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Electron Microscope Investigation of Sieve-Element Ontogeny and Structure in *Ulmus americana*

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With 32 Figures

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Summary

During advanced stages of sieve-element differentiation in *Ulmus americana* L., dispersal of the P-protein (slime) bodies results in formation of a peripheral network of strands consisting of aggregates of P-protein components having a striated, fibrillar appearance. The tonoplast is present throughout the period of P-protein body dispersal. Perforation of the sieve plates is initiated during early stages of P-protein body dispersal.

Small P-protein bodies consist of tubular components, most of which measure about 180 Å in diameter. With increase in size of the P-protein bodies narrower components appear. At the time of initiation of P-protein body dispersal, most of the components comprising the bodies are of relatively narrow diameters (most 130–140 Å) and have a striated, fibrillar appearance. Both wide and narrow P-protein components are present throughout the period of sieve-element differentiation and in the mature cell as well, and a complete intergradation in size and appearance exists between the two extremes. Both extremes of P-protein component have a similar substructure: an electron-transparent lumen and an electron-opaque wall composed of subunits, apparently in helical arrangement. The distribution of P protein in mature sieve elements was quite variable.

The parietal layer of cytoplasm in mature *Ulmus* sieve elements consists of plasmalemma, endoplasmic reticulum cisternae in two forms (as a complex network closely applied to the plasmalemma and in stacks along the wall), mitochondria, and plastids.

1. Introduction

In an earlier study (EVERT *et al.* 1969), the ontogeny and structure of the sieve elements in *Ulmus americana* were considered in detail at the light microscope level. The present article deals with the results of a similar investigation at the ultrastructural level.

2. Materials and Methods

The materials used in this investigation were obtained from the trunks of apparently healthy *Ulmus americana* L. trees 15 or more years old growing in the Eagle Heights area of the University of Wisconsin campus and in the University of Wisconsin Arboretum at Madison,

during spring and summer of 1967 and 1968. The method of obtaining tissues was similar to that of the light microscope study (EVERT *et al.* 1969). Upon removal from the tree, bark samples about 8 cm long and 4 cm wide were immersed in fixative. Soon afterward most of the outer bark was removed from each sample with a sharp razor and discarded. The remaining tissues were cut radially into longitudinal strips and the median part of each strip was diced into pieces about 2 mm square for further processing. Throughout this procedure the tissues were continuously flooded with fixative. Some tissues were fixed in 6% glutaraldehyde and postfixed in 2% osmium tetroxide, others were fixed in glutaraldehyde-formaldehyde and postfixed in 2% osmium tetroxide (KARNOVSKY 1965). Similar results were obtained with both fixatives. All tissues were dehydrated in acetone and embedded in Araldite-Epon. Sections were cut on a Sorvall MT-2 ultramicrotome with diamond knives, stained with uranyl acetate and lead citrate, and viewed and photographed with a Hitachi HU-11 C microscope.

In the present article, the term P protein is used to refer to sieve-element slime, as proposed by ESAU and CRONSHAW (1967), except where use of the term slime was judged to be preferable.

3. Observations

3.1. The P Protein

At the light microscope level, the P protein in elm is first apparent in the cytoplasm as distinct, amorphous bodies. As far as could be detected, at the ultrastructural level, the P-protein material is first discernible as small groups of parallel tubules. The tubules in such groups range in diameter from 170 to 230 Å, the diameters of most measuring about 180 Å. With continued development, progressively more tubules are added to the previously-formed groups and this results in formation of the P-protein bodies (Fig. 1). As the P-protein bodies increase in size, more and more relatively narrow tubules are encountered within them, so that, as the bodies approach full size they consist proportionately of narrower tubules than younger bodies. For example, in one large, apparently nearly fully-formed P-protein body the tubules ranged in diameter from 145 to 220 Å, with most measuring less than 180 Å in diameter.

The tubular nature of the substance comprising the P-protein bodies is apparent in Figs. 1, 3, and 31, each tubule consisting of an electron-transparent lumen and an electron-opaque wall. The tubular nature of the P protein is less apparent in the P-protein body of Fig. 4, where some of the components (especially some in the upper part of the body) have a more or less fibrillar appearance.

At the initiation of P-protein body dispersal most of the components of the bodies have the appearance of conspicuously banded or striated fibrils and range in diameter from 90 to 170 Å. However most measure between 130 and 140 Å in diameter. During P-protein body dispersal the P-protein components form aggregates which then spread throughout the parietal layer of cytoplasm and give rise to a complex, three-dimensional network. Fig. 5 shows P-protein bodies in early stages of dispersal and Figs. 2 and 7 show

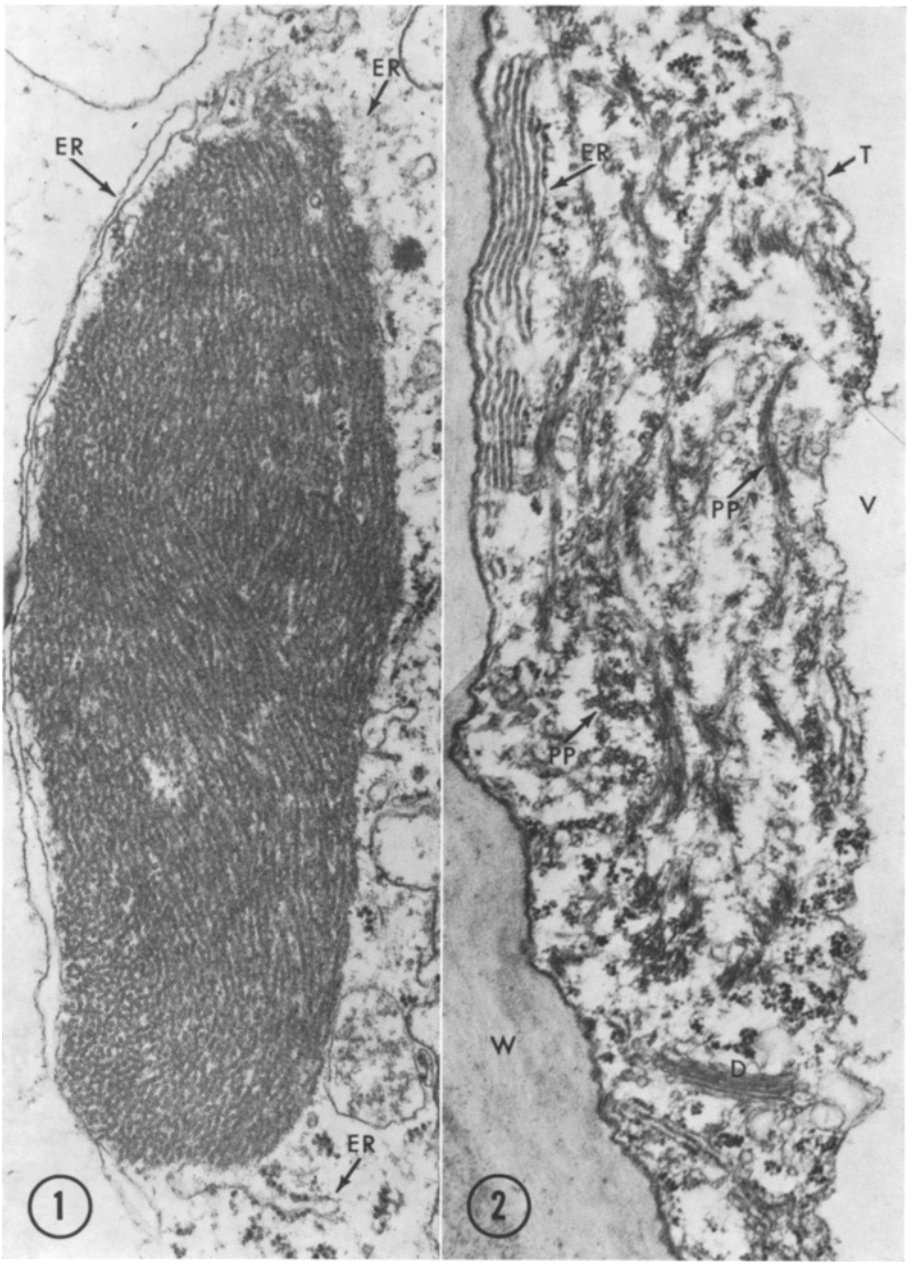

Fig. 1. Longitudinal section of young sieve element showing a P-protein body. Tubular components comprising the body are seen in both longitudinal and transverse views. Some cisternae of endoplasmic reticulum (*ER*) closely parallel the body. ×32,500

Fig. 2. Transection of differentiating sieve element showing network composed of aggregates of striated, fibrillar P-protein components (*PP*). The free surface of the orderly stack of endoplasmic reticulum cisternae (*ER*) is still associated with ribosomes. *D* = dictyosome, *T* = tonoplast, *V* = vacuole, *W* = wall. ×27,000

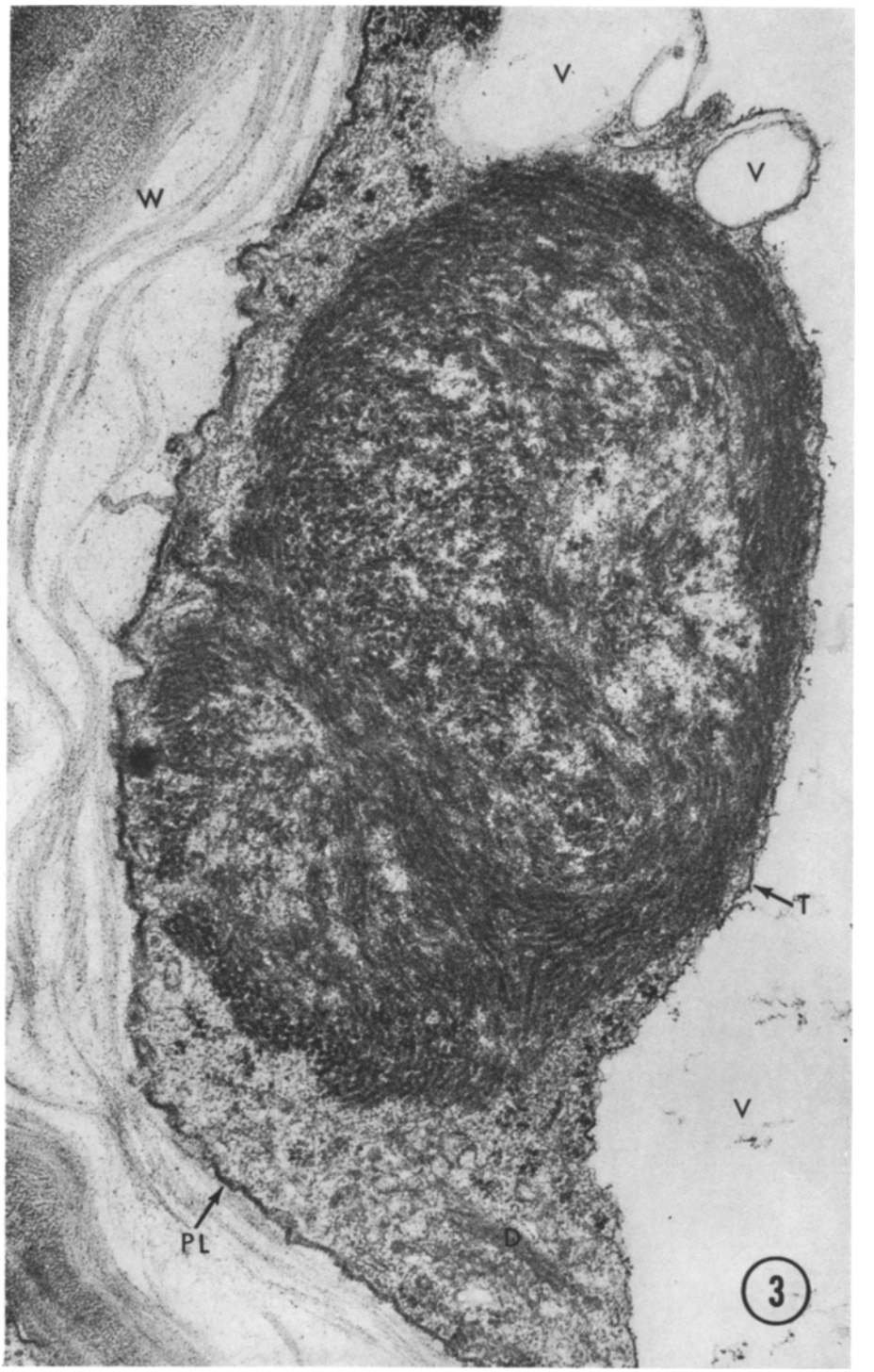

Fig. 3. Transection of differentiating sieve element showing P-protein body composed mostly of tubular components. Portion of large central vacuole is at right, and two smaller vacuoles are seen in parietal cytoplasm. Inner wall layer has loosely lamellate appearance. *D* = dictyosome, *PL* = plasmalemma, *T* = tonoplast, *V* = vacuole, *W* = wall. ×38,000

advanced stages in formation of the network. The P-protein aggregates, or "strands", in Figs. 2 and 7 are composed of P-protein components that have a striated, fibrillar appearance.

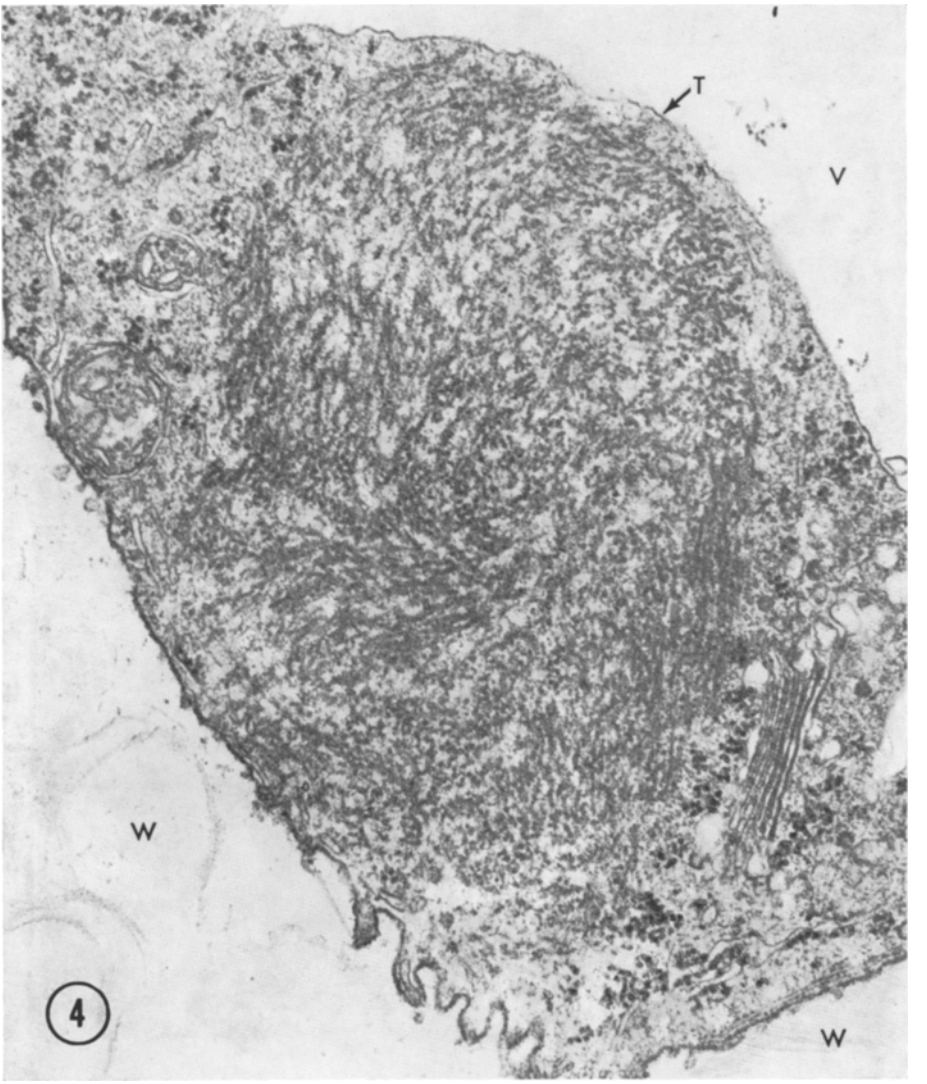

Fig. 4. Transection of differentiating sieve element showing P-protein body composed of tubular and fibrillar components. The surrounding cytoplasm contains organelles and membrane systems typical of sieve elements at this stage of development. *T* = tonoplast, *V* = vacuole, *W* = wall. ×35,500

During the light microscope study, two "types" of P-protein bodies were distinguished in the sieve elements of elm on the basis of differences in (1) the time of their initial appearance within the cytoplasm, (2) their ultimate

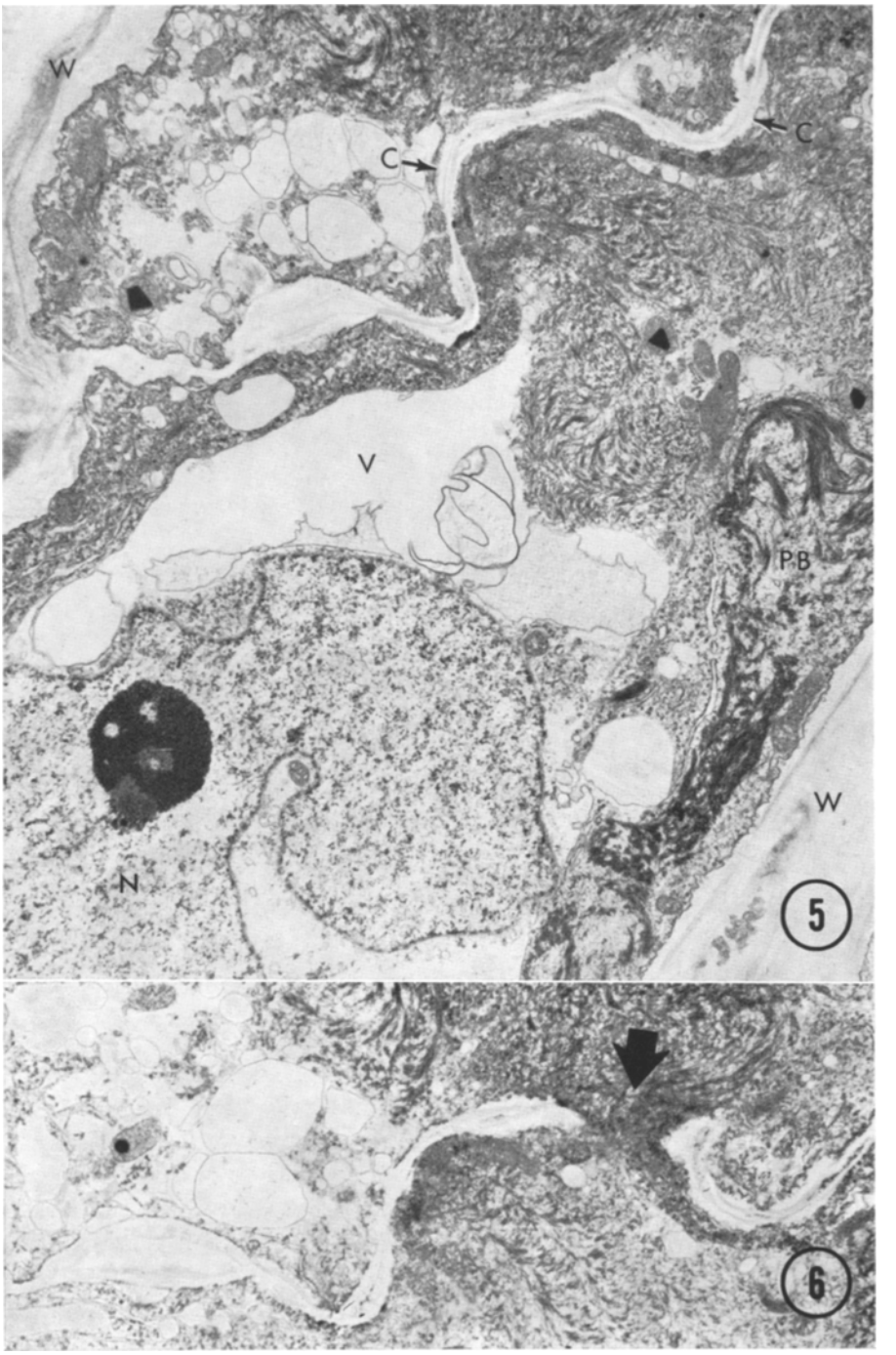

Fig. 5. Longitudinal section through two differentiating sieve elements and developing sieve plate. Callose platelets (C) mark sites of future sieve-plate pores. P-protein bodies (*PB*) are in early stages of dispersal. *N* = nucleus, *V* = vacuole, *W* = wall. ×7,500

Fig. 6. Longitudinal section through same differentiating sieve elements and sieve plate shown in Fig. 5, but from different section of series. Here, one of the pore sites is already perforated (arrow). ×9,000

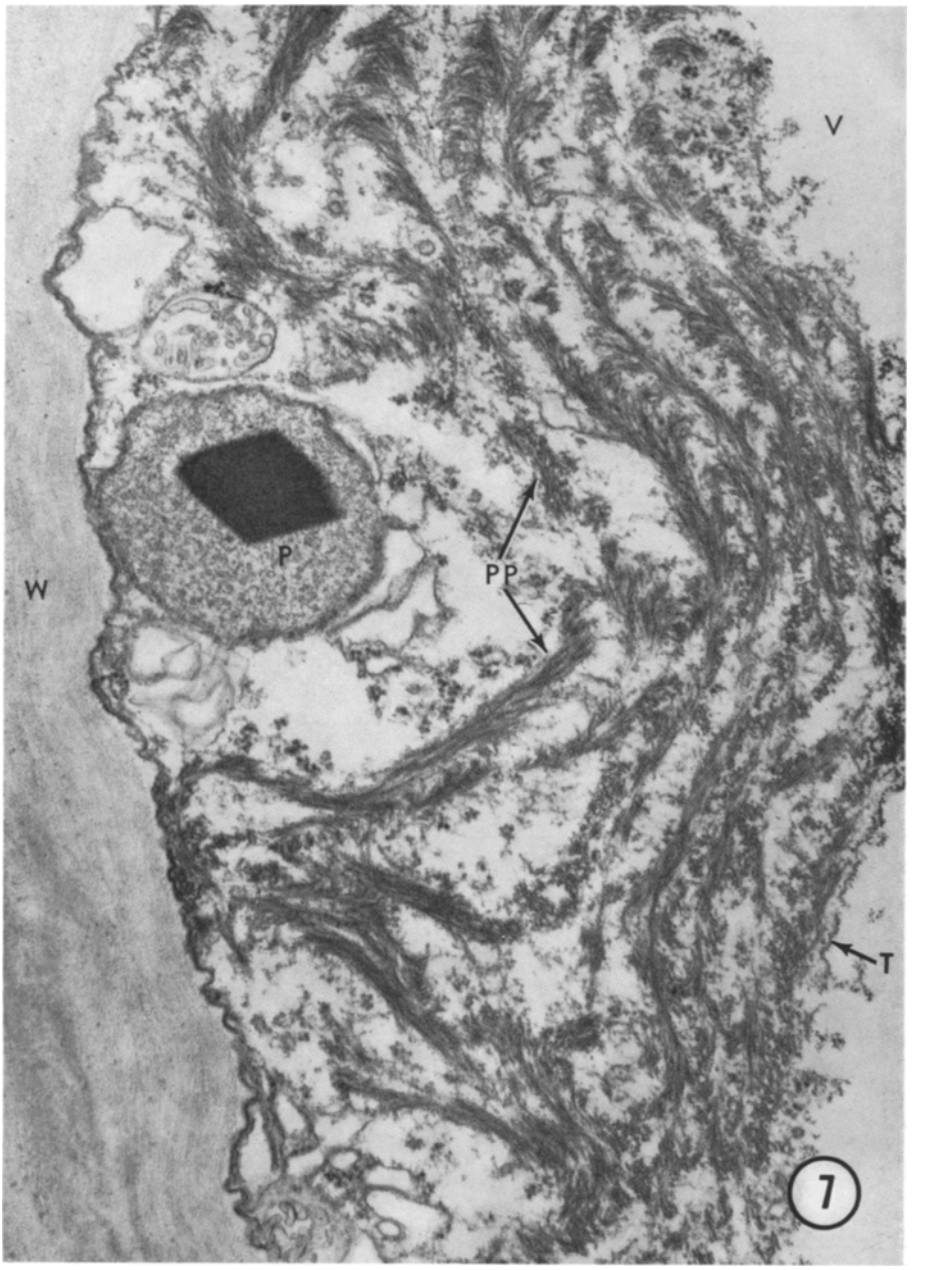

Fig. 7. Transection of differentiating sieve element showing network composed of aggregates of striated, fibrillar P-protein components (*PP*). *P* = plastid, *T* = tonoplast, *V* = vacuole, *W* = wall. ×37,000

size, and (3) the time of initiation of their dispersal. The first P-protein body (sometimes the first two) that appears in each sieve element becomes much larger than numerous, subsequently-formed P-protein bodies, and undergoes dispersal after dispersal of the small bodies is well underway. Both P-protein bodies give similar reactions to various cytochemical stains, viz., positive reactions for protein and negative reactions for lipids and carbohydrates.

During the present investigation, no difference was apparent in the composition of the two bodies. As with the small bodies, the large bodies consist of tubular components of varying diameters. During dispersal of the large bodies the central regions of such bodies were found to consist of relatively wide components ranging in diameter from 185 to 225 Å, while the aggregated striated components of the same bodies measured mostly 130 to 140 Å in diameter. A single large P-protein body in early stages of dispersal is shown in Fig. 8, and part of a second dispersing large body is shown at greater magnification in Fig. 9. The area on the right in Fig. 9 is sectioned through the center of the body.

Throughout the period of P-protein body development and dispersal described above the parietal layer of cytoplasm is clearly delimited from the large central vacuole by the tonoplast (Figs. 2–4, 5–8, 10, and 11). However, as is often the case with highly vacuolate parenchyma cells, some sieve elements were encountered in all stages of development in which the tonoplast had been ruptured during manipulation and fixation of the tissue. In these sieve elements, P-protein bodies and aggregates, or strands, of fibrillar components often disaggregate when exposed to the contents of the vacuoles, the individual components scattering throughout the cell. Other immature sieve elements were encountered in which parts of the tonoplast had become separated from the parietal layer of cytoplasm (Fig. 11), a phenomenon frequently observed during the light microscope study and of value in determining the presence of the tonoplast at that level of resolution.

During the light microscope investigation, the question was raised as to whether the tonoplast normally disappears from the sieve element as the latter approaches maturity, for many mature sieve elements were found with a membrane-like structure delimiting the parietal layer of cytoplasm, including its network of slime strands, from the vacuolar region of the cell. This question is obviously intimately related to the problem of the normal distribution and form of P protein within the mature sieve element.

Fig. 12 shows a sieve element in which P-protein body dispersal is obviously completed and in which the tonoplast, shown at higher magnification in Fig. 13, is still intact. Judging from the overall appearance of this cell, perforation of its sieve plates, which was not observed, must either have been completed or have been near completion. This sieve element lay near the cambium and its sieve plates were probably at most only recently perforated.

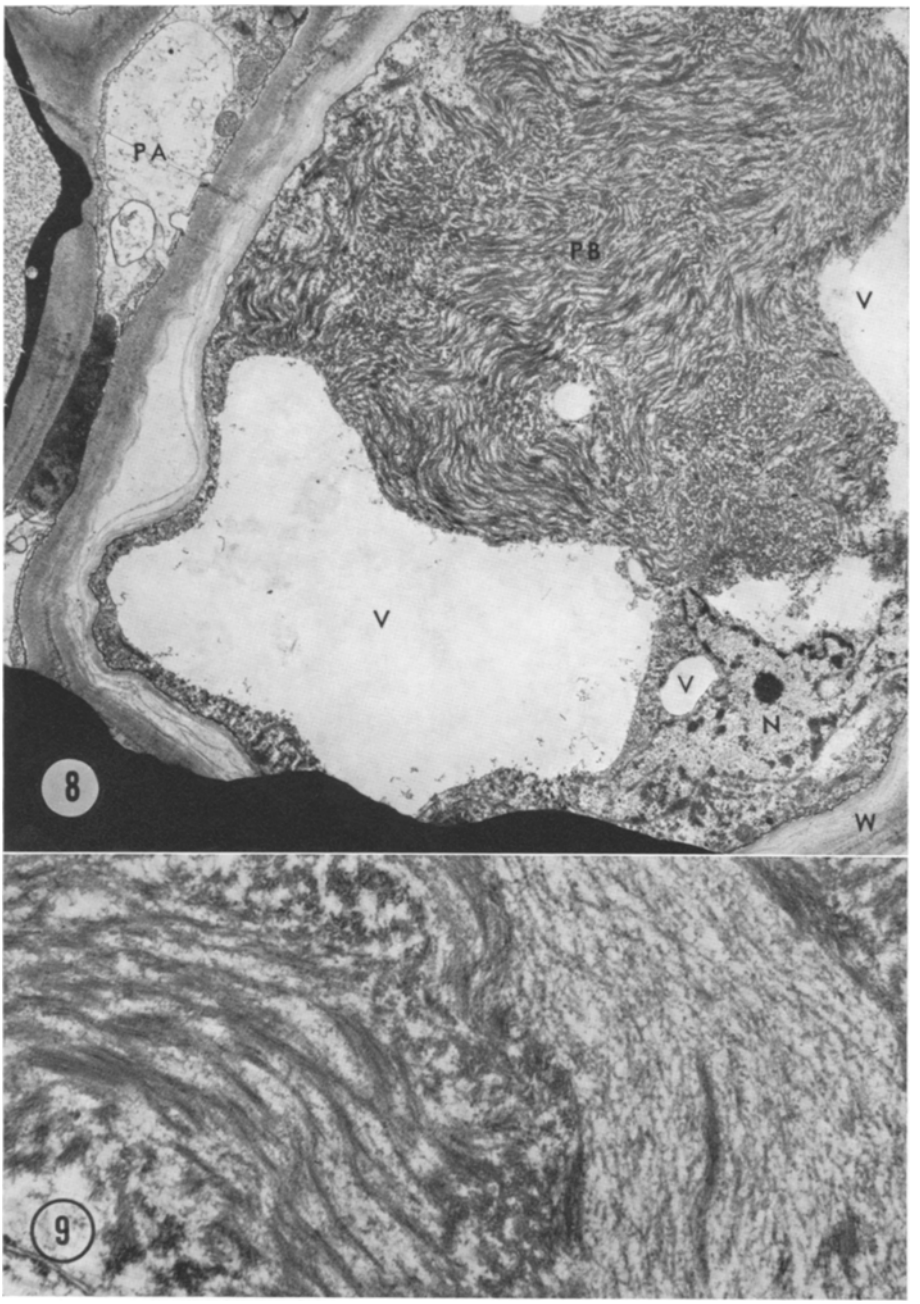

Fig. 8. Transection of differentiating sieve element showing nucleus (*N*) and a large P-protein body (*PB*) in early stages of dispersal. *PA* = parenchyma cell, *V* = vacuole, *W* = wall. ×7,000

Fig. 9. Transection showing part of large P-protein body undergoing dispersal. Aggregates of narrow striated, fibrillar P-protein components are shown on the left, mostly wider P-protein components from central region of the body on the right. ×22,000

Several sieve elements, similar to that in Fig. 14, were encountered during the present investigation in which the P protein was entirely peripheral in distribution and in which one-half to three-quarters of the peripheral contents were still delimited from the vacuole by the tonoplast. Judging from the

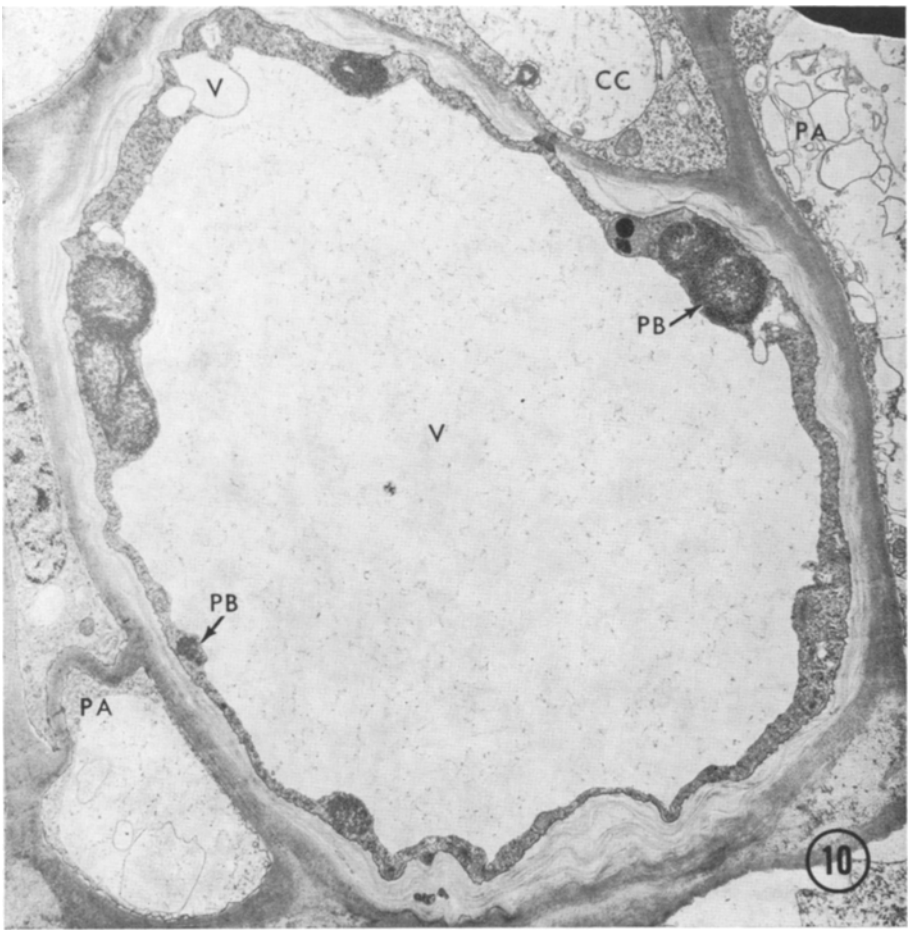

Fig. 10. Transection of differentiating sieve element showing parietal layer of cytoplasm, with several P-protein bodies (*PB*), surrounding large central vacuole. The inner wall layer of sieve element has loosely lamellate appearance. *CC* = companion cell, *PA* = parenchyma cell, *V* = vacuole. ×5,500

distance of these sieve elements from the cambium they were probably fully-differentiated elements. However, as in the case of the sieve element in Fig. 12, none of their sieve plates was observed. Part of the peripheral network of P-protein strands is still in evidence in the sieve element in Fig. 14.

Variation in the distribution of the P protein of mature sieve elements examined during the present investigation was almost as great as that en-

countered during the light microscope study. In transverse sections some mature sieve elements were almost devoid of P protein, while others were almost filled with the substance. It is likely that both sieve elements con-

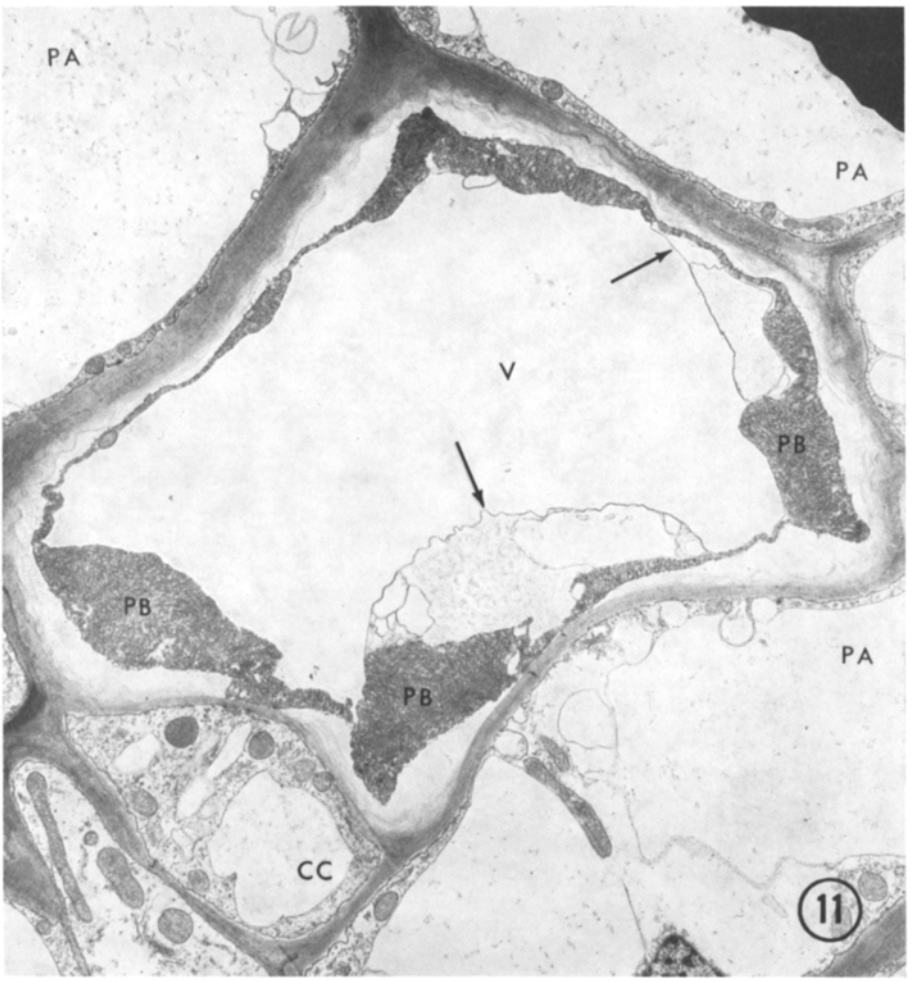

Fig. 11. Transection of differentiating sieve element showing parietal layer of cytoplasm with three full-size P-protein bodies (*PB*). At places (arrows) the tonoplast has separated from the cytoplasm. *CC* = companion cell, *PA* = parenchyma cell, *V* = vacuole. ×4,000

tained slime plugs, the elements devoid of P protein being sectioned some distance from the plugs, those filled with P protein very near or through the plugs. Portions of slime plugs from longitudinal sections are shown in Figs. 15, 20, and 23. In Fig. 23, the P-protein components are mostly randomly distributed. However, in Figs. 15 and 20, many of the P-protein components are arranged in either loose (Fig. 15) or relatively compact

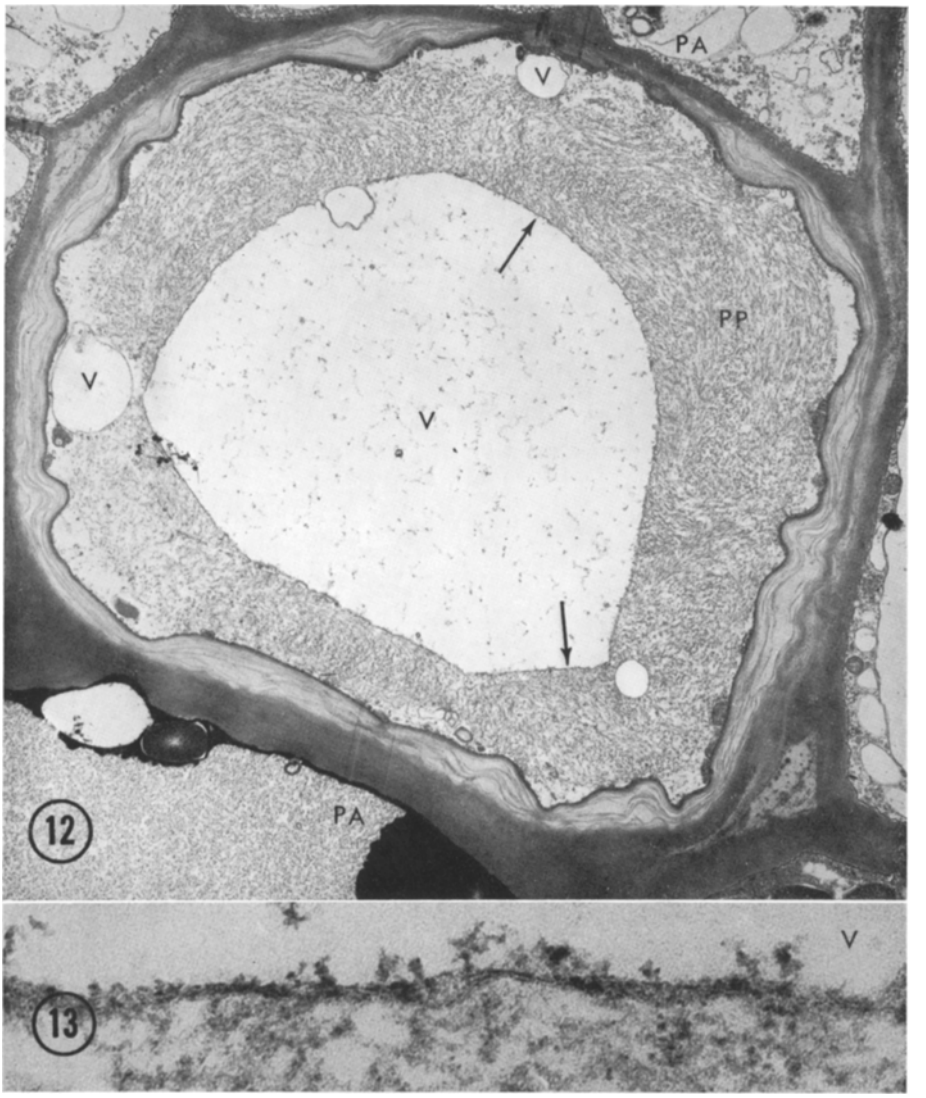

Fig. 12. Transection of sieve element in which P-protein body dispersal is obviously completed and in which the tonoplast (arrows) is still intact. Inner layer of sieve element wall has loosely lamellate appearance. *PA* = parenchyma cell, *PP* = P protein, *V* = vacuole. ×6,000

Fig. 13. Portion of tonoplast of sieve element in Fig. 12 at higher magnification. *V* = vacuole. ×110,500

(Fig. 20) aggregates. In some sieve elements the P protein was more or less evenly distributed throughout the lumina, having the appearance of a very fine network (Fig. 19).

During the light microscope investigation many of the sieve elements encoun-

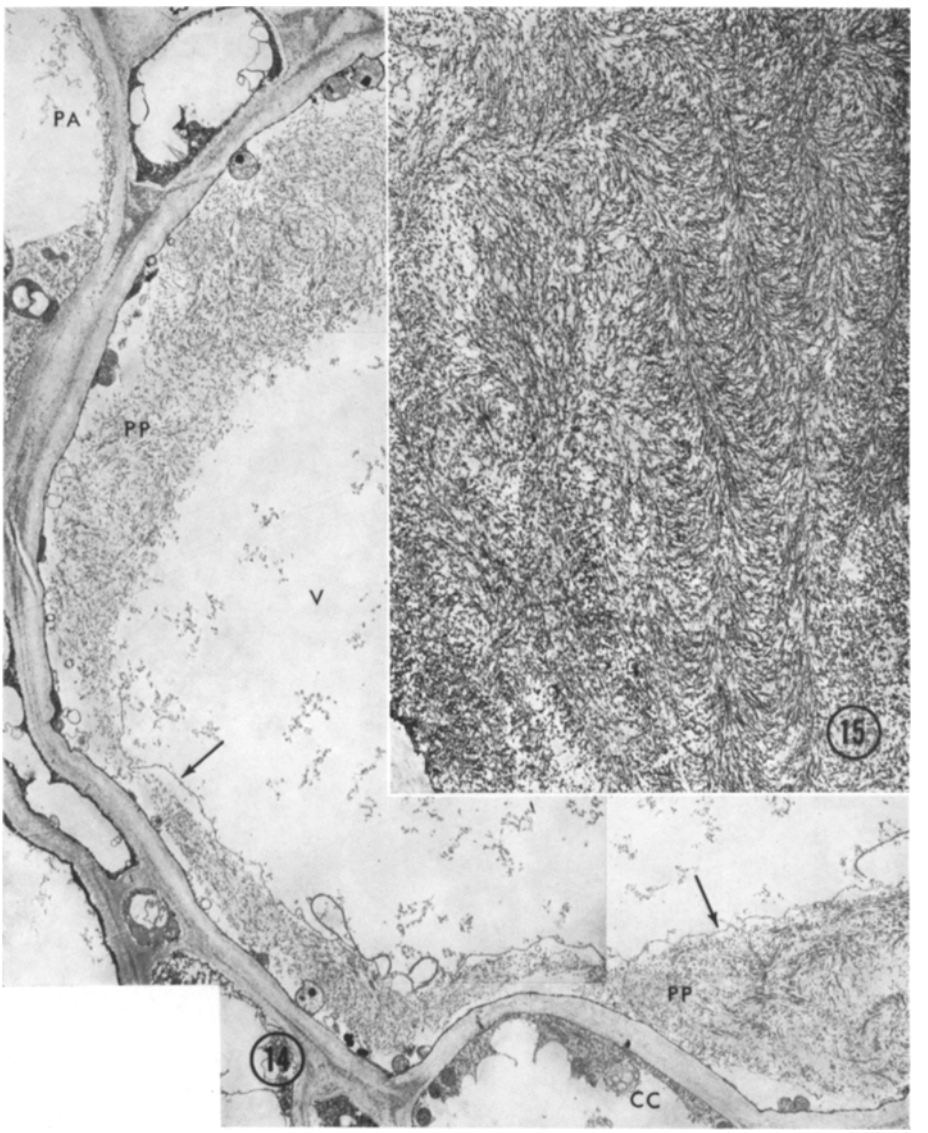

Fig. 14. Transection of portion of likely mature sieve element with parietal layer of cytoplasm and P protein (*PP*) still partly separated from large central vacuole (*V*) by tonoplast (arrows). Some of the P-protein components are still in aggregates. *CC* = companion cell, *PA* = parenchyma cell. ×4,000

Fig. 15. Longitudinal view of portion of slime (P-protein) plug composed of fibrillar components, many of which are in loose aggregates. ×8,500

tered contained slime sacs, saclike protrusions of P protein that extend through the pores of the sieve elements. Some slime sacs appeared to be delimited by a membrane-like structure. Fig. 16 shows several obliquely-sectioned slime

sacs that extended through pores of the sieve plate from the upper element into the lower one. Two of the slime sacs seem to be delimited by a membrane (arrows). Figs. 17 and 18 reveal the nature of the membrane-like structures

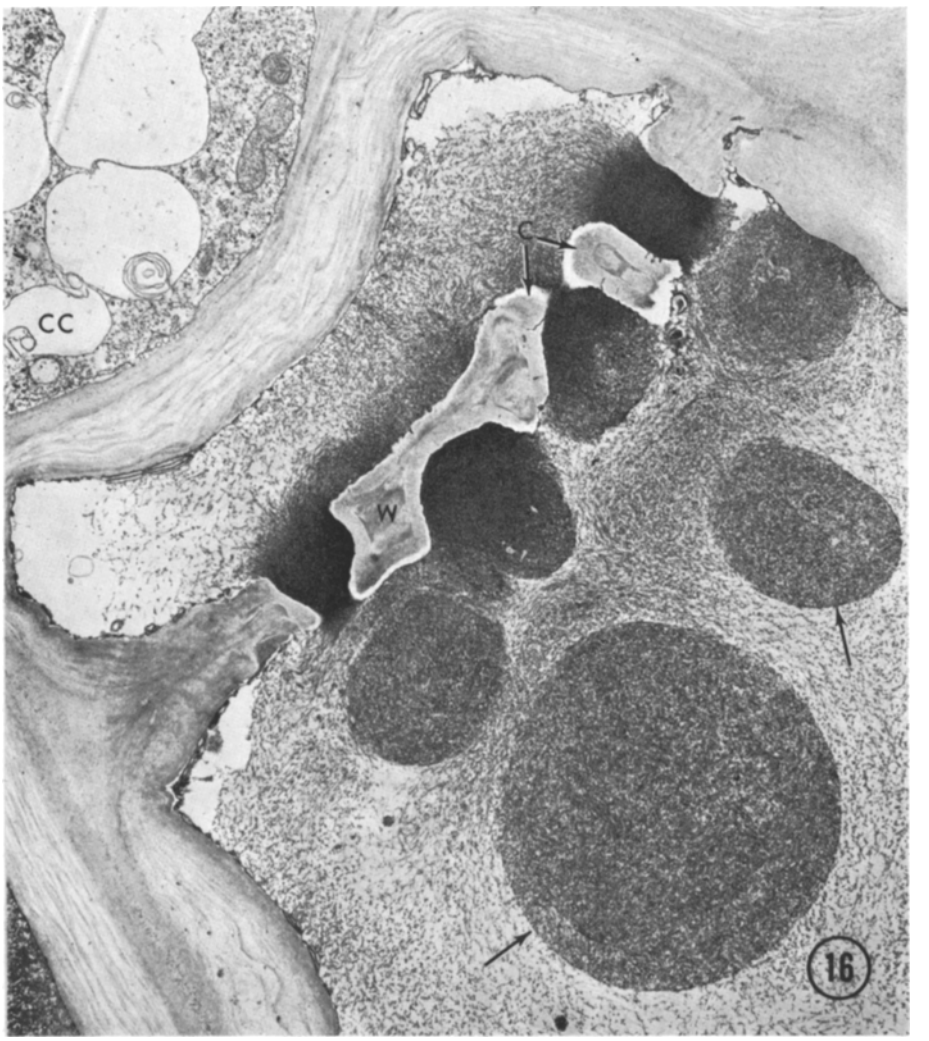

Fig. 16. Oblique section of portions of two sieve elements and a sieve plate. The lower sieve element contains several obliquely-sectioned slime (P-protein) sacs, two of which seem to be delimited by membranes (arrows). *C* = cellulose, *CC* = companion cell, *W* = wall. ×7,000

delimiting the slime sacs in elm sieve elements. They are not membranes, but aggregations of compactly interwoven P-protein components.

It is pertinent to point out that the P-protein components encountered in mature sieve elements vary considerably in size. Some are as wide as the

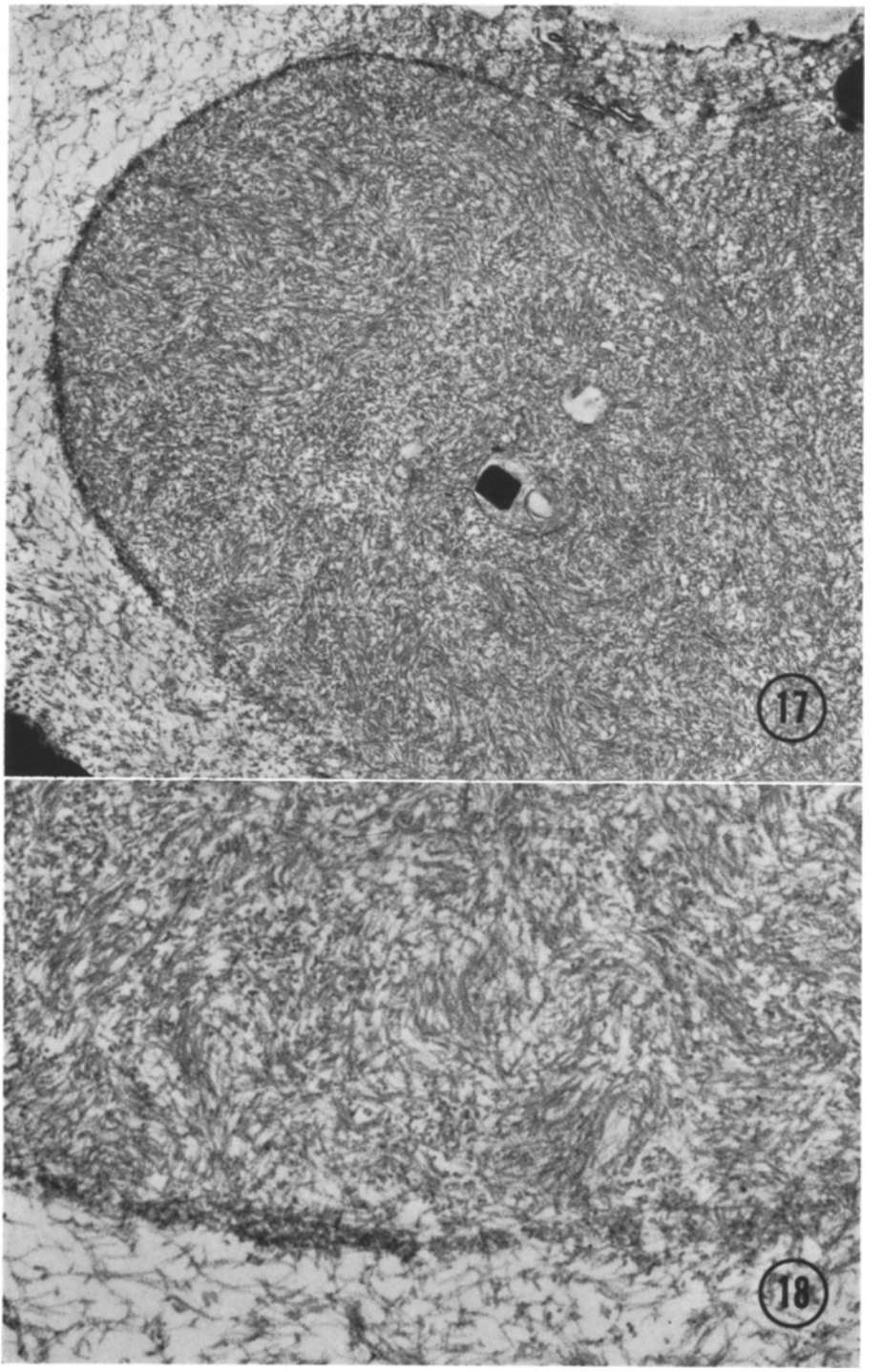

Fig. 17. View of obliquely-sectioned slime (P-protein) sac partly delimited by aggregation of compactly interwoven P-protein components. ×16,000

Fig. 18. Portion of slime sac of Fig. 17 at higher magnification. The P-protein components comprising the sac have a striated, fibrillar appearance. ×43,500

widest tubules encountered in developing P-protein bodies. Most are of relatively narrow diameters and generally have either a striated or beaded appearance (Figs. 18, 20, and 23). In mature sieve elements, the wider components are almost always encountered in groups or aggregates (Figs. 21 and 22), the narrower ones sometimes in aggregates (Figs. 15 and 20), but most often randomly arranged (Figs. 18 and 23). In some mature elements, both wide and narrow P-protein components have a decidedly tubular appearance (Figs. 22 and 23, respectively) and exhibit some evidence of substructure in their walls, especially as seen in transection.

Evidence of substructure in the walls of the P-protein components in elm can also be found in immature sieve elements, especially with the larger components. Figs. 24 and 25 show, at greater magnifications, transverse and longitudinal views of such components from the slime body of Fig. 1. The subunits are apparent in both views. In transection, the walls of the tubules seem to consist of six to eight subunits (see insert, Fig. 24). In longitudinal views (turn Fig. 25 at right angle for proper orientation), the tubules appear to be crossbanded, the crossbands being obliquely oriented. This pattern suggests that the tubules have a helical structure. Sometimes the narrow components have a decidedly helical appearance in longitudinal views (Fig. 22, arrows) with a center-to-center (repeat) distance between gyres of from 50 to 75 Å. These distances are quite similar to the center-to-center distances between striations of P-protein components of similar diameters, the latter distances ranging from 50 to 70 Å. It was not possible to measure with accuracy the center-to-center distances between crossbands of the larger components.

3.2. Organelles and Membrane Systems

Initially, the nucleate sieve-element protoplast of elm is similar in appearance to that of other derivatives of the vascular cambium. However, during the period of P-protein body development and early stages of dispersal the cytoplasmic ground substance is generally quite dense in appearance, compared with that of contiguous parenchymatous elements (Figs. 10 and 11). The cytoplasmic ground substance contains numerous free ribosomes and many cisternae of mostly rough-surfaced endoplasmic reticulum (Fig. 4). Mitochondria, plastids, dictyosomes, which give rise to numerous coated

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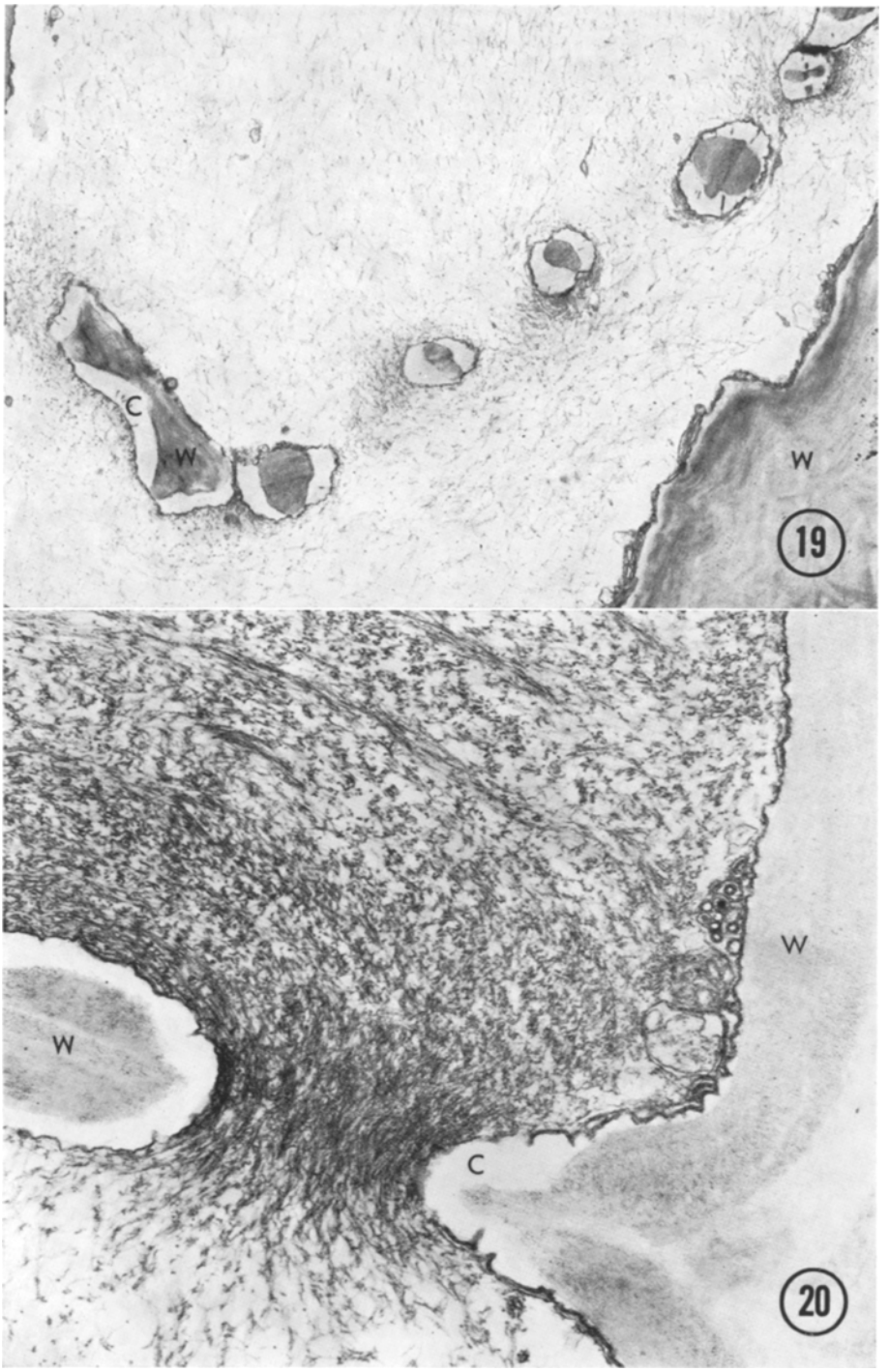
Fig. 19. Longitudinal section of portions of two sieve elements and a sieve plate, with more or less evenly distributed P protein. Part of network of endoplasmic reticulum membranes can be seen bordering the sieve-element wall on the right. *C* = callose, *W* = wall. ×23,500

Fig. 20. Longitudinal section of portion of slime (P-protein) plug and sieve plate. Some P-protein components of plug are aggregated into strands, and the sieve-plate pore is occluded with striated, fibrillar-appearing components. *CC* = companion cell, *W* = wall. ×22,500



Figs. 19 and 20

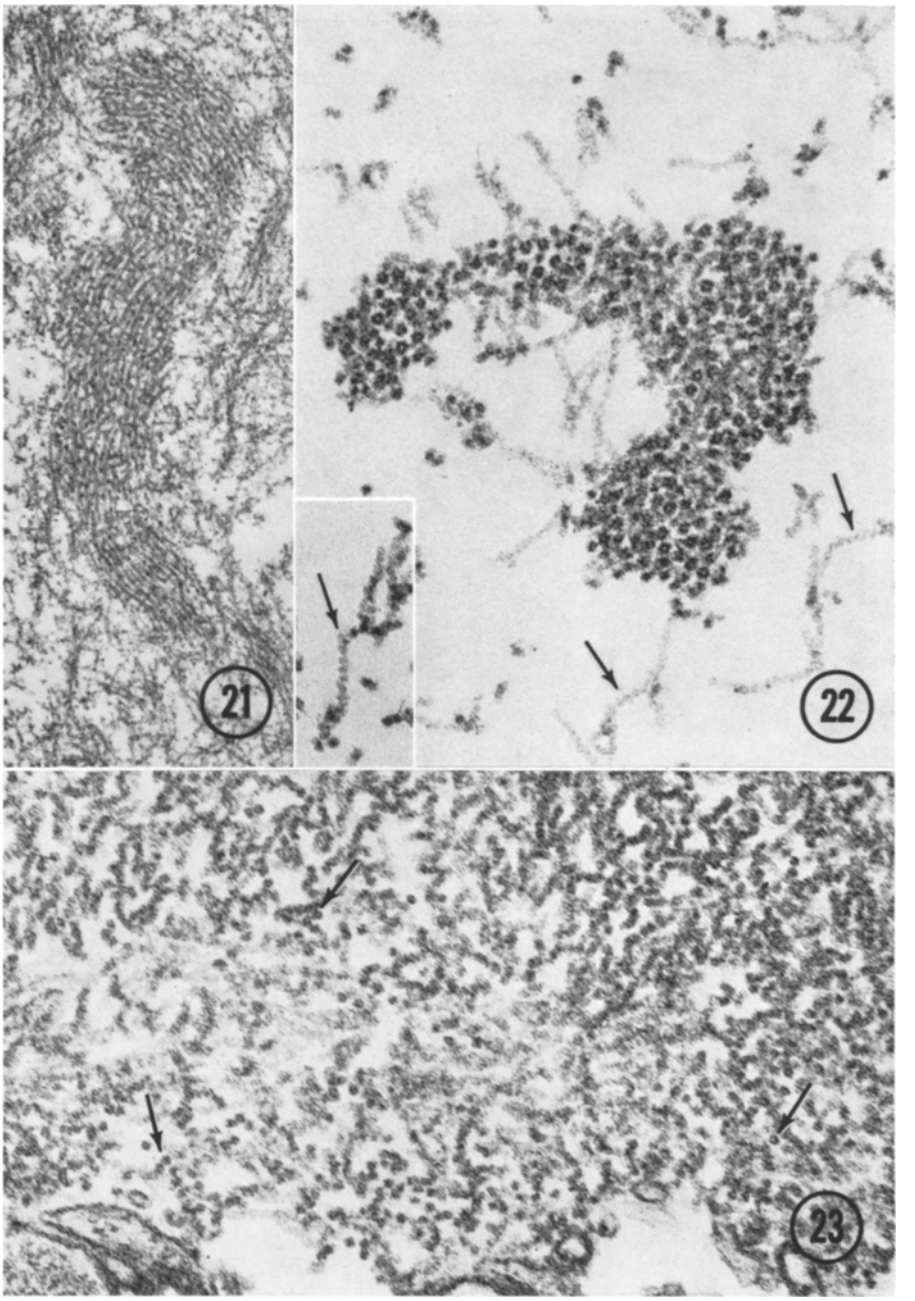


Fig. 21. Longitudinal view of aggregate of wide, tubular P-protein components in mature sieve element. ×35,500

Fig. 22. Transverse view of aggregate of wide, tubular P-protein components in mature sieve element. Narrow P-protein components are scattered and in longitudinal views have a helical appearance (arrows). ×85,500. Insert ×95,000

Fig. 23. Portion of slime (P-protein) plug composed of narrow P-protein components, many of which have tubular appearance (arrows). ×93,500

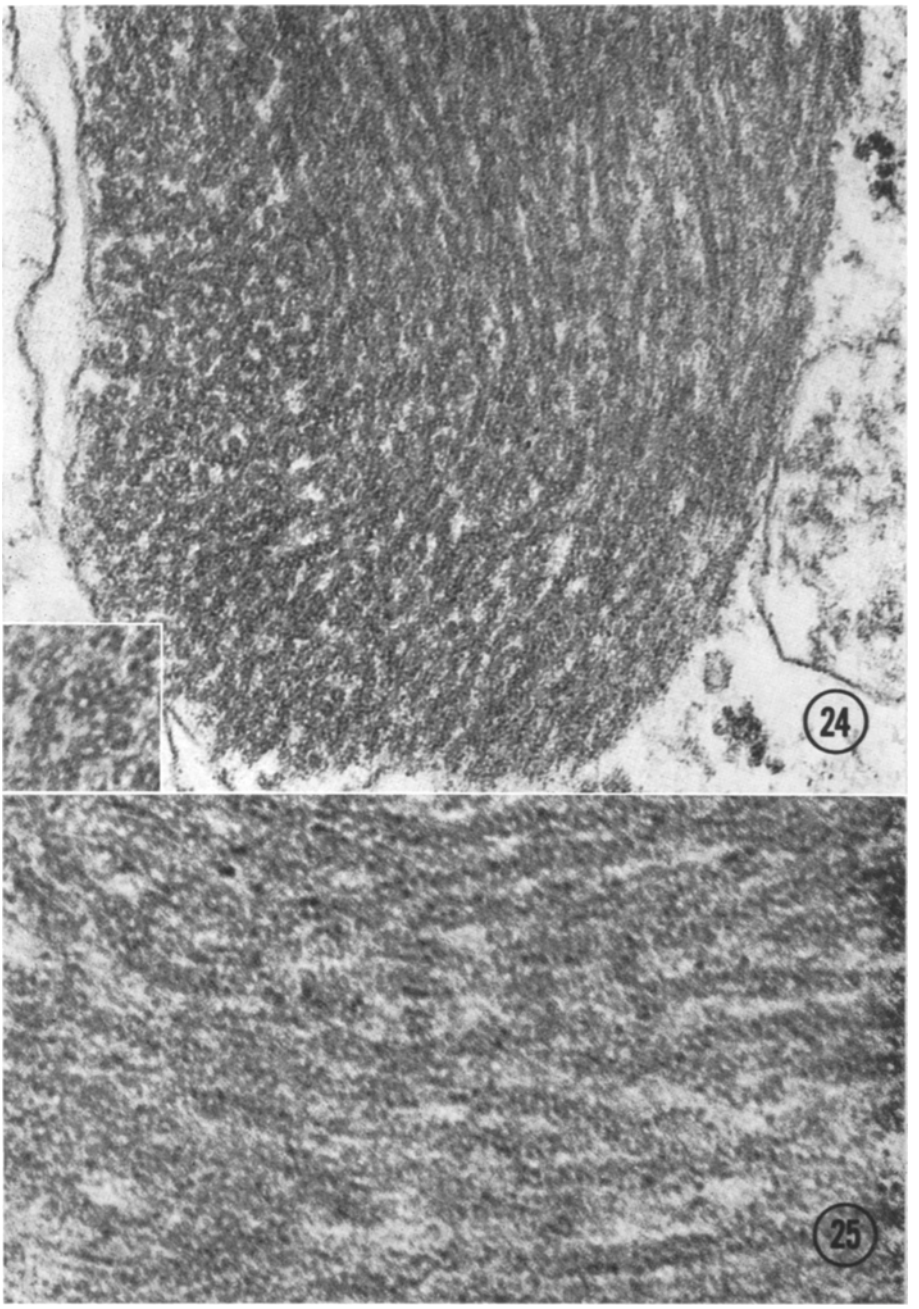


Fig. 24. Longitudinal section showing portion of P-protein body of Fig. 1 at higher magnification. The walls of these tubular components exhibit a substructure. Insert at higher magnification shows transverse view of wall of tubular component consisting of six subunits. ×84,500. Insert ×180,000

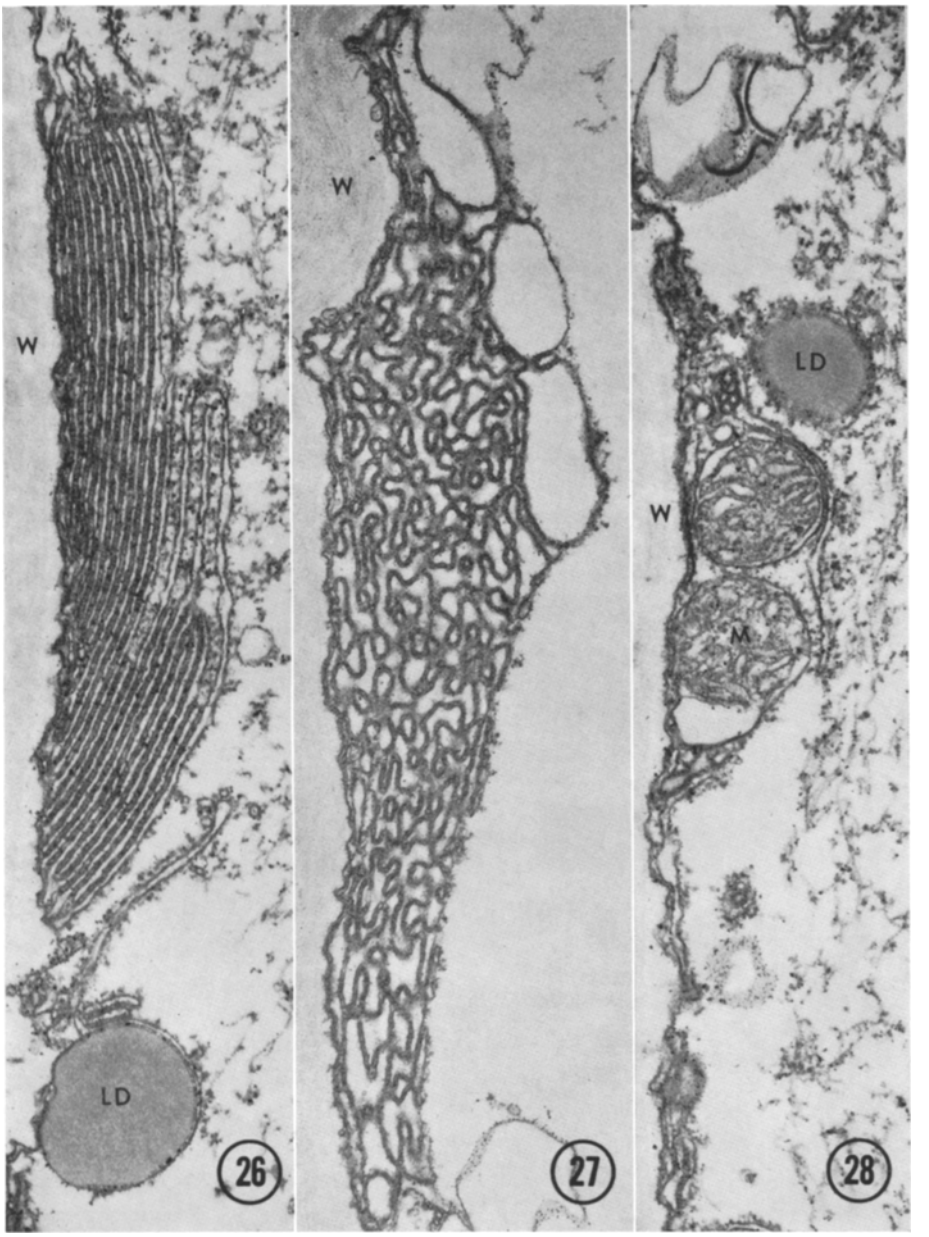
Fig. 25. (Turn at right angle for proper orientation.) Higher magnification of portion of P-protein body of Fig. 24 showing longitudinal view of tubular components. The subunits comprising the walls appear to be helically arranged. ×180,000

vesicles, and microtubules bordering the cell wall are also present during this period of development. Frequently, segments of endoplasmic reticulum closely parallel the surfaces of the P-protein bodies for considerable distances (Fig. 1). Some P-protein bodies are almost completely bounded by cisternae of endoplasmic reticulum. Except for occasional bridges of cytoplasm traversing the lumina of the sieve elements, generally at the sites of the large slime bodies and nuclei (Fig. 8), the developing sieve element consists of a parietal layer of cytoplasm surrounding a large central vacuole (Figs. 10 and 11). Small vacuoles also occur within the parietal layer of cytoplasm (Figs. 3, 8, and 10), which is bounded on its outer surface by the plasmalemma.

Concomitant with the dispersal of the P-protein bodies, certain protoplasmic components undergo profound changes. Especially notable are the changes or modifications that occur to the endoplasmic reticulum. Whereas the endoplasmic reticulum ramifies throughout the cytoplasm of young elements, it eventually becomes confined to the proximity of the walls as the sieve element approaches maturity. In mature sieve elements, one or more cisternae of endoplasmic reticulum lie parallel to the wall (Figs. 19 and 28). The outermost layer of endoplasmic reticulum membranes becomes so closely applied to the plasmalemma that often it is difficult to distinguish between plasmalemma and contiguous membrane of endoplasmic reticulum. Various views of these parietal membranes reveal that they comprise an extensive and complex network.

Other endoplasmic reticulum cisternae become stacked along the wall, the outermost membrane of each stack becoming closely appressed to the plasmalemma. In some stacks, the cisternae have a very orderly arrangement (Fig. 26), in others, the cisternae are much coiled and form a complex maze (Fig. 27). Orderly stacks of cisternae are common in mature sieve elements of elm; those with an irregular arrangement of cisternae are sparse. As mentioned, the endoplasmic reticulum membranes in young sieve elements are generally associated with ribosomes. Those in mature sieve elements are entirely smooth-surfaced. During formation of the stacks, the ribosomes apparently disappear first from the surfaces of adjacent cisternae and last from the free surface of the cisterna facing the lumen of the cell (Fig. 2). Although the intracisternal spaces are often variable in width, the widths of the intercisternal spaces are uniform, mostly about 85 Å (range: 50–110 Å). And whereas the intracisternal spaces are relatively clear in appearance (Figs. 26 and 27), the intercisternal spaces contain an electron-opaque substance (Figs. 26 and 27). Mitochondria, plastids and lipid droplets (the latter have been observed in mature sieve elements only) are often sheathed by segments of endoplasmic reticulum in mature sieve elements (Figs. 26 and 28).

The plastids of very young elm sieve elements are often difficult to distinguish



Figs. 26–28. Sections of mature sieve elements showing arrangement of endoplasmic reticulum cisternae along walls. Fig. 26 ×34,000, Fig. 27 ×27,500, Fig. 28 ×33,500

from mitochondria, for in such elements both organelles contain a dense matrix and are often similar in size. In addition, in certain planes of section the internal membranes of the plastids are numerous and often resemble those of mitochondria.

Most plastids produce a dense crystalline inclusion relatively early in their development (Figs. 29 and 30). Then, shortly before the initiation of P-protein body dispersal another dense inclusion, round in outline, appears in the plastid (Fig. 31). Similar round inclusions have been interpreted by other workers as sieve-tube starch (ESAU 1968, ZEE and CHAMBERS 1968). As the sieve element approaches maturity the plastid matrix becomes less electron-opaque (Fig. 31) and in mature elements the plastid matrix frequently is quite electron-transparent (Fig. 32). The mitochondria undergo little or no structural modification during the process of sieve-element development (compare mitochondria in Figs. 4 and 28).

Mature elm sieve elements lack ribosomes, dictyosomes, and microtubules. The microtubules apparently disappear shortly before P-protein body dispersal is well underway, for none was observed in any sieve elements during or after development of the network of P-protein aggregates. However, ribosomes and dictyosomes disappear from the protoplast sometime after P-protein body dispersal has resulted in formation of the network. Fig. 2 shows part of a sieve element in a relatively advanced stage of P-protein body dispersal. Note the presence of a dictyosome and numerous ribosomes in this area.

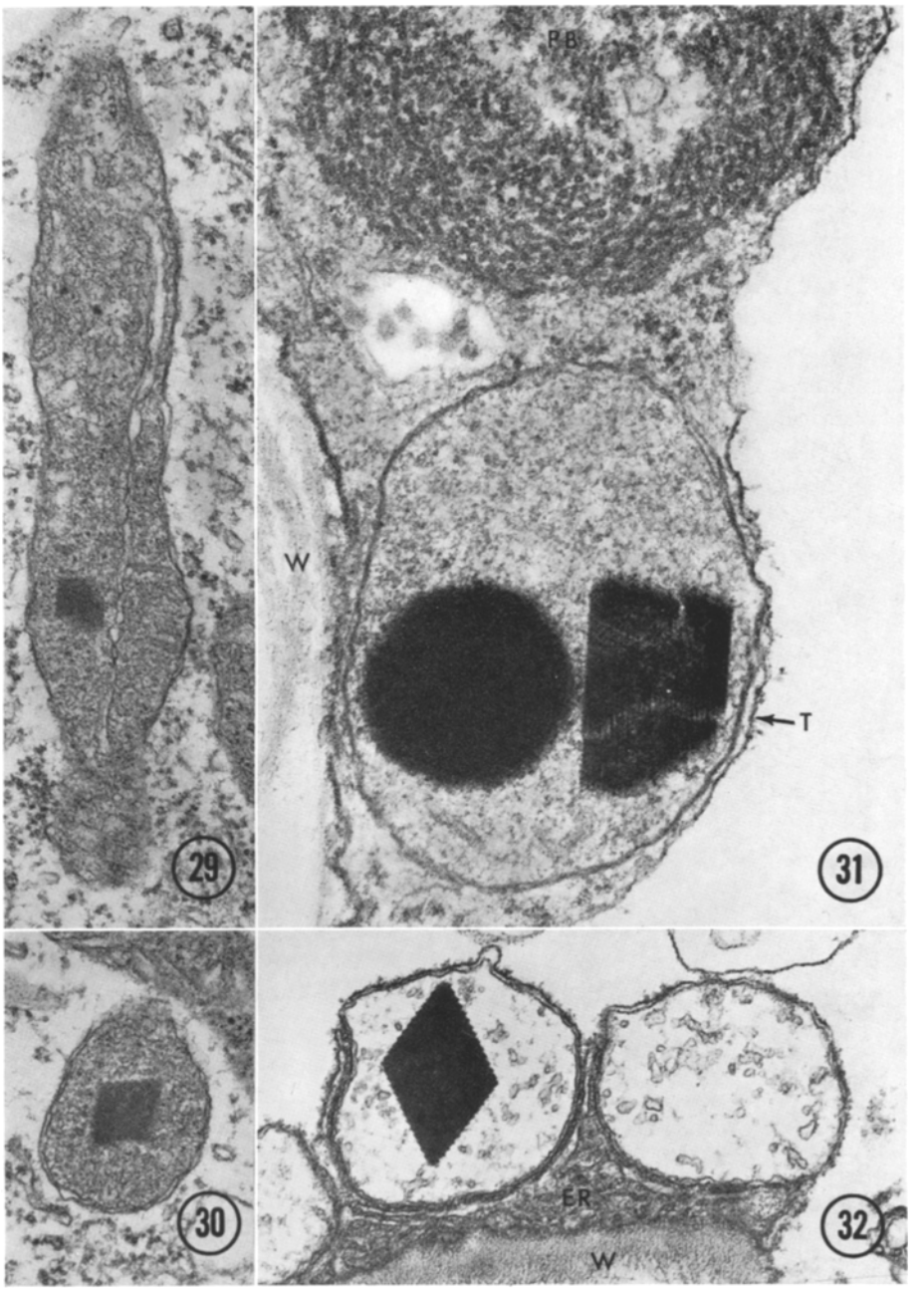
As mentioned, during the light microscope study many mature sieve elements were encountered with nuclei. During the present study, only one mature sieve element was encountered with a nucleus. The protoplast of that sieve element was poorly preserved, its P protein accumulated at one end of the cell. The nucleus itself was much distorted. It contained a single, large nucleolus.

3.3. The Sieve Plate

The many developing sieve plates encountered during the light microscope study revealed that much variation exists in the time of perforation of the pore sites of individual sieve plates and that perforation of the plate is a relatively slow process. The present study added little to our knowledge of sieve-plate development in elm, for few developing sieve plates were encountered with the electron microscope.

The most significant aspect of sieve-plate development encountered during the present study is illustrated in Figs. 5 and 6. Fig. 5 shows a developing sieve plate between sieve elements with P-protein bodies in early stages of dispersal. Pairs of callose platelets mark the sites of the future pores. Fig. 6 is a view of the same sieve plate as that in Fig. 5, but from a different section of a series. In Fig. 6, one of the pore sites is already perforated. The relevance of Figs. 5 and 6 is that they illustrate, at the ultrastructural level, that perforation of the pore sites in elm occurs while nucleus and tonoplast are still present.

None of the mature sieve plates encountered during the present study lacked callose. Not only were the pores lined with callose, but all surfaces of the



Figs. 29 and 30. Longitudinal and transverse views of plastids in young sieve element. Each plastid contains a crystalline inclusion and a very dense matrix. ×38,500

Fig. 31. Transection of portion of differentiating sieve element showing a plastid, with crystalline inclusion and grain of sieve-tube starch, and part of a P-protein body (*PB*) consisting of tubular components. The plastid matrix is less dense here than in those of the younger sieve element of Figs. 32 and 33. *T* = tonoplast, *V* = vacuole, *W* = wall. ×56,500

Fig. 32. Portion of a mature sieve element showing two plastids with electron-transparent matrix. *ER* = endoplasmic reticulum, *W* = wall. ×36,000

pectocellulose meshwork of the plates were generally lined with callose (Figs. 16, 19, and 20). This is in sharp contrast with results of the light microscope study, in which it was observed that many mature sieve plates lacked detectable callose.

At maturity, each sieve-plate pore is lined with a plasmalemma and sometimes with a single cisterna of endoplasmic reticulum, which is closely appressed to the plasmalemma. The remainder of the pore is traversed by P protein, the quantity of P protein present varying with its distribution within the sieve elements. Sieve elements with either slime plugs or slime sacs generally exhibit sieve-plate pores plugged with slime (Figs. 16 and 20). The contents of such pores are sometimes so dense that little or no structure can be seen within them (Fig. 16). In others, the nature of the P protein plugging the pores is still apparent (Fig. 20). When P protein is more or less evenly distributed throughout the sieve-element lumina, the P protein within the pores is similar in distribution and appearance to that within the lumina (Fig. 19).

At the light microscope level, the walls of most elm sieve elements appeared relatively thin, regardless of age or treatment of the tissue. Occasional sieve elements had relatively thick walls reminiscent of nacreous walls, but they were not "nacreous walls" in the restricted use of the term, for they did not consist of an inner wall layer morphologically distinct from the rest of the wall (ESAU and CHEADLE 1958). During the present study it came as a surprise that many elm sieve elements were encountered at all stages of development with a distinct inner wall layer (Figs. 3, 8, 10–12). This wall layer, with its loosely lamellate appearance, was quite wide in some sieve elements. Other sieve elements, both immature and mature, lacked any evidence of the presence of such a wall layer (Figs. 5, 7, 14, 19, and 20).

It seems likely that the distinct, and sometimes very wide inner wall layers encountered during the present investigation represent artifacts resulting from a loosening of the wall in processing of the tissue. Although they are probably abnormal, the wider walls are instructive in that they reveal the lamellate nature of the inner wall, an aspect not apparent in walls with a more normal appearance. It would be interesting to determine the degree to which the sieve element wall in elm resembles the thick, lamellate secondary walls of sieve elements of the *Pinaceae* (SRIVASTAVA 1969).

4. Discussion

The present investigation has confirmed observations of the light microscope study of the development of a network of P-protein (slime) strands during advanced stages of sieve-element ontogeny in elm and of the presence of a tonoplast throughout that period of development. At the ultrastructural level the strands are seen to consist of aggregates of P-protein components having

a striated, fibrillar appearance. P-protein aggregates of similar appearance have been recorded in sieve elements of other species (CRONSHAW and ESAU 1967, STEER and NEWCOMB 1969), but not as part of an extensive, three-dimensional network, as reported herein for elm. In addition, the electron microscope supports the view obtained with the light microscope, that in elm, both nucleus and tonoplast are present at the time of perforation of the sieve plates.

The light microscope investigation indicated that the parietal network of P-protein strands formed during P-protein body dispersal persists in the mature sieve element. In addition, some evidence was found in mature sieve elements, with the light microscope, for the presence of a membrane which delimits the parietal layer of cytoplasm, including its network of slime strands, from the vacuolar region of the cell.

During the present study, several, likely mature sieve elements were encountered with one-half to three-quarters of the parietal layer of cytoplasm delimited from the central vacuole by the tonoplast. Unfortunately, the sieve plates of these elements were not observed and consequently, it was not possible to determine unequivocally that the pertinent sieve elements were mature, *i.e.*, that their sieve plates were fully perforated. Hence the present study has not solved the problem of the nature of the membrane-like structure delimiting parietal cytoplasm from vacuole in many mature sieve elements of light microscope preparations. It has demonstrated that the tonoplast is present until at least very late in maturation of the sieve elements in elm and points to the need for a thorough reexamination of the "tonoplast-free" state of mature sieve elements in general.

Several workers (BUVAT 1963, LA FLÈCHE 1966, NORTHCOTE and WOODING 1966) who have reported that the tonoplast disappears during sieve-element differentiation have reported that the tonoplast first becomes detached from the parietal cytoplasm, then retracts into the vacuolar space and eventually disappears. Two of these workers (BUVAT 1963, LA FLÈCHE 1966) have shown relatively young sieve elements with their tonoplasts detached from the parietal cytoplasm. This phenomenon was also encountered in elm sieve elements with both light and electron microscopes, but it could not be established as a normal, ontogenetic event. Although some young elm sieve elements exhibited tonoplasts separated from the parietal cytoplasm, the great majority did not. Similarly, the great majority of sieve elements in advanced stages of P-protein body dispersal still had tonoplasts firmly attached to the parietal cytoplasm.

Earlier it was pointed out that when either P-protein bodies or aggregates are exposed to the vacuolar contents of immature sieve elements, as a result of the tonoplast being ruptured during manipulation and fixation of the tissue, the P-protein components commonly disaggregate and disperse throughout the cell. If the tonoplast is normally present in mature sieve

elements, but becomes ruptured during manipulation and fixation, it might be expected that the P-protein components of any existing network of strands would similarly disaggregate and disperse. Possibly the aggregates of P-protein components frequently observed in mature sieve elements during the present study reflect the normal association of P-protein components in undisturbed sieve elements.

Although the electron microscope has contributed relatively little to our understanding of the normal distribution and form of P protein in mature sieve elements, it has contributed much to our understanding of the nature of the P-protein components. Several workers have now reported that, after fixation with glutaraldehyde-osmium tetroxide, the P-protein components have tubular, fibrillar or filamentous appearances (LA FLÈCHE 1966, NORTHCOTE and WOODING 1966, TAMULEVICH and EVERT 1966, CRONSHAW and ESAU 1967, BEHNKE and DÖRR 1967, ESAU and CRONSHAW 1967, ESAU *et al.* 1967, WOODING 1967 a, BEHNKE 1968, CRONSHAW and ESAU 1968, JOHNSON 1968, 1969, STEER and NEWCOMB 1969), and some investigators (NORTHCOTE and WOODING 1966, CRONSHAW and ESAU 1967, WOODING 1967 a, STEER and NEWCOMB 1969) have suggested that in some species one form of P-protein component gives rise, ontogenetically, to another form. In each of the latter cases, it is a relatively wide, tubular-appearing component that gives rise to a relatively narrow, striated, fibrillar-appearing component: in *Acer pseudoplatanus,* 190–200 Å fibers apparently fray apart and form 80–100 Å fibrils (WOODING 1967 a); in *Nicotiana tabacum,* 231 Å tubules are reportedly reorganized into striated 149 Å fibrils (CRONSHAW and ESAU 1967); and in *Coleus blumei,* 190–220 Å tubules seemingly undergo a gradual reduction in size until 70 Å fibrils are formed (STEER and NEWCOMB 1969).

The young P-protein bodies in elm also consist of relatively wide, tubular P-protein components (170–230 Å). As the P-protein bodies increase in size, the diameters of components contributing to this size increase are narrower than those formed previously, so that, at initiation of P-protein body dispersal most of the components comprising the bodies are of relatively narrow diameters (90–170 Å, with most 130–140 Å). However, the entire range of diameters of P-protein body components is still represented in the fully-formed body and later, in the mature sieve element. As might be expected from the foregoing account, most of the P-protein components encountered in mature sieve elements are of relatively narrow diameters.

CRONSHAW and ESAU (1967) and STEER and NEWCOMB (1969) have reported that the striated fibrils in sieve elements of *Nicotiana* and *Coleus,* respectively, often exhibit a light-staining core, suggesting a tubular structure. In addition, CRONSHAW and ESAU have noticed that occasionally the tubular form of P protein in *Nicotiana* shows indications of cross-striations and they used this feature to relate the tubular form of P protein to the striated, fibrillar form. In elm, many of the narrow P-protein components exhibit electron-

transparent centers and both wide and narrow components give evidence of a similar substructure, *viz.*, of a wall composed of subunits, apparently in helical arrangement. Thus the P-protein components in elm, although of varying diameters, apparently are similar morphologically to one another and may be similar morphologically to other cytoplasmic tubules which frequently have been implicated with cytoplasmic streaming (LEDBETTER and PORTER 1964, BURTON 1966, O'BRIEN and THIMANN 1966, SABINS und JACOBS 1967).

CRAFTS (1968) has proposed that the fibrillar or filamentous components of mature sieve-tube elements are not slime, or P protein. He believes slime to be dead material that breaks down to molecular form and disappears from functioning sieve elements. According to CRAFTS, the fibrillar or filamentous substance of mature sieve elements co-exists with slime during sieve-tube maturation but is apparent only in functioning sieve tubes because there it is no longer obscured by either slime or plasmatic contents. This is clearly not so in elm sieve elements, where tubular- and fibrillar-appearing components comprise the P-protein bodies and occur together in mature sieve elements.

Although it is quite clear that P protein arises in the cytoplasmic ground substance, its relation to organelles or membrane systems remains uncertain. Several workers have reported that cisternae of endoplasmic reticulum frequently parallel the surfaces of developing P-protein bodies, as recorded herein for elm (NORTHCOTE and WOODING 1966, WOODING 1967 a, b, CRONSHAW and ESAU 1967, 1968), and sometimes completely surrounded them (NORTHCOTE and WOODING 1966). EVERT *et al.* (1966) reported that the slime bodies in *Cucurbita maxima* were frequently membrane-bound and interpreted the bounding membranes as limiting membranes of the slime bodies, not as cisternae of endoplasmic reticulum. Since then, CRONSHAW and ESAU (1968) have suggested that the membranes bounding the slime bodies in the micrographs of the EVERT *et al.* article are probably cisternae of endoplasmic reticulum. We concur with CRONSHAW and ESAU. It seems quite likely that the endoplasmic reticulum plays an important role in P-protein synthesis, as suggested by other workers (BOUCK and CRONSHAW 1965, WOODING 1967 b, STEER and NEWCOMB 1969).

In addition to endoplasmic reticulum, numerous coated vesicles are generally encountered in the immediate vicinity of developing P-protein bodies (NORTHCOTE and WOODING 1966, WOODING 1967 a, CRONSHAW and ESAU 1968), and elm sieve elements are no exception. However, there is no evidence that such vesicles are involved in P-protein synthesis. NEWCOMB (1967) and STEER and NEWCOMB (1969) have implicated "spiny" vesicles in P-protein formation in *Phaseolus* and *Coleus* phloem. Similar vesicles have been noted by CRONSHAW and ESAU (1968) in the region of P-protein synthesis in phloem parenchyma cells and sieve elements of *Cucurbita.*

Complexes of endoplasmic reticulum membranes similar to those encountered in mature sieve elements of elm have been reported in mature sieve elements

of a wide variety of seed plants, ranging from herbaceous (BOUCK and CRONSHAW 1965, EVERT *et al.* 1966, TAMULEVICH and EVERT 1966, ESAU *et al.* 1967, ESAU and CRONSHAW 1968 a, ZEE and CHAMBERS 1968, JOHNSON 1969) and woody (NORTHCOTE and WOODING 1966) dicotyledons to the monocotyledons (BEHNKE 1968) and conifers (KOLLMANN and SCHUMACHER 1964, SRIVASTAVA and O'BRIEN 1966), but apparently vary greatly in their abundance from one species to the next. WOODING (1967 b) has noted that, whereas aggregates of endoplasmic reticulum having a quasi-crystalline arrangement are quite common in immature sieve elements of *Acer*, they are rare in mature sieve elements. BOUCK and CRONSHAW (1965) and NORTHCOTE and WOODING (1966) have suggested that the system of membranes bordering the walls of mature sieve elements consists of membranes derived from both the endoplasmic reticulum and the nuclear envelope. Judging from the number of mature sieve elements encountered with nuclei in elm tissues of light-microscope preparations (EVERT *et al.* 1969), it would seem that at least in elm, the nuclear envelope does not contribute regularly, if it contributes at all, to the membrane systems of those cells. As mentioned, only one mature sieve element was found with a nucleus during the present study, but then, with the electron microscope it was neither possible to survey very large numbers of sieve elements nor possible to focus through intact elements in search of nuclei, as was done with the light microscope.

Among the more consistent features of sieve-element differentiation that seem to have been well established by electron microscopy are the disappearance of dictyosomes, ribosomes, and microtubules and the loss in electron density of the plastid matrix as the sieve element approaches maturity (ENGLEMAN 1965, WOODING and NORTHCOTE 1965, BEHNKE 1967, 1969, O'BRIEN and THIMANN 1967, ESAU and CRONSHAW 1968 b, ZEE and CHAMBERS 1968). WOODING and NORTHCOTE (1965) and NORTHCOTE and WOODING (1966) have reported that the plastids in developing *Acer* sieve tubes are sheathed over all or part of their surfaces by endoplasmic reticulum, but that plastids of mature elements are not. This has led WOODING and NORTHCOTE to suggest that the sheathing endoplasmic reticulum might play a role in dedifferentiation of the plastid.

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