THE FINE STRUCTURE AND DEVELOPMENT OF THE COMPANION CELL OF THE PHLOEM OF *ACER PSEUDOPLATANUS*

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ABSTRACT

At maturity the companion cell of the phloem of the sycamore *Acer pseudoplatanus* has a large nucleus, simple plastids closely sheathed with rough endoplasmic reticulum, and numerous mitochondria. The cytoplasm contains numerous ribosomes, resulting in a very electron-opaque cytoplasm after permanganate fixation. Bodies similar to the spherosomes of Frey-Wyssling *et al.* (4) are collected in clusters and these also contain bodies of an unidentified nature similar to those found by Buttrose (1) in the aleurone cells of the wheat grain. The pores through the wall between the companion cell and sieve tube are complex and develop from a single plasmodesma. Eight to fifteen plasmodesmata on the companion cell side communicate individually with a cavity in the centre of the wall which is linked to the sieve tube by a single pore about twice the diameter of an individual plasmodesma. This pore is lined with material of an electron opacity equivalent to that of material bounding the sieve plate pores. The development of the cell organelles, the possible role played in the phloem tissue by the companion cell, and the function of the complex pores contained in its wall are discussed.

INTRODUCTION

Most of the investigations of the organisation of phloem tissues have dealt with the structure of the sieve tube, since this, in its various forms, is a common feature of all phloem tissues examined. The companion cell, which only in the angiosperms is an invariable constituent of the phloem, is considered to play an important part in the maintenance of the mature, anucleate sieve tube as an efficient functional unit. It is generally held that the sieve tube is not an inert conduit as is the xylem vessel, but forms with the companion cell and phloem parenchyma a living translocation system (2, 5). As a component of such a functional tissue the companion cell would be expected to be physiologically specialised, with a corresponding specialisation in the organisation of its fine structure.

The present report arises from a more general study of the development of the woody tissues of sycamore. The differentiation of the xylem vessel has already been reported (10).

MATERIAL AND METHODS

FIXATIVE 1 Potassium permanganate (2 per cent) unbuffered, containing a mixture of sodium, potassium, and calcium chlorides to a final total concentration of 0.4 M. Dehydration in alcohol series.

FIXATIVE 2 Glutaraldehyde (12 per cent) in sucrose (10 per cent) followed by osmium tetroxide (1 per cent) in Veronal buffer in sucrose (10 per

cent) also. Dehydration in acetone; 15-minute staining with potassium permanganate (1 per cent) in 100 per cent acetone.

Segments (1 to 2 mm) of year-old stems of *Acer pseudoplatanus* (approximately 2 mm diameter) were cut into the above fixatives, dehydrated, embedded in Araldite, and sectioned, using diamond knives, on a mechanical advance microtome. Sections were picked up on copper grids with a carbon supporting film and were stained with uranyl acetate (saturated solution in 50 per cent alcohol) and/or Millonig's lead hydroxide (10). The sections were examined in a Philips EM 100 at 80 kv.

RESULTS

In sycamore each companion cell and its associated sieve tube develop from the same cambial initial. The companion cell does not increase in size but remains a very elongated cell of small diameter running the full length of the associated sieve tube, the one-to-one relationship being strictly maintained (Fig. 25). The development of the organelles of the companion cell follows a course which produces the mature cell at the same time as the completion of perforation of the sieve plate pores in the adjacent sieve tube.

Immediately after division of the cambial initial, the organelles in the twin sieve tube and companion cell are very similar (Fig. 1), and the cells can only be differentiated by examination of the end wall for evidence of sieve plate pore formation, which starts very early in development.

In the cell which will eventually become the companion cell the mitochondria are small round to oval structures with few cristae. The outer membranes of the mitochondria are less densely stained after permanganate fixation than those of the plastids. The plastids are somewhat larger in size initially than the mitochondria but similarly lack any internal structure (Fig. 1). Both mitochondria and plastids are frequently seen in shapes suggesting division by constriction at this stage. Profiles of the endoplasmic reticulum are numerous and are found mostly in association with the newly formed cell wall. The nucleus is large, and

Abbreviations

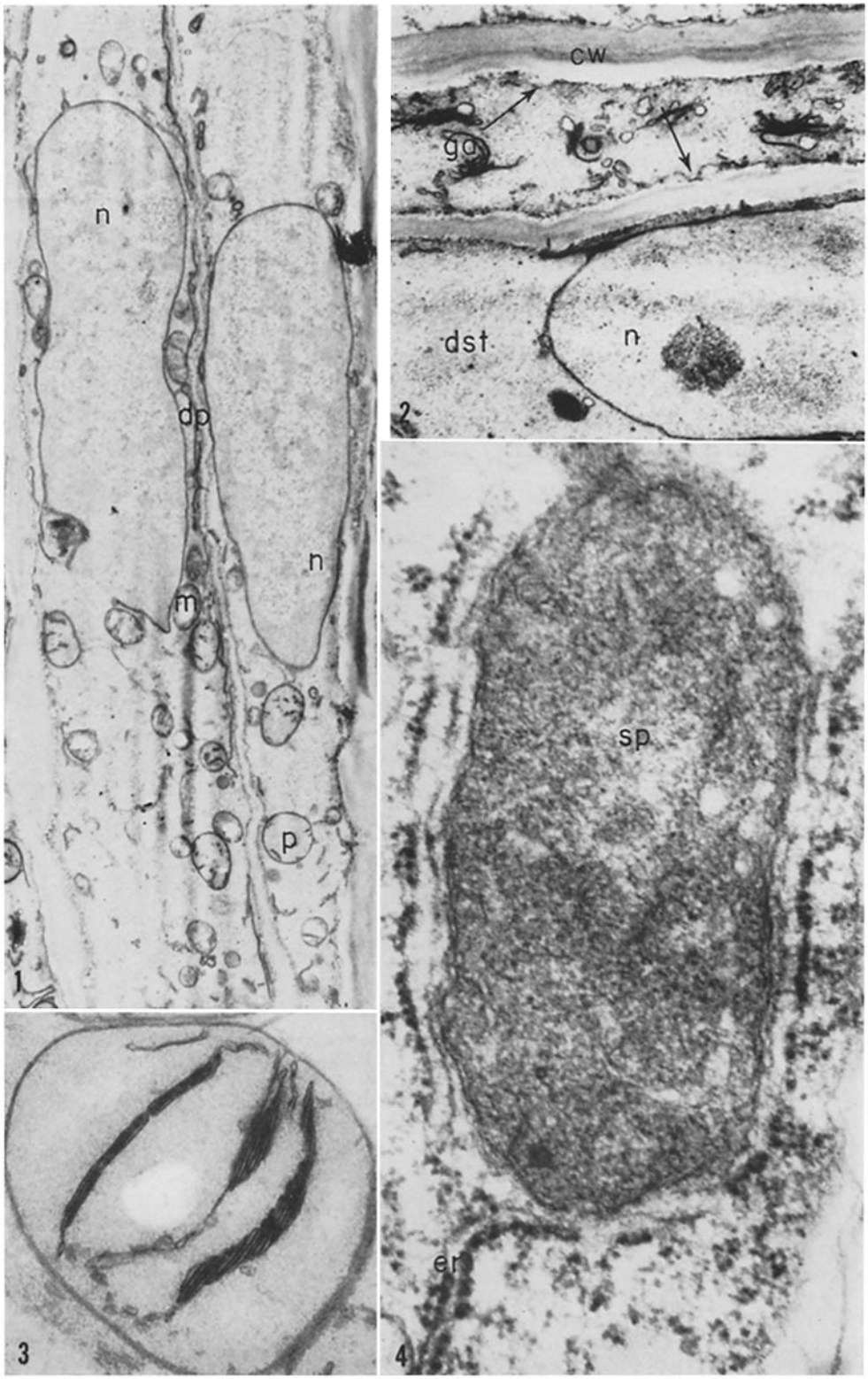
dcc, (developing) companion cell
dst, developing sieve tube
cw, cell wall
cc, companion cell
dp, cell pair which will develop into sieve tube and companion cell
er, endoplasmic reticulum
ga, Golgi body
m, mitochondrion
mp, modified plasmodesma between companion cell and sieve tube
n, nucleus
p, plastid
pd, plasmodesma
pm, plasmalemma
s, spherosome or granular body
sp, plastid—sheathed with endoplasmic reticulum
st, sieve tube
ub, unidentified body
v, vacuole
xv, xylem vessel

FIGURE 1 Recently divided cambial cell; one daughter will become a sieve tube, the other a companion cell. They cannot be differentiated at this stage. Permanganate fixation. × 5650.

FIGURE 2 Young companion cell. Numerous Golgi bodies are present in the cytoplasm, and their associated vesicles can be seen apparently depositing material on the thickening cell wall; *e.g.*, at the position indicated by an arrow. Permanganate fixation. × 12,500.

FIGURE 3 Young plastid from the phloem parenchyma. Compare the internal membranous organisation with that seen in the plastids of the companion cell, Figs. 4 to 7. Permanganate fixation. × 34,000.

FIGURE 4 Mature companion cell. Plastid completely sheathed by rough endoplasmic reticulum, which is continuous with a cytoplasmic profile of endoplasmic reticulum. There are no ribosomes on the surface of the endoplasmic reticulum adjacent to the plastid outer membrane. Glutaraldehyde fixation. × 77,500.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its outer membrane is frequently connected to the endoplasmic reticulum. The cytoplasm is fairly dense, and has no vacuoles. The numerous Golgi bodies are a characteristic feature of this early stage in development, and they produce large vesicles of low electron opacity which bear a close similarity to the material immediately underneath the plasmalemma in the developing cell wall (Fig. 2); only an occasional Golgi body is seen in the mature cell.

The nucleus is the only structure which does not undergo any obvious developmental change, retaining substantially the same appearance at all stages of cell maturation. The number and especially the length of the mitochondrial cristae increase during development, and there also appears to be an increase in the total number of mitochondria per cell, although this has not been statistically substantiated.

The young plastids display little internal membranous structure. At the most a few tubules or vesicles may be present but these never develop any organisation equivalent to the lamellar stacks seen in the plastids of the adjacent phloem parenchyma cells (Fig. 3). At the early stages, profiles of the endoplasmic reticulum are seen in close relation to the plastid outer membrane and at maturity the plastid is usually entirely sheathed by this reticulum. The micrographs of glutaraldehyde-fixed material demonstrate that it is rough-surfaced endoplasmic reticulum which surrounds the plastids, and that the surface of the endoplasmic reticulum immediately adjacent to the plastid outer membrane is free of ribosomes (Fig. 4). No connections between the membranes of these adjacent organelles have yet been observed. This sheath of endoplasmic reticulum is continuous with profiles of endoplasmic reticulum free in the cytoplasm and can occasionally be seen in direct connection to the outer nuclear membrane, or to the modified plasmodesmata through the wall between sieve tube and companion cell (Figs. 5 to 7).

The plastids never contain non-membranous inclusions such as starch nor anything similar to the darkly staining inclusion seen in the sieve tube plastids. All profiles of the endoplasmic reticulum so far observed have been of the rough-surfaced type, and these are not numerous in the mature cell except for the elements sheathing the plastids and associated with the modified plasmodesmata.

Micrographs of permanganate-fixed material show a very dense granular cytoplasm which can be seen in sections of glutaraldehyde-fixed tissue to consist mainly of numerous free ribosomes (Fig. 8).

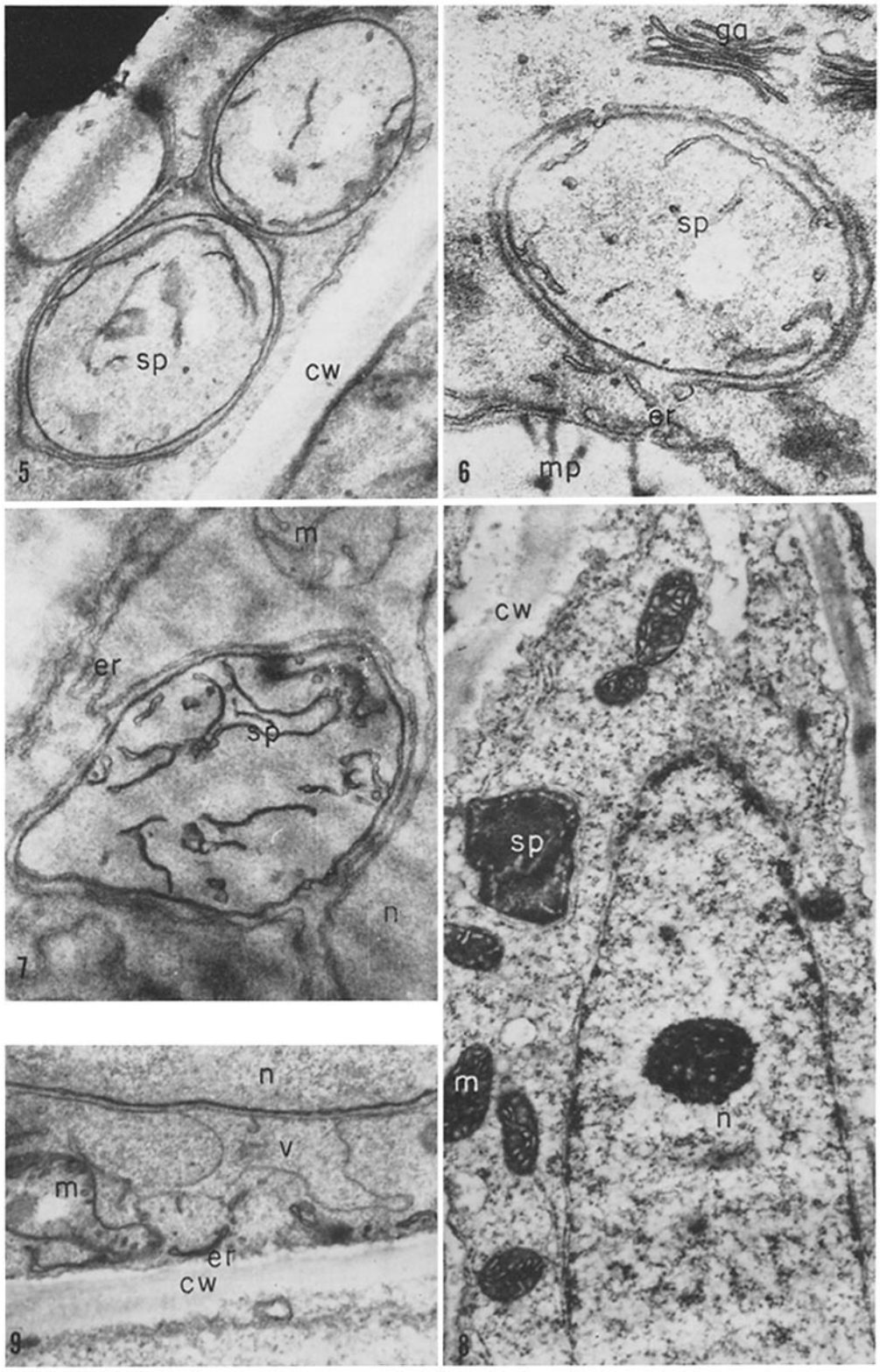
In the mature companion cell, vacuoles are occasionally seen. These are usually small and are close to the nuclear membranes. The tonoplast is thinner than a single element of the nuclear membrane, and initially the vacuole has slender projections from the central area, which are definitely thinner than the profiles of the nuclear membranes or the endoplasmic reticulum seen in Fig. 9.

Small oval or spherical bodies, 0.1 to 0.2 μ in diameter, are frequently seen in clusters in the companion cell cytoplasm (Fig. 10). They are bounded by a single membrane and enclose a uniformly granular substance which is made apparent after permanganate fixation. These organelles are very similar to the 'spherosomes' described by Frey-Wyssling *et al.* (4) and have a similar close relationship to the endoplasmic

FIGURES 5, 6, and 7 Mature companion cells. Endoplasmic reticulum sheathing the plastids. Fig. 6 demonstrates continuity of the sheath with the endoplasmic reticulum associated with the modified plasmodesmata between companion cell and sieve tube; Fig. 7, continuity with the nuclear membrane. Fig. 5 shows a common feature, a plastid sheathed over only part of its surface. Permanganate fixation. Fig. 5, × 30,000; Fig. 6, × 25,000; Fig. 7, × 20,500.

FIGURE 8 Mature companion cell. Numerous ribosomes in cytoplasm. Glutaraldehyde fixation. × 18,000.

FIGURE 9 Development of companion cell vacuole. Compare the vacuolar membrane with the profiles of the endoplasmic reticulum and nuclear envelope. Permanganate fixation. × 15,000.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reticulum (Figs. 10 and 11). Bodies of a similar size, with more particulate than granular contents, are seen after glutaraldehyde fixation. These are associated with the rough-surfaced endoplasmic reticulum and occasionally contain a large, possibly crystalline inclusion (Fig. 13). Such bodies are seen throughout the tissues of the sycamore stem, but only in the companion cell are they found in aggregations like that shown in Fig. 10. In these aggregations a further, as yet unidentified, body is found which is closely associated with the spherosomes (Fig. 10). This has a characteristically irregular outline and has no definite outer membrane but rather a densely stained peripheral zone, which fades gradually to a central area of very low electron opacity. These bodies are also associated with the endoplasmic reticulum (Fig. 12). Similar bodies have been seen in the xylem ray cells and the cells bordering the schizogenous resin canals of *Pinus pinea* (Figs. 14 and 15). They have not been observed, however, in any tissue of sycamore other than the phloem companion cell.

The new cell wall which divides the companion cell from the sieve tube thickens continuously during the early stages of development. This thickening is apparently by apposition, and the plasmalemma can frequently be seen to be very irregular, with material immediately underneath it of low contrast equivalent with that of material seen in the Golgi vesicles, which are numerous at this stage (Fig. 2). It is probable therefore that the cell wall is built up by a method of Golgi vesicle apposition similar to that demonstrated in the formation of the spiral thickenings of the xylem vessels of sycamore (10).

The plasmodesmata through the young cell wall are at first simple and appear to connect the long profiles of endoplasmic reticulum which lie parallel to the wall at this stage (Fig. 16). They develop on the companion cell side of the thickening wall to give initially a branched plasmodesma which in longitudinal section appears as a Y-shaped structure (Fig. 17). At maturity there are usually two to four plasmodesmata on the companion cell side which can be seen in longitudinal sections to connect *via* a small cavity in the centre of the wall to a single larger pore on the sieve tube side, which is lined with material similar to that lining the sieve plate pores (Fig. 19). Sections across the top of such a pore on the companion cell side (Fig. 20) show that there are eight to fifteen plasmodesmata per single sieve tube pore, the latter being about twice the diameter of each plasmodesma.

The plasmodesmata on the companion cell side occasionally appear to branch or anastomose with each other but they usually form separate channels from the cavity in the centre of the wall with which the sieve tube pore communicates (Figs. 19, 21, and 22).

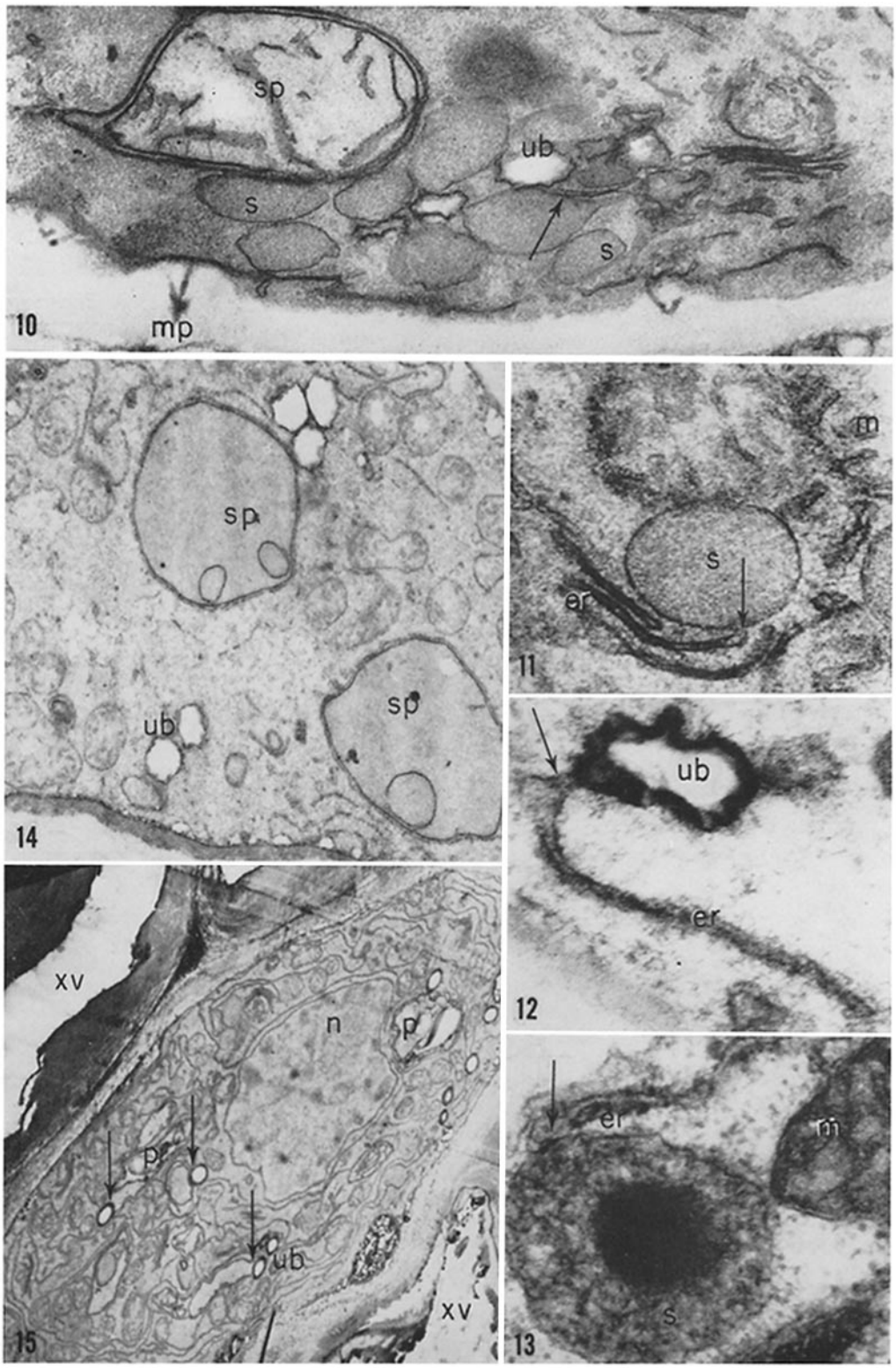
On the companion cell side the plasmodesmata are usually closely associated with either elements of the endoplasmic reticulum or the outer nuclear membrane (Figs. 17 to 19, 21, and 22). If the nucleus is a short distance away from the wall, short profiles of the endoplasmic reticulum can be

FIGURE 10 Mature companion cell. Aggregation of spherosomes, containing small unidentified bodies. Note close association of the endoplasmic reticulum with a spherosome at the arrow. Permanganate fixation. × 30,000.

FIGURES 11, 12, and 13 Close association of the endoplasmic reticulum with a spherosome in Figs. 11 and 13, and with an unidentified body in Fig. 12. Sites of possible connection between the organelles are arrowed. Fig. 13 shows a large inclusion body in the spherosome which appears to have an ordered structure. Fig. 11, permanganate fixation. × 42,000. Fig. 12, permanganate fixation. × 54,000. Fig. 13, glutaraldehyde fixation. × 72,000.

FIGURE 14 *Pinus pinea.* Unidentified bodies in a cell of the resin duct. The plastids are closely sheathed with endoplasmic reticulum and contain little trace of organized membrane structures. Permanganate fixation. × 17,000.

FIGURE 15 *Pinus pinea.* Unidentified bodies in a xylem ray cell, apparently associated with the plastids (at arrows). The cell is packed with mitochondria, endoplasmic reticulum, and Golgi bodies. Permanganate fixation. × 5400.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seen to run from the outer nuclear membrane directly to the plasmodesmata (Fig. 23).

Tenuous links between plasmodesma contents and the membranes immediately above it are occasionally visible (Figs. 18, 19, 22, and 23). On the sieve tube side the areas of material which display a hexagonal or vesicular pattern can be seen closely associated with the pores (Fig. 25). The wall is slightly swollen at the sites of these modified plasmodesmata, and if the thin section does not include a continuous pore the location of the pore may be recognised by the grouped plasmodesmata on the companion cell side which end apparently in mid-cell wall.

In contrast to this specialised pore structure the intercellular connection of companion cell and phloem parenchyma is *via* the usual simple plasmodesma (Fig. 24).

After permanganate fixation the nucleus shows little internal structure; the pores in the outer membrane are simple holes (Fig. 21). The image with glutaraldehyde fixation is, however, more complex. The structures of nucleolus and chromosomes are preserved and the nuclear pores, seen in surface section, show a considerable complexity, equivalent to the structures seen in animal cell nuclei after fixation with osmium tetroxide (Fig. 26).

DISCUSSION

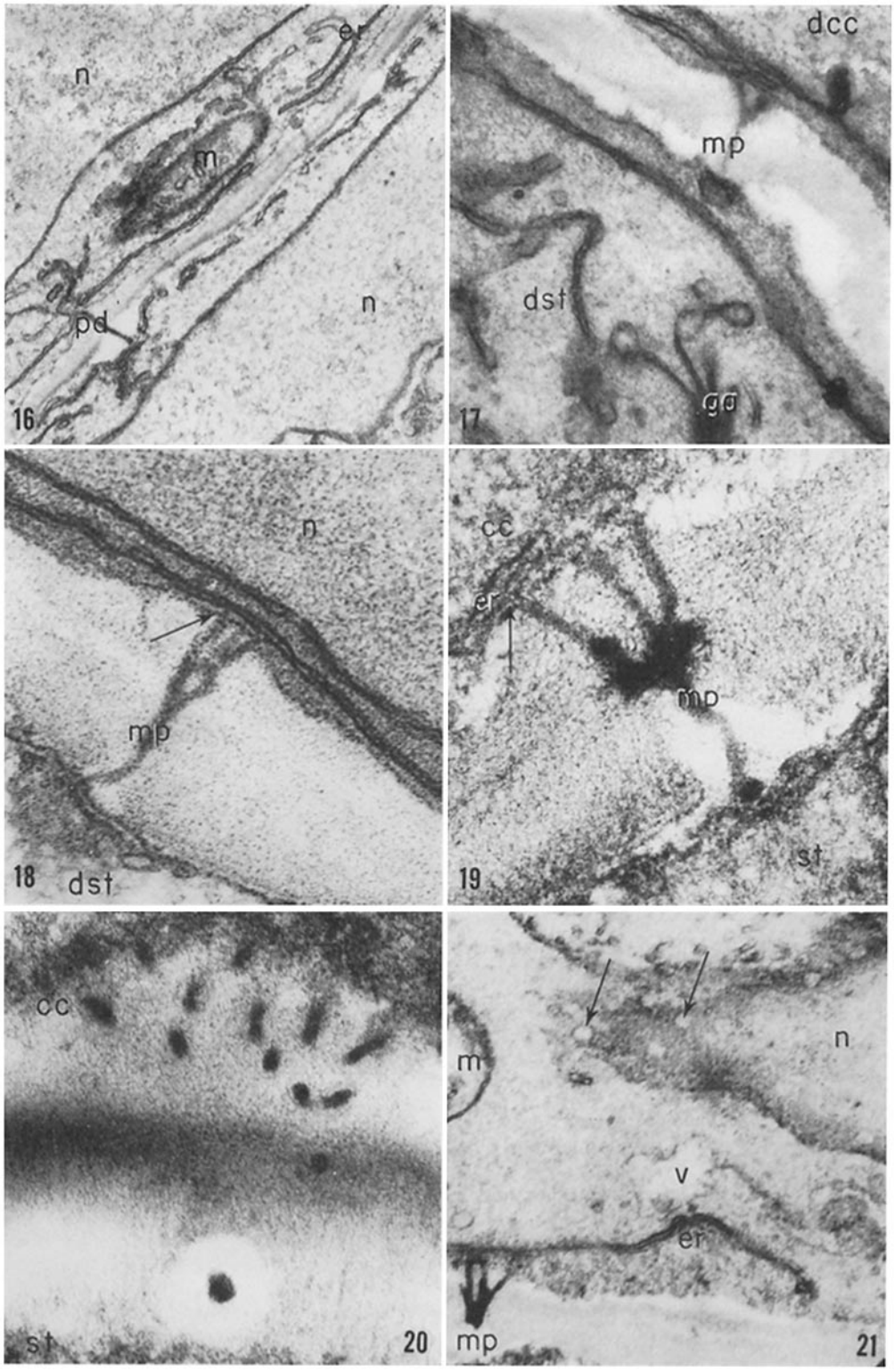
The most notable feature of the companion cell is the dense cytoplasm with its closely packed organelles. The density of the cytoplasm is due mainly to the large numbers of free ribosomes, which indicates that an active protein synthesis occurs in the companion cell. There are numerous mitochondria and plastids present which are sheathed with endoplasmic reticulum. These organelles are thought to play a major role in the carbohydrate metabolism of the cell. The presence of the aggregates of spherosomes suggests that the cell is also capable of high levels of lipid synthesis since according to Frey-Wyssling *et al.* (4) the spherosomes are the plant organelles mainly concerned with this function.

The companion cell would therefore seem well equipped to play the major metabolic role in the maintenance of a fully functional sieve tube transport system. Phloem translocation has been shown to be oxygen dependent and sensitive to metabolic poisons (9) and, since the mature sieve tube contains only a few plastids and a semipermeable limiting membrane, this would suggest that the companion cell either is directly concerned with translocation, as suggested by Spanner (7), or acts to preserve the functional integrity of the sieve tube. The latter action would be to maintain the semipermeability of the sieve tube membrane

FIGURES 16 to 19 Development of the modified plasmodesma between companion cell and sieve tube. Fig. 16 shows a single plasmodesma between two recently divided cambial daughter cells. Fig. 17 demonstrates the initial branching of the pore on the companion cell side. The first indication of development of the white material around the plasmodesma on the sieve tube side can be seen in Fig. 18; this is of greater extent in Fig. 19. These last two figures indicate the increase in number of channels on the companion cell side, and the development of a central cavity (Fig. 19). Endoplasmic reticulum is closely associated with the companion cell side of the pore at all stages of development. Tenuous links between endoplasmic reticulum and one channel of the pore are indicated by an arrow in Figs. 18 and 19. Permanganate fixation. Fig. 16, × 22,000; Fig. 17, × 42,500; Fig. 18, × 53,000; Fig. 19, × 66,000.

FIGURE 20 Surface view of the modified plasmodesma on companion cell side (top) and sieve tube side (bottom). The white "halo" around the latter is obvious, as is the multiplicity of channels comprising the former. The juxtaposition of the surface views is coincidental; the pores on each side in this section do not intercommunicate. This micrograph is a detail from Fig. 25. Permanganate fixation. × 64,000.

FIGURE 21 Association of endoplasmic reticulum and modified plasmodesma. Compare the surface view of the simple nuclear pores (at arrows) with that shown in Fig. 26 of glutaraldehyde-fixed material. Permanganate fixation. × 32,000.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and possibly to produce enzymes such as those which synthesize the massive callose deposits rapidly laid down on the sieve plate after the tissue is wounded.

The only pores between the sieve tube and contiguous cells are the modified plasmodesmata (Fig. 25). These link the sieve tube to the companion cell. Sucrose is the molecule usually transported in the sieve tube, and this may require an active process for its transfer across living cell membranes. It seems likely that the modified plasmodesmata are the route by which the massive amounts of carbohydrate required during the cambial synthesis of secondary tissue in the stem (8) pass to the developing cells from the sieve tube. The sucrose would thus pass out of the sieve tube *via* the companion cells, which do have the usual simple plasmodesmata linking them to adjacent cells. It is improbable that the chloroplasts of the cortical tissues of the stem and the phloem parenchyma can provide sufficient carbohydrate for the local demand, so that, unless the sucrose passes across the sieve tube wall and its associated membranes with no localisation of this transport, the modified plasmodesmata would be the only possible route.

Duloy, Mercer, and Rathgeber (3) have shown similar multiple fine pores on the companion cell side of the wall which separates this cell from the sieve tube in *Cucurbita*. There is a large discontinuity in the wall on the sieve tube side of each pore in *Cucurbita*, which suggests that the wall never becomes thickened at these sites. In sycamore the wall is uniformly thick or even thicker at the position of the pore. It is possible therefore that the single pore on the sieve tube side of the wall is formed secondarily in a manner similar to that in which the sieve plate pores are formed.

A developmental sequence for the pores between sieve tube and companion cell is shown in Figs. 16 to 19.

The special structure of the mature modified plasmodesma may be related to its secretory or absorptive function; or it may be a relic of the evolutionary process. The evolutionary advance is generally considered to have proceeded from the multiple fine channel type of pore found in gymnosperms; *e.g.*, *Metasequoia* (6), to the single wide channel type seen in angiosperms; *e.g.*, *Cucurbita*. This wide channel pore would allow a more efficient translocation through the sieve plates across which the main flow occurs, and the multiple fine channels are presumably sufficient for the limited local absorption or secretion of material between sieve tube and companion cell.

The close sheathing of the plastids by the endo-

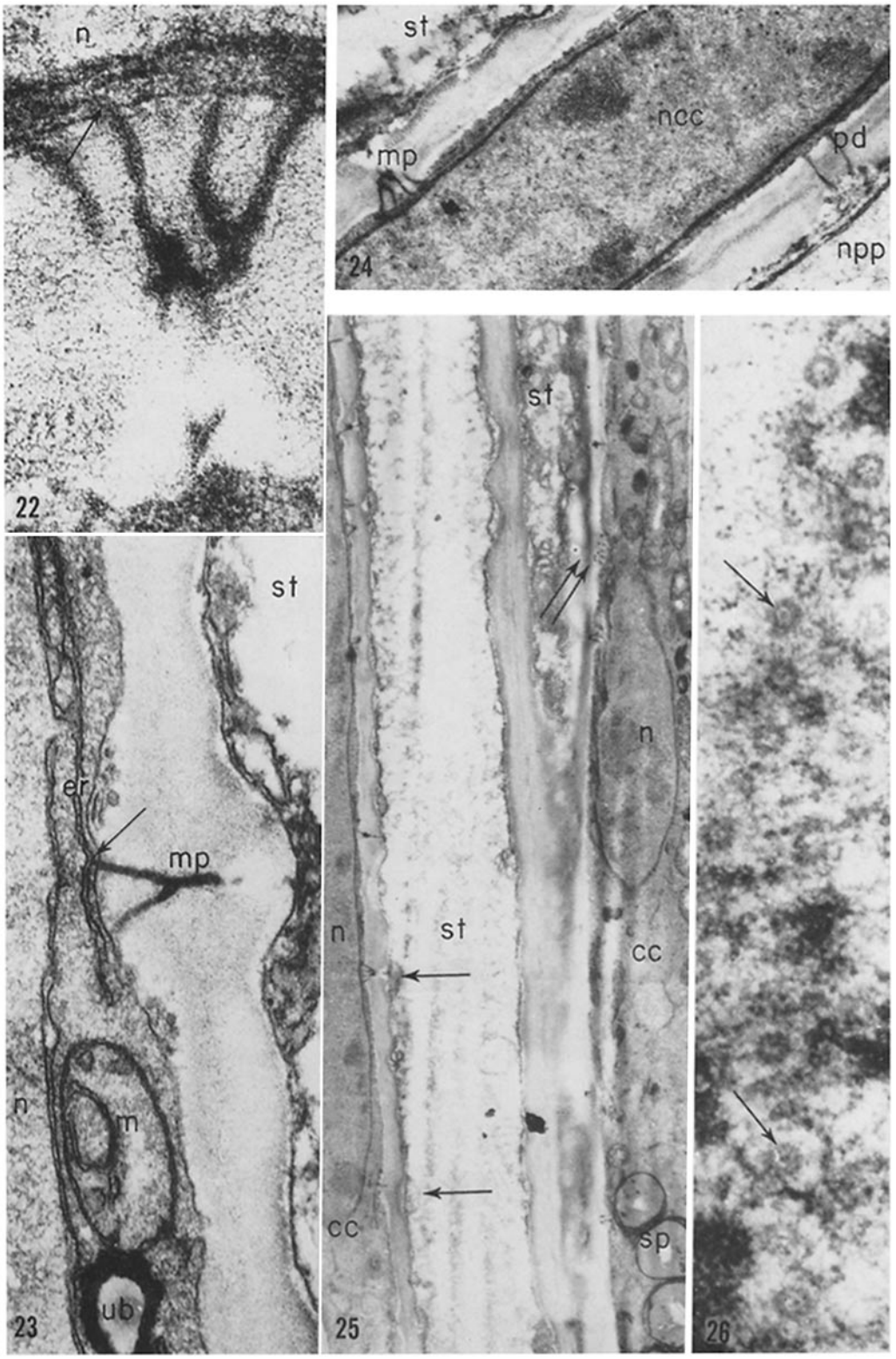
FIGURE 22 Direct association of the nuclear membrane and a modified plasmodesma. A tenuous link between nuclear membrane and one channel of the pore can be seen at the position indicated by the arrow. Permanganate fixation. × 88,000.

FIGURE 23 Connection of a modified plasmodesma to the nuclear membrane by a short profile of endoplasmic reticulum. A tenuous link between endoplasmic reticulum and a single channel of the pore is indicated by the arrow. Permanganate fixation. × 50,000.

FIGURE 24 Mature companion cell. Compare the form of the modified plasmodesma between companion cell and sieve tube with that of the two simple plasmodesmata between companion cell and phloem parenchyma. There is a marked difference in the electron opacity of the companion cell (*n cc*) and phloem parenchyma (*n pp*) nuclei. Permanganate fixation. × 18,500.

FIGURE 25 Mature companion cells and sieve tube cell. Small deposits of material which display a hexagonal pattern at higher magnification can be seen at the sieve tube end of each modified plasmodesma (indicated by arrows). The double arrow indicates the area shown in higher power in Fig. 20. Permanganate fixation. × 6500.

FIGURE 26 Surface view of nuclear membrane. The complex structure of the pores (indicated by arrows), striated annuli with a central granule, contrasts markedly with the simple holes seen after permanganate fixation (Fig. 21). This structure is typical of glutaraldehyde-fixed material and is not specific to the companion cell. Glutaraldehyde fixation. × 54,000.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plasmic reticulum, which is also structurally modified by the lack of ribosomes on the surface adjacent to the plastid, indicates some close functional relationship (Fig. 4). If the endoplasmic reticulum is regarded as an intracellular conduit it would seem likely that the material transported from the sieve tube *via* the modified pores passes directly, *via* the lumen of the endoplasmic reticulum, to the plastid. No direct interconnection of the lumen of the endoplasmic reticulum either with the lumen between the plastid outer membranes or with the central plastid area has yet been observed. Presumably the transfer process, if it occurs, is an active one across the membranes.

Frey-Wyssling *et al.* (4) have proposed that the spherosomes are sites of lipid synthesis, and that structures very similar to the unidentified bodies shown in Fig. 10 are probably fat droplets. The unidentified bodies have also been seen in *Pinus* xylem ray cells and the cells producing the resinous secretion of that tree. The xylem ray cells have a transport and maintenance function for the living tissues internal to the xylem, and the resin canal cells are very active synthetically. Buttrose (1) has also demonstrated the occurrence of similar bodies in the aleurone cells of wheat grain. He suggests that they may be a non-starchy carbohydrate reserve, and he claims that they are not lipid because of the way they react with osmium tetroxide. However, in the companion cell they are associated with the spherosomes, and this would suggest that they may have some relationship to lipid metabolism. Unfortunately they have not yet been observed with glutaraldehyde-osmium tetroxide fixation.

The relationship of Golgi bodies, Golgi vesicles, and thickening cell walls, and the apparent incorporation of vesicular material during the secondary cell wall growth would indicate that in this tissue, as in the xylem (10), the apposition to the material of the cell wall depends upon the activities of the Golgi bodies.

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REFERENCES

1. BUTTROSE, M. S., Ultrastructure in the developing aleurone cells of wheat germ, *Australian J. Biol. Sc.*, 1963, **16**, 768.
2. CRAFTS, A. S., and CURRIER, H. B., On Sieve tube function, Protoplasma, 1963, **57**, 188.
3. DULOY, M., MERCER, F. V., and RATHGEBER, N., Studies in translocation. II. Submicroscopic structure of the phloem, *Australian J. Biol. Sc.*, 1961, **14**, 506.
4. FREY-WYSSLING, A., GRIESHABER, E., and MUHLETHALER, K., The origin of spherosomes in plant cells, *J. Ultrastruct. Research*, 1963, **8**, 506.
5. EAMES, A. J., and MACDANIELS, L. H. In An introduction to Plant Anatomy, London, McGraw Hill Publishing Co. Ltd., 1951.
6. KOLLMAN, R., and SCHUMACHER, W., Uber die Feinstruktur des Phloems von Metasequoia glyptostroboides und seine jahreszeitlichen Veranderungen. IV. Weitere Beobachtungen zum Feinbau der Plasmabrucken in den Siebzellen, *Planta*, 1963, **60**, 360.
7. SPANNER, D. C., The Translocation of sugar in sieve tubes, *J. Exp. Bot.*, 1958, **9**, 332.
8. THORNBER, J. P., and NORTHCOTE, D. H., Changes in the chemical composition of a cambial cell during its differentiation into xylem and phloem tissue in trees, *Biochem. J.*, 1961, **81**, 449, 455.
9. ULLRICH, W., Zur Sauerstoffabhangigkeit des Transportes in den Siebrohren, *Planta*, 1961, **57**, 402.
10. WOODING, F. B. P., and NORTHCOTE, D. H., The development of the secondary wall of the xylem in *Acer pseudoplatanus*, *J. Cell. Biol.*, 1964, **23**, 327.