasomes, cytoplasmic fibrils, mitochondria, lipid bodies, nuclei, and vacuoles.

3.2. Endoplasmic reticulum

The structure of the endoplasmic reticulum varies. It can either have an irregularly shaped vesicle form (vesicular), or it can appear as more flattened vesicles (cisternal). In both forms the endoplasmic reticulum can be either smooth or rough, depending on the absence or presence of ribosomes on the surface.

In class 0, in which no cell wall thickenings are present, the endoplasmic reticulum has a vesicular form, and no or very few ribosomes are attached to its outer surface (*plate II, figs. 1* and *2*). In class 1 of the formation of the cell wall thickening, ribosomes are attached to the endoplasmic reticulum and it gradually assumes a more cisternal form (*plate II, fig. 3*). In classes 2 and 3 the endoplasmic reticulum is chiefly present in a rough cisternal form (*plate II, fig. 4* and *plate III, fig. 1*). In class 3, the vesicular form seems to reappear; both forms of the endoplasmic reticulum in class 3 have a rough appearance. In class 4, the endoplasmic reticulum is chiefly cisternal (*plate III, fig. 2*), although irregular-shaped arrangements are also present (*plate III, fig. 3*). Both rough and smooth surfaces can be found (*plate III, fig. 2*).

3.3. Ribosomes

Ribosomes are small electron-opaque granules and can be grouped as follows: a. the ribosomes may be attached to the membranes of the endoplasmic reticulum (especially in classes 1 to 4), or b. free in the cytoplasm (especially in class 0). The ribosomes in the cytoplasm can be dispersed or arranged in chains called polyribosomes or polysomes (*plate II, figs. 1* and *2*).

Progressing from class 0 to 4 the number of scattered ribosomes seems to increase, whereas the number of polysomes decreases.

3.4. Golgi bodies

Golgi bodies appear in the form of a stack of more or less compressed closed cisternae with associated peripheral tubules and vesicles (dictyosome) arranged parallel to each other. A dictyosome shows very little variation in form. In all classes distinguished 4 or 5 cisternae are present per stack. They are usually flat and always electron-opaque. In class 2, however, the cisternae seem to be more compressed in the middle than at the periphery. The Golgi or dictyosome vesicles can be electron-transparent or electron-opaque. The electron-transparent vesicles often contain an electron-opaque dot (*plate II, fig. 4* and *plate III, fig. 4*). Sometimes large vesicles containing two or more dots are also observed (*plate III, fig. 4*). These larger vesicles should probably be regarded as fusions of two or more single-dotted vesicles. Furthermore, it appeared that the Golgi vesicles exhibit a very special size range in the different classes of wall thickenings.

In class 0 the dictyosomes do not seem to give rise to vesicles, although sometimes a few electron-opaque vesicles with a diameter of 390 Å are found in the vicinity of the dictyosomes (*plate II, figs. 1* and *2*). In class 1, the diameter of the vesicles is about 1170 Å. The vesicles have mostly electron-transparent contents, although sometimes the contents seem to be slightly electron-opaque (*plate IV, fig. 1*). In class 2, the vesicles have a diameter of about 1560 Å. These vesicles are always electron-transparent, but most of them contain an electron-opaque dot (*plate II, fig. 4* and *plate III, fig. 4*). In class 3, the dictyosome vesicles are smaller than in classes 1 and 2. Here two types of vesicles are present: vesicles of about 390 Å diameter and vesicles of approximately 780 Å diameter. The contents of both types of vesicles are evenly electron-opaque, but the smaller vesicles seem to be more electron-opaque than the larger ones (*plate III, fig. 1* and *plate IV, fig. 2*). In class 4 two types of vesicles are also observed; one type which is similar to the smaller vesicles found in class 3, and larger vesicles (diameter 975 Å) with almost electron-transparent contents (*plate III, figs. 2* and *3*).

A frequency distribution histogram of diameters of Golgi vesicles in the different classes is given in *fig. 2*.

3.5. Plastids

The plastids in the tracheary elements are probably amylo-leucoplasts. They contain some osmiophilic globules, sometimes a few electron-transparent vesicles, and a relatively simple system of paralleling lamellae (*plate III, fig. 1*).

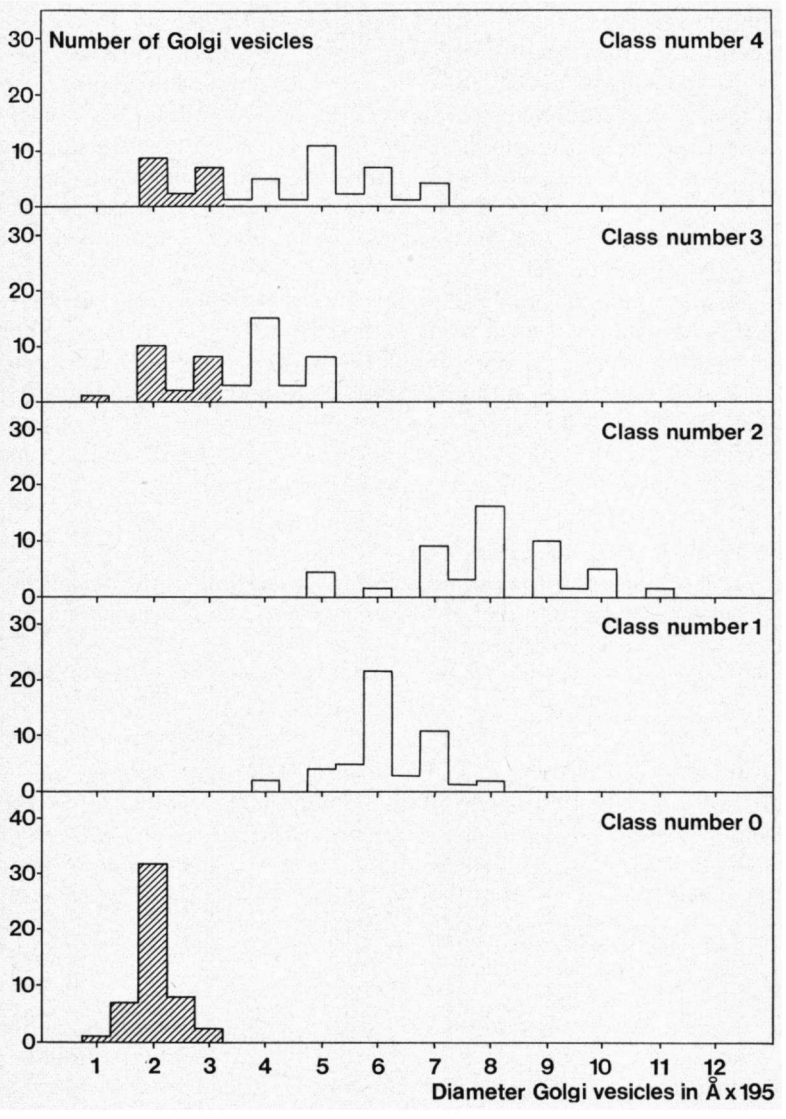


Fig. 2. Frequency distribution histogram of Golgi vesicles in the various classes. The hatched blocks represent vesicles with electron-opaque contents.

Only in class 0 are starch grains always found in the plastids (*plate II, figs. 1* and *2*).

The morphology of the plastids, however, does not markedly differ from one class to the other.

3.6. Microtubules

Peripheral or cortical microtubules are present and appear in radial sections of tracheary elements as small closed circles with a diameter of approximately 250 Å. These microtubules lie parallel to each other in a single row in the cytoplasm close to the plasmalemma. Both the distances between the microtubules (± 350 Å) and between each microtubule and the plasmalemma (± 170 Å) are constant. Connections between a microtubule and the plasmalemma are sometimes observed (*plate V, fig. 3*). Peripheral microtubules are oriented perpendicular to the axis of the cell.

In class 0 the peripheral microtubules are distributed more or less evenly along the plasmalemma (*plate V, fig. 1*). In classes 1 and 2, the microtubules are found exclusively along the cell wall thickenings (*plate V, fig. 2*). This also holds for class 3 (*plate V, fig. 3*), although a few microtubules are occasionally found between the cell wall thickenings. In class 4 the microtubules are again evenly distributed along the plasmalemma and, therefore, found both along and between the cell wall thickenings (*plate V, fig. 4*).

3.7. Lomasomes

The term lomasome is used for complex structures found at the periphery of the cell. The lomasome consists of an invagination of the plasmalemma in which tubules and/or vesicles are present.

Lomasomes are frequently found in class 0 (*plate VI, fig. 1*) and rarely in the other classes.

3.8. Cytoplasmic fibrils

Bundles of paralleling fibrils are observed only in the cytoplasm of tracheary elements in class 0 (*plate VI, fig. 2*). Some characteristics of the fibrils: a diameter of about 90 Å; a traceable length of 2 microns; and a sinuous shape. The width of the bundle is 600 Å to 1500 Å. The fibrils lie parallel to the axis of the cell.

3.9. Some final remarks

The cell organelles not mentioned, such as mitochondria and lipid bodies, are not conspicuously different in their morphology or their topography in the various size classes of the cell wall thickenings. A summary of the morphology described for the cell organelles according to thickening classification is given in *fig. 3*.

4. DISCUSSION

4.1. General discussion

In this section we shall try to establish a causal relationship for the morphological changes in the endoplasmic reticulum, the ribosomes, and the Golgi bodies; the changes in localization of the microtubules; and the presence or

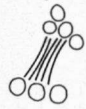
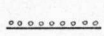
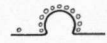
	0	1	2	3	4
endoplasmic reticulum	S.E.R.$_V$	R.E.R.$_C$ R.E.R.$_V$	R.E.R.$_C$	R.E.R.$_C$ R.E.R.$_V$	R.E.R.$_C$ S.E.R.$_C$
ribosomes	–	+	+ +	+ + +	+ + +
polysomes	+ + +	+	+	+	–
Golgi bodies morphology					
Golgi vesicles diameter in Å	390	1170	1560	390 780	390 975
plastids					
microtubules					
lomasomes		–	–	–	–
fibrils		–	–	–	–

Fig. 3. A summary of the morphological characteristics of cell organelles in xylem elements according to the various size classes of cell wall thickenings.

absence of the lomasomes and cytoplasmic fibrils during cell wall material deposition in the thickenings.

The manner in which the various cell organelles can be involved in cell wall formation differs. A cell organelle can have a role in the synthesis of cell wall precursors, cell wall substances, or enzymes. It may on the other hand serve as a substratum for enzymes. A cell organelle can have a function in the transport of cell wall precursors, cell wall substances, or enzymes. It may also exhibit a combination of these possibilities. A cell organelle can also cooperate with other cell organelles in any of these functions.

We shall now discuss the possible functions of the various cell organelles, the plastids excepted, in the same sequence as in section 3.

4.2. Endoplasmic reticulum

In class 0, in which the cell wall thickenings are still absent, the endoplasmic reticulum is smooth, whereas afterwards it is predominantly rough (classes 1 to 4). The possible function of the smooth endoplasmic reticulum is less well established than that of the rough endoplasmic reticulum.

BURGESS & NORTHCOTE (1968) assume that the smooth endoplasmic reticulum is involved in aggregating and arranging subunits for the formation of microtubules.

This assumption is not contradicted by our observations that microtubules and smooth endoplasmic reticulum are both present in stage 0. It might be that

the formation of microtubules which started in a pre-stage of stage 0 is still in progress. Then (classes 1 to 4) the microtubules remain and the smooth endoplasmic reticulum largely disappears. However, in stage 4, in which the number of microtubules increases again (for we now find the microtubules along the whole plasmalemma and no longer only around the cell wall thickenings), an increase in smooth endoplasmic reticulum is observed.

Another possible function of the smooth endoplasmic reticulum seems to be the transport of hydroxyproline-rich protein from the cytoplasm to the cell wall (ROBERTS & NORTHCOTE 1972).

Rough endoplasmic reticulum is generally assumed to be involved in the synthesis of proteins. The rough appearance is caused by ribosomes which are attached to the outer surface of the endoplasmic reticulum.

The increase in the size of the thickenings (from stages 1 to 4) indicates that cell wall substances are being deposited. Therefore, it could be that the rough endoplasmic reticulum contributes to this deposition by producing proteins – especially enzymes required for cell wall synthesis.

Another possible way for the rough endoplasmic reticulum to contribute to the formation of the thickenings is by producing the hydroxyproline-rich cell wall protein. This, however, does not easily fit in with the findings of ROBERTS & NORTHCOTE (1972). If the smooth endoplasmic reticulum does transport hydroxyproline-rich protein, it is unlikely that the cell wall protein is synthesized while the means of transport (i.e. the smooth endoplasmic reticulum) is lacking (stages 1 to 4). However, Roberts and Northcote's finding does not seem to eliminate the possibility that rough endoplasmic reticulum also has a transport function.

In addition to the difference in appearance of the surface of the endoplasmic reticulum, we have mentioned that it can be vesicular or cisternal in shape. The vesicular form, which predominates in class 0, seems to suggest that vesicles are budded off. In fact it is probable that all of the vesicles in the cytoplasm in class 0 have an endoplasmic reticulum origin, since the dictyosomes seem to be scarcely, if at all, budding off vesicles in this stage. These endoplasmic reticulum vesicles might be involved in the transport of their contents to or through the plasmalemma towards the thickenings (see also 4.7 lomasomes). On the other hand, cisternal endoplasmic reticulum does not seem to be involved to a major extent in the formation of vesicles. Nevertheless, it is possible that the cisternal endoplasmic reticulum is involved in transport, although in a different manner than is the vesicular form. The flattened and elongated form is particularly suitable for directing transport in the cisternae, or for directed transport along the cisternae (*plate II, fig. 4* and *plate VII, fig. 1*).

4.3. Ribosomes

The ribosomes in the cytoplasm are grouped in different ways in the various size classes of the thickenings. In class 0 they are not attached to the membranes of the endoplasmic reticulum, in contrast to all other classes. Therefore, no protein synthesis is associated with the endoplasmic reticulum membranes in

class 0. But free ribosomes in the cytoplasm, dispersed or arranged as polysomes, can also synthesize proteins. The difference in protein synthesis in these three groupings of ribosomes is unknown. It is possible that differences do exist. In class 0 the grouping of the ribosomes differs considerably from the other classes. This is in accordance with the deposition of the substances for the cell wall thickening that begins in class 0 and is only later in full progress.

4.4. Golgi bodies

The morphology of the Golgi vesicles in the various stages of differentiation varies in two aspects. The diameter of the vesicles differs, and the contents of the vesicles differ in electron density. The changing morphology of the Golgi vesicles might point to a changing role during the formation of the thickenings.

PICKETT-HEAPS (1968) and WOODING (1968) concluded from autoradiographical studies that Golgi vesicles transport hemicelluloses, pectin, and lignin, or precursors of these cell wall substances, from the cytoplasm to the sites of the thickenings.

HEPLER, RICE & TERRANOVA (1972) demonstrated the presence of the enzyme peroxidase in the Golgi vesicles. Peroxidase plays a role in the formation of lignin.

The transport of the different substances contributing to the formation of the thickenings by Golgi vesicles might be separated in time. Pectic substances should be deposited principally in the beginning of wall material deposition in the thickenings (MAJUMDAR 1940). Lignin will incrust the thickenings after the main deposition of cellulose and hemicelluloses has been taken place (HEPLER, FOSKET & NEWCOMB 1970).

The changing morphology of the Golgi vesicles which we described must be the result of the different contents of the vesicles at different stages. It is premature, however, to relate the observed morphology of a Golgi vesicle and the deposition of a certain cell wall compound at this time.

4.5. Microtubules

The distribution of the cortical microtubules varies in the different stages of the formation of the thickenings. If the microtubules have a function in the formation of the thickenings, this changing topography can perhaps be understood. PICKETT-HEAPS (1968) assumed a guiding function for the microtubules in the movement of vesicles of different origin from the cytoplasm to the cell wall. This assumption is based on photographs in which vesicles are found in the vicinity of microtubules. For the microtubules of the spindle an attraction of Golgi vesicles seems to have been proved (LEDBETTER & PORTER 1963). Another conclusion that has been drawn in relation to a possible attraction of microtubules and vesicles is that a certain distribution of microtubules can determine the thickening pattern (HEPLER & NEWCOMB 1964; PICKETT-HEAPS 1967; MAITRA & DE 1971 and HEPLER & FOSKET 1971).

If these findings are connected with the distribution of microtubules which we described for the various size classes, the following events might occur: a. in

class 0 vesicles might be transported along the microtubules to the areas of the cell wall between the lomasomes, b. in classes 1, 2, and 3 vesicles might be transported to the sites of the thickenings, and c. in class 4 to the sites of the thickenings as well as to sites between the thickenings because of the guiding function of microtubules. Indeed, we sometimes found vesicles in the vicinity of the microtubules at these sites. However, we also found vesicles, and more of them, at sites in the cytoplasm near the plasmalemma or in areas outside the plasmalemma where no cortical microtubules are observed.

For example, in stage 0 they were found in the lomasomes (*plate VI, fig. 1*), and in stages 1, 2, and 3 between the thickenings (*plate VII, fig. 2*). Especially in the corners of the "basis" of the thickenings, many vesicles are observed (*plate VII, figs. 3* and *4*). In stage 4 fewer vesicles are present. This is understandable in view of the fact that the formation of the thickenings is nearly completed. Besides, the orientation of the microtubules in the cytoplasm does not seem to be very favourable for movement of vesicles towards the cell wall, since the microtubules are arranged parallel to the plasmalemma. A more adequate arrangement for movement towards the plasmalemma would be an orientation of the microtubules perpendicular to the plasmalemma, pointing from the axis of the cell outwards. Furthermore, the size of the Golgi vesicles (390–2000 Å) is larger than the distance between the microtubules (350 Å) and it is not clear how the vesicles penetrate the row of microtubules. Golgi vesicles are often observed in the vicinity of microtubules as already described, but these photographs never show Golgi vesicles wriggling through the row of microtubules.

Our results point to a barrier function of the microtubules for Golgi vesicles rather than to an attraction followed by conducting the vesicles. A possible function for the microtubules in patterning the thickenings still remains. Our conclusion is that the contribution of vesicles to the thickenings in stages 1, 2, and 3 is mainly the deposition of cell wall precursors and cell wall enzymes between the thickenings.

LEDBETTER & PORTER (1963) related the microtubules and the formation and orientation of the cellulose microfibrils in the secondary cell wall to the mirroring orientations of microtubules and cellulose microfibrils. This role in directing the orientation of the cellulose microfibrils was confirmed by HEPLER & FOSKET (1971). It is probable that the precursors of cellulose are deposited at the sites where the microtubules are present.

The lignification of the thickenings starts at the point of attachment ("basis") of the thickening to the primary cell wall (HEPLER, FOSKET & NEWCOMB 1970) and supports our hypothesis. If precursors for lignin are present in the Golgi vesicles, it is more suitable that they are deposited between the developing thickenings, since lignification starts from the "basis" of the thickenings.

The discussed barrier role seems to be restricted to Golgi vesicles containing cell wall precursors and cell wall enzymes of cell wall substances other than cellulose.

4.6. Lomasomes

If the lomasomes play a role in the formation of the thickenings, it must be in the initiation of the thickenings. Lomasomes are found almost exclusively in stage 0, the stage in which the formation of the thickenings begins. If they have a function in the formation of the thickenings, it would be a more or less indirect one via the endoplasmic reticulum. The vesicles in the lomasomes, or their contents, possibly have an endoplasmic reticulum origin. Stage 0, however, would be the only stage in which the endoplasmic reticulum contributes to the thickening formation by budding off vesicles.

4.7. Cytoplasmic fibrils

The fibrils, which we found only in stage 0, are more or less similar to those described by HEPLER & NEWCOMB (1964). Both types of fibrils have a sinuous shape and the same length (2 microns traceable). They differ in the diameter of about 90 Å which we found (Hepler and Newcomb: 120–150 Å) and the fact that we did not observe an association of the fibrils with the endoplasmic reticulum. Hepler and Newcomb assumed a function for the fibrils in the formation of the thickenings which was based on four arguments: a. their abundance in the xylem elements, b. their length in relation to the cellulose microfibrils in the cell wall, c. their proximity to the thickenings, and d. their localization within the endoplasmic reticulum.

In our material, arguments for abundance and association with the endoplasmic reticulum are completely lacking. We have no evidence for assuming a role for these fibrils in the formation of the thickenings from any of our electron microscope photographs.

The cytoplasmic microfilaments found in many elongating cells, including protoxylem elements described by PARTHASARATHY & MÜHLETHALER (1972), resemble the cytoplasmic fibrils which we found, but the diameter of these cytoplasmic microfilaments is only between 50 and 60 Å, while lengths up to 12 microns were measured. No association with other cell organelles was observed. These authors postulate a function for the microfilaments in cytoplasmic streaming.

5. CONCLUSION

During the formation of the cell wall thickenings, morphological changes in a number of cell organelles (endoplasmic reticulum and Golgi vesicles), changes in the position of cell organelles in relation to the thickenings (microtubules), or the presence of organelles at certain moments (lomasomes) in the cytoplasm, could be observed.

These cell organelles are probably involved in the deposition of cell wall material during the formation of the thickenings. The changes in form and localization are interpreted as indications of a function for these cell organelles in cell wall thickening formation. Possible functions were discussed.

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List of the abbreviations used in figures and photographs

D	dictyosome	P	plastid
ER	endoplasmic reticulum	PCW	primary cell wall
ER_c	cisternal endoplasmic reticulum	Pl	plasmalemma
ER_v	vesicular endoplasmic reticulum	Pr	polyribosomes
F	(cytoplasmic) fibrils	R	ribosomes
G	Golgi body	RER	rough endoplasmic reticulum
Gv	Golgi vesicle	S	starch
L	lomasome	SER	smooth endoplasmic reticulum
Li	lipid body	T	cell wall thickening
M	mitochondrion	V	vesicle
Mt	microtubule	Va	vacuole
N	nucleus		

List of the fixation and staining used per photograph

	Fixation	Staining		Fixation	Staining
Plate I	1a	U/V	Plate V, fig. 1	1a	U/V
Plate II, fig. 1	1a	U/V	fig. 2	2	U/V
fig. 2	1a	U/V	fig. 3	2	U/R
fig. 3	2	U/R	fig. 4	1b	U/R
fig. 4	1b	U/R	Plate VI, fig. 1	1a	U/R
Plate III, fig. 1	2	U/R	fig. 2	1a	U/R
fig. 2	1b	U/R	Plate VII, fig. 1	2	U/R
fig. 3	1b	U/R	fig. 2	2	U/R
fig. 4	1b	U/R	fig. 3	2	U/R
Plate IV, fig. 1	2	U/R	fig. 4	2	U/R
fig. 2	2	U/R			

Plate I

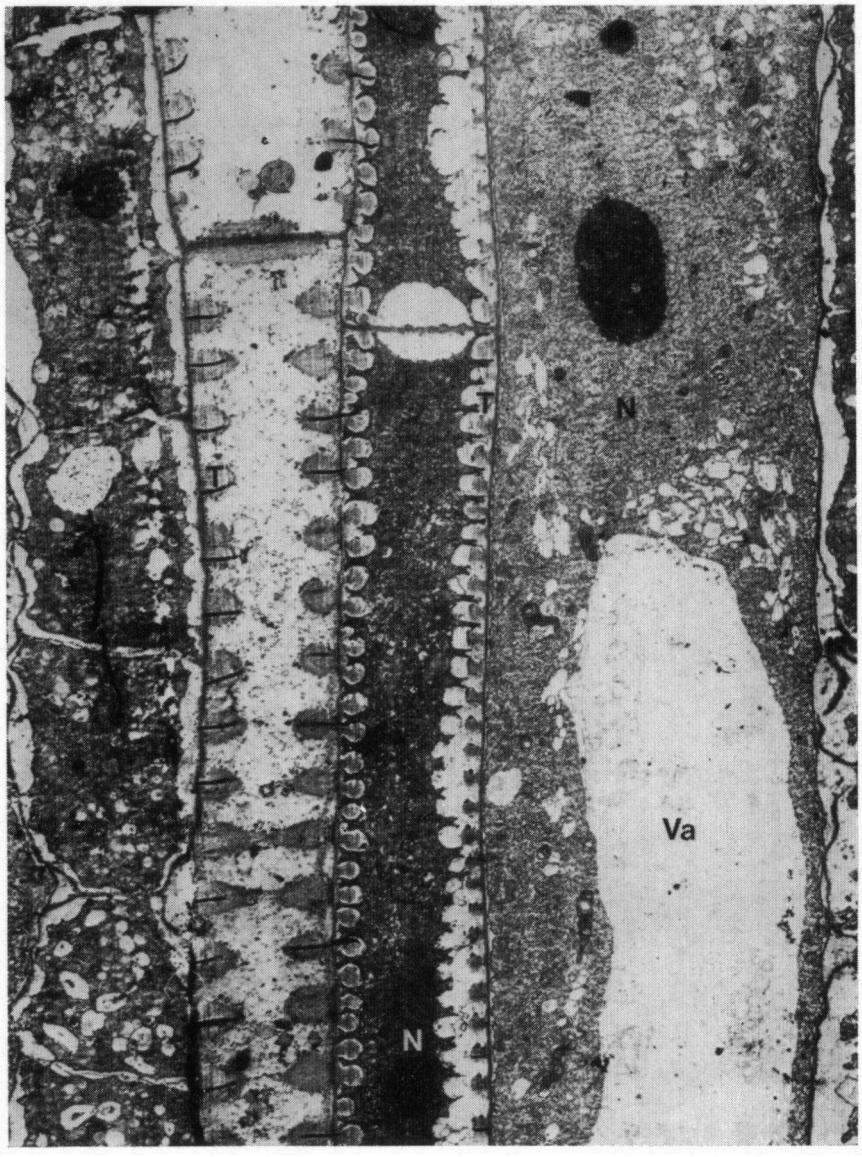


Plate I. A radial section through some tracheary elements. From the left element to the right, the cells represent classes 5, 2, and 0. The middle tracheary element shows plasmolysis. The dark bands on the thickenings of the left element are artifacts caused by ultrathin sectioning, × 2,900.

Plate II

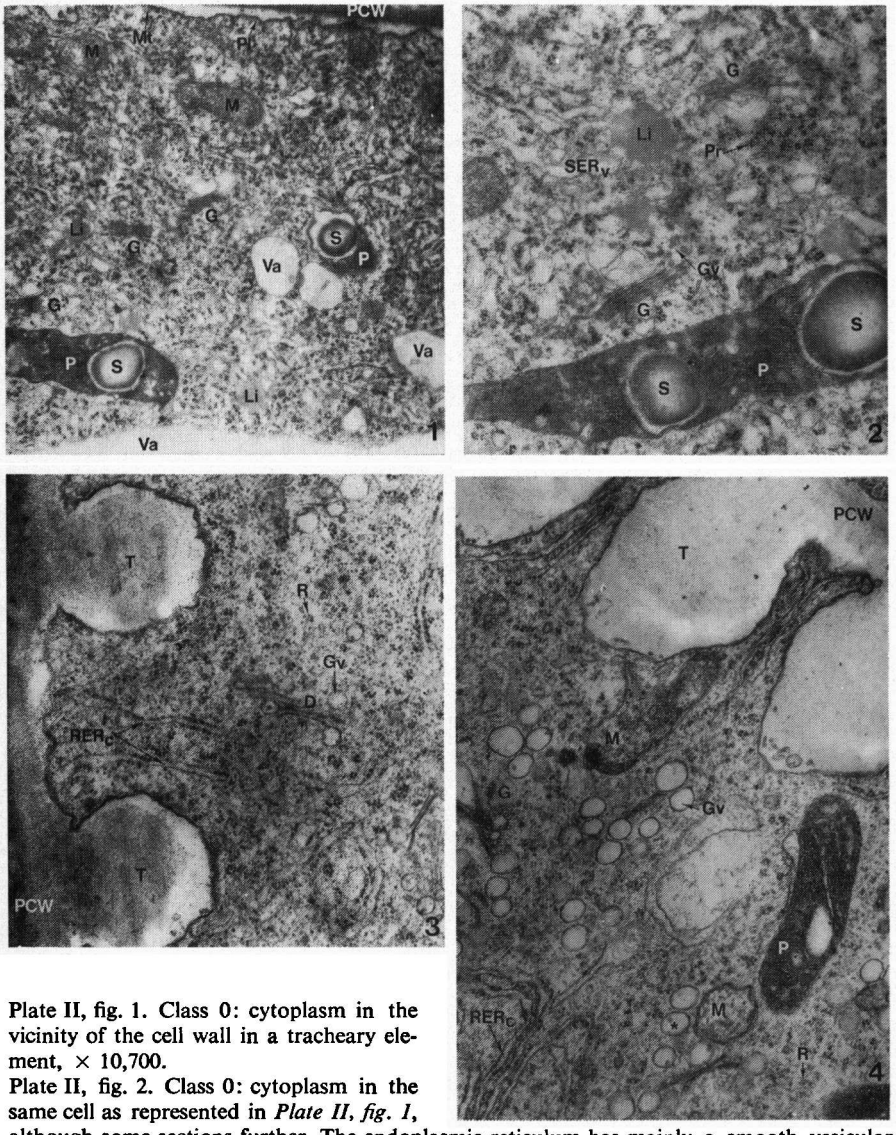


Plate II, fig. 1. Class 0: cytoplasm in the vicinity of the cell wall in a tracheary element, × 10,700.

Plate II, fig. 2. Class 0: cytoplasm in the same cell as represented in *Plate II, fig. 1*, although some sections further. The endoplasmic reticulum has mainly a smooth vesicular form. The ribosomes are grouped as polysomes. Close to the dictyosomes, small electron-opaque vesicles are visible. In the plastid, starch grains are present, × 19,800.

Plate II, fig. 3. Class 1: a zone of cytoplasm in a tracheary element showing both rough cisternal and rough vesicular endoplasmic reticulum. Ribosomes are also present, dispersed in the cytoplasm. Dictyosomes and electron-transparent Golgi vesicles are also present, × 20,600.

Plate II, fig. 4. Class 2: cytoplasm area close to the cell wall thickenings. The endoplasmic reticulum has a rough cisternal form. Note the scattered ribosomes in the cytoplasm and also the dots in the Golgi vesicles; one vesicle is marked with an asterisk, × 17,700.

Plate III

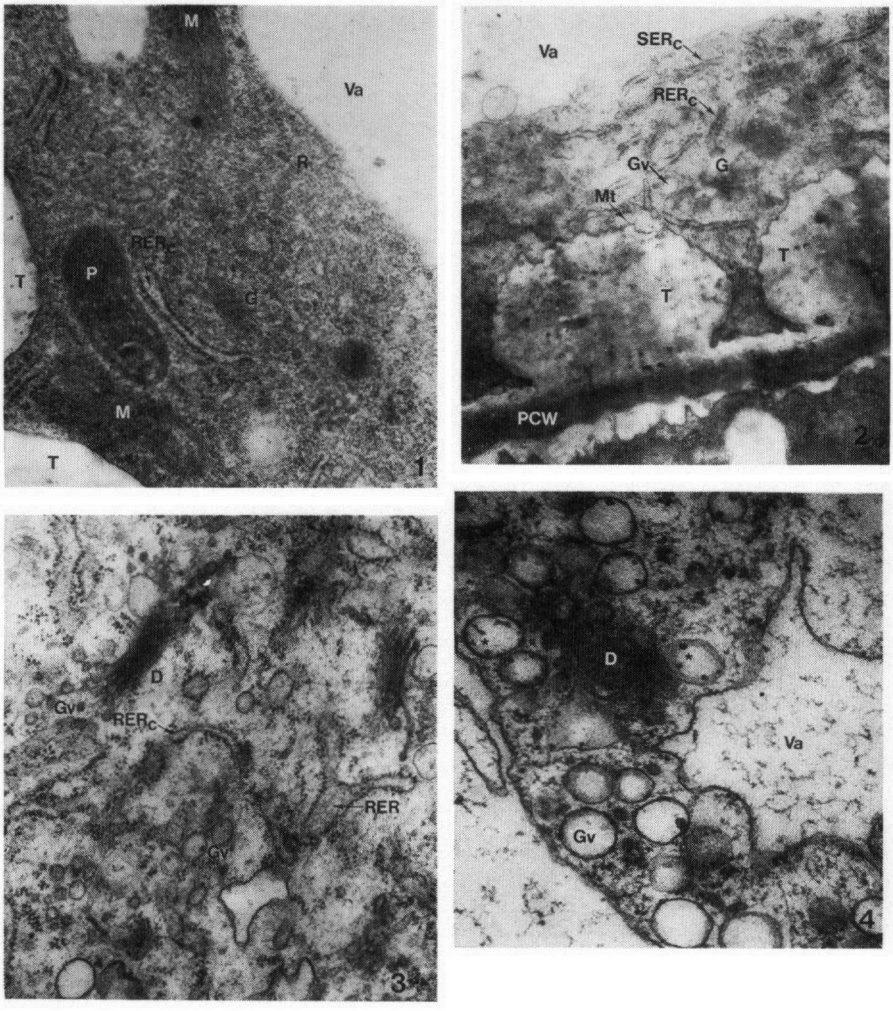


Plate III, fig. 1. Class 3: cytoplasm area next to two cell wall thickenings. Rough cisternal endoplasmic reticulum is present. Ribosomes are present dispersed in the cytoplasm. A plastid without starch grains is seen close to the thickenings. A part of the central vacuole can be observed, × 20,600.

Plate III, fig. 2. Class 4: general view of a tracheary element with some cell wall thickenings. The endoplasmic reticulum is chiefly cisternal; rough and smooth outer surfaces of the endoplasmic reticulum are visible, × 13,900.

Plate III, fig. 3. Class 4: cytoplasm area close to the cell wall of a tracheary element with smooth and rough endoplasmic reticulum in a cisternal form, but more irregularly shaped endoplasmic reticulum is also present. Two stacks of cisternae are present and Golgi vesicles are dispersed in the cytoplasm, × 24,700.

Plate III, fig. 4. Class 2: a dictyosome, with vesicles in close proximity, in the cytoplasm close to the cell wall. Some large vesicles contain two or more dots; two vesicles are marked with an asterisk, × 38,300.

Plate IV

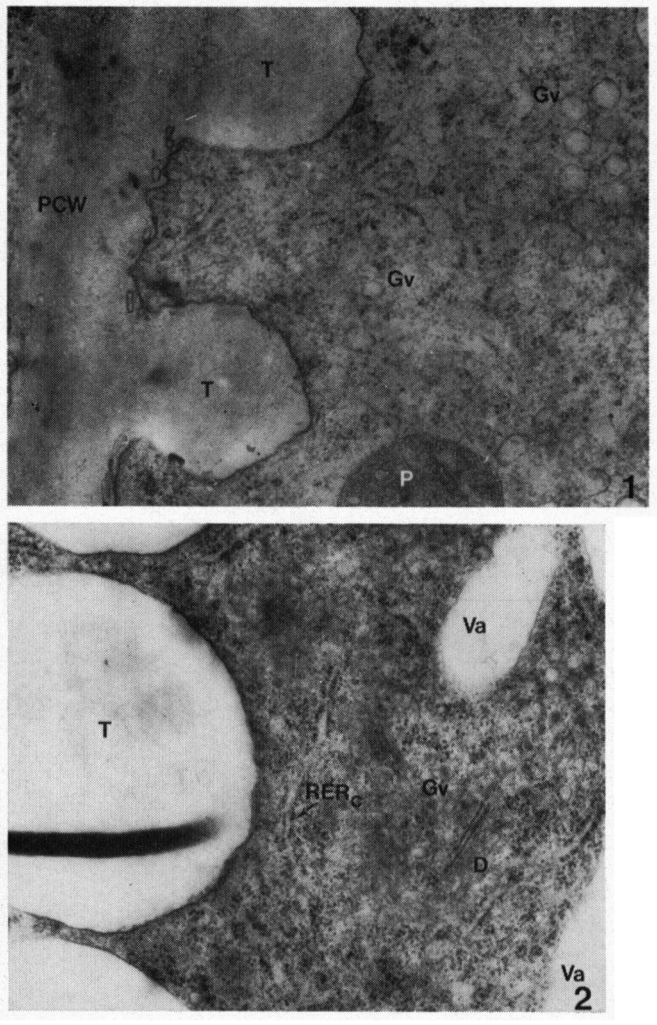


Plate IV, fig. 1. Class 1: electron-transparent Golgi vesicles close to the cell wall thickenings. A part of a plastid also can be observed, × 24,500.
Plate IV, fig. 2. Class 3: dictyosomes and Golgi vesicles, both electron-opaque and electron-transparent. Cytoplasm densely packed with ribosomes. A part of the central vacuole can be observed, × 25,700.

Plate V

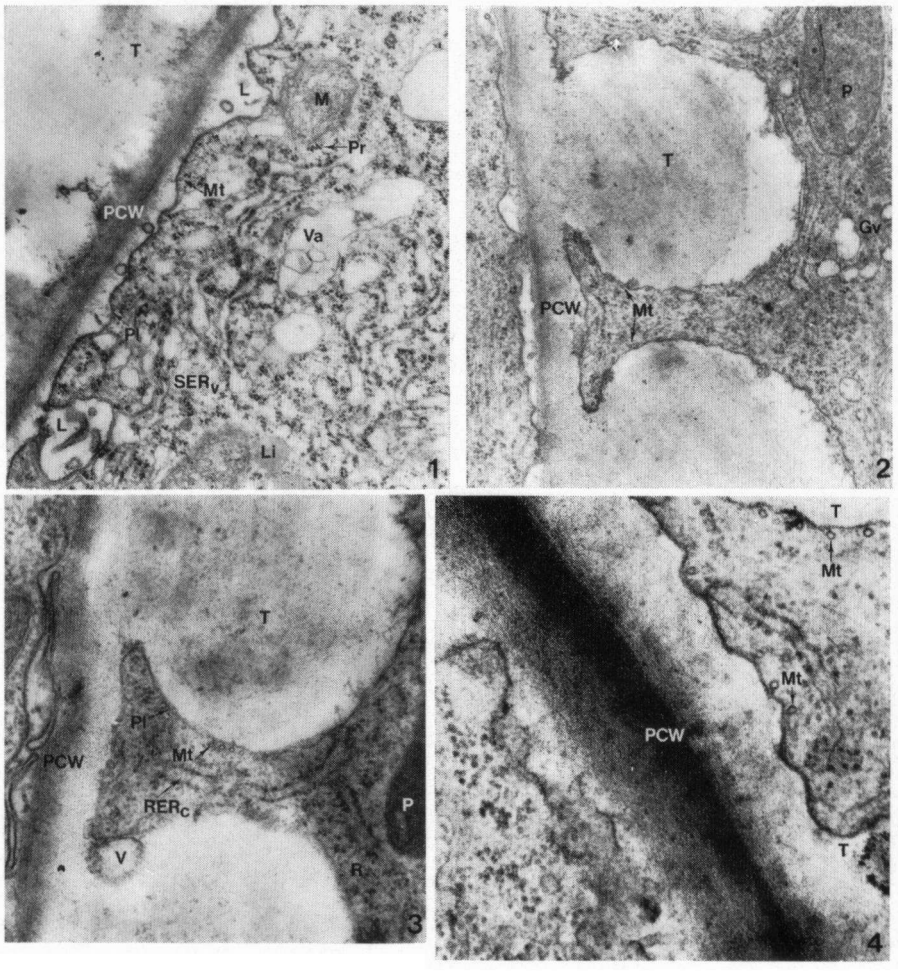
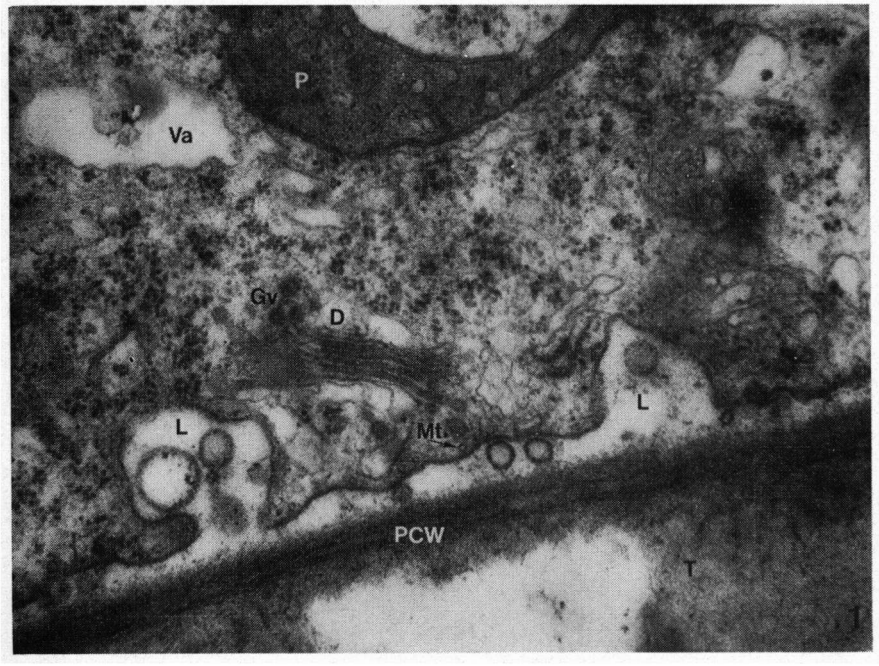


Plate V, fig. 1. Class 0: distribution of cortical microtubules along the plasmalemma. No microtubules are present around the lomasomes, × 18,800.
Plate V, fig. 2. Class 2: microtubules along the plasmalemma around the cell wall thickening, × 20,000.
Plate V, fig. 3. Class 3: part of two cell wall thickenings and the surrounding cytoplasm. Some microtubules are clearly visible along a part of the plasmalemma surrounding the thickening. Connections from the microtubules to the plasmalemma are visible, × 28,600.
Plate V, fig. 4. Class 4: part of a cell wall between two cell wall thickenings. Microtubules are present close to the plasmalemma between and surrounding the thickenings, × 47,600.

Plate VI



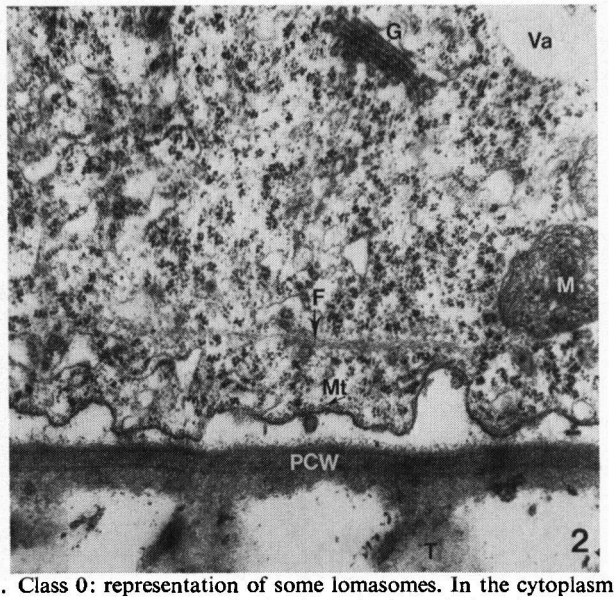


Plate VI, fig. 1. Class 0: representation of some lomasomes. In the cytoplasm a Golgi body lies close to the cell wall. Note the distribution of microtubules along the plasmalemma, × 45,600.

Plate VI, fig. 2. Class 0: bundle of fibrils in the cytoplasm close to the cell wall and parallel to the cell axis. There is no association of the bundle with other cell organelles, × 27,300.

Plate VII

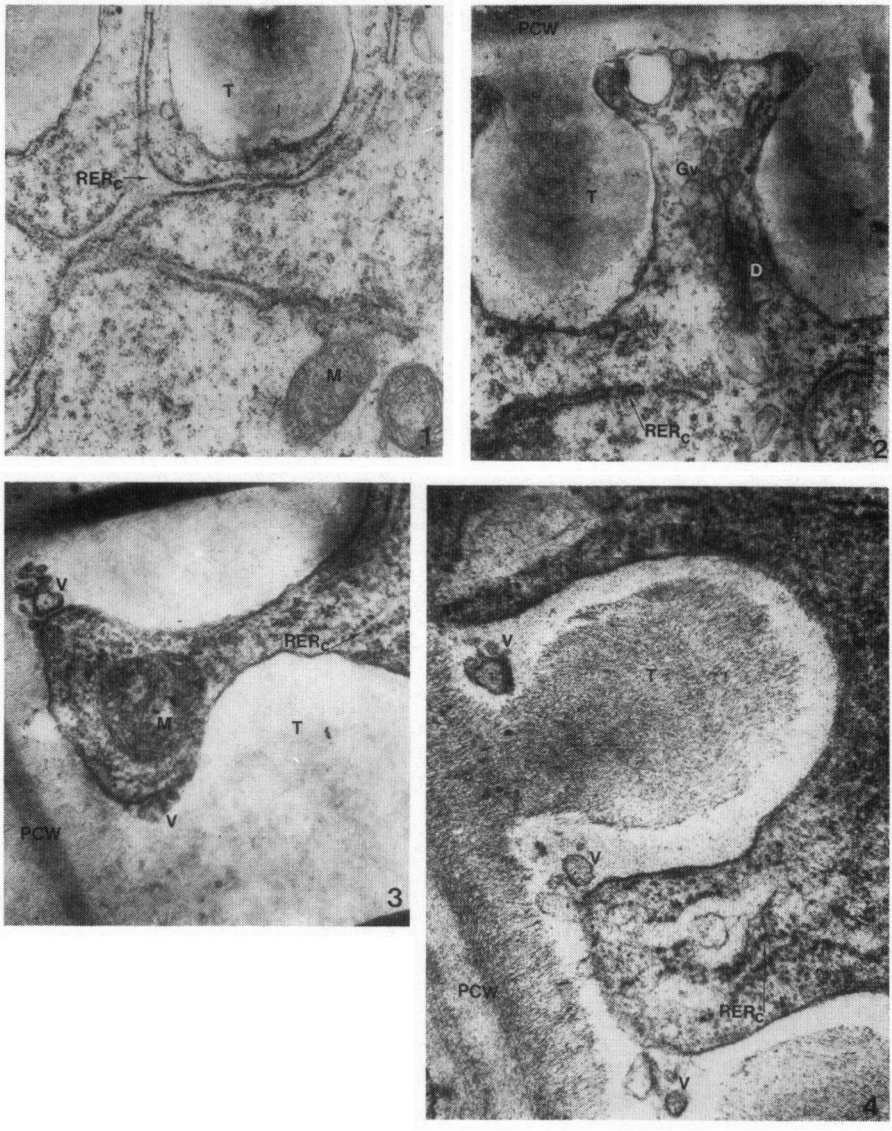


Plate VII, fig. 1. Class 2: rough cisternae of the endoplasmic reticulum close to the cell wall thickenings. Note the flattened, elongated form, × 20,600.
Plate VII, fig. 2. Class 2: a dictyosome, with many vesicles in the vicinity, between the cell wall thickenings, × 20,600.
Plate VII, fig. 3. Class 3: vesicles in the corners of the sites of attachment ("basis") of the cell wall thickenings to the primary cell wall. No microtubules are present in these places, × 29,200.
Plate VII, fig. 4. Class 1: vesicles in the corners of the sites of attachment ("basis") of the thickenings to the primary cell wall, × 36,800.