

A STUDY BY ELECTRON MICROSCOPY OF THE DEVELOPMENT  
OF THE SECONDARY PHLOEM OF ASH (FRAXINUS EXCELSIOR)

by

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ABSTRACT

The ontogenetic development of the secondary sieve elements in ash, Fraxinus excelsior is traced by using mainly conventional electron microscopy. In addition, negative staining of the exudate and light microscopy have also been used. No major differences from the established trends have been observed in the differentiation of these sieve elements.

There is evidence that the P-protein in the sieve elements originates from helical polysomes found scattered in the dense cytoplasm of young sieve elements. These polysomes also give the appearance of having been derived from rough ER cisternae.

Reorganisation of the fibrillar P-protein into the tubular form also seems to take place in the larger P-protein bodies. In negatively-stained preparations, a suggestion of a tightening of the double helix of the P-protein fibrils is discernible. This reorganisation is however not consistent. The usual banding of the fibrillar P-protein is prominent.

Most of the mature sieve plates show occluded pores either callosed or not. The P-protein frequently appears to fray out on the downstream side and sometimes on the upstream side as well. This has a bearing on the nature of functioning sieve plates and does not seem to be due to artifacts.

Empty spaces which represent probably a peculiar artifact are observed immediately above most of the mature sieve plates. P-protein seems to pile above these "barrier" spaces while the sieve plates remain occluded with P-protein.

Other formations of peculiar interest are the plasmalemmal invaginations ("blebs") found both in the sieve elements and in the parenchyma cells. In the sieve elements, their formation seems to be in an endocytic (pinocytotic) direction. In terms of the sieve tubes, these "blebs" have a possible significance in the uptake of solutes and ions.



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ABBREVIATIONS

|                 |   |                       |
|-----------------|---|-----------------------|
| a               | - | axial parenchyma      |
| ca              | - | callose               |
| cc              | - | companion cell        |
| cr              | - | crystal               |
| cw              | - | cell wall             |
| d               | - | dictyosome            |
| er              | - | endoplasmic reticulum |
| f               | - | phytoferritin         |
| fi              | - | fusiform initial      |
| ld              | - | lipid droplet         |
| m               | - | mitochondrion         |
| mc              | - | median cavity         |
| n               | - | nucleus               |
| nu              | - | nucleolus             |
| p               | - | plastid               |
| pb              | - | P-protein body        |
| pd              | - | plasmodesma           |
| pg              | - | plastoglobulus        |
| pm              | - | plasmalemma           |
| pp              | - | P-protein             |
| pp <sup>1</sup> | - | diffuse material      |
| r               | - | ribosomes             |
| rc              | - | ray cell              |
| ri              | - | ray initial           |
| se              | - | sieve element         |
| sp              | - | sieve plate           |
| sg              | - | starch grain          |
| sw              | - | sandwich layer        |
| t               | - | tonoplast             |
| th              | - | thylakoid             |
| v               | - | vacuole               |
| ve              | - | vesicle               |

NUMBERING OF FIGURES

The numbering of the figures in the thesis is based on the following.

All figures occur in Chapter III. Here, there are three sections: 1, 2, and 3. Of these only section 1 is further subdivided. Its subdivisions, i, ii, iii and iv are again individually subdivided ((a), (b), (c) etc.)

To avoid cumbersome referencing, figures in section 1 begin with the subdivision. They are then numbered in sequence within these subdivisions i, ii, iii or iv.

e.g.        i.1, i.2, i.3 ....  
             ii.1, ii.2, ii.3 ....

Within sections 2 and 3, the figures again are numbered in sequence

e.g.        2.1, 2.2, 2.3 ...  
             3.1, 3.2, 3.3. ...

Thus a reference beginning with an italic number belongs to section 1; figures belonging to sections 2 and 3 begin with these numbers in roman.

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CHAPTER IINTRODUCTION

Since their discovery in bark by Theodore Hartig more than a century ago, work on sieve tubes both physiologically and structurally has been fairly continuous. In the early twenties, workers on phloem transport could not come to an agreement about the structure of sieve tubes. Mason and Maskell, Dixon and Ball and others believed that the sieve pores were blocked by 'cytoplasm' making pressure flow impossible. Münch, however, thought that the sieve pores were continuous and this facilitated pressure flow. The reason for this uncertainty was due to the sensitivity of the sieve tubes to manipulation as well as to the small size of the critical channels. This uncertainty still exists.

Even now, with such advanced scientific techniques for probing into the complexities of the sieve tubes as are now possessed, there is still no final conclusion about many important features of the structure and function of sieve tubes, such as the state of the functioning sieve plates and the occurrence of bidirectional streaming in the same sieve tube.

The aim of the present project is thus not to solve the problem but to throw a little more light on it, in fact to trace the developmental changes and to elucidate the mature structure of the sieve tubes of Fraxinus excelsior, the common ash. It is hoped that some of the observations made in this study will help in some way to discriminate between the theories on mechanisms which have been suggested to account for transport in the sieve tubes.

Fraxinus, a woody dicotyledon, was specially chosen for this study, to gain experience in dealing with a tissue that is comparable to the rubber-bearing tissue of Hevea brasiliensis. Fraxinus also exudes well and this has a special interest to workers in the physiology of phloem transport. Another characteristic of this common ash is that the sieve tubes are fairly short and wide and this promised the facility of examining them whole in longitudinal sections.

Few detailed studies have been done on the ultrastructure of the sieve tubes of dicotyledonous trees. The latter include Acer pseudoplatanus (Northcote and Wooding 1966, Lawton 1976), Ulmus americana (Evert and Deshpande 1969), Salix caprea (L.) (Mishra and Spanner 1970), Tilia americana (Evert and Murmanis 1965). This indicated the desirability of working on a tree, especially as it is on trees that the physiological problems of transport is most acute.

Work on Fraxinus has been mostly concentrated on physiological aspects (Zimmermann 1957, 1958, 1960a), even though on a different species of Fraxinus, F. americana. Gill (1932), however, studied the differentiation and seasonal variation of the phloem of F. excelsior, using only the light microscope.

2. PLAN OF THE WORK

The work reported in this thesis comprised the following. Firstly, a review of the literature on the ultrastructure of the phloem of dicotyledonous trees to provide a background for the present study. Secondly, an investigation by light microscopy designed to provide information on gross characteristics of the phloem of Fraxinus and its seasonal variation.

Thirdly, (and this comprises the bulk of the work) an ultrastructural study of the phloem of Fraxinus and particularly the sieve tubes and their contents. Finally a review of the results obtained in connection with the problem of phloem transport.



### 3. REVIEW OF THE ULTRASTRUCTURE OF THE PHLOEM IN DICOTYLEDONOUS TREES

Literature on the electron microscopy of the phloem is voluminous. Reviews have appeared periodically e.g. by Kollmann (1964), Esau (1969) and Srivastava (1975). The monograph by Esau (1969) was very extensive and went back to the very beginning; the review by Srivastava (1975) covered the ultrastructure and development of sieve elements in angiosperms and gymnosperms from 1968 onwards. The structure of the sieve element in relation to its function has also been reviewed a number of times separately, for instance by Weatherly and Johnson (1968), Eschrich (1970) and recently by Kollmann (1975).

Since Fraxinus excelsior has been the subject in the present work, it was decided that this review should cover dicotyledonous trees only. Accordingly, work on Tilia americana, Robinia pseudoacacia, Acer pseudoplatanus, Salix caprea and Ulmus americana has been drawn upon. Studies on other trees have been done but only on particular aspects of the sieve elements (see for instance Evert et al 1970 who considered only the nuclei in 13 species of woody dicotyledons). Particular aspects of phloem ultrastructure occurring in plants other than the dicotyledonous trees will however be reviewed where appropriate.

Tilia americana has been much studied both by ordinary light microscopy (Evert 1962) and by phase- and fluorescent-microscopy (Evert and Derr 1964a and 1964b). The ultrastructure of the secondary phloem was first investigated by Evert and Murmanis (1965). They observed the "absence in the mature sieve tubes of a tonoplast and of ER either in the form of tubules or cisternae". The former point is in line with the well-known characteristic of the sieve tubes of herbaceous plants like Cucurbita but the second is rather different.

Numerous vesicles which occupied mostly the parietal position were interpreted as the remains of degenerating ER and other organelles. They also reported the presence of one to four "extruded nucleoli" per sieve element whose outer cortex was sculptured. Deshpande and Evert (1970) in their re-evaluation of these "extruded nucleoli" in four species including Tilia americana challenged this interpretation and regarded them as of extra-nuclear origin and connected rather with P-protein formation.

Evert and Murmanis were however more interested in the ultra-structure of the "slime strands" seen in the previous light microscope investigation. Fixing the material variously with  $\text{KMnO}_4$ ,  $\text{OsO}_4$  and glutaraldehyde, they were able to conclude that the true basic form of the slime, which was fibrillar, was observed best in sieve tubes fixed in  $\text{OsO}_4$  or glutaraldehyde. Normally the fine constituent fibrils were organised into coarser strands that traversed the whole cell, the sieve-plate pores and the lateral sieve areas.

The structure of P-protein bodies was not discussed in these earlier investigations. Evert and Deshpande (1970) however, observed the presence of P-protein bodies both in the cytoplasm and remarkably also in the nuclei of differentiating sieve elements of the same species. These P-protein bodies were made up of tubular P-protein components ( $180\text{\AA}$  in diameter) and showed some evidence of substructure. Fibrillar components ( $130\text{\AA}$  in diameter in both the nucleus and cytoplasm) occurred in aggregates and arose more or less simultaneously in the nucleus and cytoplasm. The tubular forms were thought to precede the fibrillar ones as was suggested by other workers in their studies (Northcote and Wooding 1966; Cronshaw and Esau 1967 and Steer and Newcomb 1969). Older sieve element nuclei apparently contained only fibrillar P-protein. Only about half of the nuclei observed in differentiating sieve elements

contained P-protein components of either tubular or fibrillar form.

A year later Evert and Deshpande (1971) reported the presence of plastids in sieve elements and companion cells of Tilia americana, contrary to the report made earlier by Evert and Murmanis (1965). The plastids in young sieve elements contained a moderately electron dense matrix and numerous internal membranes. The matrix became electron transparent, the internal membranes were fewer and the plastids contained only a single starch grain when the sieve elements matured. Plastids were scarce in companion cells and underwent little or no structural modification during cellular differentiation.

In Robinia pseudoacacia, Evert and Murmanis (1966) observed the usual contents of the enucleate sieve element: the wall lined by plasmalemma and one or more cisterna-like layers of ER; numerous mitochondria and proplastid-like structures with electron-dense inclusions. "Persistent slime bodies" were not observed. Fibrillar slime which was also evenly distributed throughout the central cavity of the cell sometimes suggested that this material normally forms fine strands which run from cell to cell through the sieve-plate pores. Like the sieve-plate pores, the lateral sieve-areas were also traversed with slime.

Of the 13 species of woody dicotyledons that Evert et al (1970) investigated for the presence in the mature sieve elements of nuclei, only Robinia, Ulmus and Vitis contained some with apparently normal nuclei.

Northcote and Wooding (1966) gave a detailed account of the development of sieve elements in Acer pseudoplatanus. They recognised five stages in the differentiation of the sieve tube by observing the development of the sieve plates. The sequence of development of

the sieve-plate pores was found similar to that described for the herbaceous Cucurbita (Esau et al 1962). Also from their autoradiographic results, they were able to determine that some of the callose formed at the sieve plate was deposited after the general formation of the wall and thus they were led to challenge the suggestion of Esau (1969) for Cucurbita that the callose platelets "sink" into the middle lamella. Deshpande (1975) also re-examined the development of the sieve plate in Cucurbita and rejected Esau's interpretation. Agreeing with Esau however, Northcote and Wooding reported for Acer that ER cisternae were also found to be functionally related to this sieve plate formation, as well as to the other organelles like plastids. At maturity the ER sheathing the plastids disappeared.

The slime bodies in Acer were described as fibrous masses consisting of "closely packed regularly arranged fibres" (180 - 240<sup>0</sup>Å diameter), "loosely organised fibrils" (90 - 100<sup>0</sup>Å diameter) or "a mixture of both components". Bodies containing 90 - 100<sup>0</sup>Å diameter fibrils, normally also have a granular area at one end, from which fibrils appear to originate. These granular bodies, probably consisting of ribosomes were suggested as giving rise to the fibrils which were linear subunits of the fibres of the slime bodies and the sieve-plate pores. Fibrils have the characteristic alternating light and dark bands about 50<sup>0</sup>Å apart. During the dispersal of the slime bodies, the organised compact fibres frayed out into 90 - 100<sup>0</sup>Å diameter fibrils which later filled the lumen of the mature sieve tube.

The nucleus of Acer too was observed to degenerate as the sieve element differentiated. The nucleus formed narrow processes and nucleoli disappeared. This was soon followed by dissolution of nuclear contents. Nuclear envelopes were modified by vesiculation outwards into the cytoplasm and sometimes more than one pair of membranes seemed

to bound the nucleus. A mass of hexagonally-arranged vesicles was sometimes seen at points where the nuclear envelope was close to the plasmodesmata between a sieve element and its companion cell.

A variety of membranous aggregates which were not apparently artifacts of fixation was frequently found. Thus, lamellar stacks were sometimes present along the walls of sieve tubes. Again, a dense laminar core surrounded by an aggregation of small vesicles packed into a hexagonal arrangement often persisted until the sieve element matured. These were commonly located near the sieve plates.

Other species of Acer e.g. A. negundo have also been studied, but the studies have been on seasonal development of the secondary phloem using the light microscope only (Tucker and Evert 1969).

Mishra and Spanner (1970) made a study of the fine structure of the sieve tubes of Salix caprea but mainly in relation to the electro osmotic theory. Their paper concentrated more on the structure of the sieve plate pores than on the rest of the contents of the sieve element. The pores were found to be occupied by slime fibrils fraying out towards the lumen and showing periodic bandings, the period being about  $100\text{\AA}$ . As in Acer (Northcote and Wooding 1966) when fibrils came laterally together, the dark and light bands aligned themselves in transverse register giving a pattern of marked cross striations.

They also reported the presence of a prominent and persistent "nucleolus", occasionally of two (see above, where Evert and Deshpande challenged this interpretation). These nucleoli showed a radiating structure of tubules and were usually found very close to the sieve plates and sometimes on both sides. ER reminiscent of other species formed parietal lamellar stacks, with the lamellae inclined to or parallel with the wall.

Detailed studies of the ontogeny of the sieve elements of Ulmus americana have been done both with the light microscope (Evert and Deshpande 1968 and Evert et al 1969) and at the ultra-structural level (Evert and Deshpande 1969). P-protein was first observed in Ulmus as small groups of tubules whose components measured mostly about 180<sup>0</sup>Å in diameter. As these groups grew bigger, narrower tubules appeared. At the start of slime-body dispersal however, most of the components of the P-protein appeared banded or striated and measured 130 - 140<sup>0</sup>Å in diameter. During dispersal, the P-protein components formed sub-aggregates which spread throughout the parietal layer forming a shallow complex 3-dimensional network. The tonoplast was found to be present throughout the period of P-protein dispersal.

In the mature sieve elements, the distribution and form of the P-protein was variable. However, most of the components were arranged either loose or in relatively compact aggregates. In some sieve elements, it was evenly distributed like a fine network. It was also the wider components (as wide as the tubules in developing P-protein bodies) that were encountered in groups or aggregates, the relatively narrower ones (striated/beaded) were most often randomly dispersed.

Evidence of substructure in the P-protein material was also clear in both the wide and narrow components of some mature sieve elements. These, both wide and narrow, exhibited an electron transparent lumen and an electron opaque wall composed of 6 - 8 sub-units in transverse section i.e. they appeared tubular. In longitudinal section, these tubules seemed to have a helical structure.

Various configurations of the ER cisternae were also observed during sieve element differentiation. Commonly found were the orderly-arranged parallel ER stacks; less commonly the much-coiled and

convoluted types. Mitochondria and plastids were normally sheathed by ER.

Ulmus sieve tubes possessed as usual, sieve element plastids; most of the plastids here contained a dense crystalline inclusion relatively early during their development. Dictyosomes, ribosomes, microtubules disappeared as the sieve elements matured.

From this review it appears that all the dicotyledonous trees studied show a similar trend of ontogeny with a little variation here and there. Differences between Fraxinus and the species mentioned above will be referred to in the Discussion.

## CHAPTER II

### MATERIALS AND METHODS

#### 1. MATERIAL AND COLLECTION

When Fraxinus excelsior was chosen for this study, it was decided that the bark from one and two-year old branches would be sampled. Accordingly collections were made from four young trees, all of different ages and heights growing in the grounds of Bedford College. No two samples were collected from the same branch to avoid undue damage.

Samples were collected over a period of two years (1975 - 1976). In the first year when growth was very active (i.e. May to July) the bark was sampled fortnightly, but for the rest of that year samples were taken monthly. Only monthly collection was made in the second year.

During the course of the work, it was found that considerable technical problems occur in the preparation of sections of Fraxinus excelsior, for the electron microscope. One such was the splitting away of the sieve element walls when sectioning embedded material longitudinally, especially with those samples collected during the inactive season. Allied to this was the tendency of the blocks to crumble while sectioning. This was no doubt connected with the fact that many of the cells had thickened and perhaps hardened walls which naturally tended to hinder the penetration of both fixative and resin. Various modifications of the standard procedure were tried and these will be mentioned later under the appropriate headings.



2. ELECTRON MICROSCOPY(i) Method of sampling

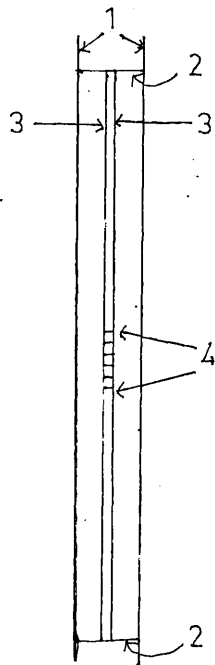
Various methods of sampling have been employed by workers on phloem in the attempt to minimise the notorious surge effects which occur only too easily when excision or chemical treatment is being carried out on the high pressure conduits (see also Chapter III, section 1.iii c).

Fraxinus, being a tree, does not allow the phloem to be excised directly under fixative while still connected to the tree as is possible in herbaceous plants. Time-delay is therefore inevitable, and will probably have some slight effect to the fixation of the tissue, even though it may not be very noticeable.

In the present work, the procedure adopted was to make two parallel longitudinal cuts about 5cm long and 1 cm apart with a sharp razor blade of rigid type. Then with two blades, transverse cuts about 5cm apart were made simultaneously to isolate a rectangle of bark. To separate this piece of bark from the branch was often quite a problem. It was found to be easier in summer when the cambium separated easily from the new wood. Samples made in autumn and winter however, were more difficult, the specimens experiencing some damage at the ends when they were being lifted from the wood. Since cambium did not part easily as in the growing season, some wood was also intentionally included when sampling to ensure that the sieve elements were not torn. The presence of wood was however not desirable for it hindered easy and rapid penetration of fixative and when possible it was removed before proceeding further.

As soon as possible the sample was quickly totally immersed in fixative (which was taken out to the site) at ambient temperature and brought at once to the laboratory. It was then subjected to vacuum for an hour to facilitate penetration of the fixative.

From the middle portion of the 5 x 1cm rectangle, a longitudinal strip 1mm wide was taken which was further cut into cubes, taking only 5mm of the middle portion of this strip. Both wood and periderm were removed to ensure better processing.



In this figure numbers  
1 - 4 indicate the  
sequence in which cuts  
were made

This latter excision was done in fresh fixative and the material selected was then kept for another 2 hours in fixative before being washed in buffer. They were then kept in buffer overnight at 4°C ready for post-fixation in osmium.

(ii) Fixative

Most of the fixative used was 5% glutaraldehyde buffered with 1/15 M Sorensens phosphate buffer at a final pH value of 7.2.

Karnovsky's (1965) fixative was also used. Since this fixative has the property of faster penetration, it was hoped that it might minimise the splitting away of the wall of the sieve tube which was a recurrent problem. The walls in fact did show some improvement after using Karnovsky's fixative.

One percent buffered osmium tetroxide was used as the secondary fixative. Samples were post-fixed in this for another two hours generally at 4°C but sometimes at laboratory temperature.

(iii) Dehydration

After post-fixation, samples were washed with distilled water and then passed through the normal graded acetone series. In a further effort to prevent the walls splitting away during sectioning, the dehydration time was prolonged to 30 minutes per stage instead of the normal 15 minutes. This proved a help.

(iv) Embedding media

TAAB resin, a commercial epoxy resin mixture introduced by TAAB laboratories, was used for most of the work. Various hardnesses can be obtained with this by varying the quantity of DDSA. The following proportions were used for most of the work:-

|            |       |
|------------|-------|
| TAAB resin | 50 ml |
| DDSA       | 25 ml |
| MNA        | 25 ml |
| DMP 30     | 2 ml  |

TAAB resin was found to be satisfactory except that splits were repeatedly encountered along the line of the sieve tube walls when sectioning longitudinally, even if resin was infiltrated very slowly

and thoroughly i.e. by keeping the samples in 10% resin for more than two days with vials on a slowly rotating inclined platform. Evacuation was also carried out during infiltration.

Being of a lower viscosity, Spurr's (1969) resin was also tried. Compared with TAAB resin, this has the advantage of better infiltration. The blocks were found easier to section and there was less splitting along the sieve tube walls. Sections, however, gave less contrast even if they were double stained, and so TAAB resin was preferred.

(v) Sectioning

Blocks were cut on a Huxley microtome Mk II using glass knives. Thin sections were picked up on copper grids coated with formvar films.

(vi) Staining

For this material, double staining was found to give better contrast than single staining. Grids were first stained with 2% uranyl acetate for an hour followed by 7 minutes in lead citrate. (Reynolds 1963). Staining procedure followed closely that of Mollenhauer (1974).

Sections were examined in both an AEI-EM6B and a Corinth 275 electron microscope.

3. LIGHT MICROSCOPY

Sections examined under the light microscope were from the same blocks used for electron microscope studies. The same sectioning procedure was followed except that thicker sections (1 $\mu$ m) were made.

Several sections in a drop of water on a slide were placed on a hot plate until the water evaporated. This flattened out the sections.

They were then flooded with 1% toluidine blue dissolved in 1% borax (Pease 1964) and put again on the hot plate. When the preparation steamed, staining could be assumed complete; stain was not allowed to dry on the sections.

Stained sections were examined and photographed with a Zeiss photomicroscope Mk II using a yellow filter and planapochromat objectives.

#### 4. NEGATIVE STAINING

The collection of exudate was not carried out until late July when the sieve tubes were fully translocating. A diagonal cut about 5 mm long was made into the bark with a sharp razor in the middle of an internode. The incision was kept shallow to avoid contamination of the exudate with xylem contents, or perhaps its loss into the xylem.

The exudate was collected in a glass microcapillary, held horizontally, the end touching the drop. A tiny droplet of the exudate was placed on a formvar coated grid, some 2% uranyl acetate added for a few seconds, and then the excess solution removed with filter paper. The grid was then dried and examined.

CHAPTER IIIOBSERVATIONS1. SIEVE ELEMENTS(i) Seasonal Variation

It is well known that woody species grown in temperate regions (e.g. Fraxinus excelsior used in this study) show periods of growth and cell division alternating with periods of relative inactivity during winter. Gill (1932) has already studied the differentiation and seasonal variation in Fraxinus excelsior. Using only the light microscope, he observed the differences between the appearance of the phloem developed during spring/summer and that towards the end of the growing season. Acer pseudoplatanus was also reported to have similar differences (Elliot 1935). Therefore this topic will be dealt with here only briefly.

Phloem produced from May to mid-August is called "summer phloem" by Gill (1932) even though its formation begins in Spring, for production of this type of phloem is most vigorous when the leaves are fully expanded. It consists of wide sieve elements each with usually one narrow companion cell (Fig i.1, arrowed region). The companion cells always lie next to a ray cell unless an axial parenchyma cell is present, when the companion cell (\*) lies on the side of the sieve element adjacent it (see also section 3 of Chapter III).

In this study, "summer phloem" was found as early as 22nd April in 1975 (Fig i.2), the black buds having already started to swell. Production continues, building up to about seven rows of sieve elements by the end of the summer season. The elements formed during this period become at first progressively larger and then progressively smaller (Fig i.3).

Samples collected by mid-August already begin to show the other type of phloem referred to as "autumn phloem" by Gill (1932). This phloem consists of sieve elements with two companion cells each, both of nearly the same size as the sieve element. Sometimes, more than two companion cells for each sieve element are also observed (see Fig 2.2). The leaves at this time are still green and active and show no signs of senescence.

By the time the leaves start to turn yellow and drop off (i.e. about mid-November) the cambium has produced a maximum of three to four rows of "autumn phloem" (Fig i.4; also compare the number of rows of "autumn phloem" in early Spring in Fig i.2). Throughout Winter until Spring, the cambium ceases its activity. The "autumn phloem" however, remains healthy. Fig. i.5 which was taken from bark sampled in late March, a month before the cambium resumes its activity, indicates a differentiated sieve element. Similarly in Quercus (Anderson and Evert 1965), the last three or four rows of late phloem over winter in an active state. No healthy sieve elements have, however, been reported in Celastrus scandens (Davis and Evert 1966) or Robinia pseudoacacia (Derr and Evert 1967) to name two other species.

With the production of the first "summer phloem" in early Spring (late April), the later formed "autumn phloem", separated from it perhaps by only a single axial parenchyma cell, still appears healthy (Fig i.6) while the earlier "autumn phloem" is on the point of collapse (see also Fig 2.32). Some "autumn phloem" has also been observed seemingly healthy well into late May (Fig i.7).

Most of the "summer phloem" however, appears to have ceased functioning, judging from the massive callose formation over their sieve pores and the congealed contents by mid-December (Fig i.8).

In this study, obliteration (i.e. crushing of the obsolescent sieve elements) of the two different types of phloem was observed to be inconsistent. Gill (1932) reported that in Fraxinus excelsior, the "autumn phloem" was not crushed until the second season of its existence. He only briefly commented that the empty "summer phloem" collapsed as a result of pressure of newly formed phloem at the end of the same season. Figs i.5 and i.6 however, indicate that some "autumn phloem" cells, probably those formed earliest, may collapse in their first season. "Summer phloem" has also been observed partially uncrushed during the second season of its existence (Fig i.7).



Fig. i.1      Transverse section ( $1\mu\text{m}$  thick) of bark sampled on 20th May, 1976 stained with toluidine blue. This shows typical "summer phloem" (within arrowed region); sieve elements being wide compared to the companion cells. Note the presence of "autumn phloem" above the enlarged summer sieve elements. Companion cells lie adjacent to ray cells, or occasionally to axial parenchyma cells (a). At this time the leaves are already expanded, x 464

Fig. i.2      Transverse section of bark sampled on 22nd April, 1975 ( $1\mu\text{m}$  thick and stained with toluidine blue). Note the onset of the "summer phloem" with large sieve elements and single companion cells. The autumn phloem (within arrows) from last season has not yet been crushed and not even those earliest formed elements. The last season's summer phloem is about to be crushed\* x 378.

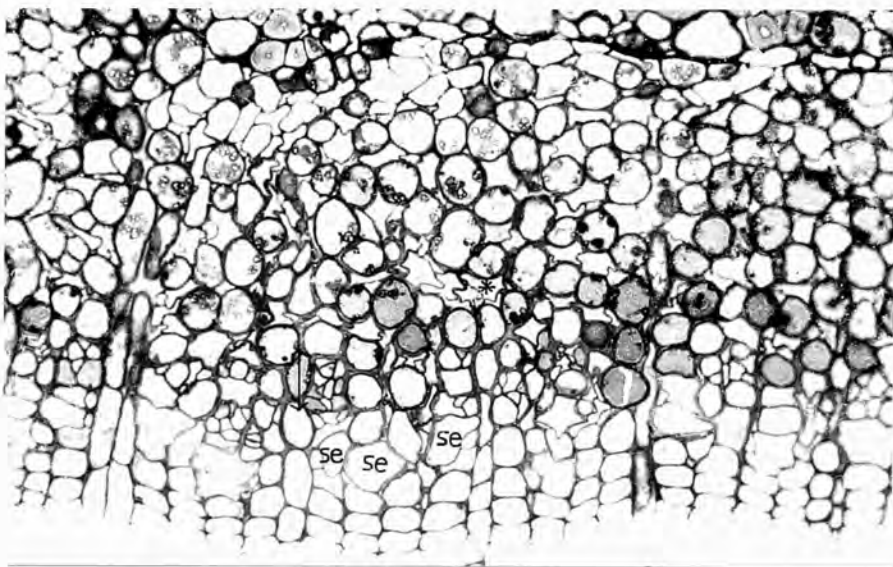
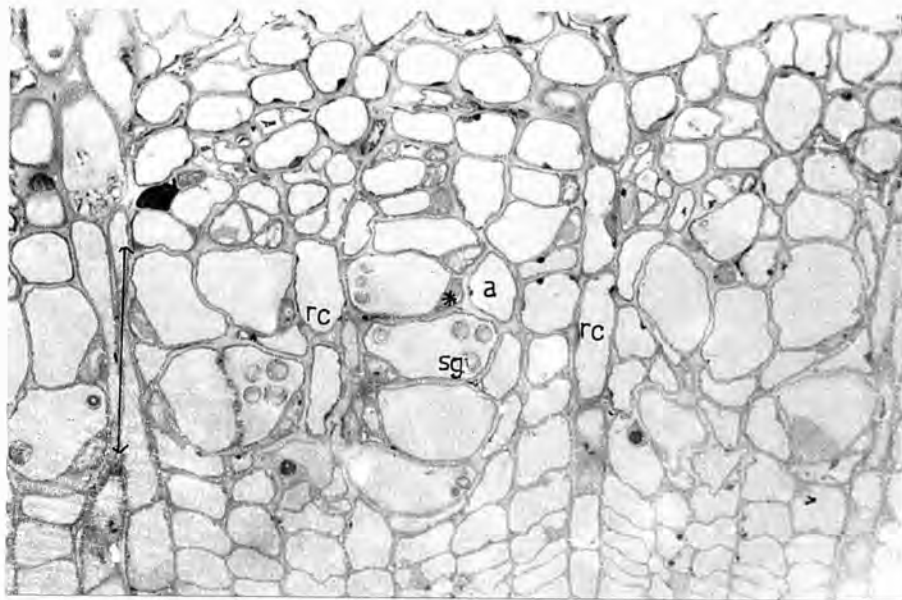


Fig. i.3      Transverse section from bark sampled in late June, 1976 (1/ $\mu$ m thick and stained with toluidine blue). There are about 7 rows of sieve elements at this time of the year (see numerals). Notice that the first and last formed sieve elements are smaller. Axial parenchyma cells (a) appear in tangential bands. x379

Fig. i.4      Transverse section of bark sampled in mid-November, 1976. There are about 3 rows of autumn phloem (arrowed region). Note that last season's summer phloem appears functional, judging from the state of the companion cells and the turgid, empty appearance of the sieve elements. x 1,300

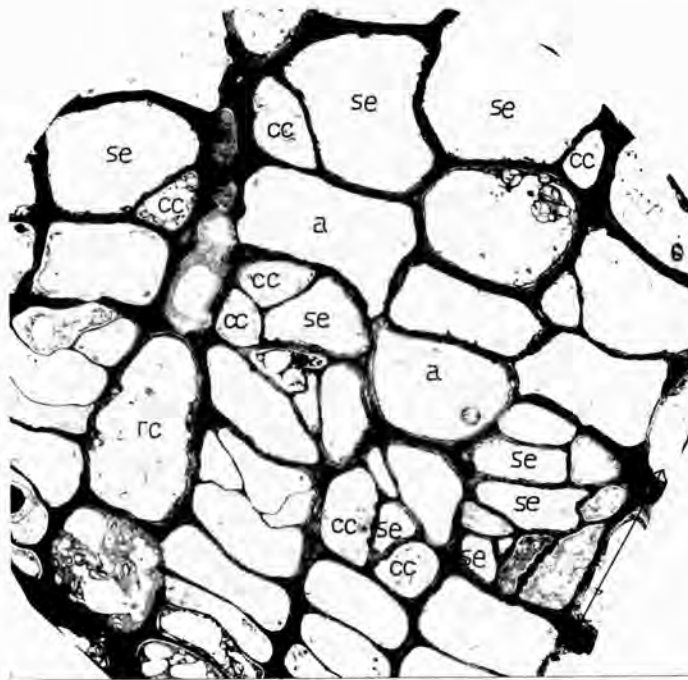
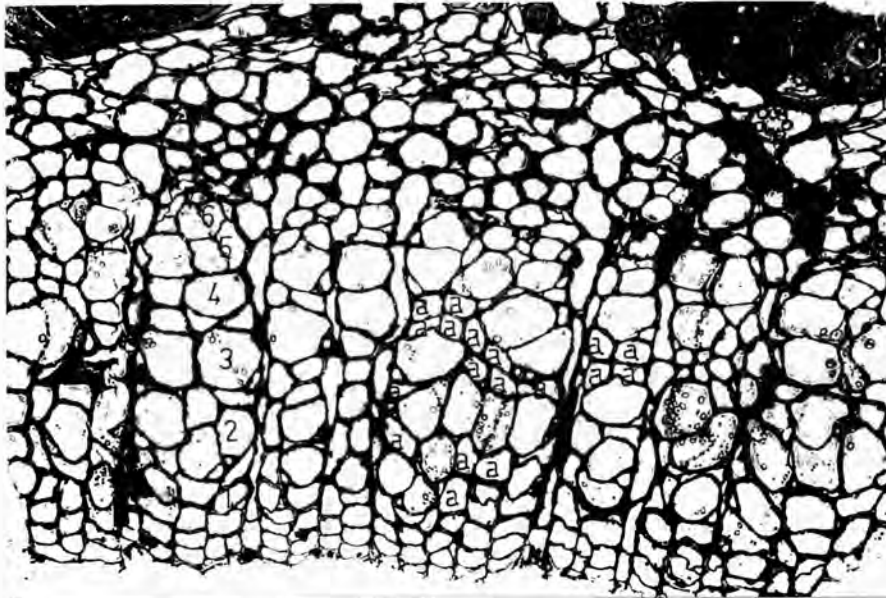


Fig. i.5      Transverse section of "autumn phloem" from bark sampled on 24th March, 1976 about a month before the onset of summer phloem. Note that the sieve element is probably healthy judging from the state of the cell. The obliterated phloem above it appears to be "autumn phloem" formed earlier. x 3,250

Fig. i.6      Transverse section of bark sampled on 23rd April, 1975, showing the onset of "summer phloem". Only one row of last season's "autumn" sieve elements appear to be still functional as judged by the turgid appearance and released starch grains; the rest are either compressed or about to be. x 1,300

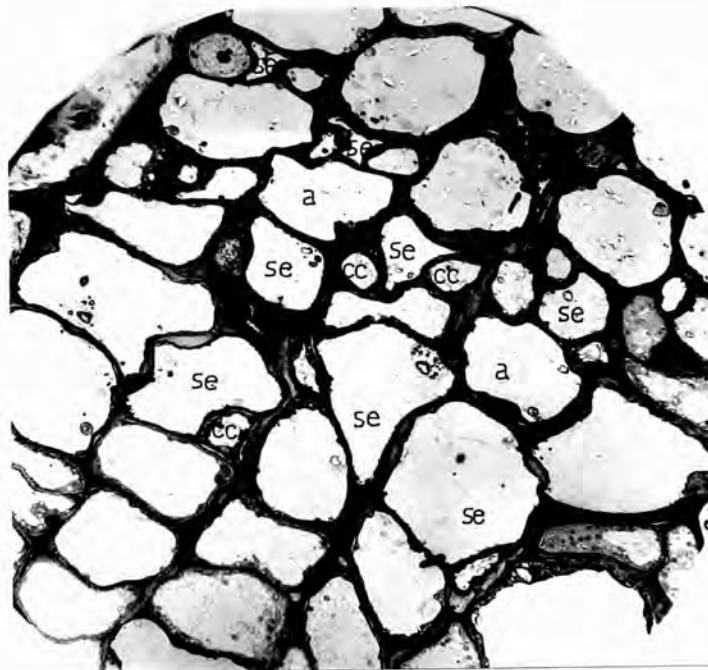
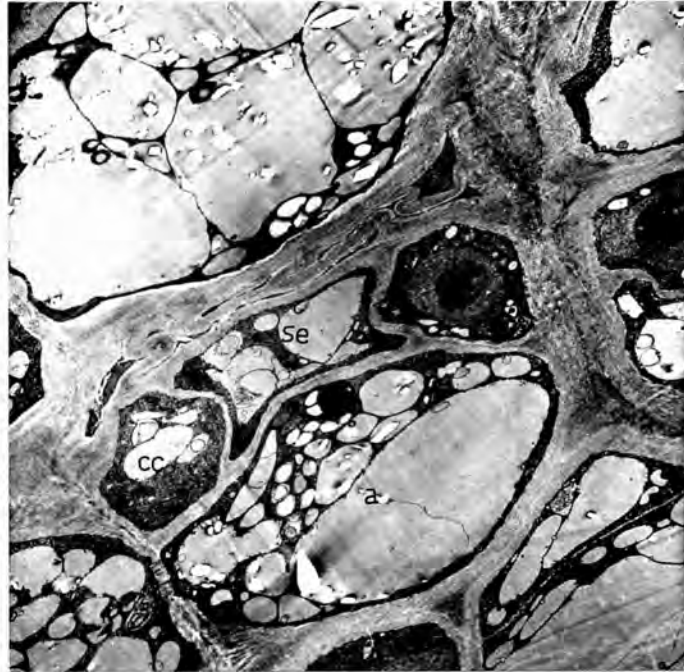
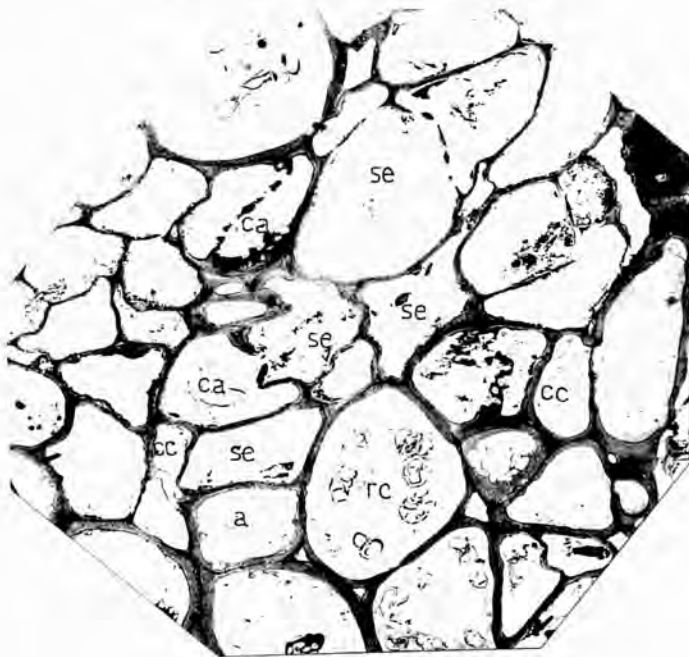
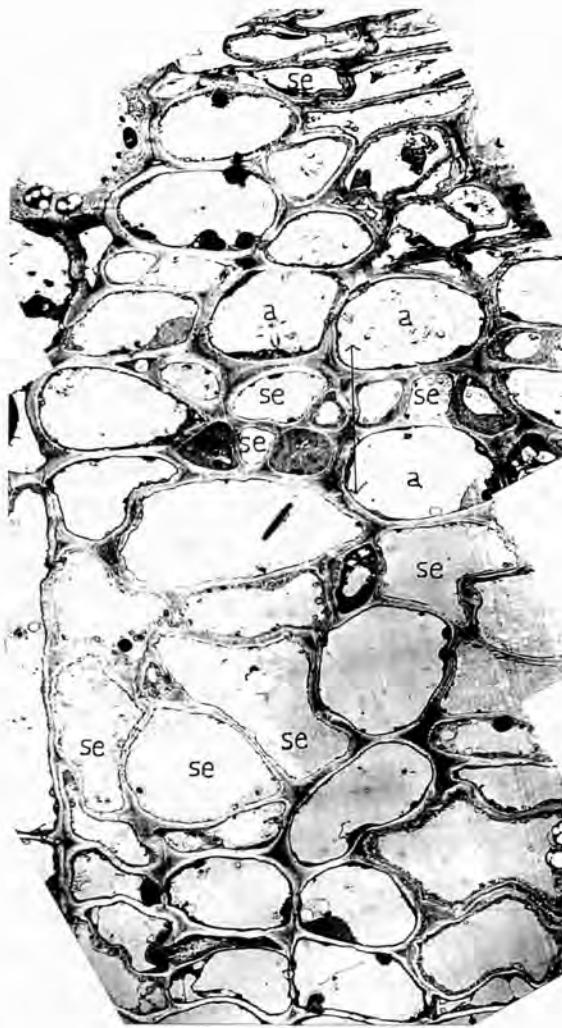


Fig. i.7      Transverse section of bark sampled in late May, 1975. Production of "summer phloem" has just begun. "Autumn phloem" (within arrows) of the last season appears to be functional. Note that the last season's summer phloem is only partially crushed.  
x 1,300

Fig. i.8      Transverse section of bark sampled in the middle of December, 1976. Note massive callose (ca) formation over the sieve plates of the summer phloem. The contents of most of these sieve elements appears congealed. That this is not due to poor fixation is suggested by the normal appearance of the parenchyma cells. x 1300





(ii) P-protein(a) Origin and early development of the material

So far little is known about the very earliest stages in the formation of P-protein at the fundamental biochemical level, though there are many speculations about the origin of the macromolecular units visible in the electron microscope. These are based mainly on the associations between the developing P-protein bodies and the ER (Bouck and Cronshaw 1965), dictyosomes (Cronshaw and Esau 1968a) and coated vesicles (Cronshaw and Anderson 1971). Spiny vesicles have also been linked with the formation of P-protein (Newcomb 1967, Steer and Newcomb 1969 and Esau and Gill 1970).

Even though Esau (1971) showed that fibrous material aggregating in young nucleate sieve elements of Mimosa pudica is the precursor of tubular P-protein, the origin of the fibrous material was not traced further back. Zee (1969a) however, suggested that the close association of polysome helices with the amorphous forms of "slime" material may possibly indicate the origin of this type of material. Similarly Behnke (1974) observed that the close contact of the helical polysomes and early P-protein and the appearance of single protein filaments near P-protein assemblies suggest a synthesis of P-protein subunits and later a formation of filaments as an assembly of several subunits.

The very early stages of development of P-protein in Fraxinus found in the present work are illustrated in the series of Figures, numbers ii.1-6.

Fig.ii.1 shows what may probably be a very young stage of P-protein development as judged by the degree of differentiation of the sieve plate. The P-protein appears as a granular mass surrounded by polysomes. It is comparable to the young P-protein bodies  $P_3$  in

Cucurbita (Cronshaw and Esau 1968a) which they described as fibrillar bodies and to the amorphous form of "slime" material in Vicia faba (Zee 1969a). Less compact material of similar structure appears elsewhere in the dense cytoplasm. It is difficult to decide whether this too is P-protein, either in the process of condensing or otherwise. It does not seem so conspicuously associated with polysomes.

Fig. ii.2 shows another young sieve tube of similar age. The presence of P-protein masses, the conspicuous fibrillar material and the thick crenelated wall are noticeable. It is apparent that the P-protein body is granular and is in close contact with the surrounding polysomes (Fig. ii.3) which seem to encircle the granular masses. It is not clear whether these polysomes have migrated from the nearby ER or elsewhere but it appears possible. Behnke (1974) has linked the disappearance of ribosomes from the ER with the first aggregation of P-protein elements. In Vicia faba, however, the polysome helices which are associated with the amorphous "slime" material seemed to be formed from free non-aggregated cytoplasmic ribosomes (Zee 1969a). Also apparent are fibrils (pp) which are probably P-protein too, and which are associated with a few polysomes.

At a higher magnification (Fig. ii.4), the fibrils seem to end on polysomes as if the latter are forming them (arrowheads). Nearby are also seen chains of polysomes arranged in a very definitely helical manner (arrows); see also Fig. ii.2. Most probably these helical chains of polysomes are connected with the formation of P-protein fibrils, themselves helical (see later).

Numerous coated vesicles (ve) which appear to arise from dictyosomes are also observed (Fig. ii.4). Cronshaw and Anderson (1971) have also reported of the presence of coated vesicles associated with developing P-protein bodies in Nicotiana. They believed that

these coated vesicles may perform the function similar to that suggested for spiny vesicles (Newcomb 1967) i.e. formation of P-protein bodies.

Fig. ii.5 which represents a somewhat more advanced stage, again shows the association of granular masses and fibrillar P-protein. Some of the fibrillar protein shows clearly the beaded or striated structure well known from studies by other workers (Northcote and Wooding 1966, Cronshaw and Esau 1968a and Parthasarathy and Muhlethaler 1969). The granular body is still surrounded by polysomes whereas the fibrillar masses are less conspicuously associated with these organelles. Possibly the two states of aggregation of what may originate as globular P-protein subunits, develop simultaneously at this stage, depending on the local density of the cytoplasm. As an indication of the age of the sieve element, the nucleus whose chromatin shows peripheral aggregation, still appears intact and the dictyosomes are still numerous and active, though not perhaps associated with the P-protein.

In Fig. ii.6, the ER cisternae without ribosomes on them are present in the vicinity of P-protein of what was assumed to be at its early stage of development. Possibly the helical chains of polysomes (arrows) found nearby the ER have been derived from the latter.

#### (b) P-protein bodies ("slime" bodies)

From the numerous ontogenetic studies already made by workers on sieve element ultrastructure, it is apparent that a P-protein body generally begins as a small accumulation which enlarges by the addition of more material in the form of granules, fibrils or tubules, until it reaches a size approximately equal to that of the nucleus as in Nicotiana (Cronshaw and Esau 1967). There are however, as noted variations between species in the morphology of the P-proteins and

their arrangement in the bodies.

In this study observations made on Fraxinus show that subsequent to its appearance, the fibrillar material aggregates together to form the large P-protein bodies. Figs. ii.7 and ii.8 show the two ends of a developing aggregate of P-protein fibrils. One end of the aggregate (Fig. ii.8) already shows some kind of alignment of the fibrils and is fairly compact; the other is less so (Fig. ii.7). In Fig. ii.7, polysomes appear to intermingle with the fibrils where the arrangement is looser and more random. This could probably indicate as suggested previously that the polysomes are the source of the fibrillar material. Dictyosomes are abundant and might conceivably also have a part to play. A rough estimate of the size of the individual fibril gives a diameter of approximately  $100\text{\AA}$ .

Fig. ii.9 shows what may be a stage later than Figs. ii.7 and ii.8. The elements which now appear more tubular than fibrillar (approximately  $130 - 165\text{\AA}$ ) are rather tightly packed and tend to be arranged parallel to one another and the sieve tube axis. Fig. ii.12 is an illustration of a rather more well-developed example of this.

Thus it appears that a well-developed P-protein body has undergone an internal reorganisation; the initial fibrillar form has been replaced by a tubular form. This conversion is however not constant; sometimes a P-protein body which appears to have completed its formation is still composed of what may be fibrillar components (Fig. ii.11). A P-protein body in Fig. ii.10 is loosely aggregated. The tonoplast is intact, the nucleolus has started disintegrating and dictyosomes and ER are in the vicinity of the large P-protein body; this sums up the state of the differentiating sieve element. It would not be altogether surprising (see Cronshaw and Esau 1967, 1968a) if the common distinction

into tubular and fibrillar forms was not altogether precise.

A smaller P-protein mass lies near the large P-protein body in Fig. ii.10. Whether this small P-protein body will fuse with the larger one or remain as it is before dispersal is uncertain.

The P-protein bodies of Fraxinus, as in most dicotyledon e.g. Cucurbita maxima (Cronshaw and Esau 1968a) are arranged in the peripheral cytoplasm and two broad types may be distinguished: a larger (Figs. ii.12 and ii.13) and a smaller (Figs. ii.14 - ii.17). Usually within a sieve element there can be found one large P-protein body and several smaller ones.

Larger mature P-protein bodies are usually ellipsoidal, elongated in the same direction as the sieve element (Fig. ii.12). Individual components are compactly arranged, parallel to one another with their long axis in the same direction as the sieve tube. Sometimes, these large P-protein bodies may also appear to be lobed. The hollowed-out appearance of the P-protein body in Fig. ii.11 suggests this. In transverse section (Fig. ii.13) they are commonly ovoidal. P-protein bodies of Solanaceae were also reported to be elongated and often sin<sup>u</sup>ous (Esau 1938). In Ricinus, the P-protein bodies are loose aggregates and do not have such compact forms (Cronshaw 1975).

Smaller P-protein bodies appear to have slightly wider tubules (maximum width of approximately 200 Å<sup>o</sup>) than those of the larger P-protein bodies. The orientation of the tubules within the smaller P-protein body is inconsistent. Only a few of the bodies have their tubules oriented in the same way as in the larger ones (Fig. ii.16); most have them oriented perpendicular to those of the latter. Figs. ii.14 and ii.15 indicate this; in longitudinal section (Fig. ii.15) the

tubules appear transversely, while in the transverse section they are cut more nearly parallel to their length (Fig. ii.14).

(c) Dispersal

Subsequent to what is regarded as their mature state, the P-protein bodies of Fraxinus disperse, as in most of the dicotyledons studied. However, in the extrafascicular sieve elements of Cucurbita maxima, the bodies often fail to disperse (Cronshaw and Esau 1968b). The initial evidence of dispersal is the swelling of the P-protein bodies as the components move apart (Figs. ii.18 and ii.19). The components segregate into tightly packed flocculi which proceed to move apart from one another (Fig. ii.21). This latter figure also indicates the state of the sieve element when dispersal begins; the still intact tonoplast, necrotic nucleus and typical mature sieve element plastids can be seen.

Higher magnification of the flocculi shows clearly the appearance of the well-known semi-crystalline banded structure (Fig. ii.22), the periodicity of the fibrils becoming prominent due to their alignment in contiguous fibrils. In the previous tubular form this periodicity was not evident.

As dispersal continues, these flocculi spread out throughout the parietal layer, while the tonoplast is still intact (Fig. ii.23). Eventually when the tonoplast breaks down (Fig. ii.24) the products of disaggregation are distributed throughout the lumen of the sieve element; smaller flocculi persisting amongst the fibrillar network. The sieve plate has not yet been perforated in Fraxinus (Fig. ii.24) in contrast to what is normally assumed in most dicotyledons studied e.g. Ulmus (Evert and Deshpande 1969). This seemed to be the case in most of the sieve tubes observed.

Instances where flocculi are not conspicuous have also been found. The P-protein bodies either disaggregate into a loose mass at once (Fig. ii.25) or assume an intermediate sort of configuration (Fig. ii.26).

Whether the smaller P-protein bodies will disperse eventually is still uncertain.

#### (d) Molecular morphology of P-protein

It is only recently that phloem exudate has been used to study the morphology of the P-protein. Sieve tube exudates are normally negatively stained or shadowed before examining in the electron microscope. Otherwise, descriptions of P-protein are based on thin stained sections of fixed materials.

Exudates from Cucurbita and Nicotiana have been examined by Kollmann et al (1970). They negatively stained them and observed two types of filaments: an elementary filament measuring  $40\text{\AA}$  in diameter and a second type measuring  $90\text{\AA}$  in diameter. Cronshaw et al (1973) also reported that the bulk of the filaments in Cucurbita and Cucumis measured  $80\text{\AA}$ ; tubules measuring  $230\text{\AA}$  were also observed. In Nicotiana however, Cronshaw et al (1973) observed that the filaments are not similar to those of Cucurbita as reported by Kollmann et al (1970). The filaments in Nicotiana are slightly wider, consisting of two helical subfibrils, each subfibril measures  $150\text{\AA}$  and an overall diameter of  $300\text{\AA}$ .

Morphology of P-protein of Ricinus communis is also well studied. Williamson (1972) observed two types of filaments; a larger type of  $200\text{\AA}$  in diameter and a smaller type of  $20\text{-}80\text{\AA}$  in diameter. Stone and Cronshaw (1973) who also examined the Ricinus exudate, observed two

types of fibrils. Fifty percent of the fibrils are made up of the larger type of fibrils ( $200\text{\AA}$ ) similar to those reported by Williamson. The second type ( $141\text{\AA}$  in diameter) consists of a central filament with lateral projections.

In this study, the exudate of Fraxinus was stained with 2% uranyl acetate and from measurements made on photographs, two types of P-protein were observed. The first type which is encountered less frequently has a diameter of approximately  $240\text{\AA} - 300\text{\AA}$  (Fig. ii.27). This type, it may be suggested, corresponds to the tubules making up the two different sized mature P-protein bodies; in fixed sections, however, these tubules vary from  $150\text{\AA} - 200\text{\AA}$  for the smaller P-protein bodies and  $120\text{\AA} - 150\text{\AA}$  for the larger P-protein bodies so there is a difference in diameter.

The second type is more frequently encountered and measures approximately  $165\text{\AA}$  (Fig. ii.28). In fixed sections, the fibrillar material possibly corresponds to this second type. Fig. ii.28 (arrow) also shows two fibrils, loosely wound in a helix. It may be conjectured that this helix gives rise to the tubular, wider form as a result of change in tightness of the helix. That P-protein can change its appearance even though remaining linear in the broad sense, is certainly suggested by the different images it presents from the earliest (Fig. ii.2) to the latest (Figs. ii.27 and ii.28). This is understandable in the light of the many suggestions that have been made that it is composed of chains of globular subunits (e.g. Lawton and Johnson 1977).



Fig. ii.1

Longitudinal section of a young sieve element sectioned obliquely near the sieve plate. A granular mass of P-protein (pb) is seen surrounded by polysomes. Some more diffuse material (pb'), perhaps also P-protein is present nearby. Note the numerous plasmalemma invaginations with enclosed fibrillar material. From bark sampled in mid-September, 1975.

x 47,500

Fig. ii.2

From a longitudinal section sampled in late April, 1975. Note the association of the different forms of P-protein; the granular mass of P-protein (pb) which is surrounded by polysomes is close to a fibrillar region. The presence of smooth ER profiles nearby possibly indicates that polysomes may have been derived from them. Arrows indicate helical chains of polysomes. x 20,000.

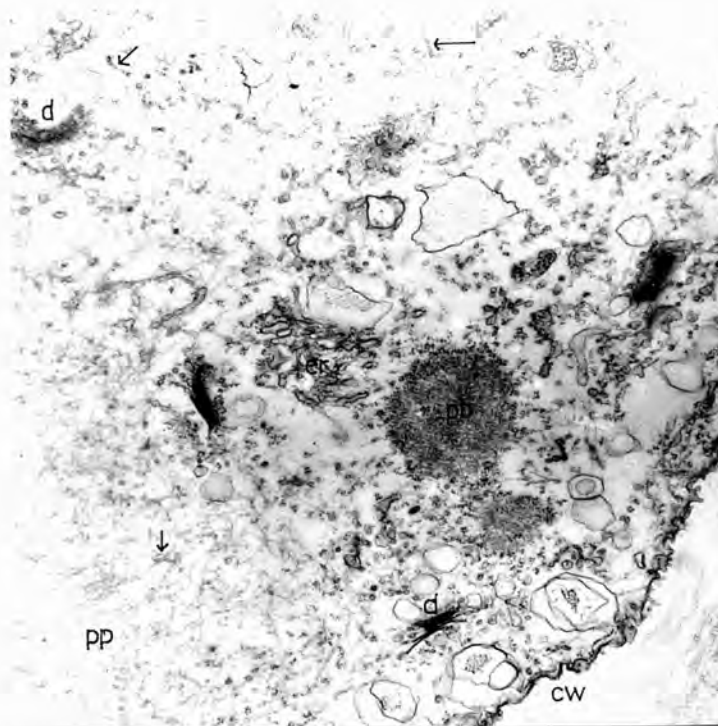
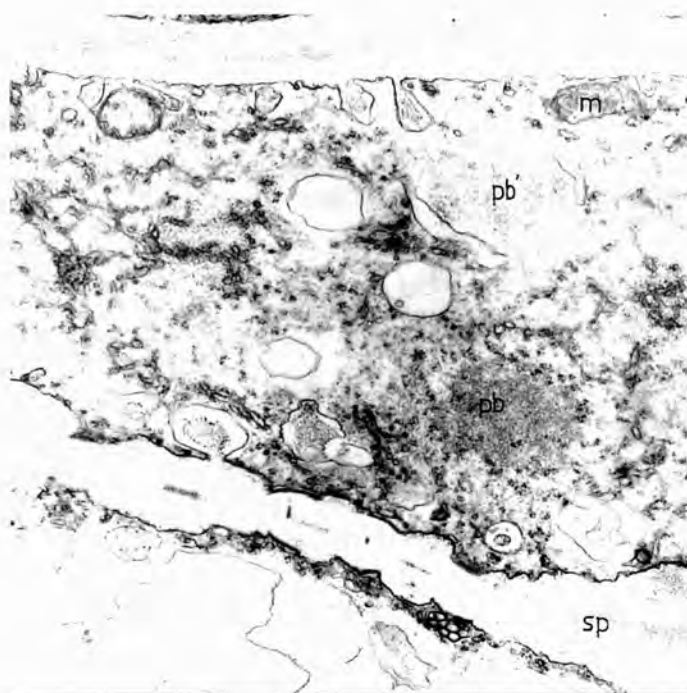


Fig. ii.3      A higher magnification of the granular mass of P-protein from Fig. ii.2. Note the close association with polysomes.  
x 50,000.

Fig. ii.4      A higher magnification of the fibrillar material from Fig. ii.2. Arrow indicates a helical chain of polysomes; arrow heads show what may be the generation of the first P-protein fibrils from the polysomes.  
x 42,000.

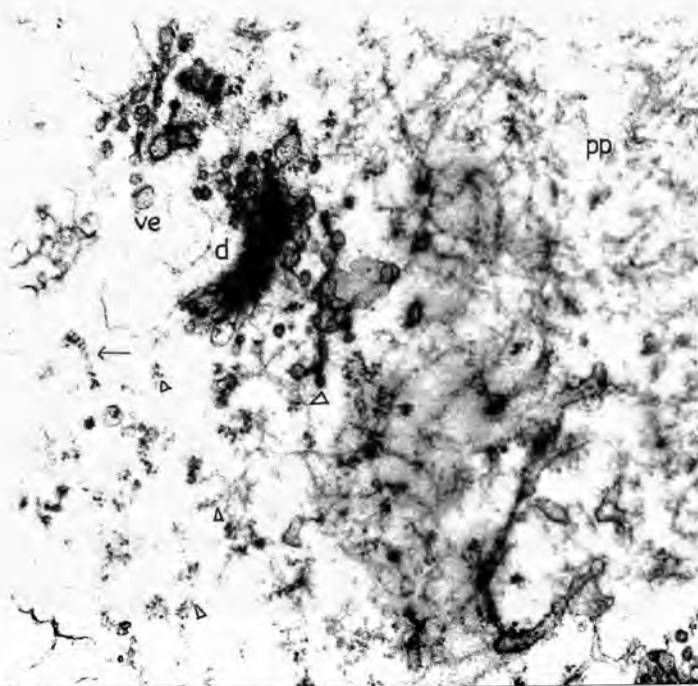
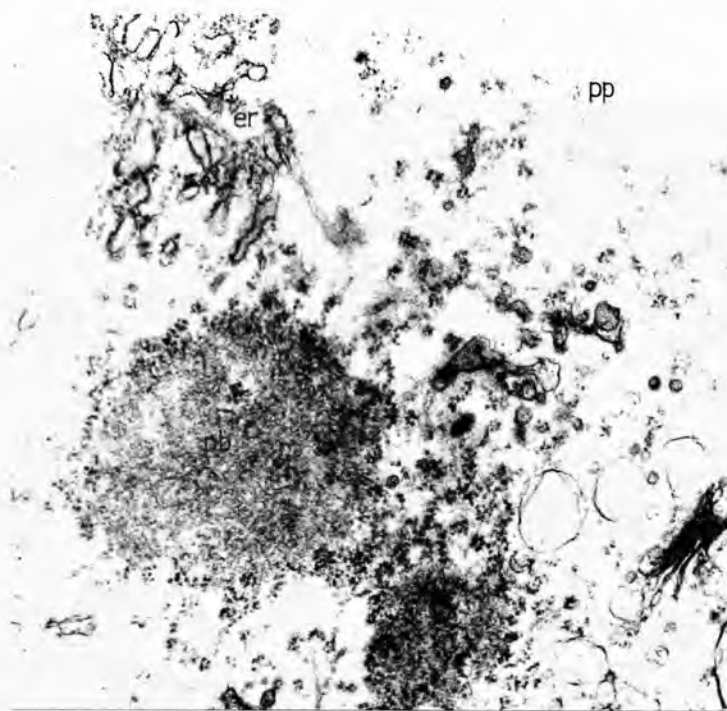


Fig. ii.5      Longitudinal section of a comparatively young sieve element sampled in early October, 1975. Note the close association of the granular mass and the fibrillar material. The beaded or striated nature of the fibrils is apparent. Polysomes surround the granular mass especially. x 12,000.

Fig. ii.6      Longitudinal section from a stem sampled in late July, 1975. Granular mass of P-protein (pb) is partially surrounded by polysomes, though there are many polysomes elsewhere as well. Nearby ER cisternae are devoid of ribosomes. Note the helical chains of polysomes indicated by arrows. Stacking of ER along the periphery has already taken place in the next sieve element. Fibrils fill the background in both the elements and in many cases seem to be associated with polysomes. x 10,200.

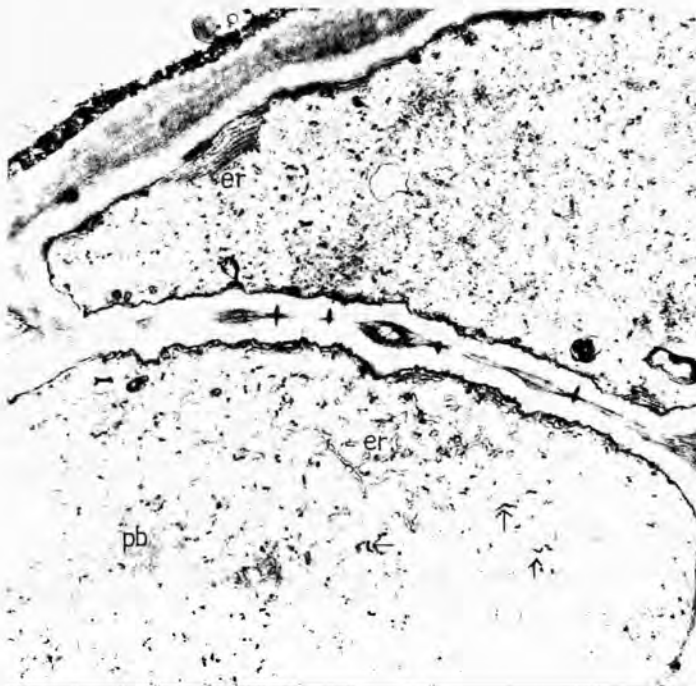
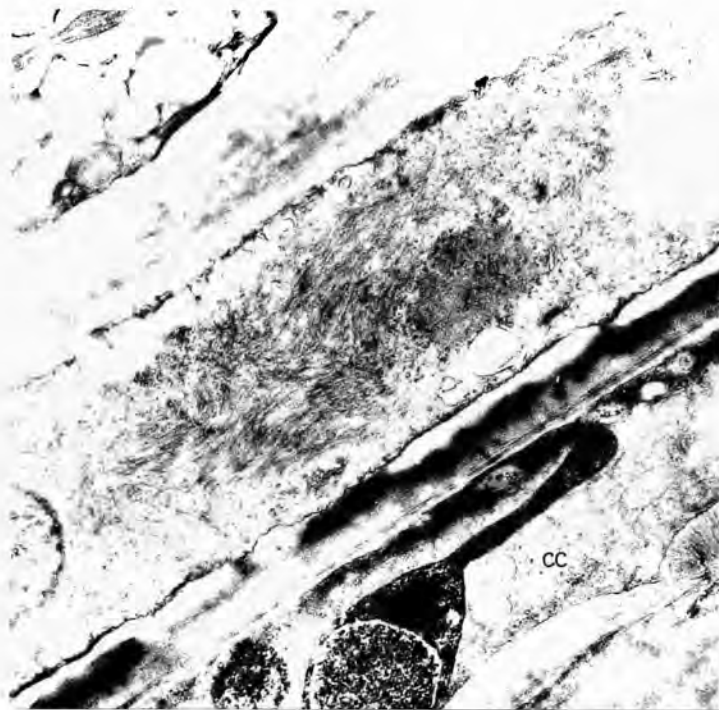


Fig. ii.7 Part of a large P-protein body at its early stage of development taken from a longitudinal section of stem sampled in late May, 1975. Fibrils which measure approximately  $100\overset{\circ}{\text{\AA}}$  in diameter are loosely and randomly arranged. Note the polysomes which are interspersed between the fibrils. x 20,000.

Fig. ii.8 The other end of the large developing P-protein body shown above. Here the elements appear compacted and tubular (approximately  $100\overset{\circ}{\text{\AA}}$  in diameter). Some degree of alignment is also indicated; parallel to the long axis of the sieve tube. Note possibly a flattened vesicle (ve) bounding the P-protein body. x 20,000.

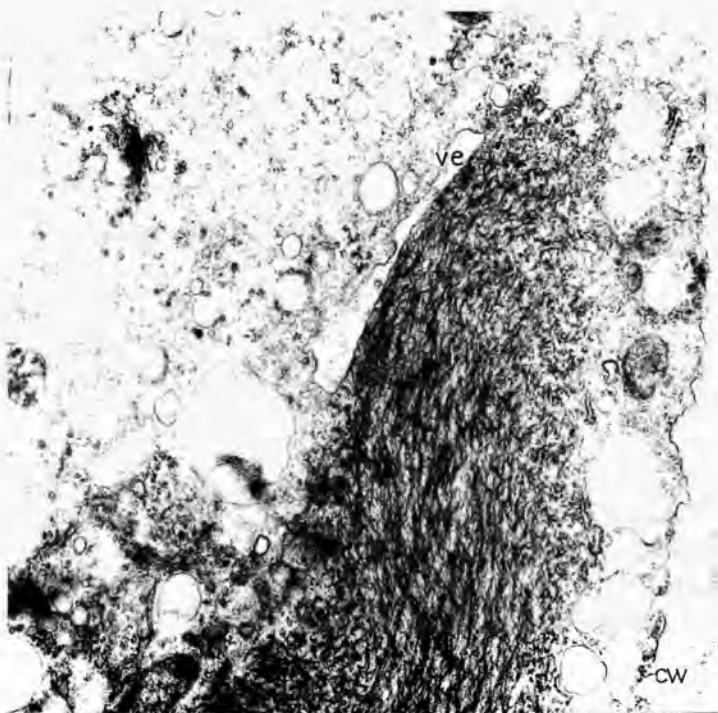
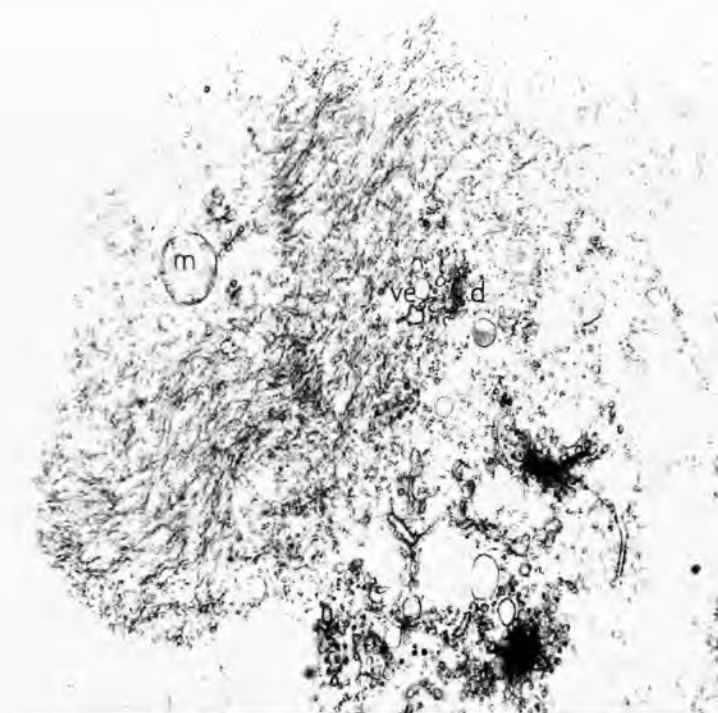




Fig. ii.9      A longitudinal section of a fairly compact region of a large P-protein body. Individual components appear tubular (approximately 150Å in diameter), and are arranged parallel to each other and to the long axis of the sieve tube. Note the presence of ER along the periphery of the P-protein body. x 30,000.

Fig. ii.10      An early stage of a P-protein body taken from a longitudinal section of stem sampled in late May, 1975. The body is composed of short fibrils (133Å in diameter) loosely aggregated. Note the intact tonoplast and the nucleus whose nucleolus is disintegrating. A smaller P-protein body (pb) is also present. ER is almost devoid of ribosomes. x 12,7500

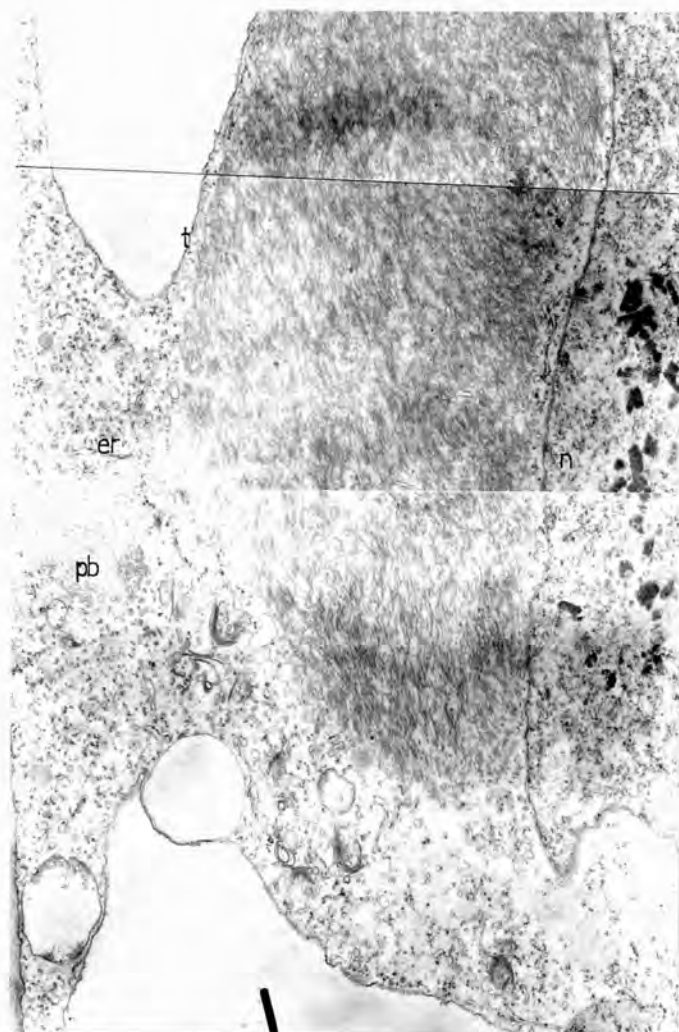
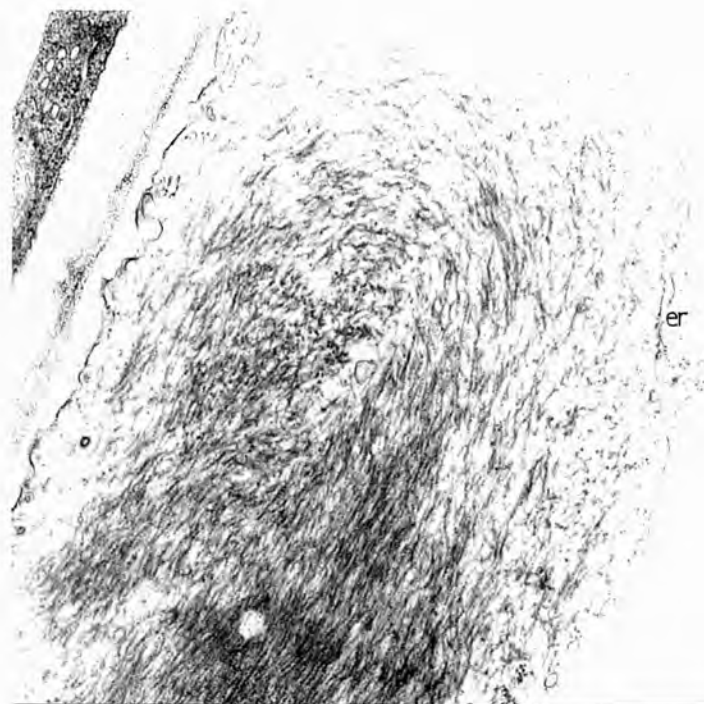


Fig. ii.11

A longitudinal section of a large P-protein body occupying almost the whole cross section of a sieve element. It is probably a glancing section which is responsible for the hollow appearance, the P-protein body being lobed. From a stem sampled in late May, 1975. x 7,400.

Fig. ii.12

A large compact P-protein body in longitudinal section. Individual components measure approximately 120-150<sup>0</sup>Å in diameter. Tonoplast appears to be disintegrating. From a stem sampled in late May, 1975. x 10,200.

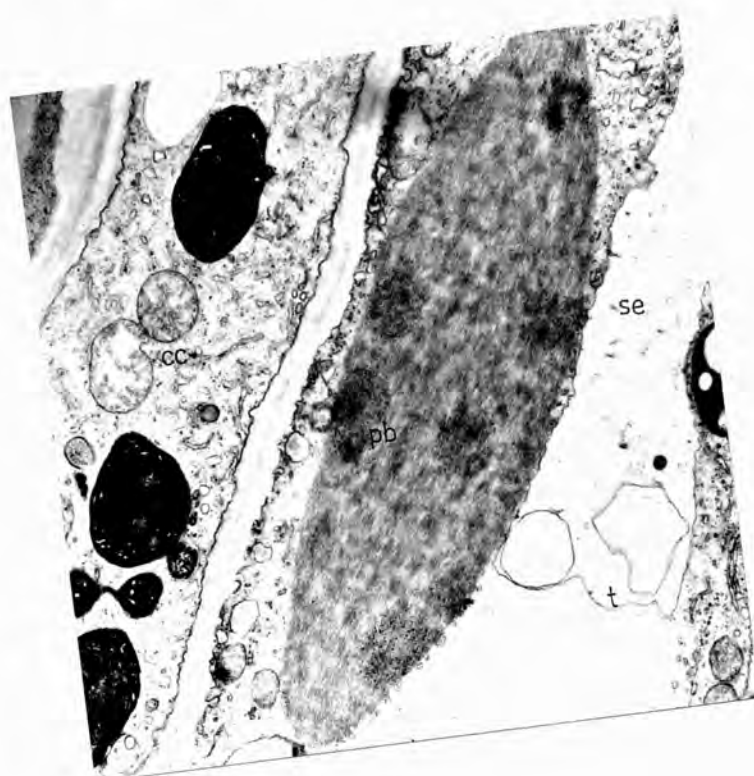
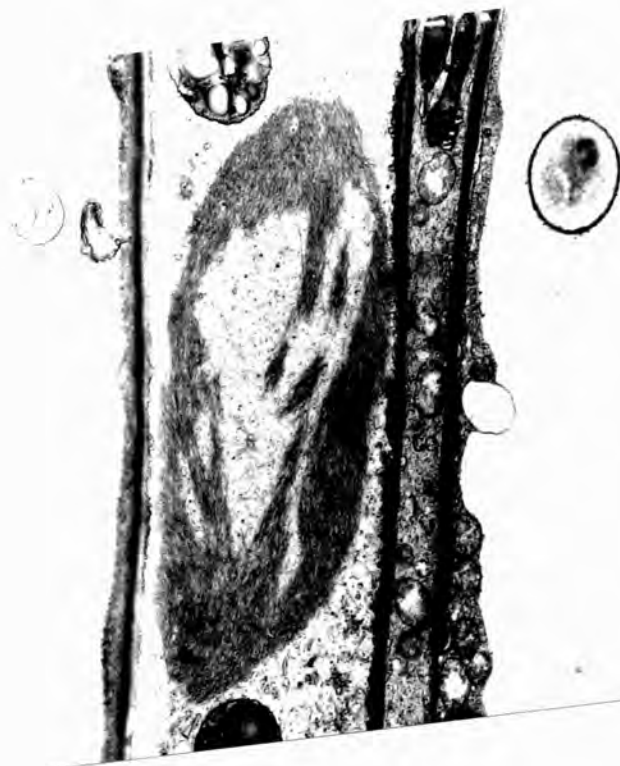


Fig. ii.13      A cross section of a sieve tube sampled in late May, 1975. The P-protein body is of the large type. Components appear tubular (approximately  $130\text{\AA}$  in diameter). The tonoplast is disintegrating. x 8,000.

Fig. ii.14      A transverse section of a sieve tube with two smaller P-protein bodies. Individual components (approximately  $150\text{\AA}$  in diameter) are tightly packed and appear to be arranged parallel to the plane of the section. Note plasmalemmal invagination of the wall filled with fibrillar material. From a stem sampled in late May, 1975. x 20,000.

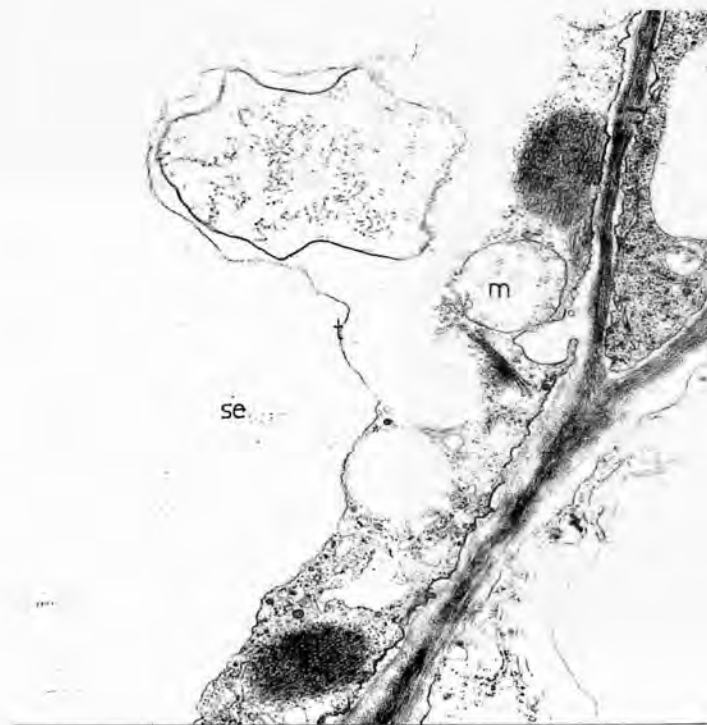


Fig. ii.15      A small P-protein body. The tubules (200<sup>0</sup>Å in diameter) appear to have been cut perpendicularly. From a longitudinal section of a bark sampled in late May, 1975. x 32,500.

Fig. ii.16      Longitudinal section of a young sieve element sampled in late May, 1975. Note the orientation of the tubules (133<sup>0</sup>Å in diameter) in the small P-protein bodies; parallel to the longitudinal axis of the sieve tube. Matrix of the typical sieve tube plastids is moderately electron dense. The tonoplast has not yet disintegrated and polysomes are much in evidence. x 30,000.

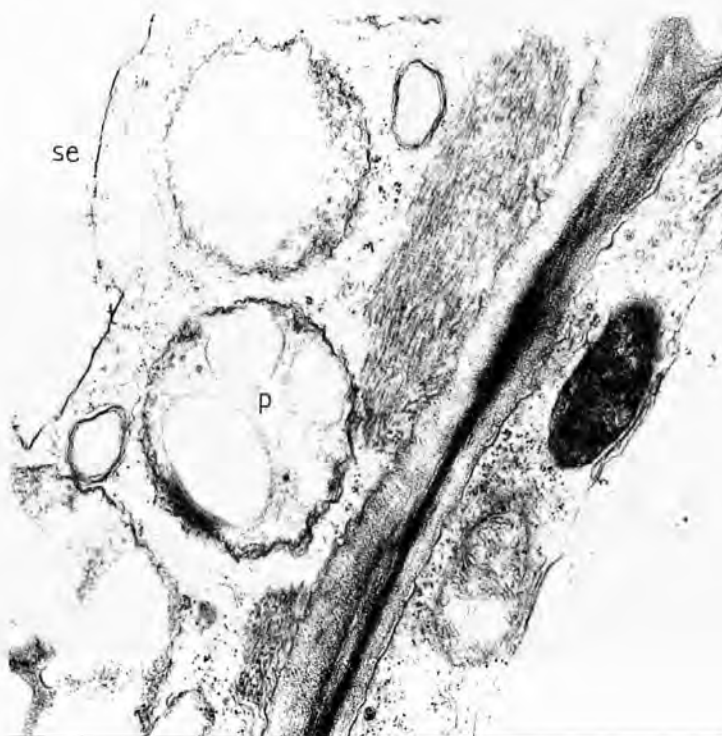
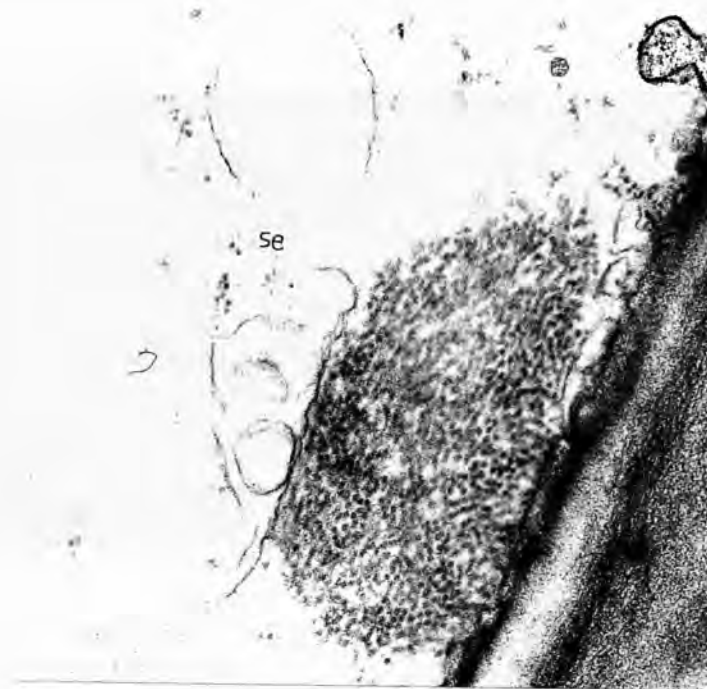




Fig. ii.17      Longitudinal section of a young sieve element from a stem sampled in late May, 1975. The tubules (approximately  $160\text{\AA}$  in diameter) have an inclined orientation, probably more nearly transverse than longitudinal. The tonoplast appears to have disintegrated indicating a later stage than in Fig. ii.16. x 50,000.

Fig. ii.18      Early stage of a P-protein body dispersal; note the flocculi. However, tonoplast is still intact. The presence of two nucleii were possibly representing a lobed nucleus. From a longitudinal section of stem sampled in late April, 1975. x 3,000

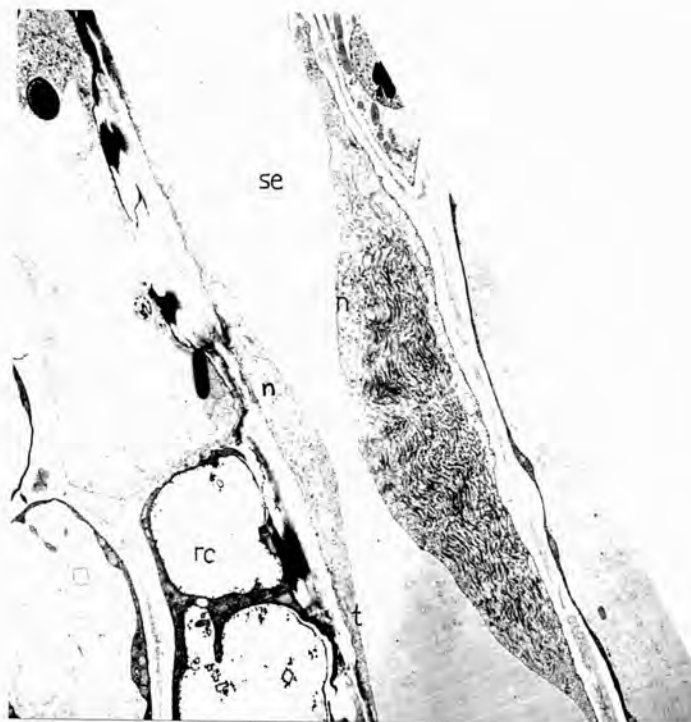
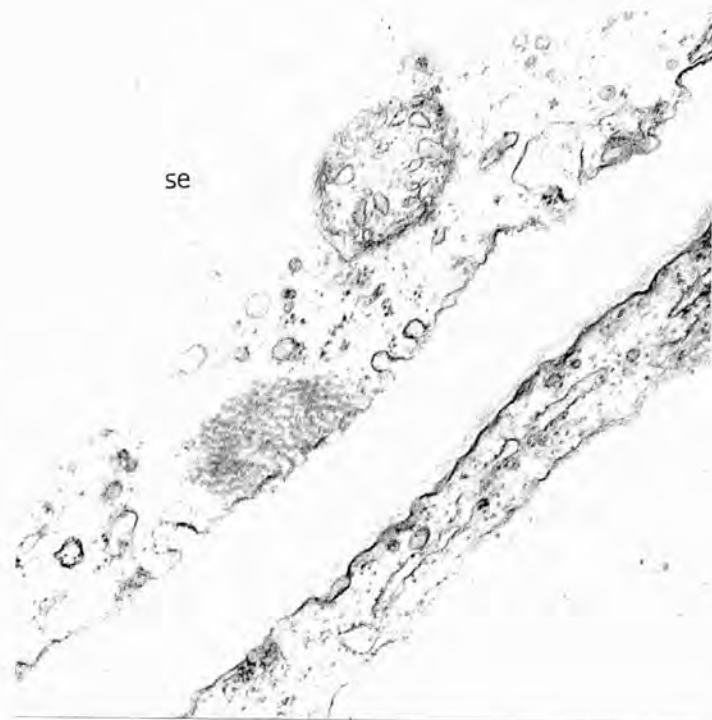


Fig. ii.19      Higher magnification of Fig. ii.18. Flocculi of P-protein clearly show the banded structure. The tonoplast seems to have persisted to a later stage than is sometimes the case (Compare Figs. ii.12 and ii.13).  
x. 12,000.

Fig. ii.20      A transverse section of secondary phloem from a stem sampled in late May. P-protein body is dispersing and is located on the periphery of the sieve tube while the tonoplast remains intact. Nucleus appears necrotic.  
x 7,000.

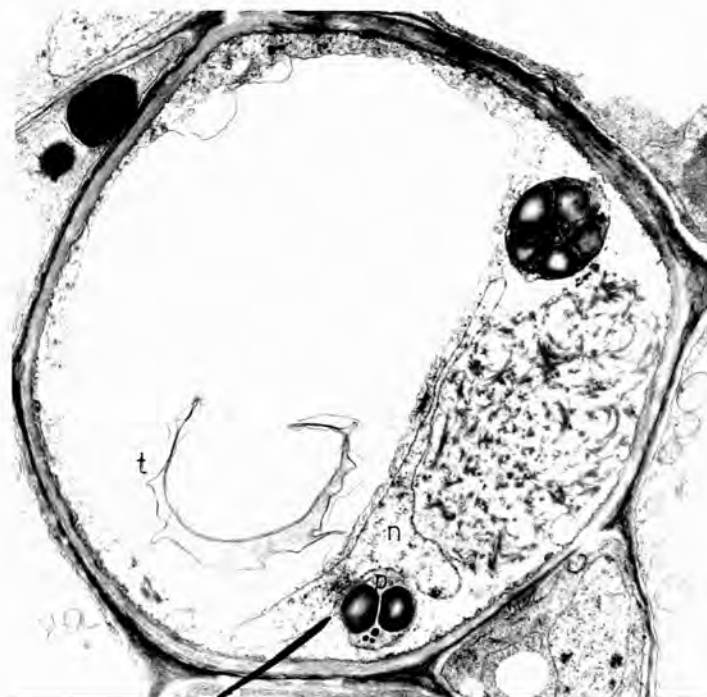
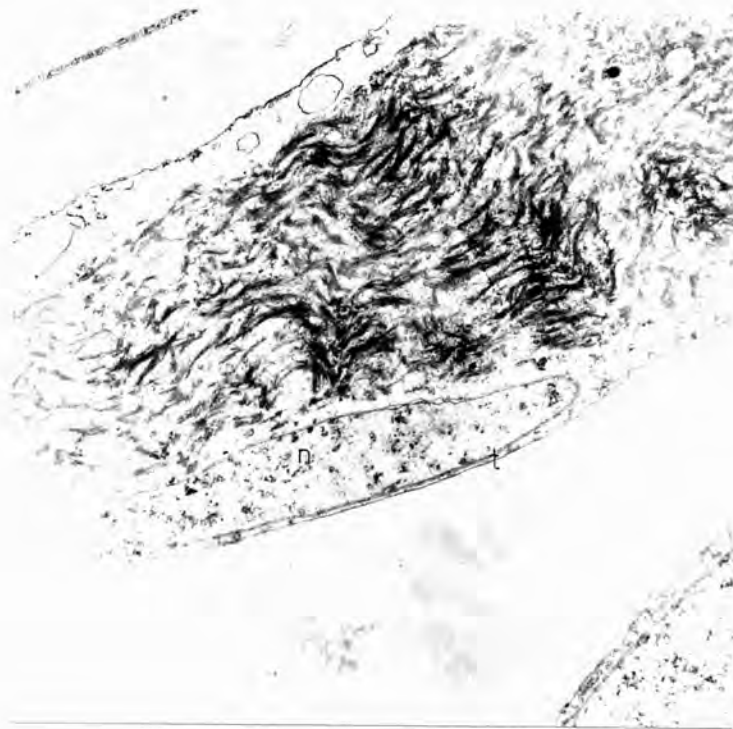


Fig. ii.21      A later stage of P-protein body dispersal. Flocculi have moved further apart and are interspersed with some single strands of fibrillar P-protein. Tonoplast is still coherent; nucleus is reduced. Plastids are characteristic of mature sieve tubes. From longitudinal section of secondary phloem of stem sampled in late May, 1975. x 5,000.

Fig. ii.22      Flocculi at a higher magnification. Note the semi-crystalline banded structure indicating the fibrillar form of P-protein. From a longitudinal section of bark sampled in late April, 1975. x 83,750



Fig. ii.23      A typical flocculus at a later stage of dispersal when these will be found mostly at the periphery of the sieve tubes. Note the semi-crystalline nature shown by the banded structure. From a longitudinal section of stem sampled in late May, 1975. x 43,500





Fig. ii.24      Montage of a longitudinal section of a sieve tube with still unperforated sieve plates sampled in late July, 1975. P-protein is distributed throughout the lumen and the tonoplast has broken down; a few flocculi of P-protein are still present in the central region of the sieve element. Nucleus (n) is disintegrating. Plastids are broken down. x 3,000



Fig. ii.25      Longitudinal section of a sieve element approaching maturity as judged from the "clear" appearance of the nucleus (see Chapter III, Section iv (b)). P-protein body is dispersing without forming flocculi, as more often observed. From stem sampled in late May, 1975. x 20,000

Fig.ii.26      Longitudinal section of a mature sieve element sampled in late May, 1975. Dispersing P-protein is in loose aggregates which appear to be of the tubular form in some places. Note the absence of the tonoplast and the sheathed mitochondria. x 20,000

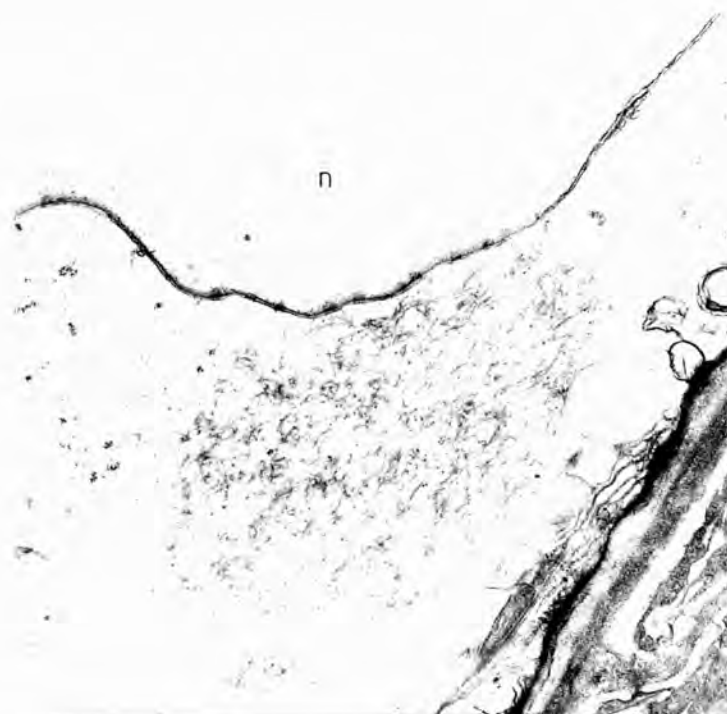
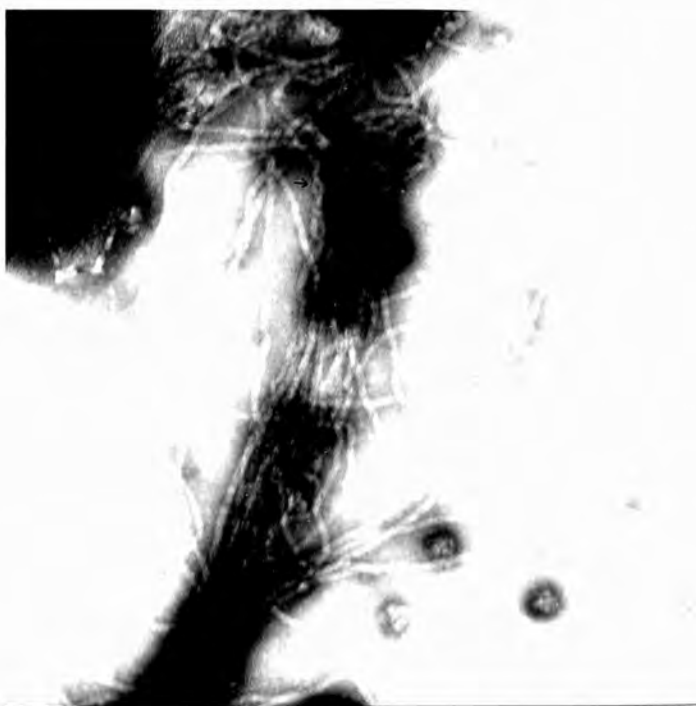


Fig. ii.27 - ii.28

Filaments from sieve tube exudate  
negatively stained with 2% uranyl acetate.  
Exudate collected in July, 1976.

Fig. ii.27      The filaments measure approximately  $240\text{\AA}^{\circ}$   
in diameter. The dimensions correspond  
with those of the tubular form of P-protein  
seen in a glutaraldehyde fixed sections.  
x 95,500

Fig. ii.28      The filaments measure approximately  
 $165\text{\AA}^{\circ}$  in diameter. Note the loosely  
wound helix of 2 filaments (arrow)  
x 54,000.



(iii) Sieve Plates(a) Development

In Fraxinus it is difficult to recognise sieve plates in their very earliest stages before callose platelets are deposited. Very often they resemble the transverse walls of parenchyma cells. The only means of detecting these plates is to rely on the presence of certain other features characteristic of sieve tubes.

A phloem mother cell in Fraxinus, gives rise to one large sieve element and a smaller companion cell in summer or to a smaller element and two companion cells at the end of the growing season. It is on this criterion that identification of most of the earliest stages of the sieve tube development was based (Figs. iii.1, iii.2). The other most easily recognised feature is the presence of a P-protein body (Fig. iii.4), but the chance of finding one in the earliest stage of the sieve tube development is rather remote. Probably the presence of P-protein initials (Fig. iii.5) is all that can be expected. Other features which have also been used to determine the earliest sieve plate stages include:- the dense cytoplasm which is due to the abundance of ribosomes (Figs. iii.3, iii.5), and the special sieve-tube plastids which have an electron dense matrix (Figs. iii.1, iii.2)

The development of the sieve plate in Fraxinus appears to be as follows: Figs. iii.2 - iii.6 show the very earliest stages in the development. Before callose platelets begin to be laid down, the plasmodesmata appear similar to those in cell walls between two parenchymatous cells (Fig. iii.3). The compound middle lamella thins out as it approaches the plasmodesmata (Figs. iii.3, iii.5). The area around the plasmodesmata appears quite distinctive from the

rest of the cell wall, though not so dense in the present preparations as that found in Saxifraga (Deshpande 1974) and Cucurbita (Deshpande 1975).

Fig. iii.6 shows that the pre-pore area seems to grow into protuberance, indicating that its growth rate is faster than that of the wall in the immediate vicinity. Whether callose is laid down at this stage is not obvious.

Usually one ER cisterna is shown to be closely opposed to these pre-pore protuberances. Invaginations formed from the plasmalemma covering the sieve plate, often with fibrillar contents, are commonly encountered (Fig.iii.6). Probably these fibrillar materials contribute to the development of the cell plate (see Chapter III, section (iv) (a)).

What happens in the next phase to these protuberances has not been ascertained. The next stage traced is the familiar appearance of a well-defined, pair of bevelled electron-lucent callose platelets, one on each side of the future sieve plate and surrounding the plasmodesmata (Figs. iii.7 - iii.10).

Initially these pairs of platelets appear to protrude above the level of the neighbouring cell wall indicating their fast growth (Fig. iii.7). The intervening wall (Fig. iii.8) probably shows by its pronounced crenulation that it is in the process of growth as well. When growth of the intervening wall overtakes that of the callose platelets, the latter may even appear depressed (Figs. iii.13 - iii.14). It is also probable at this stage that the ER begins to aggregate in stacks on the callose platelets (Figs. iii.13 and iii.14). Otherwise, only one cisterna of the ER lies in close opposition to the platelets (Figs. iii.10, iii.11). The ER may even penetrate the plasmodesmatal canal (Fig. iii.11) as has been shown by Esau and Gill



(1972) and Deshpande (1974).

Presumably, the callose platelets grow laterally by deposition on the cellulosic wall that is still growing. When this happens the growth of the cellulosic wall is checked. The angle of slope between the callose platelets and the middle lamella may indicate the relative growths of the platelets (laterally) and the adjoining wall (axially). If there are two or more plasmodesmata close to each other, the lateral growth of the callose platelets will cause the callose to meet at the edge (Fig. iii.9). Further growth of the sieve plate will mean that while the rest of the intervening wall increases in height, that part covered with callose will appear as a depressed compound sieve plate.

#### (b) Perforation

Perforation begins with the widening of the plasmodesmatal canal at the middle lamella regions, forming a median cavity (Fig. iii.14). At what particular stage of the sieve plate differentiation perforation begins, is difficult to ascertain. It has been observed in Fraxinus that these median cavities appear at different stages. Fig. iii.14 shows these cavities being formed when the intervening wall is raised above the pore sites i.e. at a fairly advanced stage of differentiation; and some have also been observed to be present when sieve pore sites are at the same level as the neighbouring wall (Figs. iii.15 and iii.17).

It is not clear what takes place at the final stage of perforation. It has been assumed that as the median cavity grows laterally along the sandwiched layer, a corresponding erosion of the callose axially along the plasmodesmatal canal also takes place. This results in the widening of the pores.

The existence of the sandwiched layer until the final dissolution of the callose suggests that its replacement by callose as suggested by Esau for Cucurbita (Esau 1969) is unlikely. Finger-like projections extending from the median cavities as seen in Saxifraga (Deshpande 1974) have also been observed (Fig. iii.17).

Whether all the callose is removed finally is not settled. Even though, most of the perforated pores encountered in Fraxinus have fairly conspicuous callose, pores with very little callose have also been observed (See Chapter III, section (iii) (c)).

The various stages of differentiation of the sieve plate, however, have not been correlated in detail with the state of the cytoplasm. However, with the few low magnification photographs available, some general idea can be derived about the corresponding states of the cytoplasm.

The tonoplast may even be present at the advanced stage of sieve plate differentiation (Fig. iii.12). The exact time of disappearance has not been observed. It is also at the advanced stage of the sieve plate differentiation that the cytoplasm begins to lose its organelles, even though some polyribosome still persist (Figs. iii.13 and iii.14).

#### (c) Structure of mature sieve plates

The true nature of some structural features of mature sieve plates, have, for a long time, been a major subject of controversy. The picture is still not clear mainly because of a) the high longitudinal conductivity of sieve tubes and b) the ability of the sieve-tube protoplast to respond to manipulation by a rapid production of callose.

Functioning sieve tubes have a high turgor pressure; Weatherly (1962) found a positive pressure of 30 atm. In the preparation of material for electron microscopy, this high turgor pressure has somehow to be released, and it is usually released longitudinally. The P-protein often seen occupying the pores of sieve plates may thus be there due to this release, as a surge artifact.

Attempts have been made to minimise these surge effects. Hepton and Preston (1960), Duloy et al (1961), Mehta and Spanner (1962) used sucrose in the fixative to lower the turgor pressure. Others have tried various methods of fixing while the phloem is still attached to the plant; Esau and Cheadle (1961) injected 4%  $\text{KMnO}_4$  straight into the hollow stem of Cucurbita maxima, Buvat (1963) removed the epidermis from stems and placed drops of fixative on the exposed surface, Kollmann and Schumacher (1962) surrounded their stems with a glass trough full of fixative before cutting, and Wark and Chambers (1965) also fixed by injecting into the pith cavity in stems of intact Pisum. Other methods tried include immersing whole tobacco plants in fixative, rapidly freezing whole plants into isopentane-methylcyclohexane mixture cooled by liquid nitrogen, before fixation (Cronshaw and Anderson 1969), and wilting by withholding water for 9 days prior to fixation (Anderson and Cronshaw 1970a). Evert et al (1973a) too, eliminated all mobile carbohydrates before fixation by cutting off the cotyledons and darkening.

Other workers including Bouck and Cronshaw (1965), Evert and Murmanis (1965), Wooding and Northcote (1965b), Northcote and Wooding (1966), Evert et al (1966) have taken rather simpler but probably effective precautions against the turgor release, merely using a narrow central portion from an excised sample, a few cm long. Yet their photographs more often than not show material occupying sieve

plate pores. In fact their results do not differ much from those of workers who took more elaborate precautions.

The question of whether the rapid production of callose is responsible for all the callose seen is still doubtful. But callose has sometimes been found to be absent from functioning sieve tubes of orange for instance (Schneider 1952) and from the sieve cells of conifers (Evert and Alfieri 1965).

Eschrich (1956, 1963) who carried out extensive studies found that rapid callose deposition is a result of injury. Zimmermann (1960b) discovered by light microscopy that callose is only found on the sieve plates when tissues are stored or fixed and not when sectioned immediately.

Many workers have shown that rapid killing techniques yield results with sieve plates showing no deposition at all; Evert and Derr (1964a) showed that the extent of callose deposition seemed to depend upon the rapidity with which the sieve element protoplast was killed after wounding and that dormancy and definite callose was a natural phenomena. Currier and Shih (1968) also prevented callose formation in Elodea leaves during fixation by freeze killing and Parthasarathy (1968) observed progressive accumulation of callose in tissue killed in liquid nitrogen at several-minute intervals after collection.

By contrast, Engleman (1965a) tried killing tissues of Impatiens rapidly by subjecting intact stems with dry ice, liquid nitrogen, boiling water, oil and crushed  $\text{CO}_2$ . He found that callose deposition was still apparent only 4 seconds later in the young sieve elements and yet more in the more mature ones. These deposits only occurred in sieve plates, not in pit fields of parenchyma cells. He argued

that if callose had already formed within four seconds of the start of manipulation, then it was most likely that this deposition was not an artifact. He concluded that callose was present naturally in sieve elements of intact plants of Impatiens.

Crafts and Currier (1963) also reported the presence of callose in mature sieve elements of rapidly killed herbaceous shoots.

Possibly there is some callose naturally present in mature pores which may be a relic from that deposited while the pores were being formed, for Esau et al (1962), Esau and Cheadle (1965), Northcote and Wooding (1966) and many others have suggested that callose deposition is a normal phenomena during the development of the sieve plates. Further, Hepton and Preston (1960), Esau (1964, 1965), Evert and Derr (1964a) have also found that callose is deposited when sieve elements become dormant in those species where they persist for another season. Perhaps only some dormancy callose disappears when sieve tubes become active again. Esau (1948, 1965) has shown the lability of callose to be associated with the developmental state of the cell and that this is responsive to internal changes as well.

Thus, there are two controversial points concerning the structure of functioning sieve plates: (1) are the pores opened or occluded with P-protein; (2) is there appreciable or very little callose present. Munch's pressure flow theory is favoured by open pores where there is a wide and clear continuity between the sieve elements, with little of either P-protein or callose. Occluded pores on the other hand traversed by P-protein constitute the favourable structure of the sieve plates for the electro-osmotic theory (Spanner 1958, 1975).

The type of fixative used has also been found to contribute to the picture of the sieve plate structures especially their contents.

$\text{KMnO}_4$  was found unsuitable for it failed to fix the non-membranous contents of sieve tubes and replaced them with coarse precipitates (Kollmann and Schumacher 1962; Johnson 1966). Esau and Cheadle (1961) have also found unplugged pores when  $\text{KMnO}_4$  was used. After the introduction of glutaraldehyde and acrolein for fixation of phloem tissues (Bouck and Cronshaw 1965), later workers preferred these fixatives followed by  $\text{OsO}_4$ .

Photographs shown in the following pages are representative of the many mature sieve plates observed. It should be noted that these samples were conventionally fixed with glutaraldehyde followed by  $\text{OsO}_4$  unless otherwise stated. Precautions against surge effects have also been taken (see Methods).

It has been repeatedly observed in the present work that most of the mature sieve plates of Fraxinus excelsior are heavily callosed and occluded with P-protein even if Karnovsky's fixative, which has high tonicity and speed of penetration was used. Figs. iii.18 - iii.29 show this type of pore. Pores as is now well known are lined with plasmalemma, which is continuous from cell to cell (Fig. iii.19). Sometimes plasmalemma may also form invaginations within the callose (Fig. iii.20). Possibly this configuration represents the remnant of the median nodule.

The P-protein within the pores is most often found to be densely packed but cases in which it is in a loosely packed state have also been observed (Fig. iii.19). Closeness of packing is probably due to the formation during fixation of thick callose cylinders compressing the sieve pore contents. The P-protein fibrils recognised by the banded structure (see Fig. iii.23) are aligned transversely giving it a semi-crystalline appearance. In Fig. iii.21 membranes from ER

cisternae also transverse the sieve pore, again a feature noticed in other species.

It is also interesting to note that in the sieve tubes whose pores are heavily callosed and occluded, the plastids near the pores tend to be broken and the starch grains released. The starch grains released are normally found above the mass of P-protein which accumulates at the sieve plates (Fig. iii.24). A few are also found on the downstream side, very close to the sieve plates (Fig. iii.25). There is not always the suggestion, however, that the latter position of the starch grains is due to surge effects forcing the starch grains through the sieve pores.

Sometimes, these starch grains become embedded in the P-protein mass (Fig. iii.27) creating a fibril-free space above the sieve plates. These starch grains appear to be trapped in between the plates and the P-protein mass.

A similar sort of space, but this time surrounding individual starch grains or a group of them, is also observed. Fig. iii.26 shows this space or halo surrounding groups of starch grains giving the appearance of an imaginary membrane of a plastid. See also Fig. iii.22.

Literature regarding sieve tube plastids and starch grains has been reviewed by Esau (1950), Engleman (1965b) and Behnke (1972). Engleman reported that the rupture of sieve tube plastids is most frequent in sieve elements near the cut surfaces. In this study, the sieve plates observed are some distance from the cut surfaces.

Some sieve pores have also been observed which are thinly lined with callose (Figs. iii.30 - iii.33). Again the packing of P-protein

within the pores is variable. Fig. iii.32 shows a loosely packed P-protein within the pores as compared to Fig. iii.31.

Open pores were also sometimes encountered but less often. Figs. iii.34 and iii.35 are examples devoid of any P-protein or other occlusion, except for one which is lined by stacks of ER. Many workers including Cronshaw and Anderson (1969), Anderson and Cronshaw (1969, 1970b) maintain that open pores like the present represent the natural functioning state.

Figs. iii.36 - iii.40 however, show a different kind of open pore. The pores are either thinly callosed or without any callose at all. Except for a few loose fibrils traversing the pores, they are empty. Even if there is appreciable P-protein accumulating near the sieve plates (Fig. iii.39) the P-protein does not actually plug the pores as in the previous examples. It was observed that in these sieve tubes with such open pores, the starch grains are seldom broken (Fig. iii.37) a circumstance which lends colour to the claim that they represent the undisturbed condition.

Figs. iii.41 and iii.42 show sieve pores probably affected by surge effects. These plates were, however, nowhere near the cut surface (compare Fig. iii.43). In Fig. iii.41 the whole mass of P-protein is seen to have pushed its way through the sieve pores trapping the starch grains in between the P-protein mass and sieve plate. The pores are blocked by starch grains although one of the starch grains has apparently managed to pass through a pore. Simultaneously, protruberances of P-protein emerging out of sieve pores (Fig. iii.42) also probably indicate a surge effect.

Fig. iii.44 has also been included to show that callose formation may lead to the complete blockage of the sieve plate. Since



the bark was sampled in late Spring (May), it seems unlikely that it was a dormancy callose. The presence of intact plastids suggests that the sieve tube was still functional and formation of callose may also be an effect of manipulation.

When extended lengths of phloem are examined, the appearance of successive sieve plates in a file is similar. There is always a greater accumulation of P-protein on corresponding sides of the plates presumably on the 'upstream' side (Figs. iii.45 and iii.46). It has previously been mentioned that there is often a space around released starch grains against the sieve plates, though sometimes it is in the lumen. The nature of this space is not altogether clear. It may give the appearance of being due to shrinkage and contraction of the P-protein mass (Fig. iii.45); or of being filled with starch grains (Fig. iii.27) or of having no probable explanation (Fig.iii.22).

Fig. iii.1

Longitudinal section in the phloem region.

The presence of the narrow cells next to the larger ones suggests that the latter form is a sieve tube with its companion cells. The sieve element has a thin layer of cytoplasm as compared to the companion cell. From a stem sampled in late May, 1975. x 7,000.

Fig. iii.2

A very young sieve tube as indicated by the presence of dense matrix plastid (p) and a narrow companion cell next to it. Cytoplasm forms a narrow dense peripheral layer. Note the large invaginations of the tonoplast, those on the right being presumably sectioned perpendicular to their "stalk". The tonoplast appears to be missing in places. From a stem sampled in early June, 1976. x 7,000.

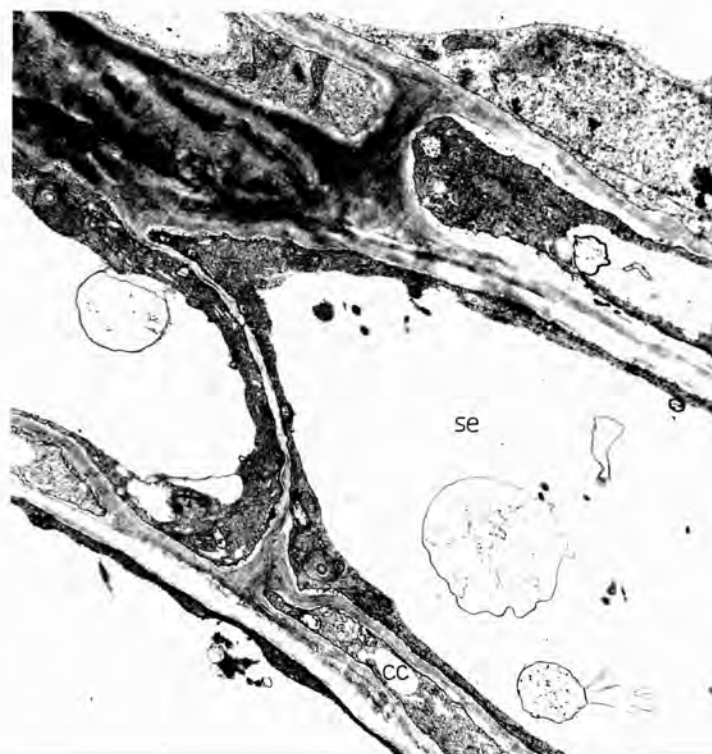
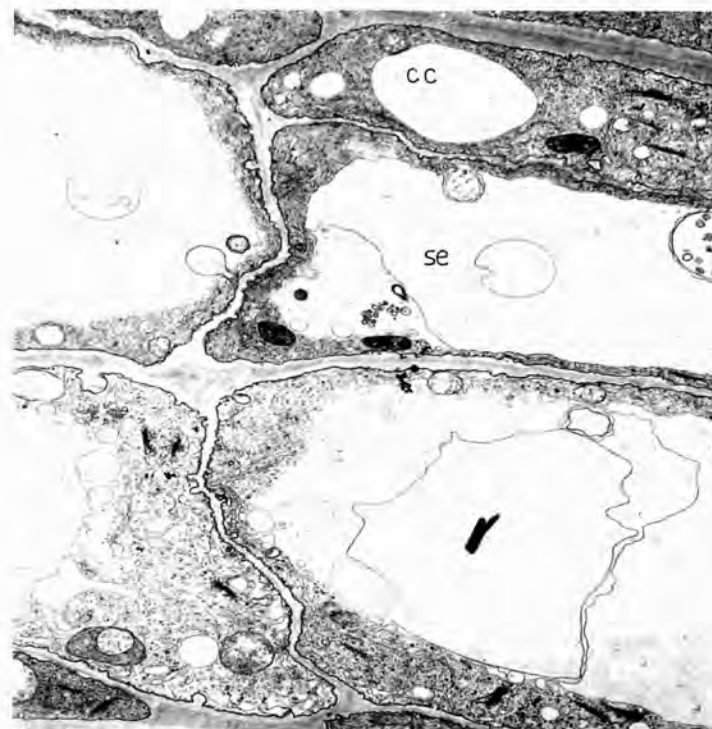


Fig. iii.3 Higher magnification of Fig.iii.2.  
Note the plasmodesma which is similar  
to one between two parenchyma cells.  
The compound middle lamella becomes  
thinner as it approaches the plasmodesma.  
Cytoplasm is rich in ribosomes.  
x 28,000.

Fig. iii.4 The presence of a small P-protein body  
suggests that this is a sieve plate even  
though plasmodesmata are not discernible.  
The ER is not yet aligned in close  
apposition to the cell plate. Invaginations  
of the plasmalemma are present on the future  
sieve plate. From a longitudinal section of  
stem sampled in late May, 1975. x 10,200.

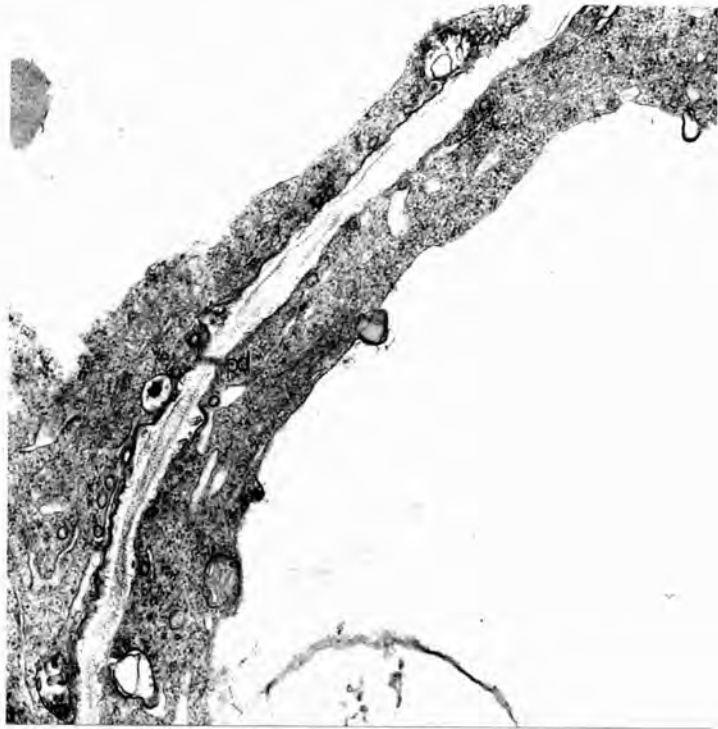


Fig. iii.5      A sieve plate at a very early stage, identified by the fibrillar mass (pb'), the initial of a P-protein body. Cytoplasm is dense with ribosomes. The area round the plasmodesmata is distinctive. Middle lamella is very thin in the region of the plasmodesmata. From a longitudinal section of tree sampled in early June, 1976. x 42,000.

Fig. iii.6      Micrograph of part of a very young sieve plate, rather out of focus. Note the formation of protruberance in the pre-pore area. Plasmalemmal invaginations appear to be filled with fibrillar material. From longitudinal section of stem sampled in late April, 1976. x 80,000



Fig. iii.7

A sieve plate showing an early stage of callose deposition. Intervening wall highly crenulated, possibly indicating rapid growth. Tonoplast still present and note the typical plastids with dense matrix. From stem sampled in late May, 1975. x 8,400.

Fig. iii.8

A young sieve plate at platelet stage. Highly crenulated intervening wall begins to overtop the callose discs. Invaginations with fibrillar material seem to develop out of these crenulations. The callose platelets are separated by only a very thin layer and are covered by ER. The tonoplast is still intact and ribosomes are abundant. From longitudinal section of stem sampled in late May, 1975. x 21,000



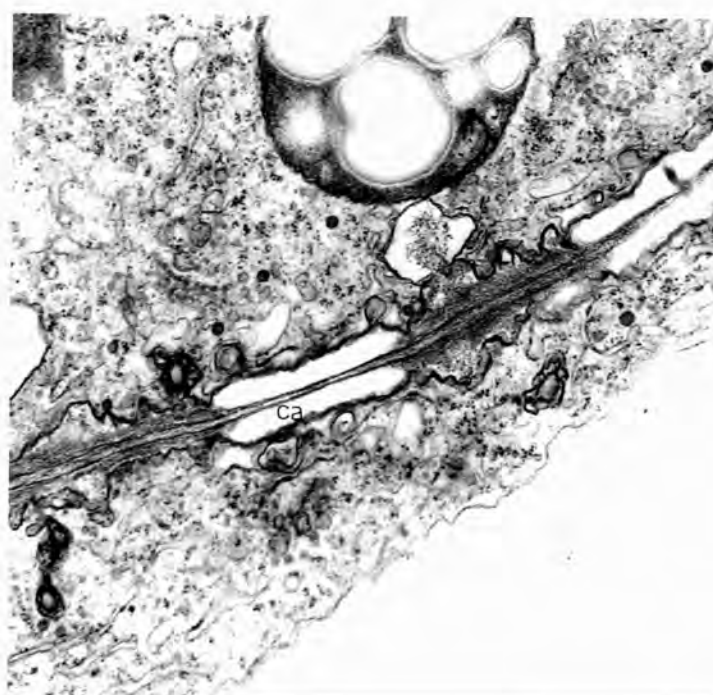
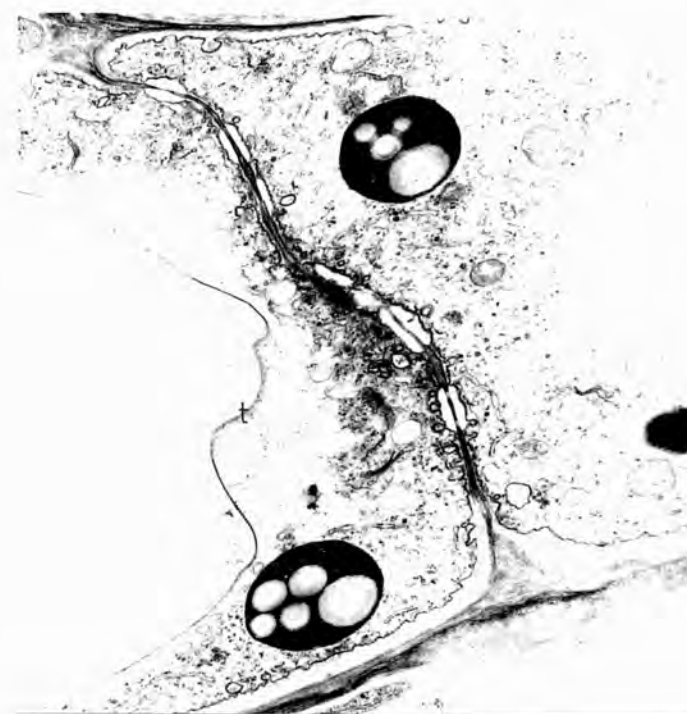


Fig. iii.9      A young sieve plate showing an almost compound sieve plate. The tonoplasts are disintegrating; the cellulose wall does not yet exceed the callose-covered areas in thickness. From a stem sampled in late May, 1975. x 8,400.

Fig. iii.10      Higher magnification of the figure above. ER cisterna (much dilated) is in close apposition to the plasmalemma lining the callose. No ribosomes are present on these ER cisternae. Note small invaginations or "blebs" formed from the plasmalemmae covering the platelets. x 35,000

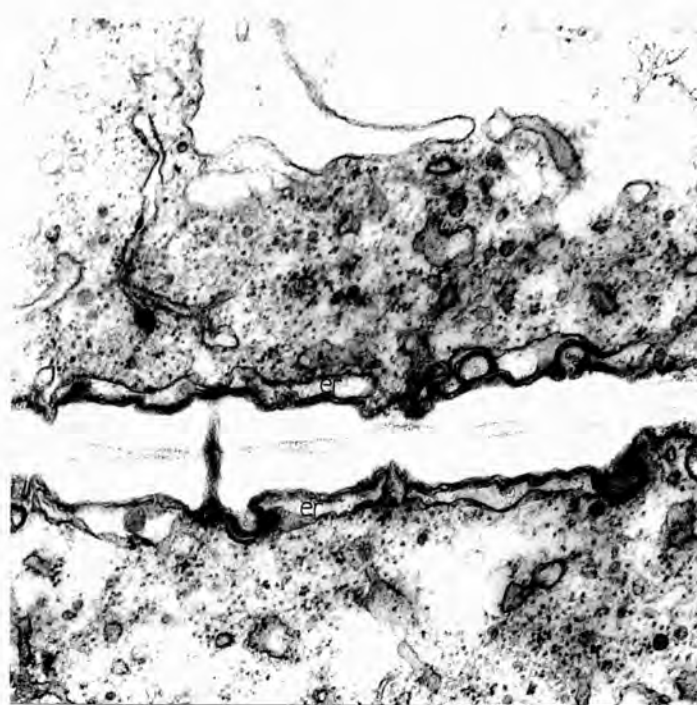
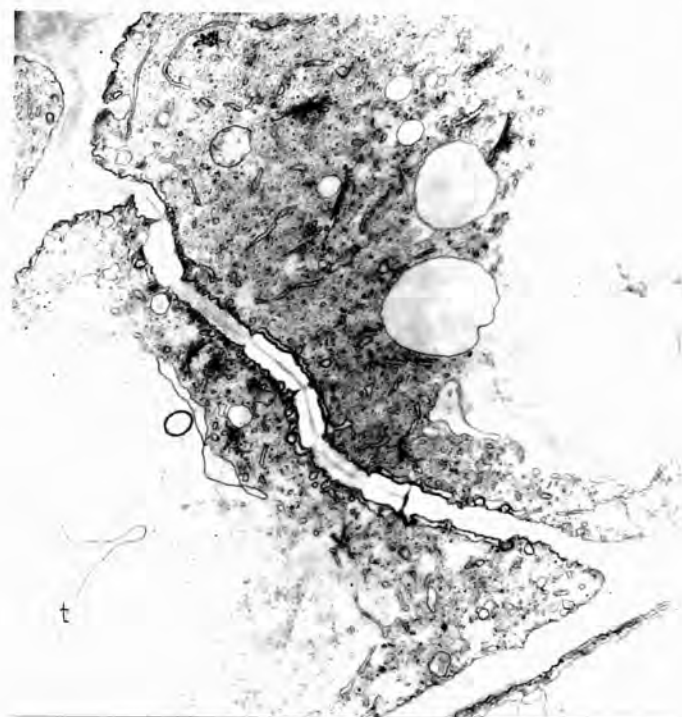


Fig. iii.11      Callose platelets merging laterally. ER is in close apposition to the platelets. Note also ER entering the plasmodesmata. From longitudinal section of stem sampled in late May, 1975. x 42,500

Fig. iii.12      A later stage of growth of the sieve plate wall. Intervening cellulose wall attains the same thickness as the callose platelets. Note the stacks of ER over the latter. Tonoplast may have been beginning to disintegrate. From a stem sampled in late May, 1975. x 8,400

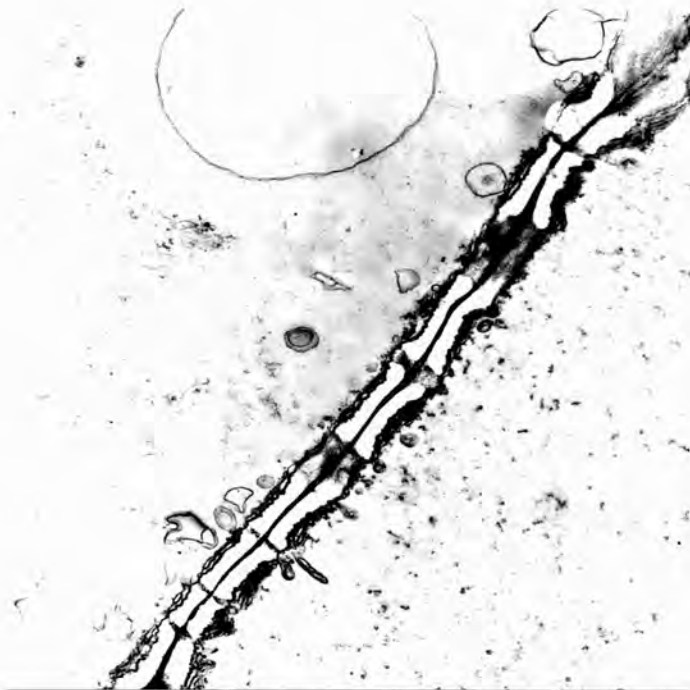
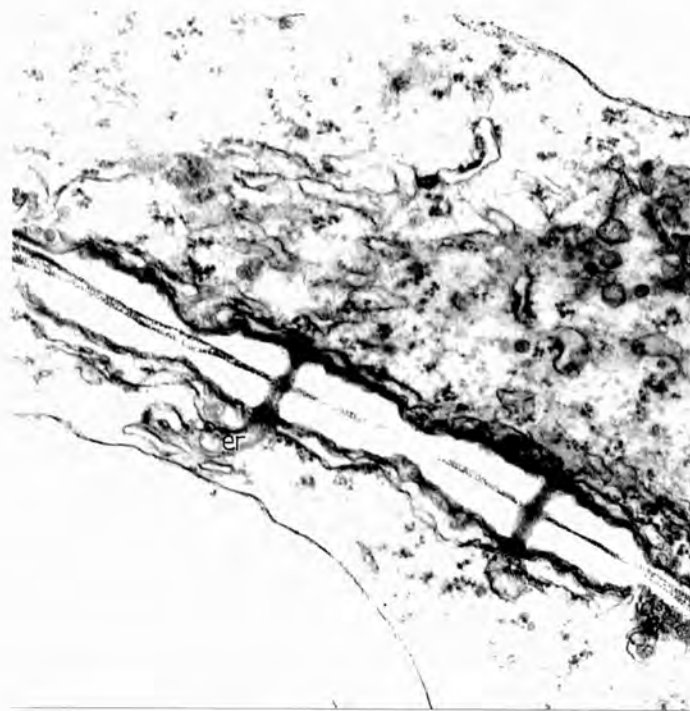


Fig. iii.13 Further growth of the intervening wall makes the callose platelets appear sunken. Stacks of ER over the platelets are now prominent (cf iii.12) and also along the longitudinal wall. Polyribosomes are still present in the lower half especially of the sieve tube. The myelin body may be connected with the disappearance of the tonoplast. From a stem sampled in late May, 1975. x 17,000.

Fig. iii.14 Beginning of perforation. Median cavity formed at the middle lamella. Note the stacks of ER over platelets and the crenulated edge of the platelet. Polyribosomes still very conspicuous. From a stem sampled in late May, 1975. x 25,000

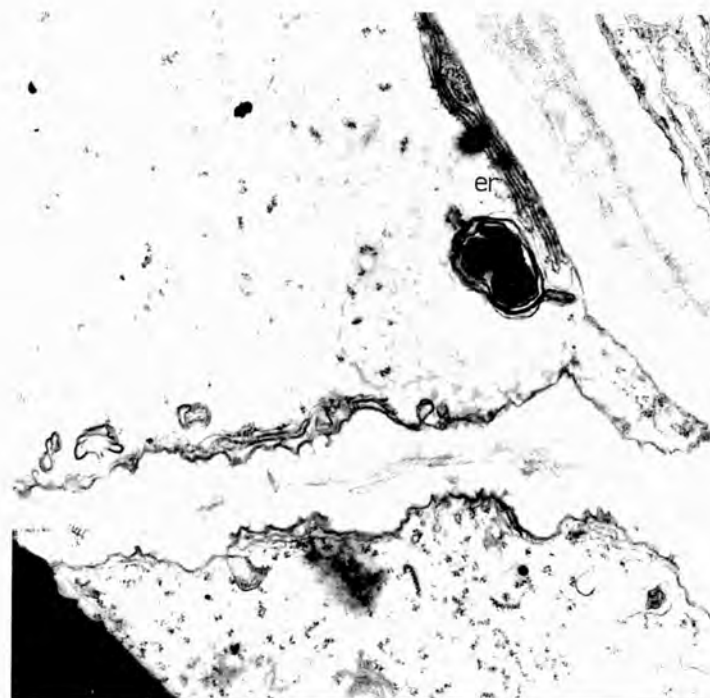


Fig. iii.15      Sieve plates with median cavities.  
Sandwiched layer still persists, plastids  
at the periphery and polyribosomes less  
evident. From a stem sampled in late  
May, 1975. x 14,000

Fig. iii.16      Early stage of perforation. Median  
cavity growing laterally along the  
middle lamella. From a stem sampled  
in late May, 1975. x 14,000



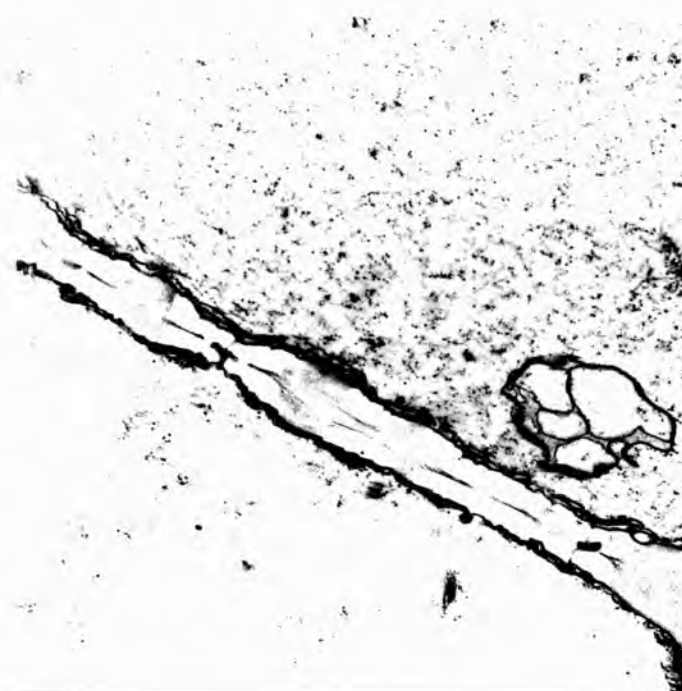
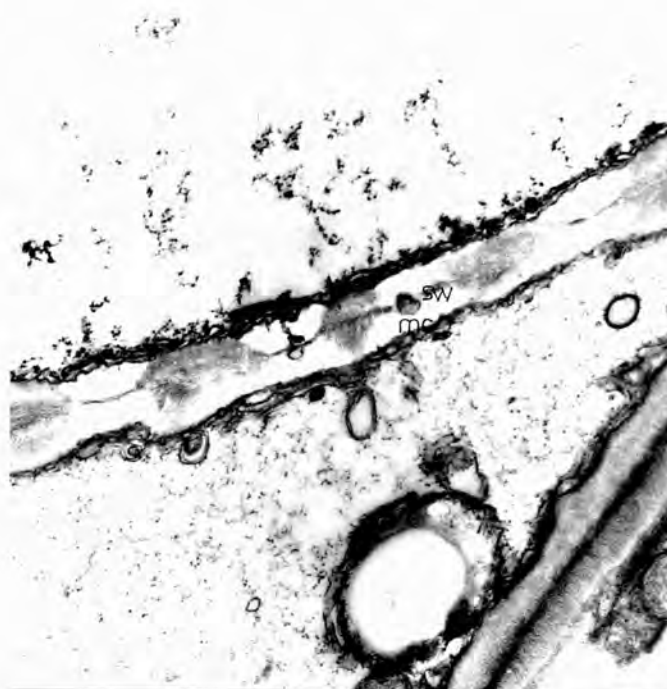


Fig. iii.17      Slightly later stage of perforation. Median cavity enlarging laterally. Projections from the median cavity extend to the outer surface of the callose platelets. Note that plasmalemma lines these projections and the median cavity. From a stem sampled in late May, 1975. x 47,500

Figs.iii.18 - iii.21 show a series of mature sieve plates in longitudinal section. Fixation was in glutaraldehyde without addition of sucrose.

Fig. iii.18      Starch grains from broken plastids are present. From stem sampled in late April, 1976. x 34,000

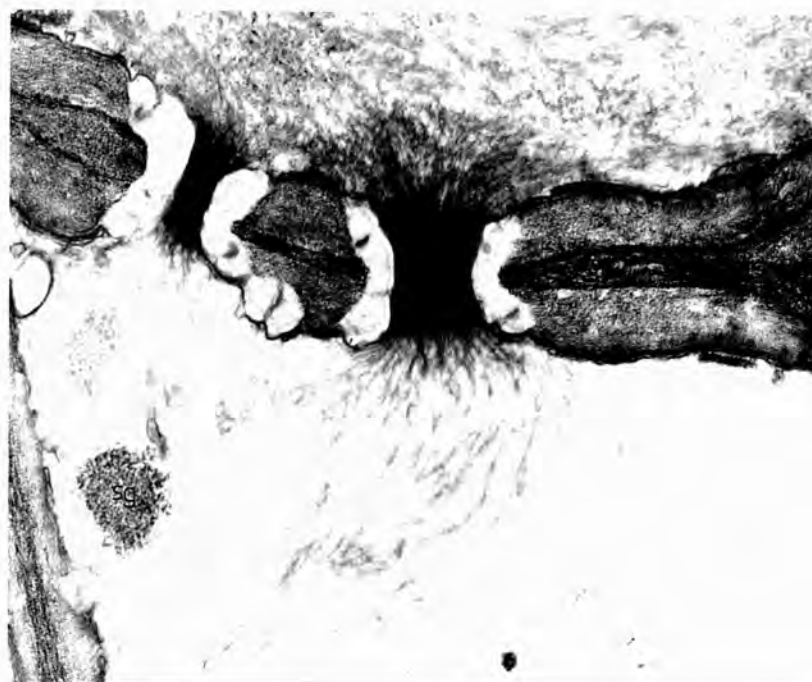
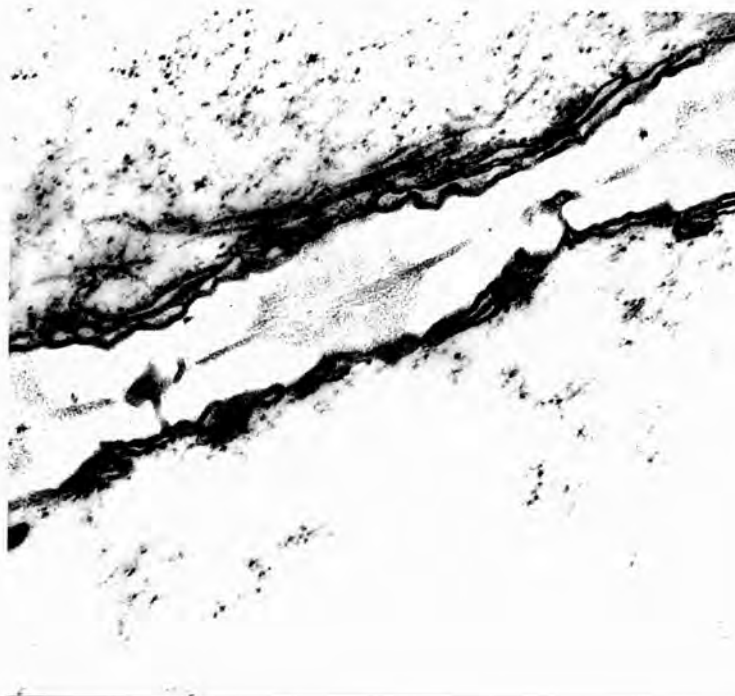


Fig. iii.19      Plasmalemma lines the sieve pores (arrows)  
P-Protein in the middle sieve pore is  
loosely packed. From stem sampled in  
early June, 1976. x 39,000

Fig. iii.20      Plasmalemma may also infold within  
the callose (arrow). From stem sampled  
in early June, 1976. x 40,500



Fig. iii.21      Note ER (or other membranous structures) running through the sieve pore. Some of the ER cisternae are swollen giving the appearance of small vesicles. From stem sampled in late May, 1975. x 35,000

Fig. iii.22      A mature sieve plate. Pores lined with thick callose (ca) are plugged with P-protein. Note the space created around the released starch grains. ER appears to line the sieve element. 5% sucrose was added to the fixative. From stem sampled in early October, 1975. x 14,000

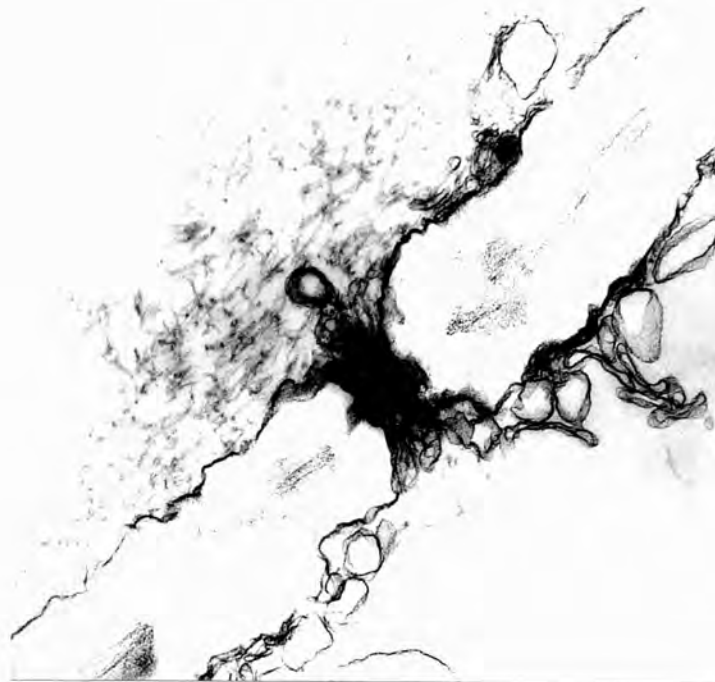


Fig. iii.23 Higher magnification of Fig. iii.22.  
P-protein is banded and laterally in  
register. x 40,000

Fig. iii.24 A mature sieve element. Pores are heavily  
callosed. P-protein accumulates on the upward  
stream and broken plastids pile on top of  
this P-protein mass. Companion cells are  
still dense. From stem sampled in late  
April, 1976. x 3,250



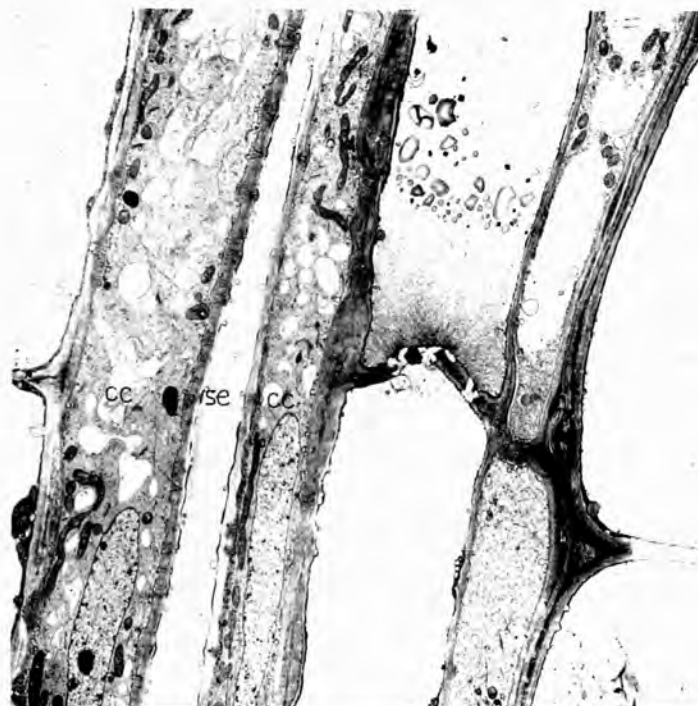


Fig. iii.25 A perforated and an unperforated sieve element. The sieve pores are heavily callosed and occluded with P-protein. Plastids in the downward stream of the sieve element are either intact or broken. From stem sampled in late April, 1976. x 3,250

Fig. iii.26 A mature sieve element whose pores are heavily callosed. The starch grains appear to be enclosed by an invisible membrane. From stem sampled in early June, 1976. x 6,500

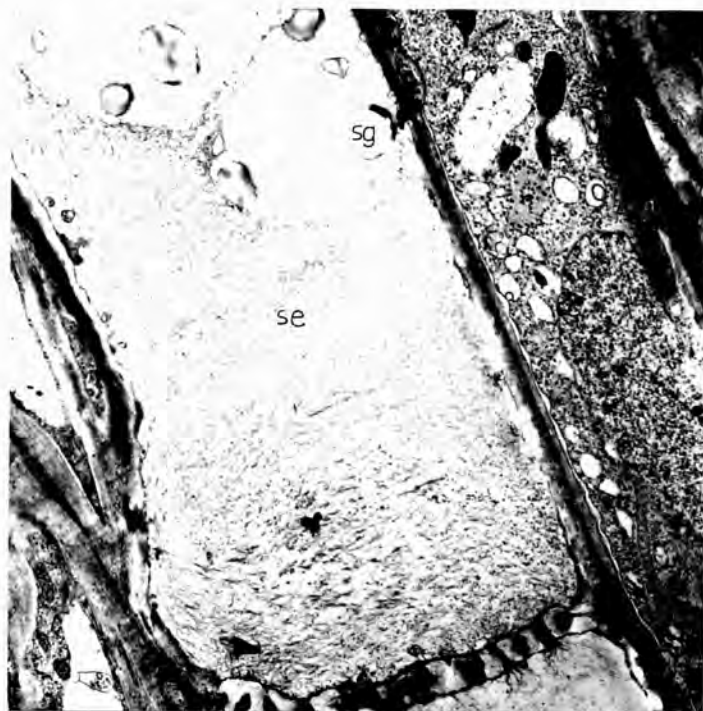
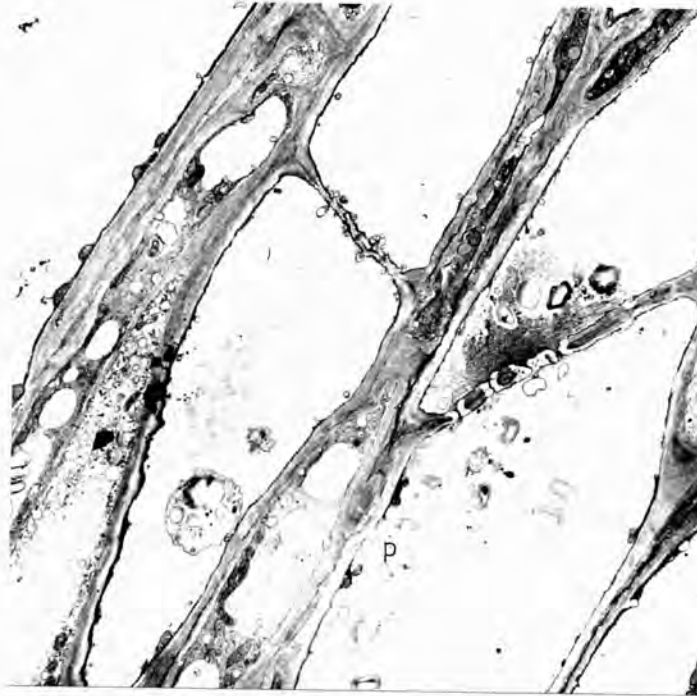


Fig. iii.27

A mature sieve element. Sieve pores are occluded with P-protein. Starch grains appear to be trapped in the P-protein mass above the sieve plate. Halos seem to surround these starch grains. From stem sampled in late April, 1975. x 6,800

Fig. iii.28

Abundant P-protein accumulates above the sieve plate. Sieve pores are callosed and occluded with P-protein. Cavities left by starch grains are apparent below the sieve plate. 5% sucrose has been added to the fixative. From stem sampled in early October, 1975. x 10,200



Fig. iii.29      Oblique surface view of mature sieve plates.  
Pores are callosed and occluded with P-protein.  
From stem sampled in late June, 1975.  
x 8,400

Fig. iii.30      Occluded pores with thin lining of callose.  
5% sucrose has been added to the fixative.  
P-protein fibrils are tightly packed within  
the pores. Note the alignment of the  
fibrils. From stem sampled in early  
October, 1975. x 17,000



Fig. iii.31 Two mature sieve plates whose pores are thinly lined with callose. The P-protein fibrils (from the sieve plates on the left) appear to trail freely on the downward stream. Note cavities representing starch grains above the left sieve plate. From stem sampled in early October, 1975.

x 5,600

Fig. iii.32 A sieve pore at a higher magnification. Plasmalemma lines the thinly callosed pore (arrows). P-protein within it is loosely packed. From stem sampled in early October, 1975. x 68,000



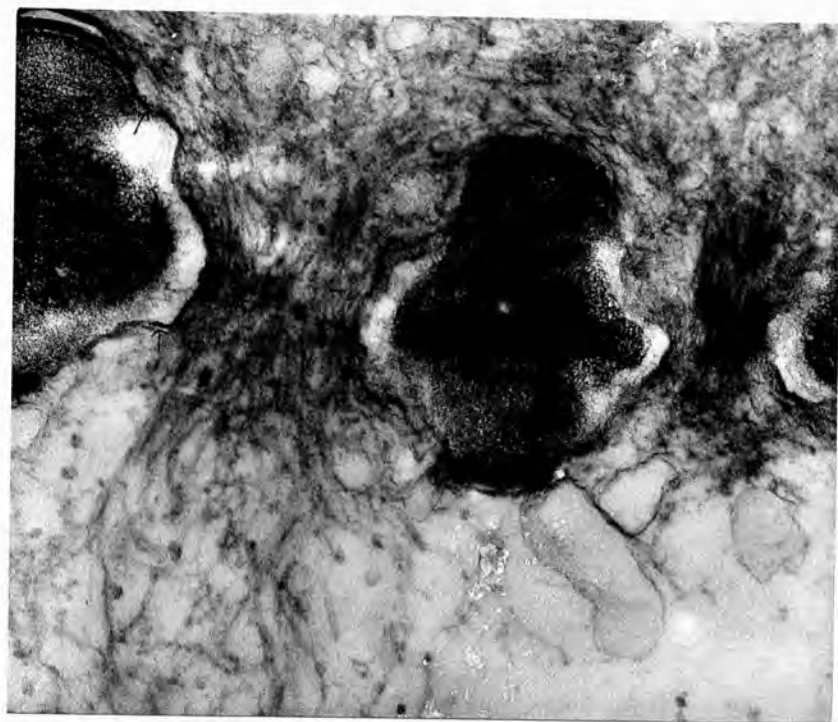


Fig. iii.33      Thinly callosed sieve pore. Cavities left by starch grains are present on the upward stream side. From stem sampled in late July, 1975. x 5,600

Fig. iii.34      Transverse section of part of a sieve plate. Sieve pores are unoccluded and callose is absent. From stem sampled in late June, 1976. x 9,750

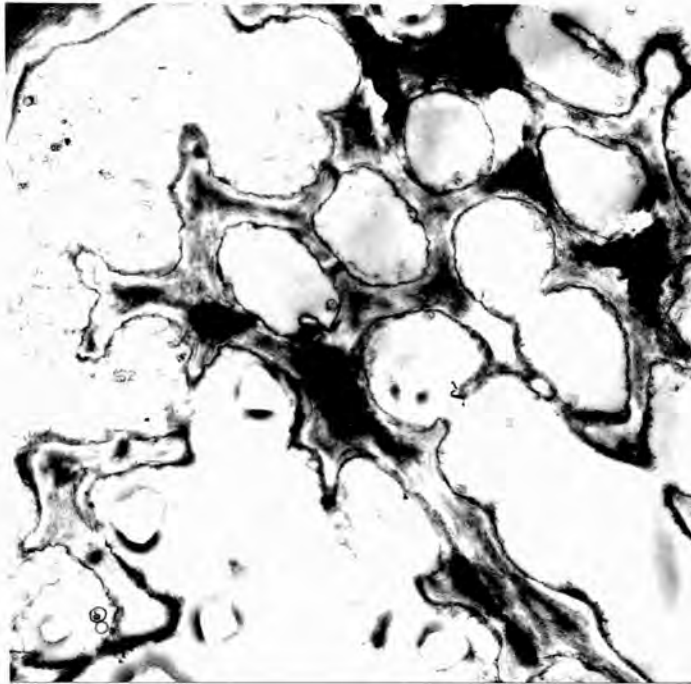


Fig. iii.35      Transverse section of part of a sieve plate. Pores appear empty and thinly lined with callose. ER stack is visible in one of the sieve pores. From stem sampled in early October, 1975. x 35,000.

Fig. iii.36      An open-pore sieve plate with no callose. Note small vesicles attached to the sieve plates. 5% sucrose has been added to the fixative. From stem sampled in early October, 1975. x 9,825

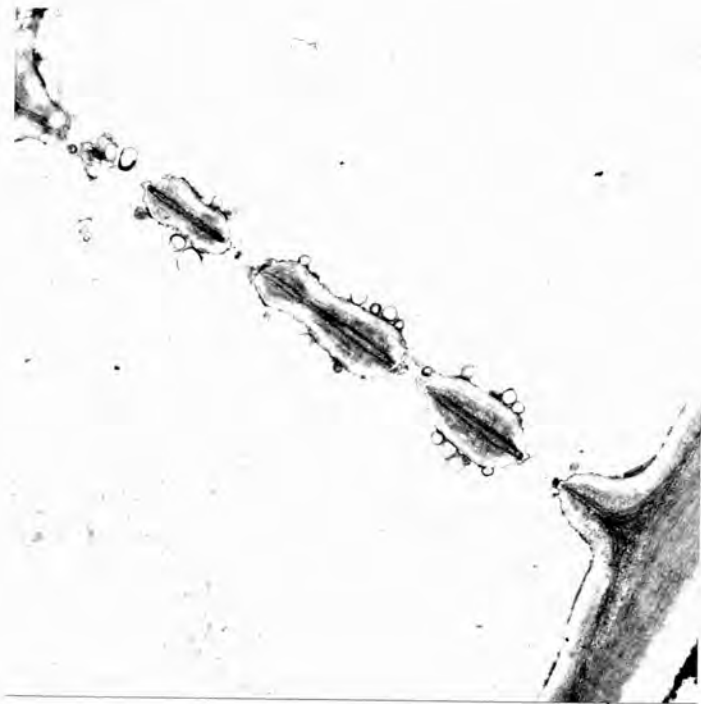
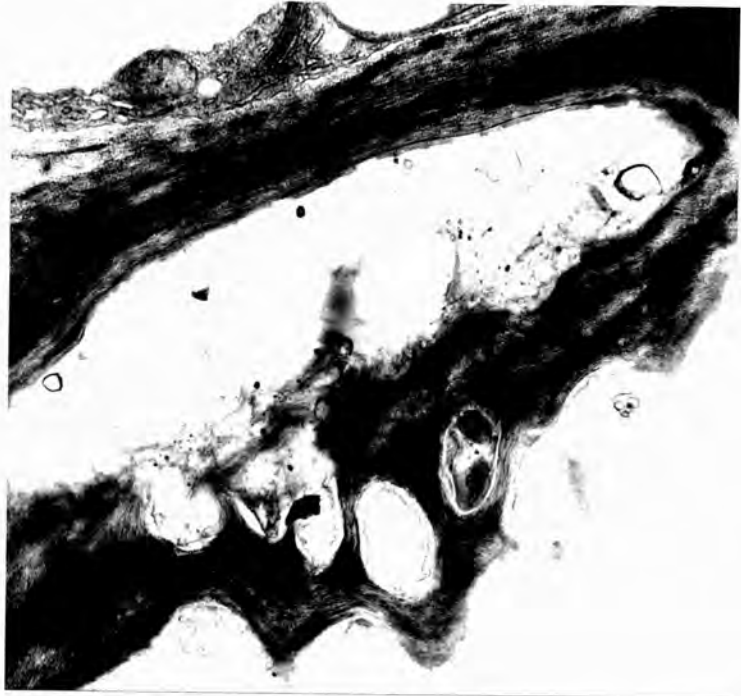


Fig. iii.37      Longitudinal section of a phloem area.  
Most of the sieve pores appear unoccluded.  
Note that the plastids are mostly intact  
and peripherally situated. From stem  
sampled in late July, 1975. x 1,700

Fig. iii.38      Sieve pores with little callose. P-protein  
is minimal; few loose fibrils appear to  
traverse the pores. Plastids below the  
sieve plate remain intact. From stem  
sampled in late July, 1975. x 6,800

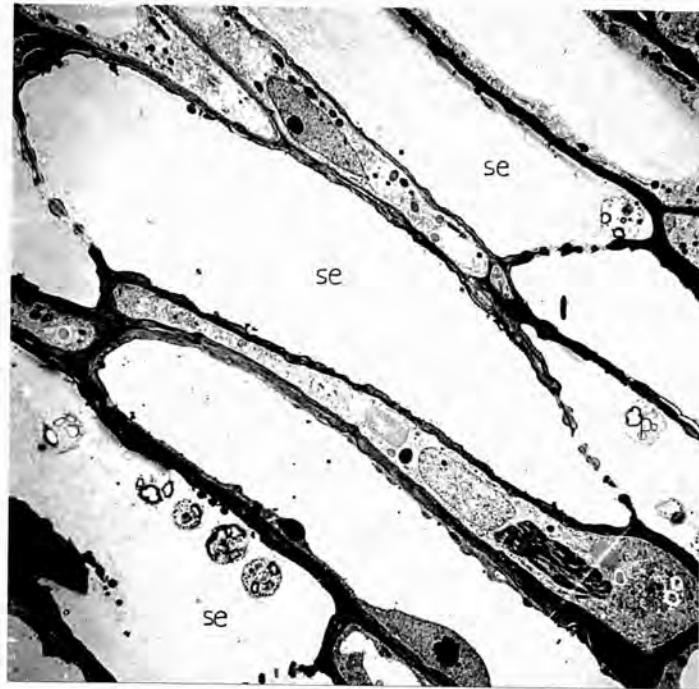
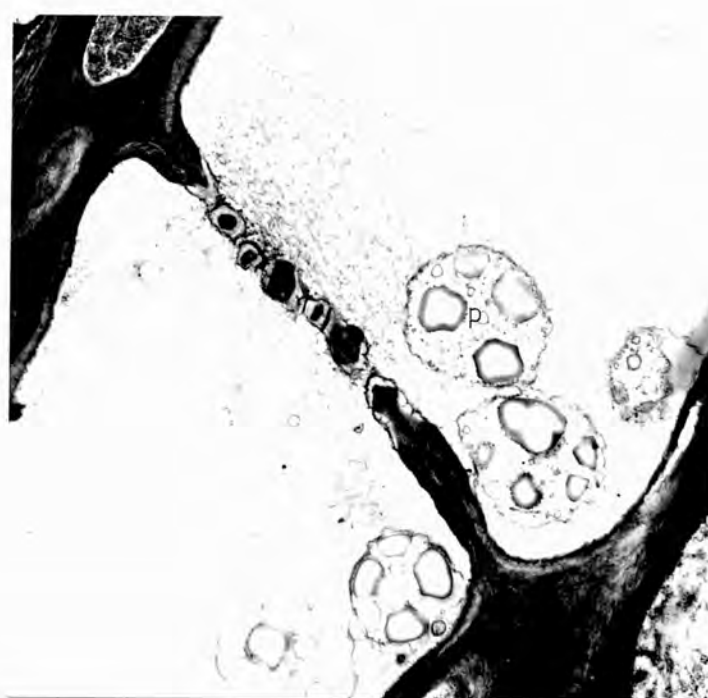
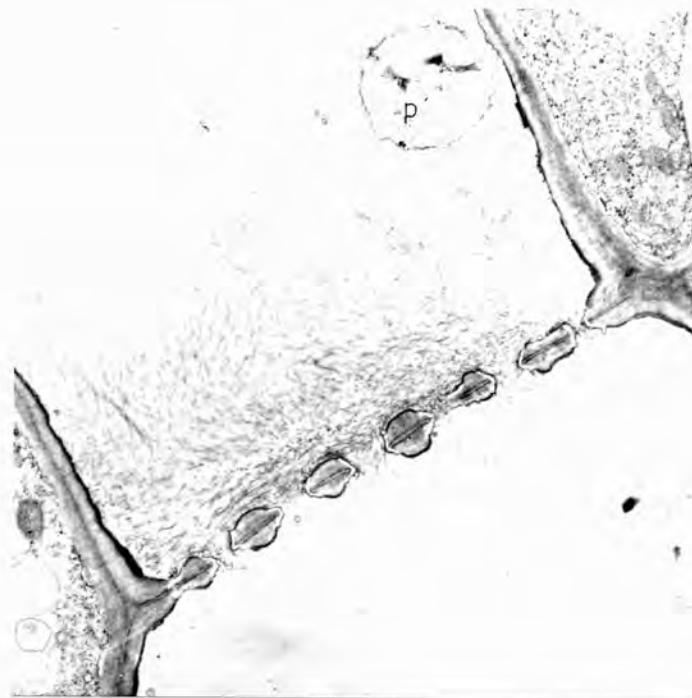


Fig. iii.39      Sieve pores are thinly lined with callose.  
Amount of P-protein near the sieve plate is  
appreciable but the P-protein does not  
actually plug the pores. Plastids remain  
intact. From stem sampled in late July,  
1975. x 6,800

Fig. iii.40      Pores are callosed but are not heavily  
plugged with P-protein. Note that the  
plastids near the sieve plate are still  
intact. From stem sampled in late July,  
1975. x 27,400





Figs. iii.41 and iii.42 Effects of surge.

Fig. iii.41      The P-protein appears to have surged through the pores. Starch grains are trapped in between the sieve plate and the P-protein mass. From stem sampled in late May, 1975:  
x 5,600

Fig. iii.42      The P-protein appears to have been pushed through the pores forming heavy slime plugs whose ends are cylindrical (arrows). From stem sampled in early June, 1976  
x25,000

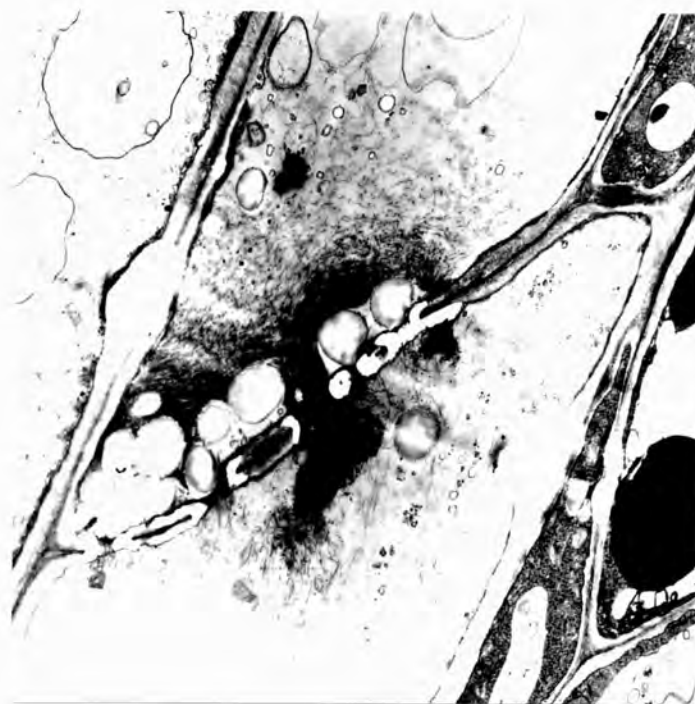


Fig. iii.43      Longitudinal section of a mature sieve plate near the cut end. The sieve pores are without callose. Starch grains are lodged in the sieve pores and some have managed to go through. From stem sampled in late October, 1976. x 4,250

Fig. iii.44      Formation of massive callose over the sieve plate. Plastids are still intact. From stem sampled in late May, 1976. x 12,750

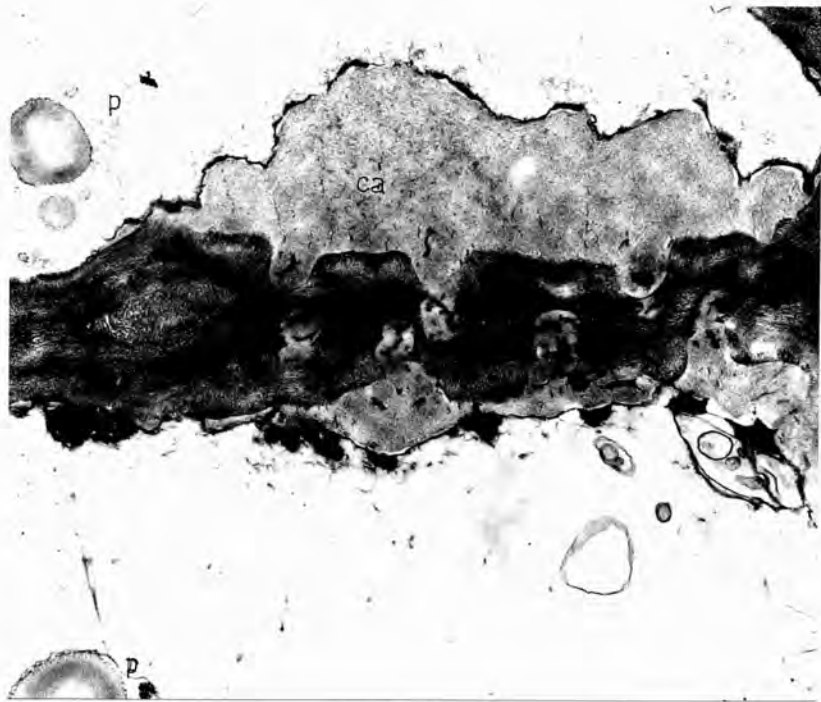
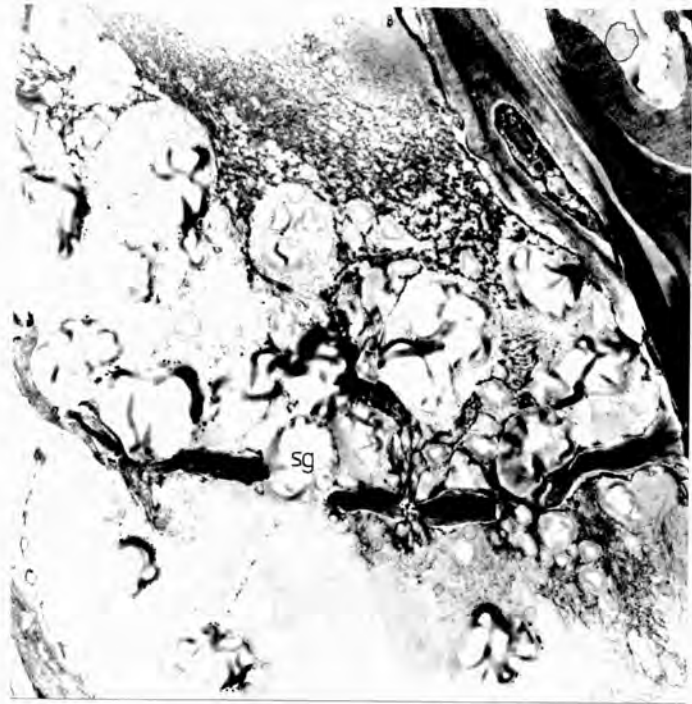


Fig. iii.45      Montage of mature sieve-plates in a  
file. Sieve pores are callosed and are  
occluded with P-protein. Note the  
space above each sieve plate. From  
stem sampled in early June, 1976.

x 2,625



Fig. iii.46      Montage of mature sieve plates in a file. Space above each sieve plate is characteristic. Most of the plastids are broken. Note the crack along the sieve tube wall which has caused a major problem during sectioning. From stem sampled in early October, 1975 and 5% sucrose has been added to the fixative.

x 1,150



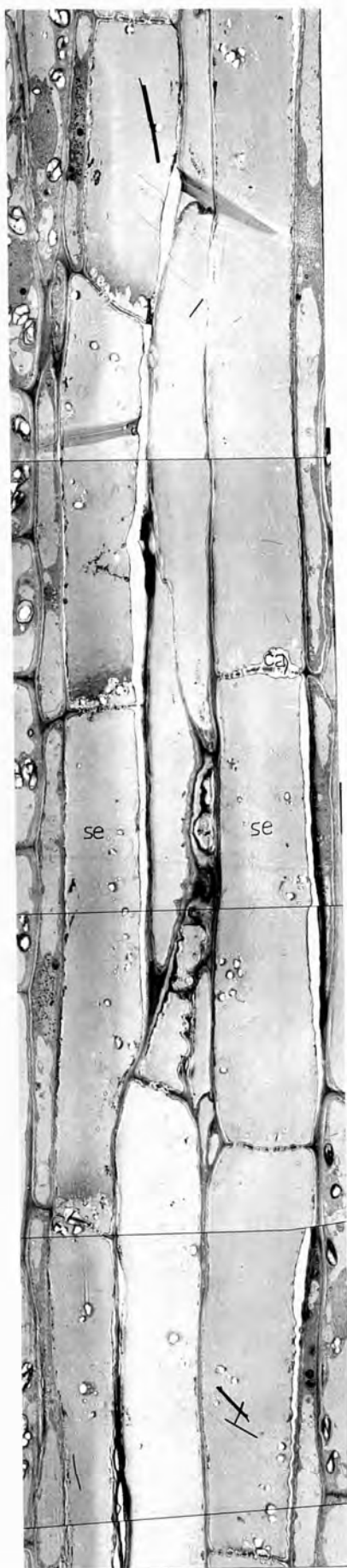


Fig. iii.47      Montage of mature sieve plates in a file. Here the space above each sieve plate is not so well defined. Starch grains are situated above the P-protein mass instead. Some of the plastids (p) are still intact. From stem sampled in late July, 1975. x 1,725



(iv) Membrane systems and organelles(a) Plasmalemma invaginations

Structures which appear to be invaginations of the plasma membrane, sometimes of complex type, have often been illustrated in plant and animal cells. Despite their common occurrences, many authors regard them as artifacts. Marchant and Robards (1968) who attempted a classification, based on the analysis of similar structures illustrated by the published results of others, came to the conclusion that these peripheral invaginations were not artifacts. There are also other opinions which support this conclusion. Mahlberg et al (1970) made comparative studies of living and fixed hair cells of Tradescantia virginiana. He interpreted invaginations in these as real. Other workers have also reported the occurrence of these structures in cultured cells of higher plants and have described them as "lomasomes" (Sutton-Jones and Street 1968) or "multivesicular bodies" (Halperin and Jensen 1967). In normal cells, these structures have also been referred to as "boundary bodies" (Esau, Cheadle and Gill 1966), "plasmalemmasomes" (Robards and Kidwai 1969), "vascular inclusions" (Thomson 1967), or "included bodies" (Fineran 1971). Marchant and Robards (1968) however, suggested that all such membrane systems associated with the plasmalemma be classified under the general term "paramural bodies", subdividing them into "lomasomes" if they are derived from cytoplasmic membranes or "plasmalemmasomes" when these are formed from the plasmalemma.

However, based on the fact of their being apparently in origin projections into the cytoplasm, some of these formations have also been called "secondary vacuoles" by Mahlberg and his co-workers to distinguish them from the primary vacuoles. Extensive studies have

been made to elucidate the dynamics of formation of these "secondary vacuoles", from studies on both living cells of Tradescantia virginiana (Mahlberg 1972) and fixed cells (Mahlberg et al 1971, 1974).

In contrast with the above view, i.e. that these bodies originate in endocytic movements, other workers have interpreted them as arising through exocytic ones. Even though some of the formations observed are similar to the early stages of development of indubitably endocytic structures, Cronshaw and Bouck (1965), Esau, Cheadle and Gill (1966) regard many formations as stages in a moving outwards, towards the cell wall, like a reversed pinocytosis. Vesicles of unknown origin within the cytoplasm of Helianthus were also interpreted by Walker and Bisalputra (1967) in this sense. Buvat (1968) regarded these vesicles as associated with degenerative processes in the cytoplasm.

Similar formations and interpretations have also been encountered in lower plants: internodal cells of Chara and Nitella (Crawley 1965 and Barton 1965) and hyphae of species of Eucomycota which Moore and McAlear (1961) have called "lomasomes".

The exocytic function is possibly related to the cell wall synthesis. Thus autoradiographic studies of celery petioles (Cox and Juniper 1973) suggest that these paramural bodies participate in the matrix polysaccharide deposition. Fowke and Setterfield (1969), however, found a negative correlation between paramural bodies and wall synthesis in auxin treated tissue disks.

In Fraxinus similar invaginations (the use of this word is not intended to prejudge the issue of endocytic vs exocytic origin) have been observed in various differentiating cells: parenchyma, sieve tube and companion cell. Xylem cells are not examined here but there are reports of the existence of similar invaginations in the xylem of

Beta vulgaris L. and Cucurbita maxima (Esau, Cheadle and Gill 1966).

There are, however, some characteristic differences between these invaginations found in the sieve tubes and in the parenchyma or ray cells.

#### Sieve elements

The frequency of these invaginations along the plasma membrane is variable. There may be many in some cells, but few in others. There is again the possibility that these vesicular formations, having originated from cytoplasmic organelles, are migrating to the plasma membrane. This seems likely in very young sieve tubes whose vesicles are relatively small and which have been observed to be closely associated with dictyosomes (Fig. iv. 1).

In sieve tubes, these formations are encountered most frequently along the longitudinal walls, both in young and mature cells (Figs. iv.2 - iv.3). Also at the early stages of the sieve plate differentiation, these apparent invaginations are common (see Chapter III, section (iii) (a)). They are formed both over the callose platelets and the intervening walls (see Fig. iii.8). Plasmalemmal invaginations were also observed over the callose deposited on the sieve tube side of a plasmatic connection between sieve tube and companion cell (Fig. iv.4)

Plasmalemmal invaginations found in sieve tubes are mostly small compared to those in parenchyma or ray cells. They are mostly within the range of  $130\text{\AA}$  to  $260\text{\AA}$  but seldom do they rise beyond  $850\text{\AA}$ . Mature sieve tubes normally have very small invaginations.

The interesting features of the invaginations in sieve tubes are their shape and contents. In both young and mature sieve tubes, they usually have fairly wide orifice regions (Fig. iv.5) and few appear

with short necks between the plasmalemma membrane and enlarged sac (Fig. iii.3). Perhaps this represents a stage which lasts only a very brief time.

Frequently closed sacs, sometimes numerous, are evident in thin sections (Figs. iv.6' - iv.8). These may possibly be represented in non-median sections. If these sacs increase in size away from the plasmalemma, and remain within the cytoplasm, it would seem that they are to be regarded as originating from the plasmalemmal invaginations, enlarging and eventually detaching themselves (Fig. iv.6).

In Fig. iv.7 the presence of an organelle, a mitochondrion in the intervening cytoplasm and the protrusion of the sac into the primary vacuole may also indicate that the latter has detached itself from the plasmalemma.

Fig. iv.8 also shows the presence of empty rounded 'vacuoles' in mature sieve tubes, in the vicinity of the smaller plasmalemmal invaginations. It is possible that these may have originated from the plasmalemmal invaginations. If these were the remains of plastid membranes (suggested perhaps by the presence of starch grains nearby), then they would probably not be intact, nor so small and with a single membrane. Again there is a possibility of these being artifacts. The thin elongated neck seen in Fig. iv.9, bridging a small invagination and an enlarged sac, certainly suggests that the formation is in the process of being pinched off having moved in an endocytic direction. Probably such pinocytosis is continuous. The smaller invagination left behind will grow again once the enlarged sac is detached.

It has also been observed, that whatever the shape and size of the invaginations or the state of the differentiation of the sieve tubes, the contents of these invaginations are frequently fibrillar

(Figs. iv.6 and iv.7). It is rare to find tubules or vesicles within them (Figs. iv.5 and iv.11). Often, these invaginations, especially those found in mature sieve tubes, are devoid of any contents (Fig. iv.3).

The fibrillar content sometimes appear to originate from the sieve tube wall itself. Figs. iv.2 and iv.10 show the continuity of the fibrillar content with the wall fibres. As for the origin of the occasional tubules, it is possible that these are derived from infoldings of the invaginations as has been suggested by Mahlberg and co-workers (1974). A few vesicles have, however, been observed very close to the cell wall possibly indicating a different origin (Fig. iv.11).

#### Phloem parenchyma

A rather different sort of formation is encountered in the parenchyma and ray cells. Most of the invaginations are fairly big compared to those found within the sieve tubes. Most are observed to lie in the range of 1000 nm to 1500 nm and occasionally bodies as large as 3000 nm are encountered.

The occurrence of rounded sacs, with various fibrillar, tubular, vesicular or laminar contents lying free in the big vacuoles of the parenchyma or ray cells, is more frequent here than in sieve tubes (Figs. iv.13 - iv.15). In fact this does not happen in the sieve tubes except when the tonoplast breaks down as the sieve tube matures. In most of these sacs, the two membranes of what may probably represent the tonoplast and plasmalemma are still visible. Mahlberg and co-workers (1971) were convinced that these represent sectional views of large invaginations that protrude into the vacuole. Robards and Kidwai (1969), however, suggested that such invaginations do actually become incorporated into the vacuoles by breaking free.



In general, the shapes of the plasmalemmal invaginations in parenchyma or ray cells are variable, unlike those in the sieve tubes. Most are rounded with fairly wide orifices (Figs. iv.16 and iv.17) and those with narrow orifices normally have a short neck (Figs. iv.12 and iv.19), and an ovate enlarged sac.

Vesicles, tubules and lamellae rather than fibrils are the usual contents of these invaginations. Internal vesicles vary in size, numbers and contents. There are many vesicles as often as few in an invagination (Figs. iv.18 and iv.19). Size up to 200 nm have been observed and these large ones are usually spherical (Fig. iv.15) although collapsed forms are also evident (Fig. iv.12). Constriction of the bigger vesicles may also result in a beaded appearance (Fig. iv.14). Frequently, these forms intergrade one another (Fig. iv.14).

Most of the contents of these internal vesicles are of low electron density; sometimes the density of the overarching membrane obscures this (Fig. iv.15). The presence of almost similar vesicles within the cytoplasm (Fig. iv.18) may give an alternative idea of origin.

The tubular contents often have a fairly uniform diameter but sometimes they are 'beaded' which suggests the effect of periodic constriction along their length (Fig. iv.14). Fig. iv.20 also shows the ends of these tubules swelling. The small spherical outline may represent the cross-section of the tubules.

Laminar membrane aggregates, if they are present as these contents, frequently look like myelin sheaths (Figs. iv.15 and iv.21).

It is still uncertain how these contents originate. The suggestion of Mahlberg and co-workers (1971, 1974) is supported by Fig. iv.19 (arrow), which seems to show that vesicles arise from an infolding of the plasmalemmal membrane at the orifice region. Other similar infoldings

(Figs. iv.15 and iv.17) appear at different regions. Fig. iv.22 shows the close proximity of the vesicles to the cell wall.

The functions of these invaginations and their contents remain to be elucidated. There are two schools of thought regarding them. As noted above, one holds that they develop in exocytic direction and the other that they develop in endocytic one. In the former case, cell wall growth is a possible function; in the latter, the uptake of solutes and ions. Certainly in the sieve tubes, it seems hardly likely that the movement is exocytic; unless the invaginations are purely artifactual, therefore, it would seem reasonable to suppose that they have a pinocytic function. Further studies are necessary.

(b) Endoplasmic reticulum

In Fraxinus, the endoplasmic reticulum (ER) of a young sieve tube has the appearance usual for such nucleate cells. The profiles show the typical elongated cisternae or short tubules and most often they are associated with ribosomes (Fig. iv.23). This roughER has no strikingly obvious distribution in the young sieve tube but the following remarks can be made. It is always present in the vicinity of the developing sieve plates. Fig. iv.23 shows the ER in close apposition to the plasmalemma of the callosed regions. The branching nature of the ER is also evident. Some ER has also been found to aggregate near granular masses (Fig. ii.6). Since P-protein is assumed to originate from these granular masses, (see Chapter III, section iia, the association of ER with them suggests some ontogenetic relationship. Cronshaw and Esau (1967) also observed the presence of ER surrounding or within the P-protein bodies of Nicotiana. It is also probable that as early as this stage some single cisternae of ER may have already positioned themselves at the periphery of the young sieve tube wall (Fig. iv.24). Presumably the single ER cisternae

seen in this figure will form part of the 'parietal' system seen in mature sieve tubes after the ribosomes are lost.

As the sieve tube differentiates, the ER in it too becomes modified. It ceases to be in the extended form or associated with ribosomes. It forms smooth stacks or convoluted associations of various kinds which will later migrate to the periphery of the sieve tube. The relative timing of these various changes in the sieve tube is difficult to determine.

Different types of flattened ER aggregation have also been reported in many other plants. Their profiles are sometimes interpreted as tubular or cisternoid arranged in stacks, parallel or perpendicular to the sieve tube wall. Thus in mature sieve tubes of Cucurbita (Esau and Cronshaw 1968a) the 'parietal' ER appears as a single layer cisterna next to the plasmalemma although stacked membranes are also encountered. Evert and Desphande (1969) also reported the common occurrence of orderly stacks of ER cisternae in mature sieve elements of Ulmus americana; those with an irregular arrangement are sparse. In Acer (Northcote and Wooding 1966), even though the 'lamellar stacks', which was finally concluded to be ER remnants, have individual lamellae equivalent in width to the plasmalemma, the triple layer of the individual lamella and the continuity of these two structures have never been resolved.

Bouck and Cronshaw (1965) observed that in mature sieve tubes of Pisum sativum, the ER is compressed to form flattened layers of cisternae parallel to the wall. These are sufficiently distinct that ER merited a different name 'sieve tube reticulum'. Srivastava and O'Brien (1966) also reported of similar findings in Pinus strobus. They suggested this name be substituted with 'sieve element reticulum' which would be applicable to all types of sieve elements. In both

cases, vesiculation stages were reported to precede the development of the mature forms. No such stages have, however, been observed in Fraxinus.

Convoluting forms are also common both in dicotyledons (Johnson 1969 and Esau and Gill 1971, to name a few) and the gymnosperms. Recent workers on gymnosperms observed that in Gnetum gnemon (Behnke and Paliwal 1973), after losing their ribosomes, the ER tubules form aggregates which lie parallel to each other but may become twisted altogether. While Evert et al (1973b) reported much branched tubular aggregates of smooth ER in Welwitschia. Even though 'spiny vesicles' have been found together with these aggregates which are clearly ER, they cannot here be related to the formation of P-protein for Welwitschia appears not to have any.

Membranous bodies with quasi-crystalline aggregates possibly formed by the association of originally randomly arranged ER cisternae, have also been observed by Wooding (1967) in Acer pseudoplatanus. Wooding also pointed this similarity of ordered aggregate in Acer to the lattice-like inclusions of Dioscorea (Behnke 1965) except for the dimensions in the spacings between the cisternae. Dioscorea has a larger spacing.

Very little information is available on the actual process of stacking or convoluting of ER in the maturing sieve tubes. Generally there can be two different ways in which stacking is probably taking place. Parthasarathy (1974) observed that in palms there is a gradual shifting of the rough ER to the periphery of the cell as the sieve tube differentiation progresses. Prior to aggregation of the ER along the walls, the ribosomes are shed off first so that aggregations are composed of only smooth ER. In most plants, however, the rough ER cisternae aggregate in pairs or more before losing their ribosomes.

By the time the stacks arrive at the periphery of the wall, they are mostly smooth ER. Primula (Tamulevich and Evert 1966) Pisum (Zee 1969c) and Phascolus (Esau and Gill 1971) have been observed to stack their ER in the second mode.

Stacking of ER in Fraxinus, apparently takes place within the cytoplasm before the stacks finally migrate to the periphery of the sieve tube. Fig. iv.25 shows a stage possibly at the beginning of stacking where three ER cisternae are packed close together. Ribosomes though present are not apparently attached to the ER cisternae.

As stacks increase in depth (Fig. iv.26), they show the usual alternating narrow intercisternal spaces with the wider ER cisternae. The intercisternal spaces are more electron dense than the cisternae. The nature of the electron dense material has not been determined. Possibly it is P-protein as the tubular form has been reported to be present in the intercisternal spaces of Vicia faba (Zee 1969a). Fig. iv.26 shows ribosomes still attached to both the free surfaces of the cisternal stack.

Other modifications to the otherwise normal flat stacking of ER are sometimes observed. A somewhat fenestrated form is shown in Fig. iv.27. Fig. iv.29 shows ER aggregating in a circular configuration.

An interesting feature in the process of stacking of these ER aggregations in Fraxinus, is their timing in relation to the differentiation of the sieve tubes. In all cases shown, there seems to be no definite time when stacking is actually taking place. Stacking can take place when the tonoplast is still intact and the plastids have an electron dense matrix (Fig. iv.26). Even when the tonoplast has broken down (Fig. iv.28) stacking is still going on. The stages in the development of the convoluted form of ER have not been traced.

The orientation of the ER stacks along the periphery of the wall does not appear to be significant. Within the same sieve tube, two different types of orientation can be present. Fig. iv.30 shows two profiles of flat cisternae in a stack, one lying parallel to the wall and the other perpendicular to it. However, the common form is the stack arranged parallel to the longitudinal wall of the sieve tube. Short perpendicular stacks are sometimes observed (Fig. iv.31). These are reminiscent of the 'brush border form' which led to the suggestion, now abandoned, that they are actually plasmalemmal material displaced from the wall (Spanner and Jones 1970). Sections cut obliquely may give a rather different image of these ER stacks (Fig. iv.32).

Even though various other aggregations of ER are observed in the mature sieve tubes, they are less common. Normally, a mature sieve tube would be lined by one or two ER cisternae next to the plasmalemma (Fig. iv.33). Very often the space inbetween the plasmalemma and the cisterna is also electron dense. These ER cisternae also give the appearance of being continuous along the sieve tube wall.

Very often ER aggregates are observed to be associated with other organelles in the sieve tube. Fig. iv.34 shows flat stacks of ER in close contact with a mitochondrion. The association of ER with the nuclear envelope is not prominent in Fraxinus (see Chapter III section (iv) (c)) unlike the cases depicted by Esau and Gill (1971). Here only vesicles or tubules appear to be found within or outside the nuclear envelope.

The significance of aggregation of ER as the sieve tube differentiates is still problematic. Aggregation is not restricted to sieve element alone but occurs in many other cells such as nectariferous plant cells which are highly active metabolically. Dexheimer (1966)

has also shown that ER aggregates can be induced in Lobelia erinus pollen by treating with chloramphenicol, a chemical agent known to inhibit protein synthesis. These changes were associated with cessation of growth of the pollen tube and hence aggregation was regarded as non functional.

In the sieve elements of Metasequoia, Kollmann and Schumacher (1964) regards the stacking of ER as an expression of specific differentiation related to the function of the cell. Similarly Dorr (1972) showed that the stacked ER in Cuscutta appear to serve for the transfer of food from host sieve elements to the parasite. ER may also be an important source of enzymes involved in autophagasis in differentiating cell as proposed by Zee (1969c). Wooding (1967), however, suggested that the aggregation of ER represents a sequestering of the membrane in an inactive form. He thought that the aggregates degenerate before the sieve element assumes the function of conduction.

#### (c) Nucleus

Most workers on the phloem structure of the higher plants agree that mature sieve tubes lack a functional nucleus, a point already established by early workers using the light microscope. They detected its disintegration as the sieve tube differentiates. The absence of a functional nucleus is observed not only in the higher plants but often in the gymnosperms and the vascular cryptograms as well (Maxe 1966).

Evert et al (1970) however, observed that the mature sieve elements of the three species of Taxodiaceae and 12 out of the 13 species of the woody dicotyledons they examined still contain nuclei. Only Robinia, Ulmus and Vitis and all the three gymnosperms contained some sieve elements with normal nuclei at the time when the former are involved

with long distance transport. Therefore, that the sieve elements are enucleate at maturity, might possibly have been induced during manipulation and fixation of the tissue.

Two broad types of nuclear degeneration have been recognised so far in the study of sieve tube differentiation (Srivastava 1975). In the first the nucleus enlarges and loses its nucleolus and chromatin; then the nuclear envelope disrupts and contents disperse. This has been shown to take place in the nucleus of Phaseolus (Esau and Gill 1971) and tobacco (Esau and Gill 1972). In Mimosa (Esau 1972), before the nucleus disintegrates completely, pieces of nuclear envelope could still be recognised by its nuclear pores. In the second type, which has been observed in Allium (Esau and Gill 1973), the chromatin is first converted into an amorphous alveolate mass and the nuclear envelope becomes chromatic. The envelope does not maintain the integrity of the nucleus, even though it was not obviously ruptured and the cytoplasmic ribosomes enter the nucleoplasm. Even when the cell matures, the nucleus is still discernible. It contains deeply stained amorphous material. Retention of the necrotic nucleus was also observed in Pinus strobus (Murmanis and Evert 1966), P. pinea (Wooding 1966) and rye coleoptile (O'Brien and Thimann 1967).

In the present work with Fraxinus, the nucleus has been observed to undergo morphological changes as the sieve tube differentiates. The nucleus of a comparatively young sieve tube, as judged by the presence of P-protein body (Fig. iv.35) or the very dense matrix of the plastid (Fig. iv.36), does not differ much from any other nucleus at interphase. It has a well defined outline but the presence of what appears to be additional small nucleus in Fig. iv.36 suggests that it is highly lobed even at the early stages of sieve tube differentiation. A single nucleolus is usually prominent. The chromatin is evenly



dispersed giving a homogenous appearance to the nucleoplasm which is comparable in density to the cytoplasm (Fig. iv.35).

The nucleolus in Fig. iv.37 appears to be splitting up into fragments. The chromatin is evenly dispersed as in the nucleus at the early stages of sieve tube differentiation. The nucleus, which is lobed, does not occupy more than 50% of the width of the lumen (compare Fig. iv.36). Vesicles with double membranes are present within the nucleus; probably these represent places where the cytoplasm is present in invaginations of the nuclear envelope, a conclusion suggested by the appearance of pores in the double membrane.

Stacking of ER along some parts of the nuclear envelope, which is observed in most of the sieve tubes studied e.g. Mimosa (Esau 1972) is less commonly found in Fraxinus (see also Chapter III, section (iv) (b)). Fig. iv.38 shows two stacks of ER cisternae in close contact with the nuclear envelope with electron dense material present between the cisternae. This section was, however, taken from a less mature sieve tube since P-protein is only about to be dispersed and both the cytoplasm and nucleoplasm are comparatively quite dense.

However, at a later stage of the sieve tube differentiation, chromatin aggregates are more conspicuous for they appear as electron dense masses randomly distributed within the thinning nucleoplasm. There is also some slight tendency towards localised aggregation along the periphery of the nuclear envelope (Fig. iv.39). This nucleus is from sieve element whose P-protein is about to be dispersed and whose sieve plates have not yet been perforated. The tonoplast is still intact but no nucleolus is evident. The plastids too appear immature.

As the sieve tube differentiates further and the tonoplast breaks down, the nucleus enlarges and loses its distinctive contents (Figs.

iv.40 and iv.42). Generally only one side of the nucleus is in close contact with the longitudinal wall of the sieve tube even though Fig. iv.42 shows the nucleus occupying the whole lumen of the sieve tube. The nuclei at this stage are however not highly lobed. Only Fig. iv.40 shows vestiges of the fragmented nucleolus; generally the cytoplasmic contents appear to invade the nuclear space (Figs. iv.40 - iv.45). Thus the nucleoplasm at this stage appears very electron lucent and more or less homogenous with the cytoplasm. At a higher magnification (Fig. iv.41) the nucleoplasm may seem to be slightly denser than the cytoplasm and to consist of uniformly dispersed fine material; but this may be a very slightly earlier stage than the one seen in Figs. iv.43 and iv.45 which give the appearance of the cytoplasm having penetrated the nuclear space even though there is no clear indication of any rupture in the envelope.

At this stage of nuclear disintegration, tubular or laminar structures are always associated with the nuclear envelope, either inside or outside the latter. In Figs. iv.43 and iv.45 those inside the nuclear envelope give the appearance of smooth elongated ER. Rounded outlines or vesicles are also common but these are probably cross-sections of tubular structures. Probably some localised changes in the nuclear envelopes during nuclear disintegration are responsible for these structures (Zee 1969a).

Quite mature sieve tubes with degenerated nuclei are seldom encountered and what is normally observed are just mature sieve tubes without any trace of nucleus. Figs. iv.46 and iv.47 are among the few photographs obtained of a disintegrating nucleus. The nuclear envelope in Fig. iv.46 which is elsewhere intact, is ruptured at one point. A nuclear pore is barely visible (arrow) and the nuclear content is now aggregated along the periphery of the nuclear envelope.

The nucleus in Fig. iv.47 also gives the appearance of the cytoplasm having penetrated through the broken envelope while the nuclear content exists as small dense aggregates. This photograph was from a sieve tube whose sieve plates were about to perforate.

In Fraxinus, therefore, disintegration of the nuclear envelope takes place after the disorganisation of the nuclear content, with the envelope still intact as in Pisum sativum (Bouck and Cronshaw 1965) and Acer pseudoplatanus (Northcote and Wooding 1966).

The ultimate fate of the nucleus in Fraxinus is conjectural. Probably it is enzymatically digested fairly completely after disintegrating further into small fragments. That may be the reason for the difficulty in finding recognisable fragments in mature sieve tubes.

In a number of woody dicotyledons nucleoli have been reported to be extruded from the nucleus and to remain in the sieve element, as early as in 1944 by Engard in Rubus. These 'extruded nucleoli' have been observed to have various external projections, well seen for instance in Salix (Mishra and Spanner 1969). Zahur (1959) has reported the existence of 'extruded nucleoli' in 41 species distributed within 12 families. However, Deshpande and Evert (1970) disagree with the conclusion that these bodies are in fact 'extruded nucleoli'. They found that they were quasi-crystalline aggregates which arose early in the sieve tube ontogeny when nucleus and nucleolus were clearly visible and intact. Their preliminary tests indicate these inclusions to be proteinaceous and similar to the P-protein in their staining properties. A search was made for such bodies in Fraxinus, but during the course of this work none were found.

(d) Plastids

Plastids of sieve elements are unique. Their morphology and inclusions differentiate them from the plastids of other cells. Behnke (1972) has even made a phylogenetic classification based on the inclusions present alone. Morphological changes seem to take place within the plastids as the sieve tube differentiates.

In a very young sieve tube of Fraxinus, the plastids have the usual double membrane, some electron dense lamellae, and a dense fibrous stroma (Fig. iv.48). The fibrous nature of this is sometimes not visible when the ground stroma is very dense. A few tubules may be present, probably indicating an unorganised internal membrane system. Most of the plastids are rounded even at this stage and are usually larger than the mitochondria. In sections, the plastids range in size from 0.8 $\mu$  to 2.0 $\mu$ ; the number of starch grains per plastid varies from one to nine. An estimate of the number of plastids in a sieve tube gives a value of 58.

Later, numerous rounded starch grains appear within the plastids. Sometimes spherical electron-dense inclusions are also present in the fibrous stroma (Fig. iv.49); these could be phenolic in nature (Gunning and Steer 1975). According to Behnke (1971), this type of plastid is classified as the S-type, as opposed to the P-type which has proteinaceous inclusions instead of, or in addition to, starch grains.

The appearance of starch varies from greyish (Fig. iv.49) to whitish (Fig. iv.50). It has been assumed that these differences are dependent upon the fixation and staining. In this study the same fixation and staining combination has been used consistently throughout consequently variation in the appearances of the starch is more likely

to be due to the plane of cut or the thickness of the sections. A halo is also sometimes present around the grains (Fig. iv.50).

As the sieve tube undergoes differentiation, the plastids gradually increase in size and appear to be less turgid, losing some of their roundedness. The outline of the plastid envelope becomes irregular (Figs. iv.51 and iv.53), the two membranes sometimes becoming separated (Figs. iv.52 and iv.53).

The stroma becomes less dense and often shows a number of tubular membrane profiles sometimes possibly derived from disintegrated thylakoids, a feature common to sieve element plastids in other species (Behnke et al 1975). Very often small dark bodies, possibly plastoglobuli, are also present (Figs. iv.52 and iv.53). Finally the stroma loses its electron opacity almost completely (Figs. iv.53 and iv.54).

The starch grains remain intact but often appear to become etched at the margin giving a fissured or even a coarsely granular look (Fig. iv.53). This has been illustrated for other species e.g. Phaseolus (Palevitz and Newcomb 1970).

The distribution of plastids in a young sieve tube is variable. The plastids are probably abundant near the sieve-plate ends when the sieve tubes are not fully vacuolated (Fig. iv.55). As the cytoplasm thins out with the formation of a large single vacuole, the plastids are located along the periphery (Fig. iv.56). In fully perforated sieve tubes, plastids are more often found disrupted especially those near the sieve plates (see Chapter III, section (iii) (c)). Starch grains released from them are accumulated near the sieve plates. This break-up of plastids probably reflects an osmotic shock while the tissue was being manipulated for examination in the microscope.

Fig. iv.1. TS of a young sieve element identified by the dense matrix of the plastid. Note the plasmalemmal invagination and large vesicles. Close by are dictyosome vesicles which may be migrating to the invagination. Some vesicles intermediate between the two types occur, perhaps indicating a connection. From stem sampled on 23rd June, 1975  
x 39,000

Fig. iv.2 TLS young sieve element showing beginnings of the invaginations (arrows). Note the apparent continuity of the fibrillar contents with the cell wall fibrils. Judged by the microtubules, the wall is seen partly in surface view. From stem sampled in late May, 1975  
x 27,000

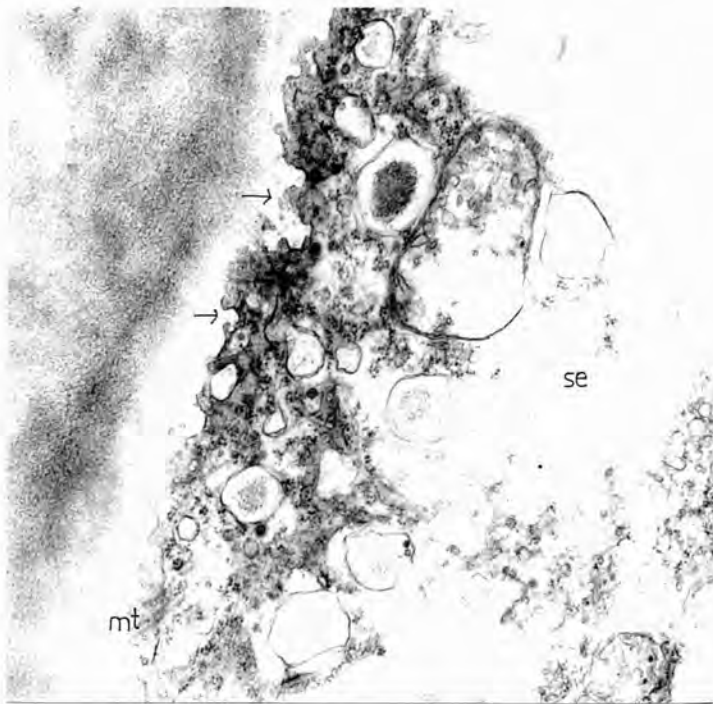
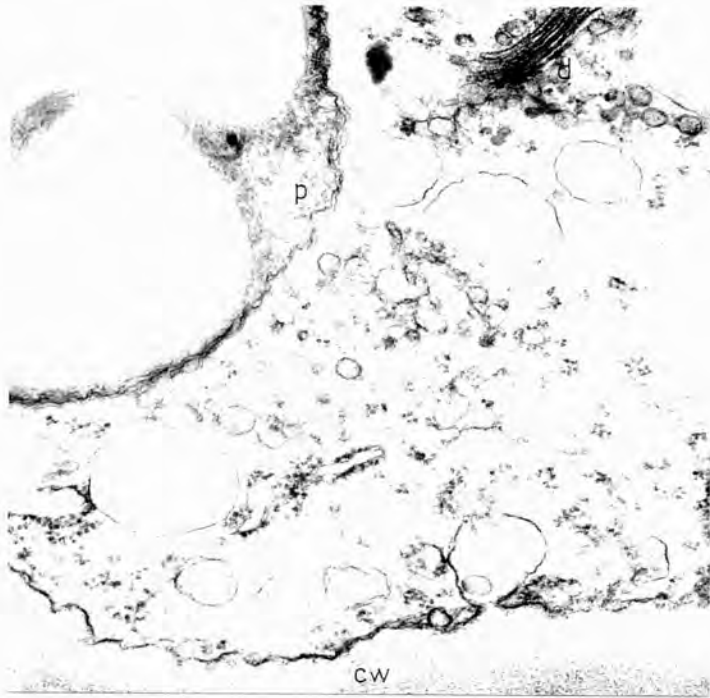


Fig. iv.3

TS mature sieve tube. Note the frequency of the invaginations and the gradations in their sizes. Some invaginations appear devoid of any contents. From stem sampled on 21st October, 1975  
x 39,000

Fig. iv.4

Formation of invagination over callose deposited at the plasmatic connection between a sieve element and a companion cell. No contents appear to be present. From TLS of stem sampled in late June, 1975 x 54,000



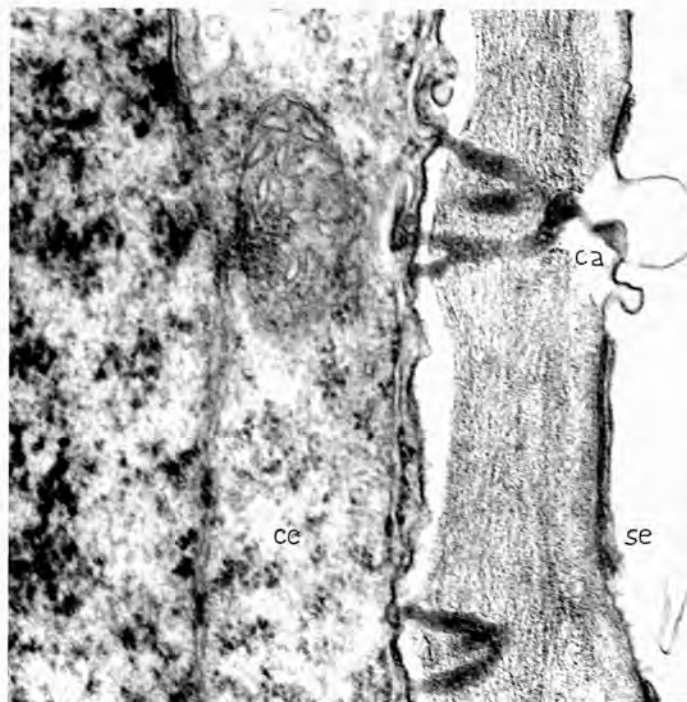
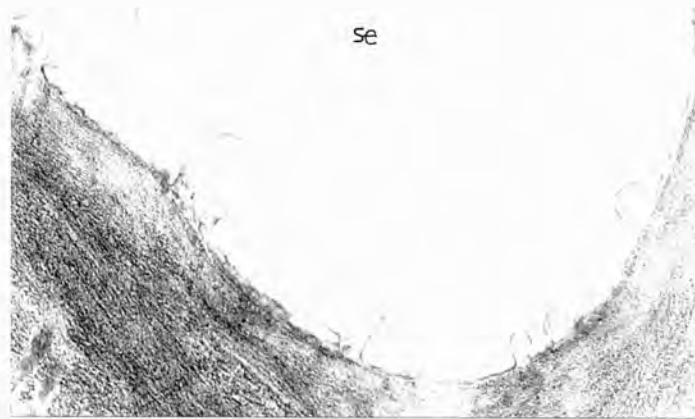


Fig. iv.5

Invagination with wide base and vesicular content in a mature sieve tube. Short tubules at the base may be non-median infoldings of the plasmalemma opening into the sieve tube lumen. From TS of stem sampled on 20th May, 1976  
x 52,000

Fig. iv.6

A young sieve element as indicated by the dense cytoplasm and thick crenulated wall. Note the beginning of the invaginations (arrow). The size of the sacs increases away from the plasmalemma. The tonoplast is still intact. From TLS of stem sampled in late May, 1975 x 18,000

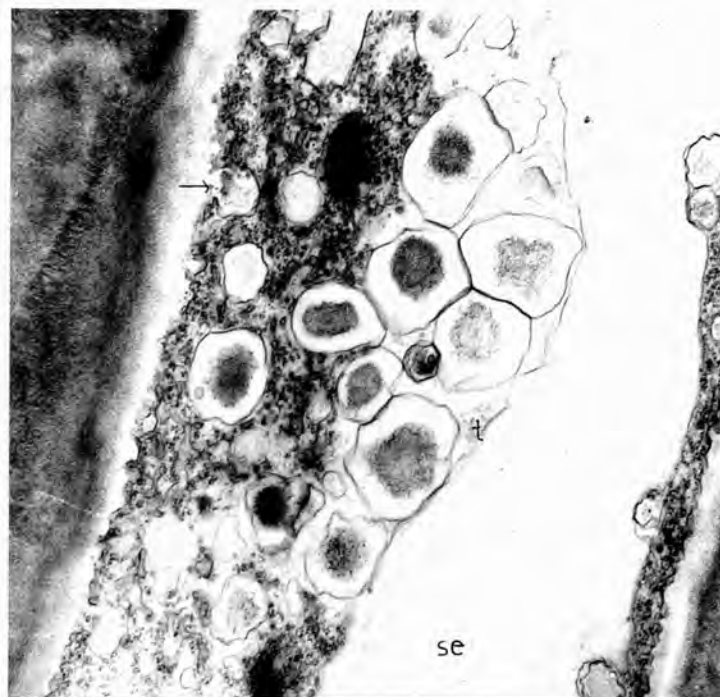


Fig. iv.7      Invagination with fairly narrow orifice and short neck. Note sac containing similar fibrils; also the presence of a mitochondrion in the intervening cytoplasm. The tonoplast is intact and the sac is within the cytoplasmic region. From TLS of a young sieve element sampled in late May, 1975      x 45,000

Fig. iv.8      Empty spherical vacuoles within a mature sieve element in the vicinity of small plasmalemmal invaginations. Note the presence of released starch grains (sg). Section has chatter marks. From TLS of stem sampled on 21st October, 1976  
x 10,800

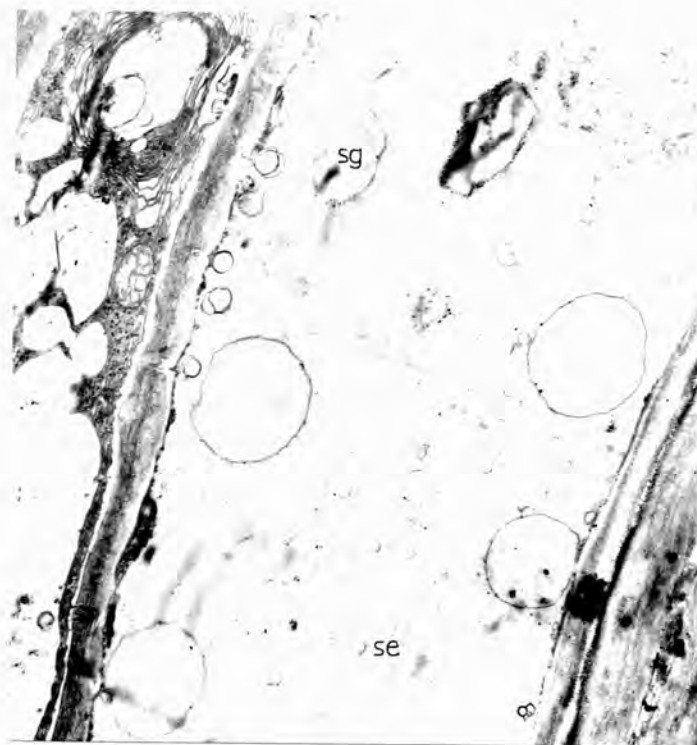


Fig. iv.9      Immature sieve element (note tonoplast) with two invaginations, one having an elongated neck. Probably it is in the process of being "pinched off". The base which remains may give rise to a new invagination. The contents are fibrillar and show some continuity with wall material. From TLS of stem sampled in late May, 1975      x 45,000

Fig. iv.10      Sieve plate at a late stage of differentiation, recognised by the callose platelets and the stacking of ER. Note the two invaginations. The fibrillar content in the bigger one appears continuous with material in the walls. From TLS of stem sampled on 23rd June, 1975  
x 52,000

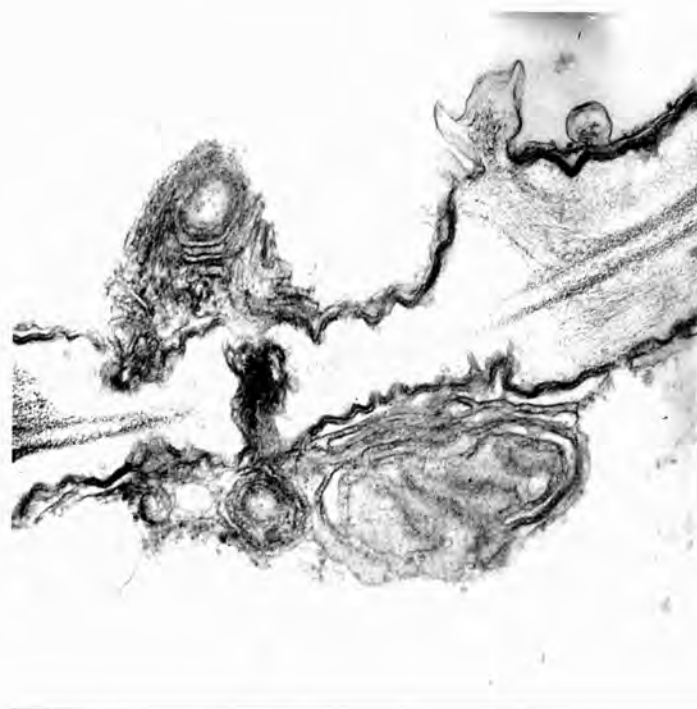
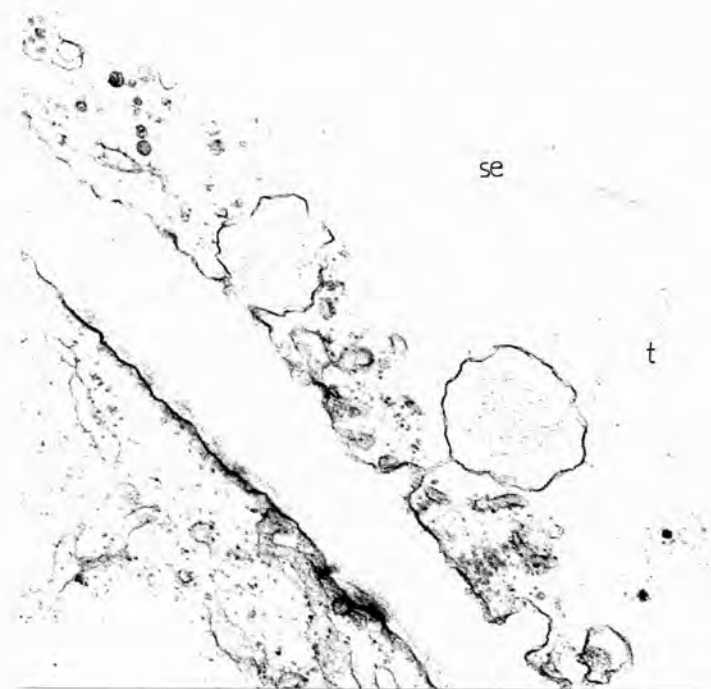


Fig. iv.11      TS of mature sieve elements. Note the small size of the invaginations and their contents. One contains a combination of fibrils and vesicles, the latter somewhat submerged in the wall. From TLS of stem sampled in mid-December, 1976      x 39,000

Fig. iv.12      A large invagination in a ray cell. Invagination is ovate with a fairly narrow base and short neck. Some of the larger vesicles within appear collapsed. Fibrils are also present. The position of the tonoplast is not obvious. From stem sampled in late May, 1975      x 26,000





Fig. iv.13      TLS of phloem parenchyma cell. Note  
the abundance of various types of sacs  
lying 'free'. From stem sampled in  
mid-September, 1975      x 10,875

Fig. iv.14      Higher magnification of the figure above.  
Note the intergradation of the contents in  
the sacs. Tubules appear beaded. The two  
membranes of what may probably represent the  
tonoplast and plasmalemma are evident at  
A.      x 39,000

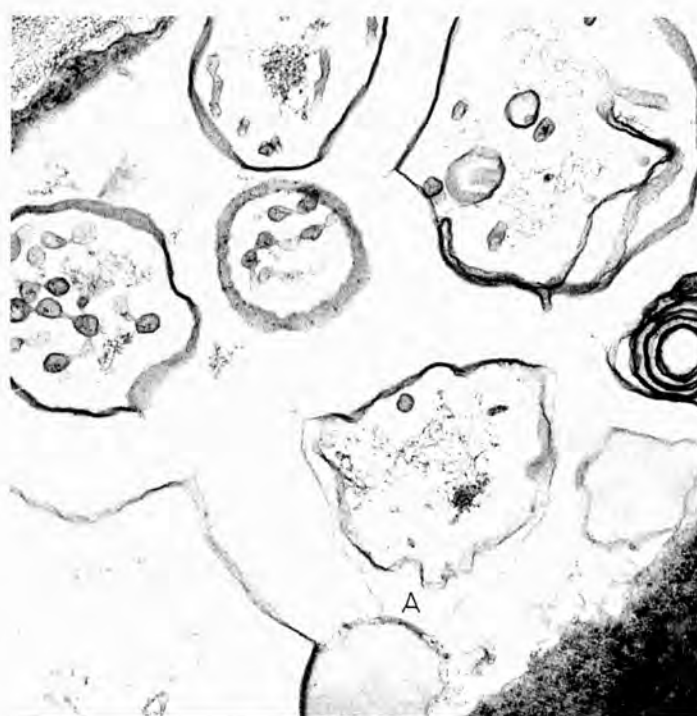
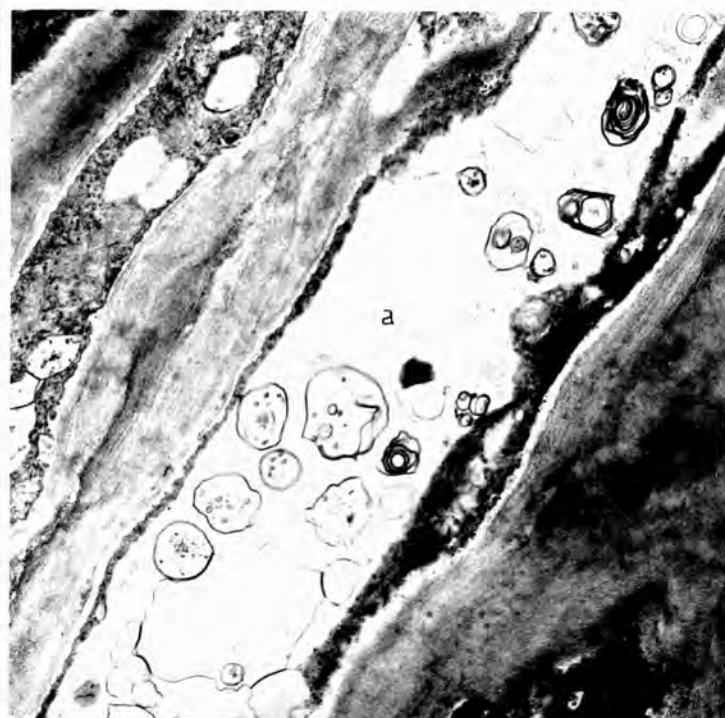


Fig. iv.15      Apparently 'free' sac in the vacuole of a phloem parenchyma cell. The sac is filled with spherical vesicles. Two membranes appear to bound this sac. From stem sampled in late May, 1975      x 32,500

Fig. iv.16      In phloem parenchyma cell. Invagination has fairly wide base and short neck. Contents are mainly vesicular. Note the intermembrane zone at A (i.e. zone between tonoplast and the plasmalemma) and the infoldings of the membrane (arrows), suggesting an origin for the vesicles seen within. From stem sampled in mid-December, 1976      x 52,000



Fig. iv.17      A wide based invagination. Vesicles are comparatively large. Note infoldings of membrane of invaginations (arrow). From TLS of phloem parenchyma cell sampled in mid-December, 1976    x 52,000

Fig. iv.18      In a phloem parenchyma cell. Invagination has numerous vesicles within. Density of vesicles appears similar to the cytoplasm but this may be due to the membrane. Note the presence of other vesicles within the cytoplasm. From stem sampled in late May, 1975    x 26,000

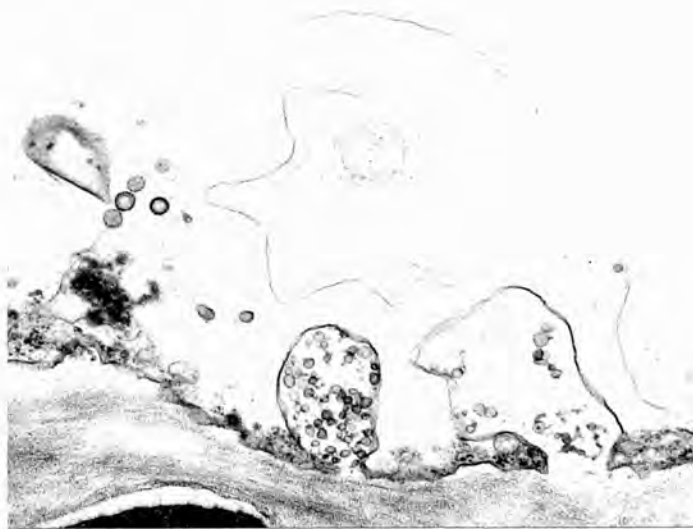


Fig. iv.19      Large invaginations in phloem parenchyma cells with vesicular and tubular contents. Invagination has ER within the intermembrane zone. Note the infolding of the membrane (arrow) in the invagination on the right. From stem sampled in late May, 1975  
x 27,000

Fig. iv.20      'Free' sac within vacuole of phloem parenchyma cell. The sac contains tubules and circular membranes. The end of the tubules appear swollen. From stem sampled in mid-December, 1976  
x 39,000



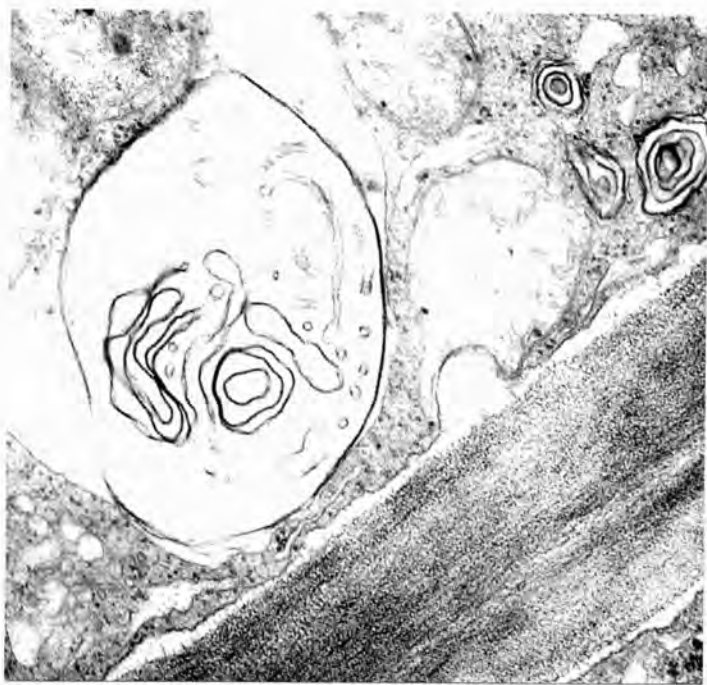
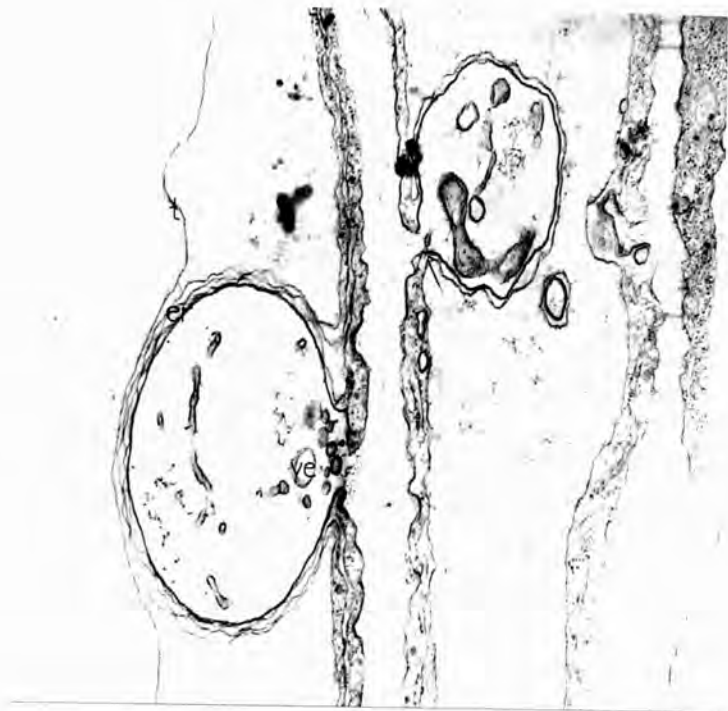


Fig. iv.21      Invagination without attachment to the  
 plasmalemma but within the cytoplasm.  
 The layered lamellae within the invagination  
 present the appearance of a myelin sheath.  
 From TLS of phloem parenchyma cell, sampled  
 in late May, 1975      x 13,000

Fig. iv.22      TS of two adjacent ray cells. Note the  
 close proximity of the vesicles in the  
 lower right invagination to the cell wall;  
 also the presence of spherical vesicles  
 in the 'paramural' area to the left.  
 From stem sampled in mid-December, 1976  
 x 39,000

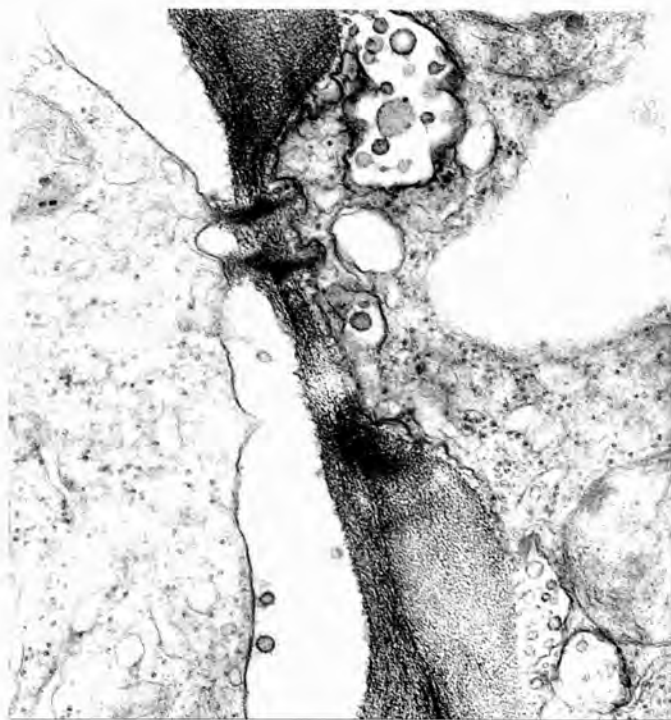


Fig. iv.23

A developing sieve plate. ER lies next to the plasmalemma of the callosed areas. The space between the plasmalemma and the cisternae is not as electron dense as that later occurring between the cisternae in ER stacks. Ribosomes are still attached to the free surface of the cisternae. From stem sampled in late May, 1975 x 52,000

Fig. iv.24

TS of young sieve element. A single ER cisterna has already aligned itself at the periphery of the wall to the left. Presumably this gives rise to the 'parietal' ER. Note the plasmalemmal invaginations with fibrillar contents. From stem sampled in late May, 1975 x 26,250

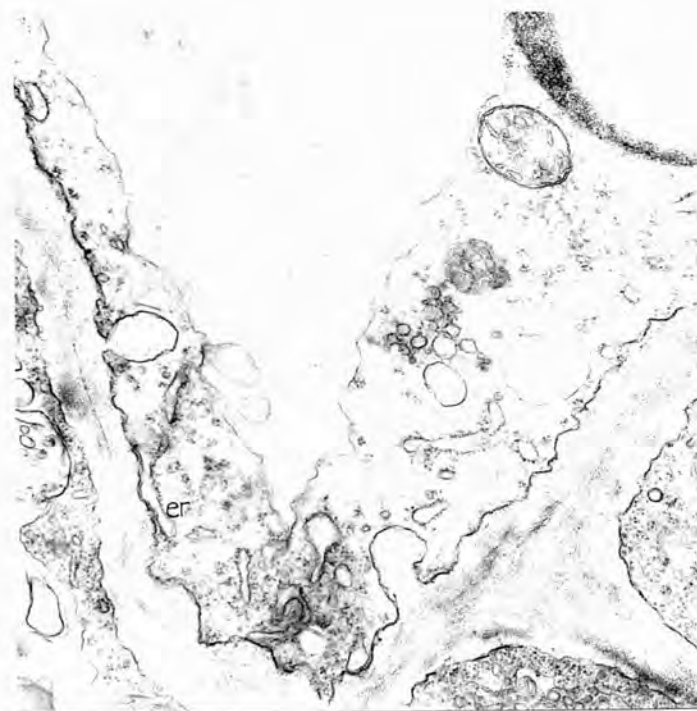
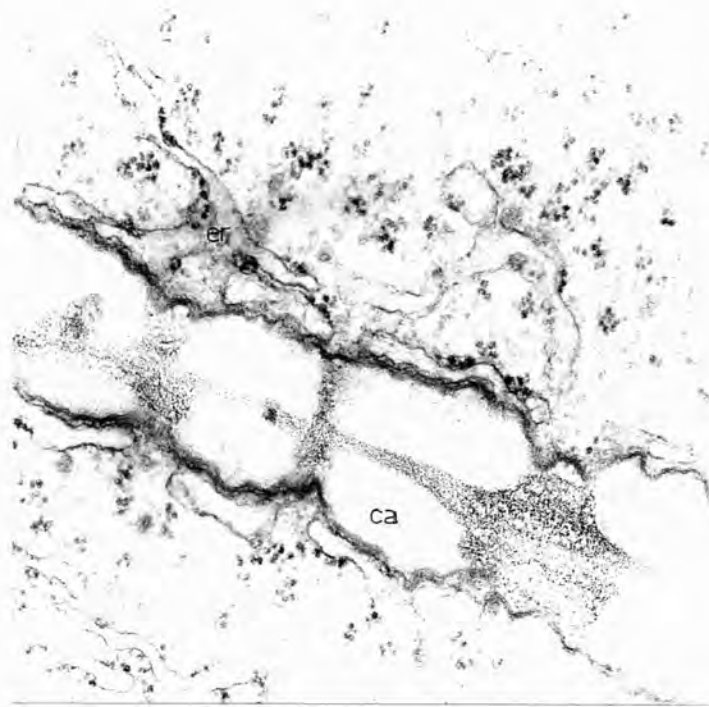


Fig. iv.25      TLS of young sieve element. The beginning of stacking of the ER cisternae. From stem sampled in late May, 1975      x 26,000

Fig. iv.26      Parallel stacks of ER cisternae. Ribosomes are still present on the free surfaces of the cisternae. Note the electron dense intercisternal spaces. The sieve element is relatively young as judged from the dense matrix of the plastids and intact tonoplast. From stem sampled in mid-September, 1975      x 17,500

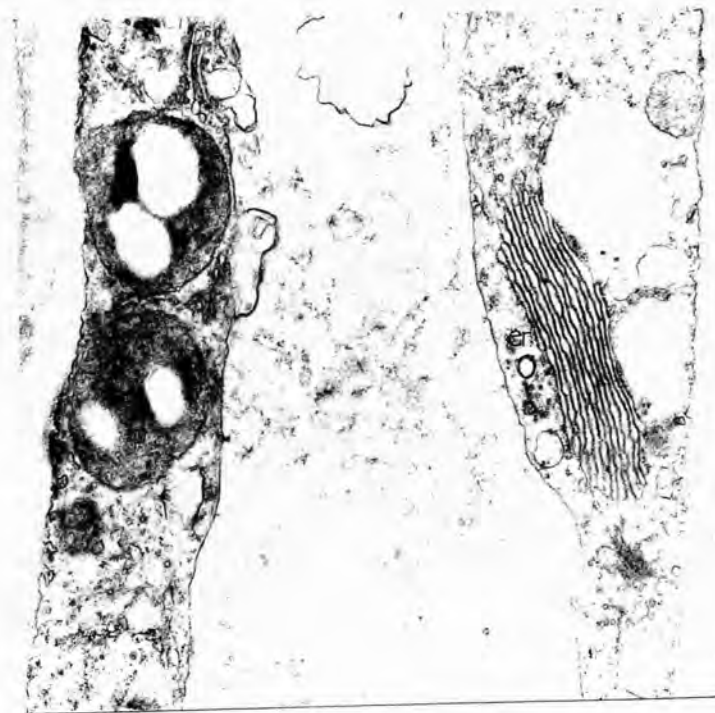
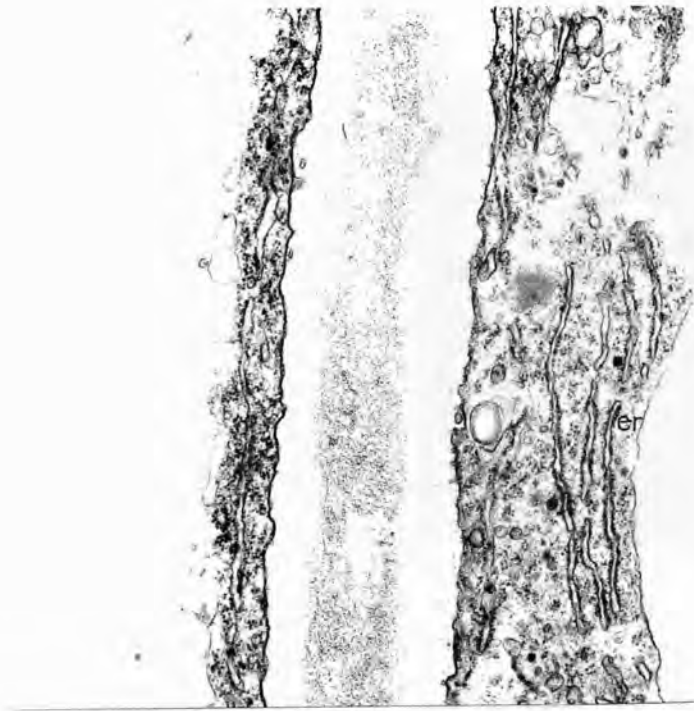


Fig. iv.27      An ER aggregate with both flattened and fenestrated cisternae. From TLS of mature sieve tube of stem sampled in late May, 1975      x 26,250

Fig. iv.28      A parallel stack of ER in a relatively mature sieve tube. Note the electron dense intercisternal spaces. One of the outer cisternae appears to be inflated. From stem sampled in late May, 1975      x 26,250



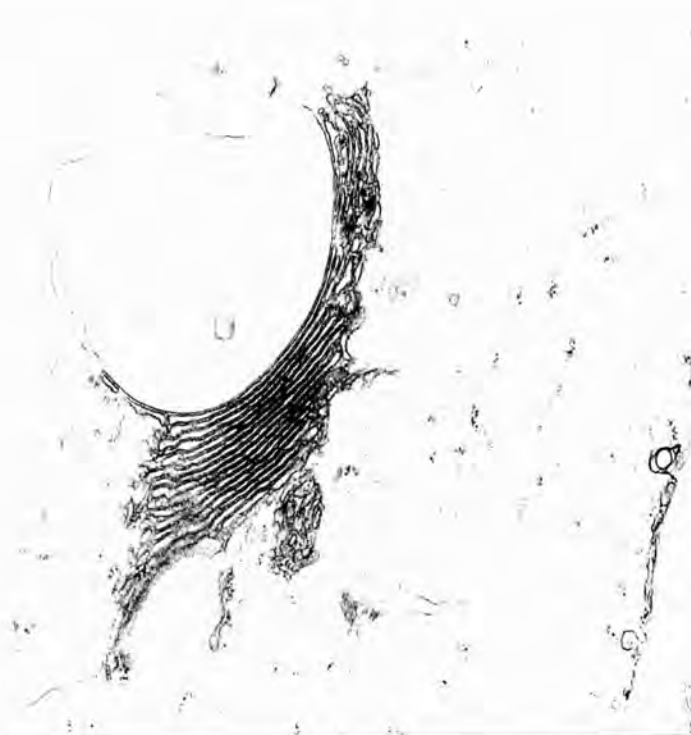
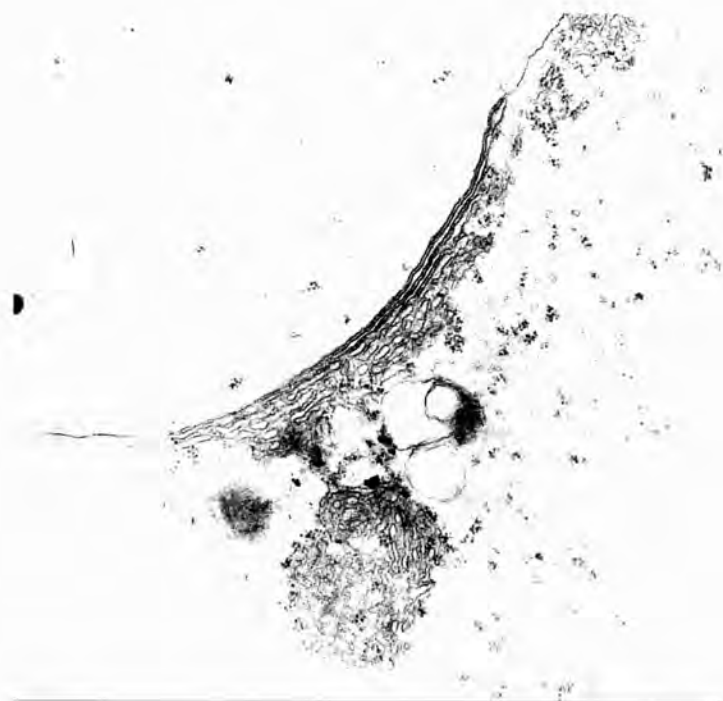


Fig. iv.29      RLS of mature sieve element. ER aggregates in a circular or spiral form. Note the 'parietal' ER. From stem sampled in late June, 1975      x 43,750

Fig. iv.30      TS of mature sieve element. The stacks of ER are oriented both parallel to and perpendicular to the wall. Notice the mitochondrion partially sheathed by stacked ER. From stem sampled in late May, 1975      x 26,250.

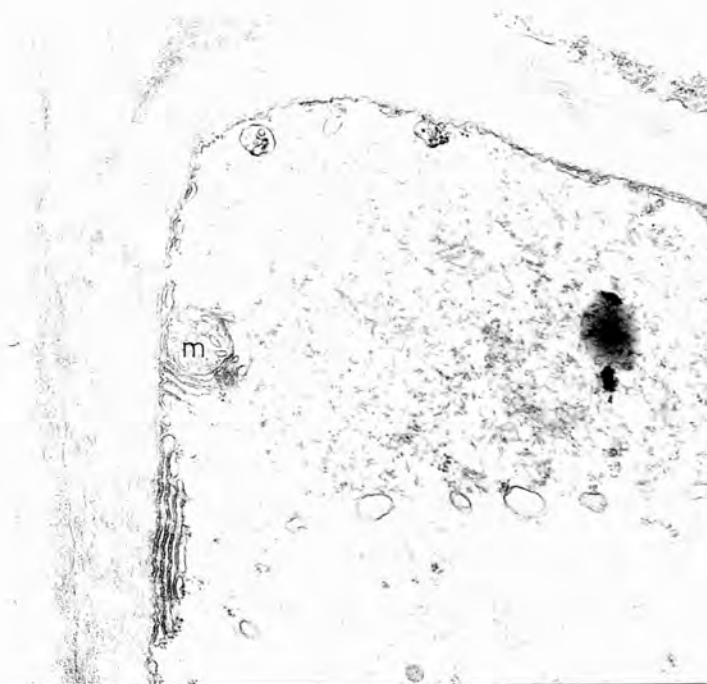


Fig. iv.31      TLS of lateral wall between two mature sieve tubes. Short ER aggregates lie perpendicular to the wall. Note the plasmalemma to the left side of the wall, and the invagination on the right. From stem sampled in early October, 1975  
x 43,750

Fig. iv.32      TS of mature sieve element. Probably an oblique section of an ER aggregate. From stem sampled in early May, 1975  
x 70,000



Fig. iv.33

TLS of mature sieve element. The 'parietal' ER is appressed to the wall of the sieve element. The former has one or sometimes two ER layers, which appears to be fairly continuous along the wall. The intercisternal space is electron dense. A median nodule is evident in the region of a plasmodesma. From stem sampled in late May, 1975 x 43,750

Fig. iv.34

TLS of mature sieve element. ER stacks sheathing a mitochondrion. Note the tubular P-protein near to it. From stem sampled in early October, 1975 x 26,000

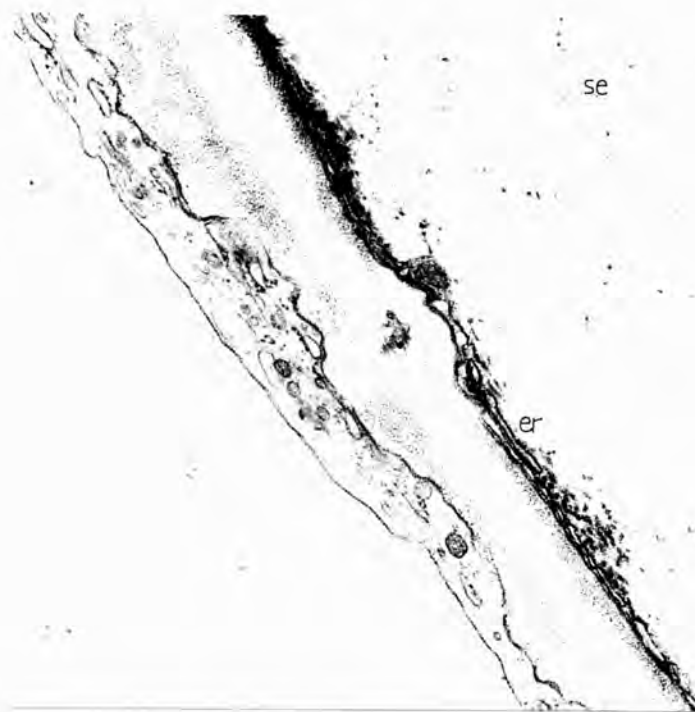


Fig. iv.35

TLS of a sieve element judged by the P-protein body and other organelles to be comparatively young. Nucleus is lobed and has prominent nucleolus. Chromatin is evenly dispersed within the nucleus. From stem taken in late May, 1975 x 14,000

Fig. iv.36

Nucleus in a sieve element recognised as rather <sup>lobed.</sup> Nucleolus is prominent. The smaller 'nucleus' is probably the section of a lobe of the nucleus. Nucleoplasm is homogenously as dense as the cytoplasm. From stem sampled in late May, 1975 x 4,625



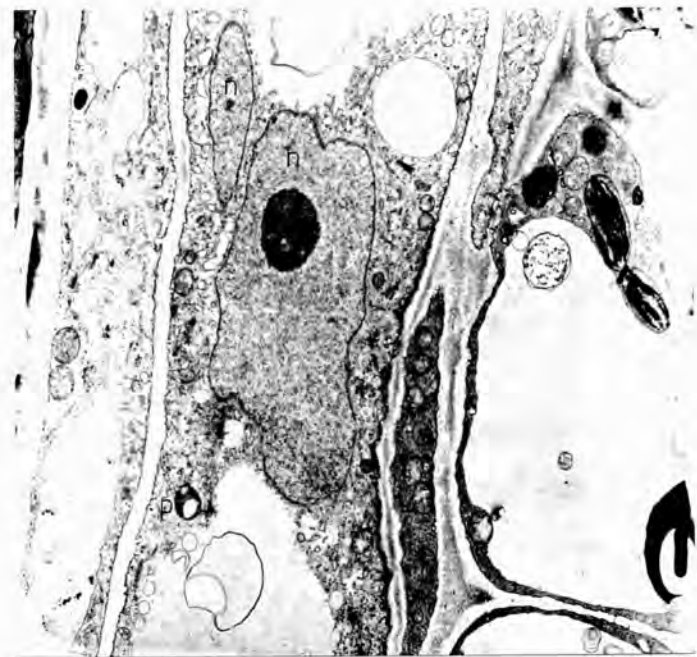
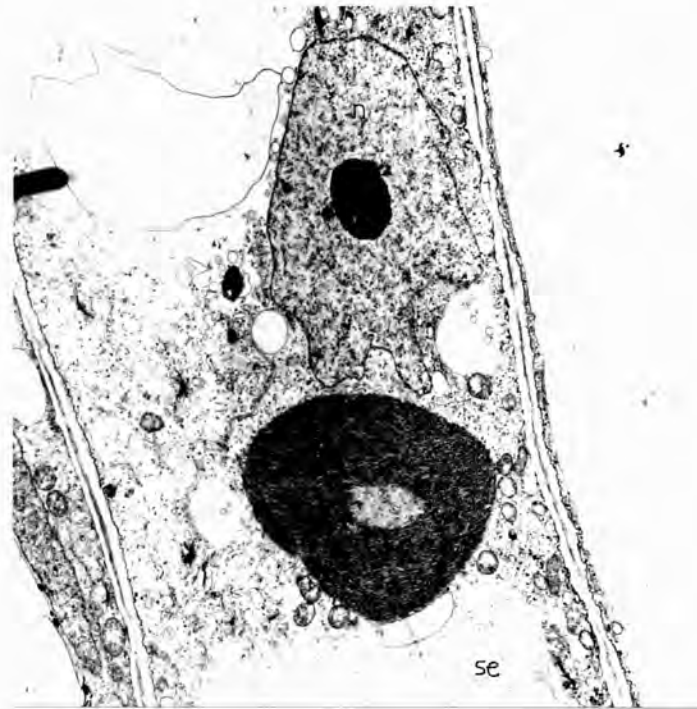


Fig. iv.37

Nucleus in an unperforated sieve element, where tonoplast is more or less intact. Nucleolus is fragmenting and nucleoplasm is as dense as the cytoplasm. Note the two double membraned 'vesicles' within the nucleus. Arrow probably indicates a nuclear pore which would suggest that the vesicles are cytoplasmic invaginations of the nucleus. From stem sampled in late May, 1975 x 7,200

Fig. iv.38

TLS of an immature sieve tube. Two ER cisternae stack is in close association with the nuclear envelope. Note the dispersing P-protein and the plastid which indicate the state of differentiation of the sieve tube. From stem sampled in late April, 1976 x 25,500

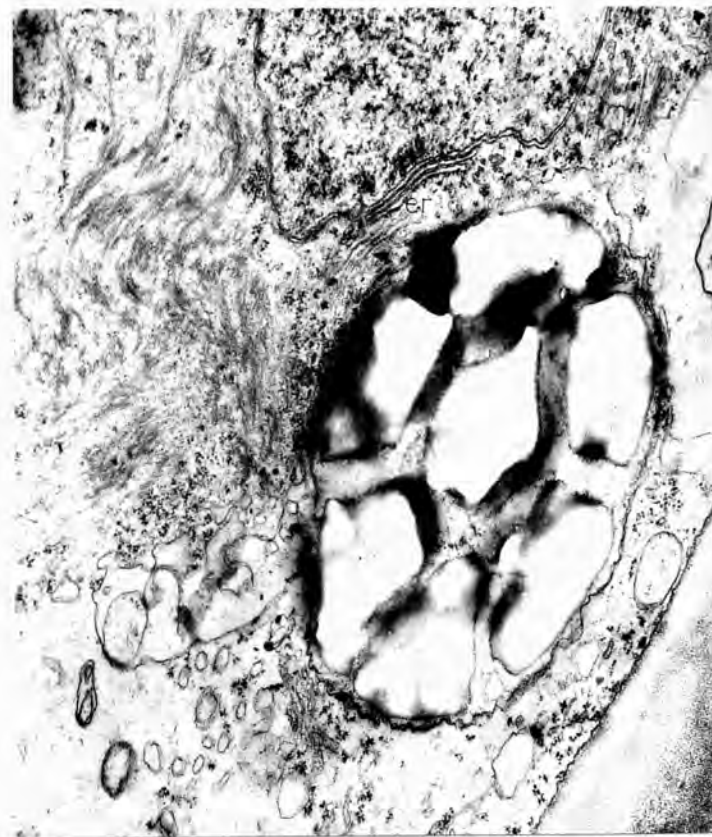
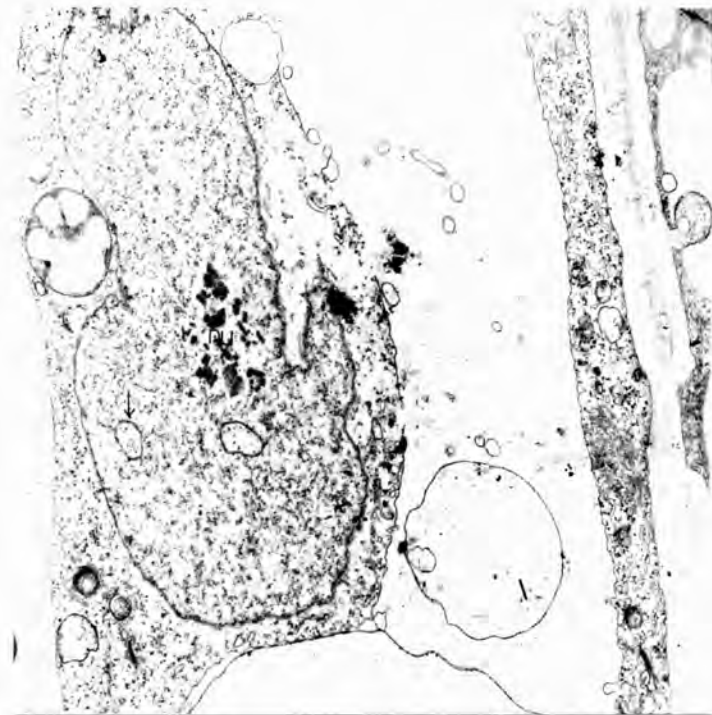


Fig. iv.39

TLS of an unperforated sieve tube older than that in Fig. iv.36. Nucleus is enlarged and nucleolus is not visible. Chromatin is visible as small dense aggregates distributed unevenly within the nucleus. There is also some tendency of localised aggregation along periphery of nuclear envelope. Note dispersing P-protein and the plastids, both of which indicate the relative stage of differentiation. From stem sampled in early June, 1976 x 3,250

Fig. iv.40

Nucleus in a fairly mature sieve tube where tonoplast has broken down. Nucleus almost fills the lumen of the sieve tube. Nucleolus is in fragments. Nuclear pores still evident (arrows). Note the few ER cisternae which are appressed to the nuclear envelope inside or outside it. From stem sampled in late May, 1975 x 7,200

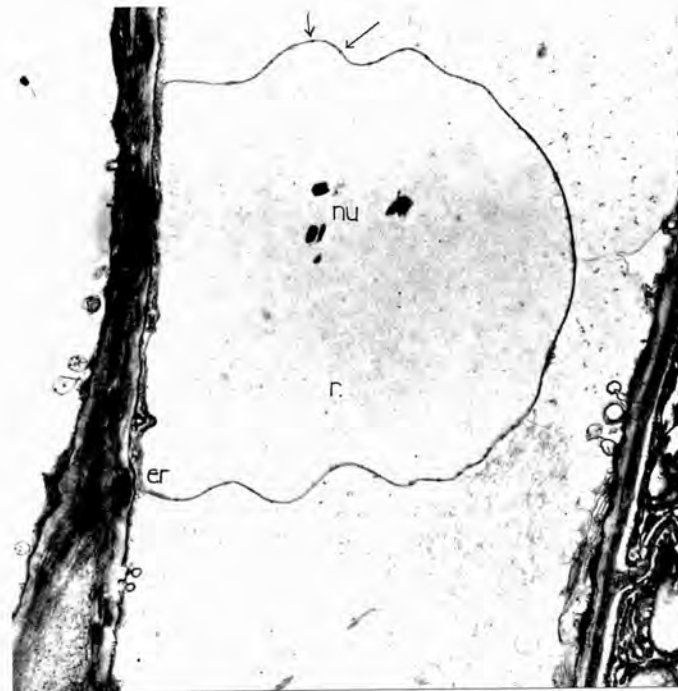


Fig. iv.41 Higher magnification of Fig. iv.40. There is a slight difference in the density of the nucleoplasm and cytoplasm. Small tubule-like structures are present within the nuclear envelope (arrowhead). Arrows indicate nuclear pore. x 27,000

Fig. iv.42 Enlarged nucleus occupying the whole lumen of a mature sieve tube in longitudinal section. Note the various shapes and sizes of the double membraned 'vesicles'. The nucleoplasm and cytoplasm appear almost alike in density. ER cisternae are appressed to the membrane or free or outside it. From stem sampled in late May, 1975 x 7,200

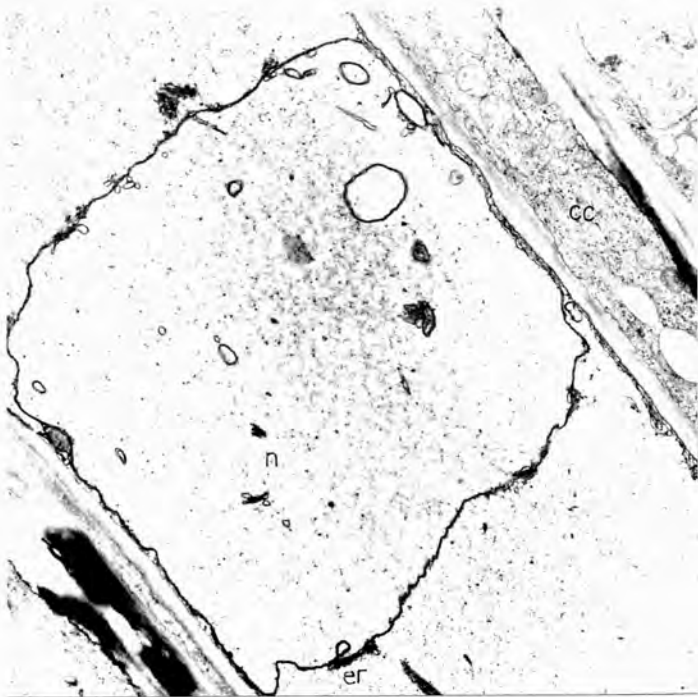
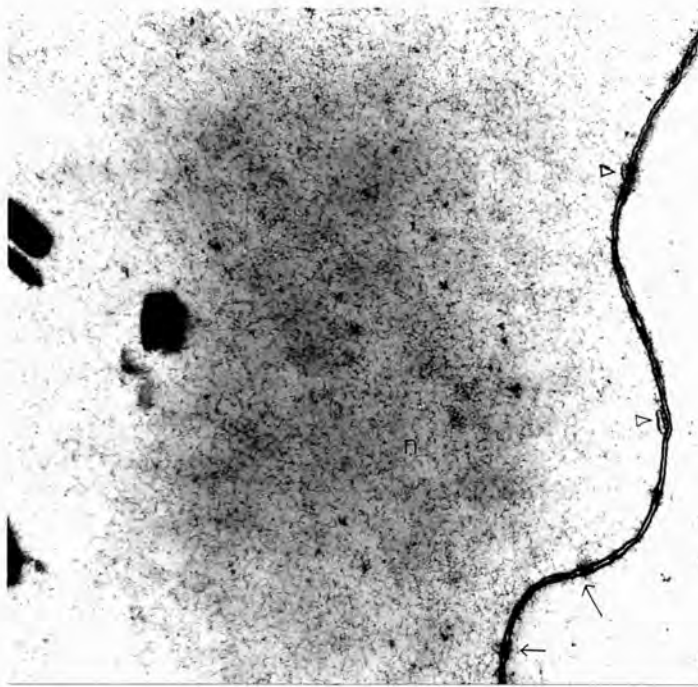


Fig. iv.43

Higher magnification of part of Fig. iv.42.

The flattened tubule or cisterna within the nuclear envelope gives the appearance of a smooth ER. Spherical tubules are also present on the right hand side. The stack of P-protein at the top left hand corner appears to be of the tubular type. Nucleoplasm is of similar density to the cytoplasm. x 27,000

Fig. iv.44

Nucleus in a perforated sieve tube.

Tonoplast is broken down, and there is a good deal of ER associated with the nuclear membrane and the sieve tube walls. The sieve tube plastid is maturing. The nucleus is irregular in outline. From stem sampled in late May, 1975  
x 10,800



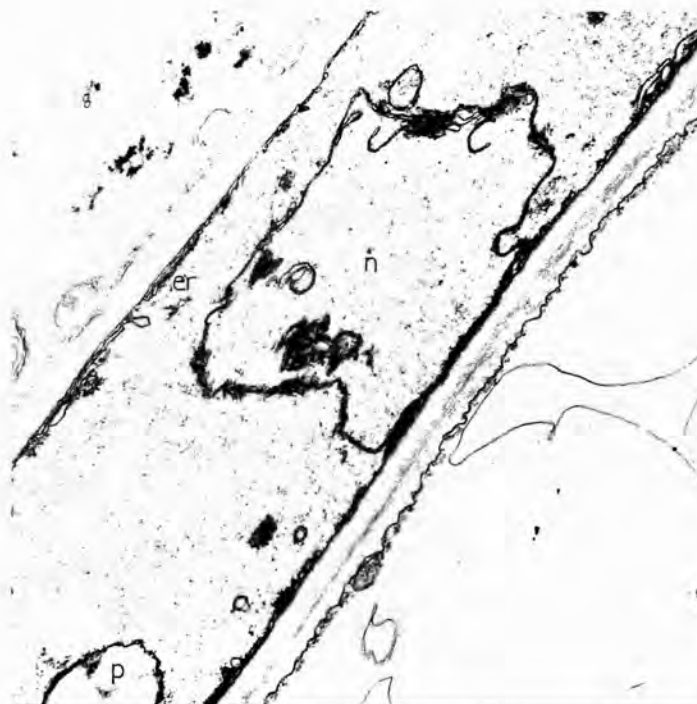


Fig. iv.45

Higher magnification of Fig. iv.44.

Nucleoplasm is of similar constituency as cytoplasm. Note the elongated tubules which suggest smooth ER. Nuclear pore (arrow) still visible.

x 45,000

Fig. iv.46

RLS of a mature sieve tube. Nucleus is in an advanced state of degeneration. Nuclear envelope is ruptured at one end whilst the rest remains intact. Nuclear content is now aggregated along the periphery of the nucleus and nuclear pore (arrow) is perhaps evident in the inner layer. From stem sampled in late June, 1976 x 42,000

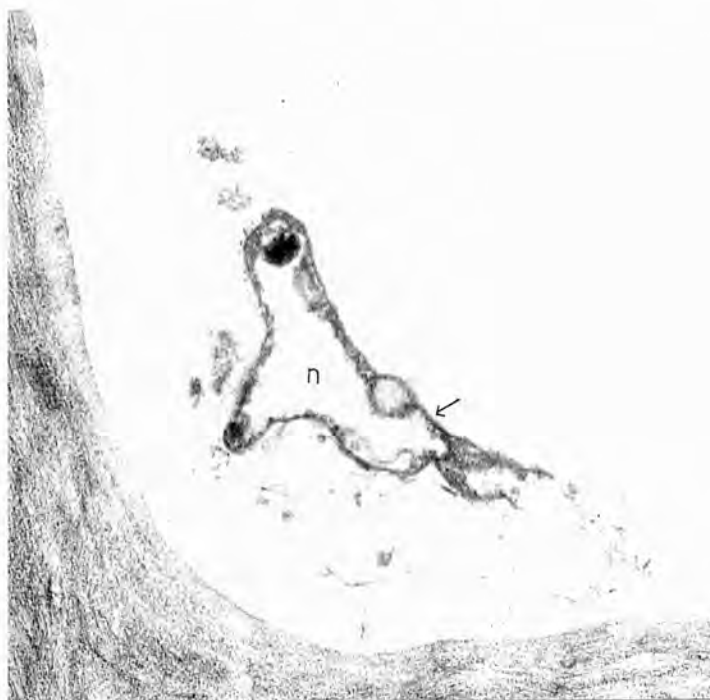


Fig. iv.47

TLS of a sieve tube which is about to perforate. Nuclear envelope is ruptured at one side and pores are still evident in some places (arrows). Appearance suggests that the cytoplasm has penetrated into the nuclear space. The nuclear material is present as dense aggregates. From stem sampled in late July, 1975 x 18,000

Fig. iv.48

Plastids are rounded and bigger than the mitochondrion. Their stroma is rather electron dense and fibrous in texture, some tubules are present probably indicating an unorganised internal membrane system. Electron dense bodies can be seen, perhaps lipid in nature. From TLS of a young sieve tube whose callose platelets have not even been laid down on the future pore sites. x 46,450

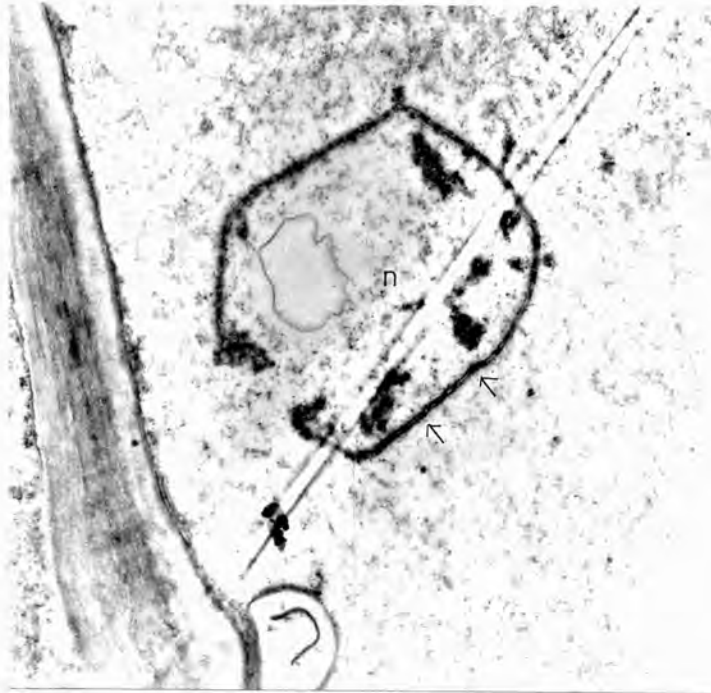


Fig. iv.49

TLS of a young sieve element. Plastids are rounded and contain numerous starch grains. Stroma is rather electron dense and fibrous or granular. An electron dense body is present in one of the plastids. The starch grains have an even boundary. From stem sampled in late May, 1975  
x 9,750

Fig. iv.50

TLS of a young sieve element. Plastids are rounded and have a very electron dense stroma. There is an electron dense membranous structure inside the plastid on the right. Starch appears whitish and has a halo. From stem sampled in late May, 1975 x 27,000

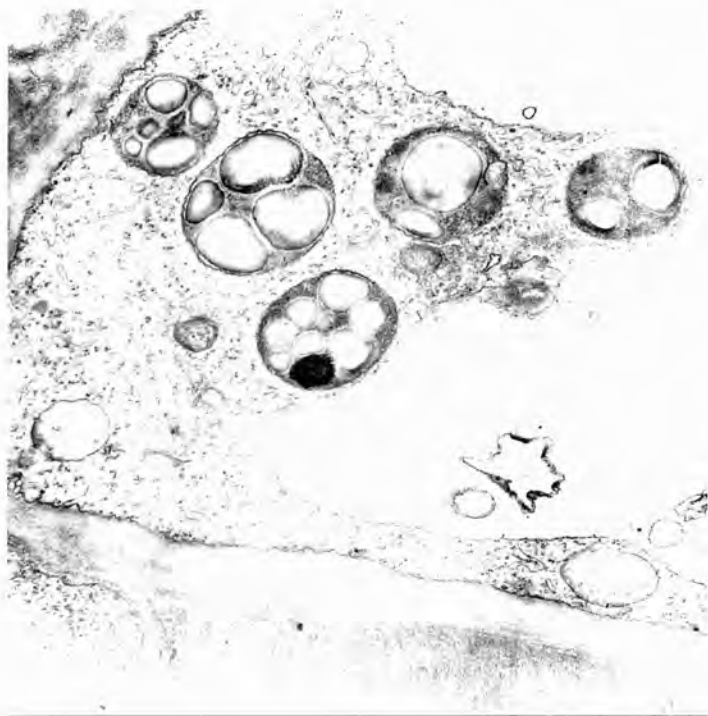


Fig. iv.51

Plastid from a mature sieve tube. The outline of the plastid is indented and the shape is a little flattened. Note the tubules within, some forming a ladder-like body. Starch grains appear white. From stem sampled in mid-September, 1975  
x 18,000

Fig. iv.52

Plastids have a stroma containing a good deal of tubular membrane structures. Some plastoglobuli are conspicuous. Note the shape of the starch grains and the separated membranes of the plastid envelope near the sieve tube wall. From a TLS of stem sampled in early October, 1975 x 27,000



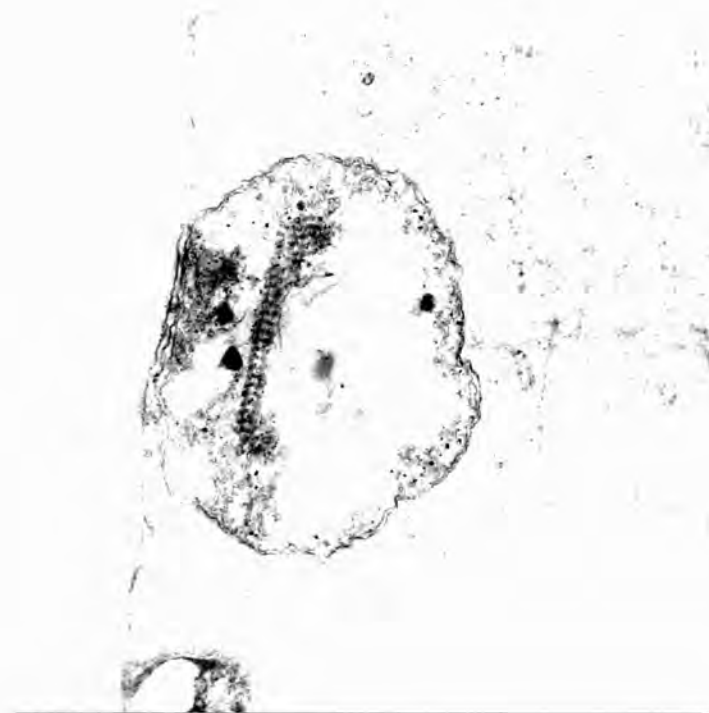


Fig. iv.53

TLS of a mature sieve tube. The membranes of the plastid at A are partially separated and its starch has a fissured margin. Starch in plastid B appears similarly coarsely granular. Tonoplast has broken down. Note the dispersed P-protein, the mitochondrion and the membrane stack. From stem sampled in late April, 1976  
x 13,000

Fig. iv.54

TLS of a mature sieve tube with fully perforated sieve plate. The stroma material has disappeared from the plastid leaving the interior electron transparent. Starch appears fissured at the margin and some appear granular when the section is glancing. Note the irregular outline of the plastid envelope. The plastids appear to have lost their turgidity. From stem sampled in late May, 1975 x 18,000

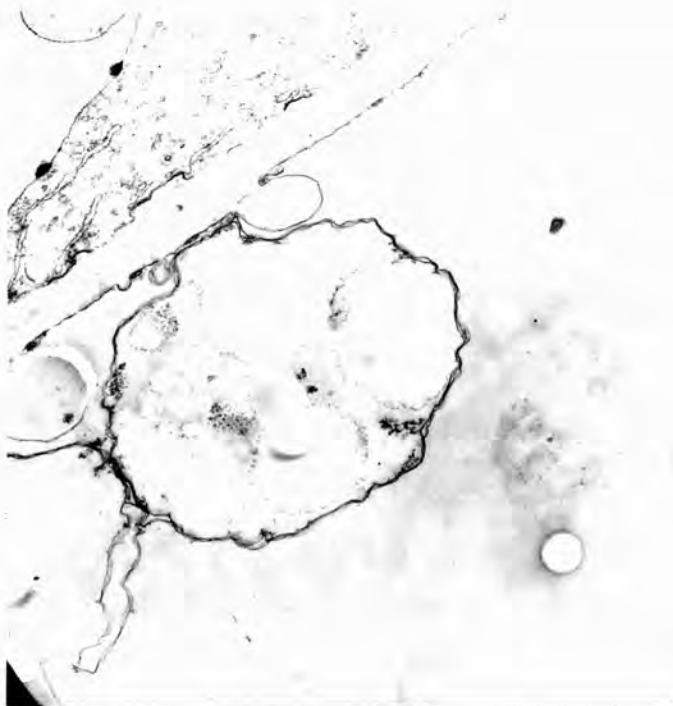


Fig. iv.55 LTS of a young sieve element. The plastids are distributed near the unperforated sieve plate. The matrix of these plastids appear electron dense. Note the dense cytoplasm. From stem sampled in late May, 1975  
x 10,000

Fig. iv.56 LTS of a young sieve tube. Plastids are now situated along the periphery of the sieve element. Note that the sieve element has a bigger vacuole. From stem sampled in mid-July, 1975  
x 3,250



## 2. COMPANION CELLS

Companion cells are a very characteristic component of the phloem tissue of angiosperms. They can therefore be used as a distinguishing feature for angiosperm sieve elements since gymnosperm sieve cells lack them.

There is a close relationship between companion cells and their sieve elements; for a cambial cell gives rise to a phloem mother cell which in turn divides unequally, usually to form a bigger sieve element and a smaller companion cell. Gill (1932) reported that the companion cell of Fraxinus was formed by a radial longitudinal wall between the two tangential walls of a phloem mother cell and not by an angled wall (but see below, Fig. 2.1) cutting off a corner of the mother cell as suggested by Eames and MacDaniels (1925) as general in angiosperms.

In Fraxinus, the size and number of the companion cells vary according to the time of the year. During the active growing season, companion cells are generally smaller and limited to one sieve element. The companion cells normally lie next to the ray cells (Fig. 2.1); occasionally a phloem parenchyma cell comes between them. Those formed at the beginning of the resting season, however, have two or more companion cells to each sieve element and the former are very similar in size to the sieve element itself (Fig. 2.2). Here the companion cells lie on either side of the sieve element i.e. are tangentially disposed. Besides cases where companion cells extend along the whole length of their sieve elements (Fig. 2.3) there are others where because of transverse divisions, a sieve element possesses a chain of companion cells (Fig. 2.4). The former tend to occur in the very early summer phloem, the latter in the phloem formed later in the season. In the minor veins and endings of the vascular bundles in leaves the relative widths of the sieve elements and companion cells are reversed

(Morrettes 1962).

Observations made on the companion cells of other species show that their cytoplasm is typically very dense. This is also true in Fraxinus. The cytoplasm appears more dense in the companion cells formed during the onset of the resting season. It is often the abundance of ribosomes (Fig. 2.2) which accounts for this denseness and probably the cytoplasmic ground substance at this time of the year also takes up more stain. Fig. 2.5 shows ribosomes which sometimes group together in clusters, usually in the vicinity of ER.

Vacuoles, delimited by a tonoplast, are small and numerous especially in newly formed companion cells and in companion cells formed over the resting season (Fig. 2.2). Vacuoles often enclose membranous material (see also Fig. 2.4).

The nucleus is also a prominent feature of companion cells for in many views it occupies a large proportion of the cell (Fig. 2.6). The nucleus usually has an even outline and its nucleoplasm appears to be slightly electron dense than the cytoplasm of the companion cell.

As the companion cell ages with the maturing sieve element, its nucleus undergoes degenerating changes. The size is relatively reduced (Fig. 2.7). Some of the nuclear envelope appears to be inflated (Figs. 2.5 and 2.8). As suggested by Esau (1973) who also reported of inflated nuclear envelopes in companion cells of Mimosa pudica, these may probably be artifacts. What happens to the nucleus when the companion cell degenerates has not been traced.

A well established fact about the companion cell is the absence (except in rare cases) of starch grains in their plastids. Starch grains have in fact only been found in a few genera e.g. Cucurbita

(Esau and Cronshaw 1968b). In Fraxinus the plastids appear to be typical i.e. none were observed to carry starch grains.

The plastids in the companion cells of the present species assume different shapes. Among those found are the ovoid (Figs. 2.9 and 2.11) and the stretched elliptical (Figs. 2.12 and 2.13). The stroma of these plastids is so electron dense, especially in young companion cells, that it sometimes obscures any internal structures present. Thylakoids, if they are present, are not organised into the grana-fret system. They appear in the simple form (Fig. 2.9), or beaded (Fig. 2.14) and may be filled with electron dense material (Figs. 2.12 and 2.14). Rounded membrane-bound and electron dense bodies are sometimes observed within these plastids (Fig. 2.11). Plastoglobuli are common, more so in plastids of mature companion cells (Fig. 2.15). Figs. 2.16 and 2.17 show a peculiar type of body regularly found in companion cells. It appears to be a plastid for it has a double membrane. Within it and occupying a large proportion of its volume are electron dense masses, which may be lipoprotein in nature as suggested by Srivastava (1966). These masses are reminiscent of the intralamellar inclusions of the plastids in the cambium of F. americana. Degeneration of these masses has also been observed (Fig. 2.17).

Mitochondria, abundant in companion cells, support a belief in their high metabolic activity. They are frequently ovoid and have well developed cristae (Fig. 2.16). Dumb-bell shapes are also common (Fig. 2.18). When companion cells are about to degenerate in step with their associated sieve tubes, mitochondria normally become swollen (Fig. 2.15) and their cristae fragment (Fig. 2.19). The latter may eventually disappear leaving the mitochondrion empty.



Plasmatic connections between sieve elements and companion cells were recognised as a special feature as early as 1908 by Hill. Their ultrastructure, however, was not revealed until 1963 by Eschrich. Observations made by many workers on other species have been found to be parallel to the present study. Where a plasmodesma connects the two cells it is always highly branched on the companion cell side. The wall of the companion cell is usually thickened at this point giving the final appearance of a delta (Fig. 2.20). On the sieve element side, only a single branch is present and this meets with the branches from the other side in the region of the middle lamella, often forming median cavities (Fig. 2.21). Each plasmodesmatal branch is lined by plasmalemma and sometimes may be penetrated by what appears to be a membranous tubular structure (Fig. 2.22). Observations also showed connections of ER cisternae to these plasmodesmatal branches from both the sieve element and companion cell sides (Fig. 2.20). Callose is only encountered on the sieve element side of the plasmatic connections (Figs. 2.22 and 2.23).

Another feature commonly observed in companion cells of Fraxinus is a profusion of smooth ER (Figs. 2.24 and 2.25). This is probably similar in nature to the ER stacks found in the periphery of mature sieve tubes. As in the mature sieve tubes, electron dense material appears between the narrow alternating spaces of cisternae.

'Paramural bodies' which appear to arise from plasmalemmal invaginations of the companion cells are also common (Fig. 2.26). These small internal vesicles seemed to be very intimately associated with the wall itself (see Chapter III, section (iv) (a)). Whether these bodies detach themselves from the wall has not been ascertained yet. However, Fig. 2.27 shows multivesicular bodies without any apparent connection to the plasmalemma. This may be, however, due merely to

the plane of section.

When a sieve element nears the end of its life, or actually ceases to function (as judged by its state or by its distance from the cambium) the companion cell too undergoes further changes. There is a general thinning of the cytoplasmic layer (Figs. 2.28 and 2.29) which is now confined to the periphery, though the cytoplasm itself may appear as dense as ever. Mitochondria, as mentioned earlier, swell and lose their internal cristae, and the ER cisternae likewise swell (Figs. 2.30 and 2.31). The fate of the plastids is still doubtful. Fig. 2.15 suggests that the plastids become swollen as well when the mitochondria swell. Plastoglobuli seemed to be more abundant in these plastids. However, Fig. 2.28 probably shows another stage of plastid disintegration. Here the type of plastid is similar to that of Fig. 2.10 in which electron dense material seemed to occupy the central portion of the plastid. Both the inner content and the dense matrix are in the process of loosening up and in Fig. 2.30, they appear to be blending with the cytoplasm as the latter disintegrates. This interpretation is borne out by the fact that the neighbouring parenchyma cell has still its tonoplast intact and appears well preserved. Tubule-like structures and vesicles seem to occupy the space opened out between the plasmalemma and the cell wall.

When the cytoplasm of the sieve tube is reduced to a congealed residue which will finally be crushed or obliterated as the cambium produces new phloem tissue (see Fig. 2.32), the companion cell contents appear to undergo the same fate. Thus, a correlation seems to exist to the end between the state of the sieve elements and that of their companion cells which underlines the interdependence of these two cell types. Whether such a correlation is present at the earlier stages of development is more difficult to determine.

Fig. 2.1

TS of the phloem region of 2-year old stem sampled on 20th May, 1976. The size of the companion cell is very small compared with that of the large 'summer sieve element'. The companion cell normally lies next to the ray-cell. Note that the 'autumn phloem' (within arrows) is still intact. In it, both of the two companion cells and their sieve elements are much more nearly equal in size. x 1,300

Fig. 2.2

TS of phloem region of 2-year old stem sampled in late March, 1976 ('autumn phloem'). Note the relative sizes of the cells. Vacuoles in the companion cells are numerous. x 9,750

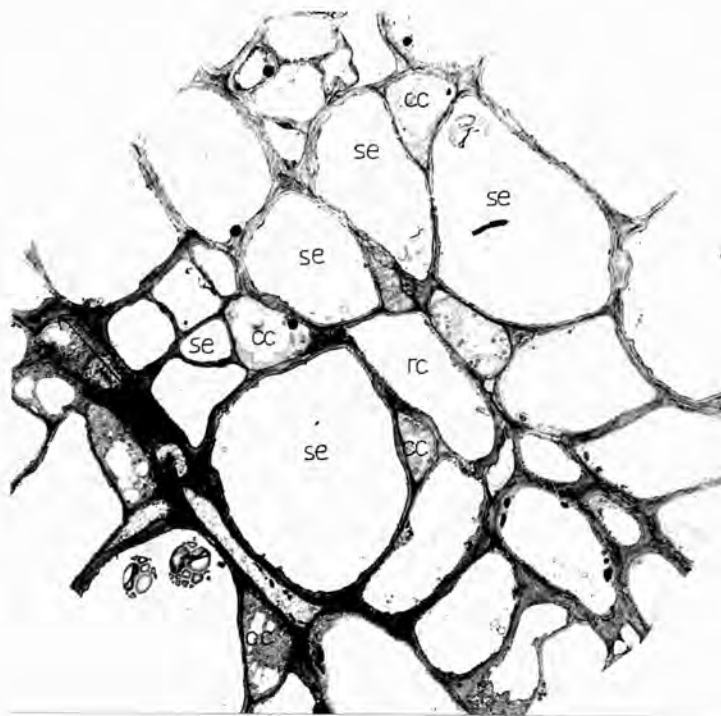


Fig. 2.3      TLS of phloem of 14 year old stem  
sampled in late May, 1975. The  
companion cell extends along the  
whole length of the sieve element.  
Note the plastids, vacuoles and  
nucleus in the companion cell.  
x 1,300



Fig. 2.4

TLS from 14 year old stem sampled in mid-May, 1976. The width of the companion cell is as large as that of the sieve tube which suggests that this is part of the 'autumn phloem'. The companion cells form a chain. No signs of disintegration of the companion cell appear yet at this stage. The dark bodies in the phloem parenchyma are probably tannin. Note the inclusions within the vacuoles of the companion cell. x 1,200





Fig. 2.6 TS of a companion cell from stem sampled in late March, 1975. The nucleus occupies a large proportion of the cell and its nucleolus appears to be double. Plastids are very electron dense. x 9,750

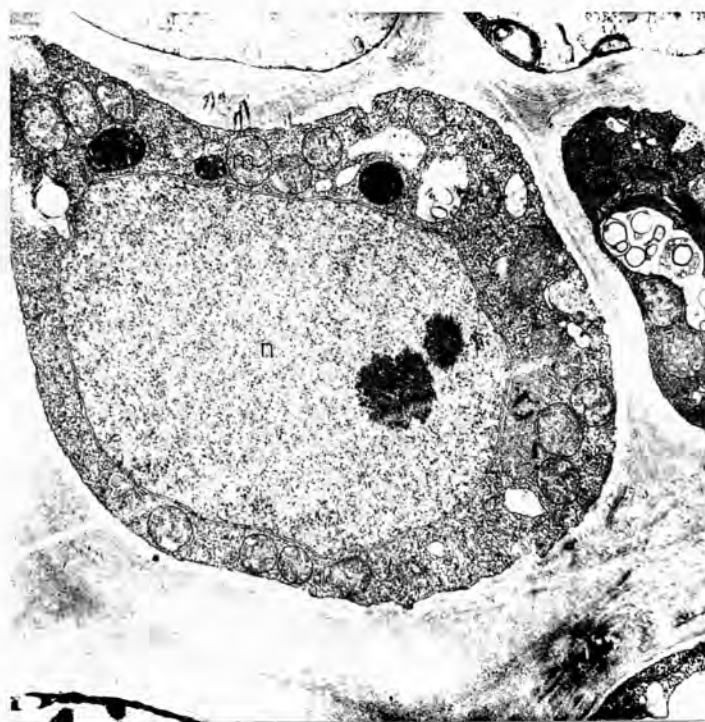
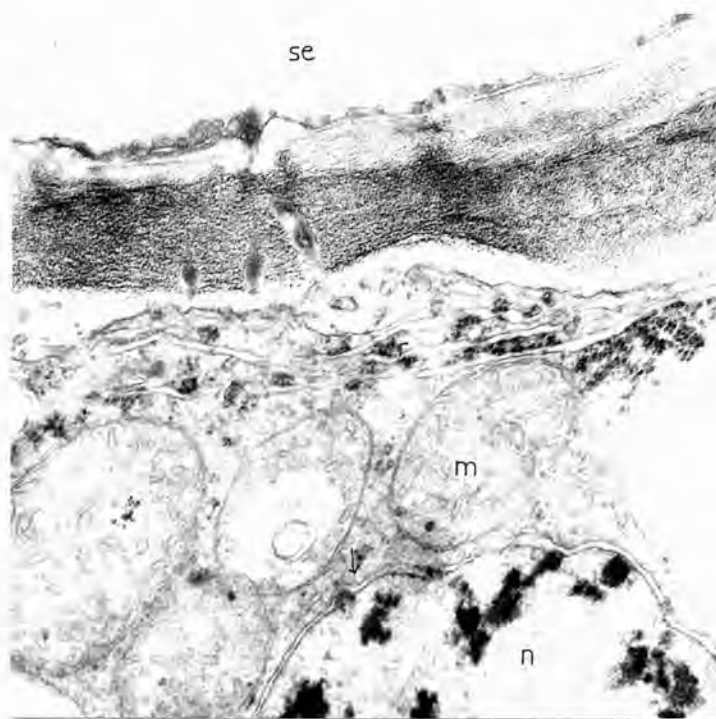


Fig.2.7

TS of a companion cell of summer phloem from stem sampled in late June, 1976. The nucleus apparently is very much reduced in size. Nucleoplasm is as dense as the cytoplasm. Arrow indicates nuclear pore.  
x 39,000

Fig. 2.8

TLS of a companion cell. The nuclear envelope appears inflated (arrow). Nucleolus is showing sign of disorganisation. Nucleoplasm almost identical in density with the cytoplasm. From stem sampled in early October, 1976 x 19,500

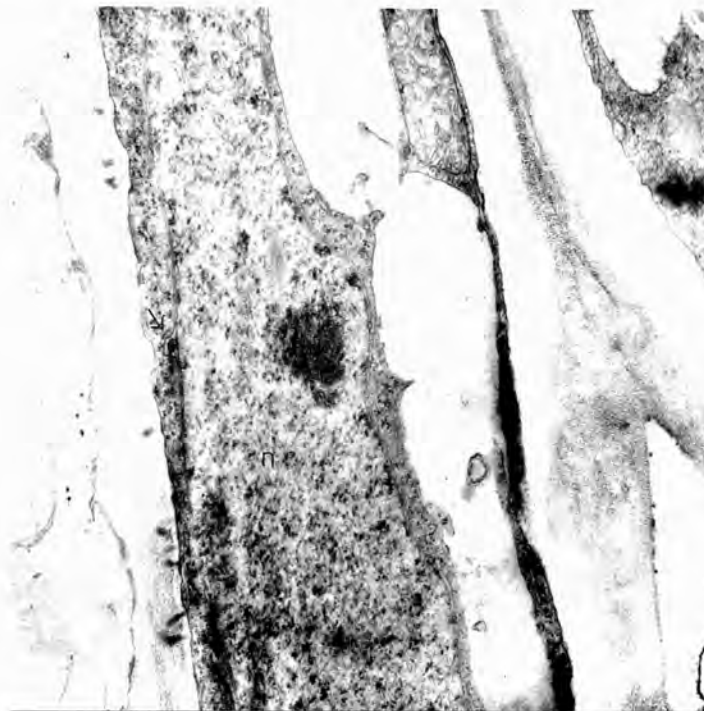
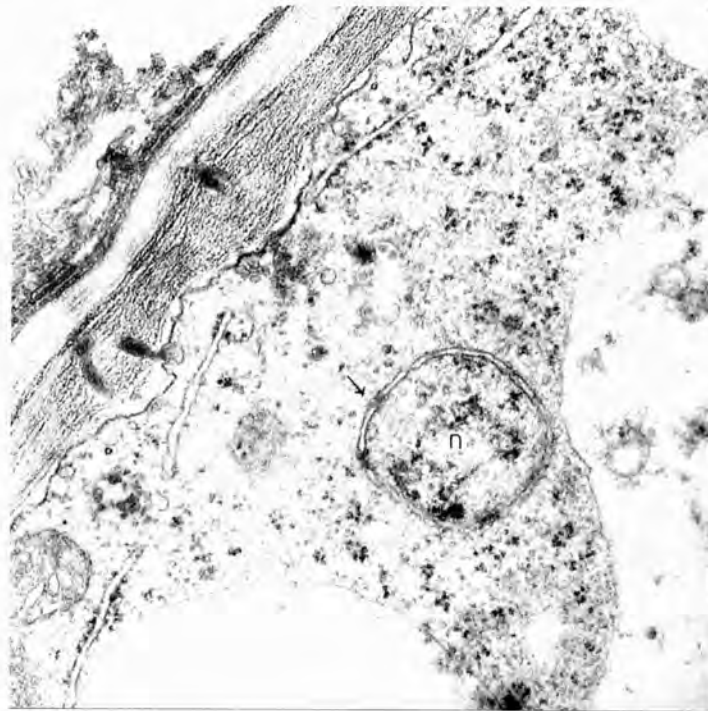


Fig. 2.9                      TLS of a companion cell showing one of the few plastids seen with simple thylakoids. Note the abundance of plastoglobuli (pg). From stem sampled in early October, 1975  
x 40,500

Fig. 2.10                     TS of a companion cell showing possibly the same type of plastid as that in Fig. 2.9 From stem sampled in late April, 1975.  
x 32,500



Fig. 2.11

TLS of a companion cell showing plastids. Note the continuity of the inner of the two membranes with the internal membrane system (arrow). Note also the presence of rounded membrane-bound electron dense bodies and thylakoids filled with dark material. From stem sampled in late May, 1975  
x 43,750

Fig. 2.12

TLS of a companion cell. Plastid is stretched-elliptical with sparse internal membrane system. Note the electron dense filled structures, possibly thylakoids. From stem sampled in late May, 1975 x 43,750

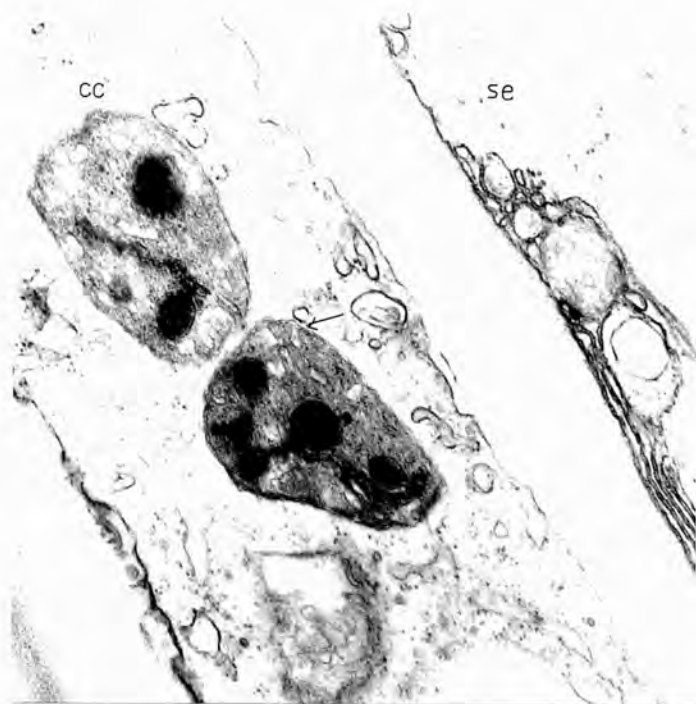




Fig. 2.13      TLS of a companion cell. Plastids  
have electron dense stroma and possibly  
thylakoid. From stem sampled in late  
May, 1975      x 19,500

Fig. 2.14      An array of plastids in companion cell in  
TS. One plastid (A) has a very electron  
dense stroma; another (B) appears to be  
cup-shaped; another (C) has plastoglobuli  
and electron dense thylakoids and in  
(D) thylakoids appear beaded. From stem  
sampled in early May, 1975      x 26,250

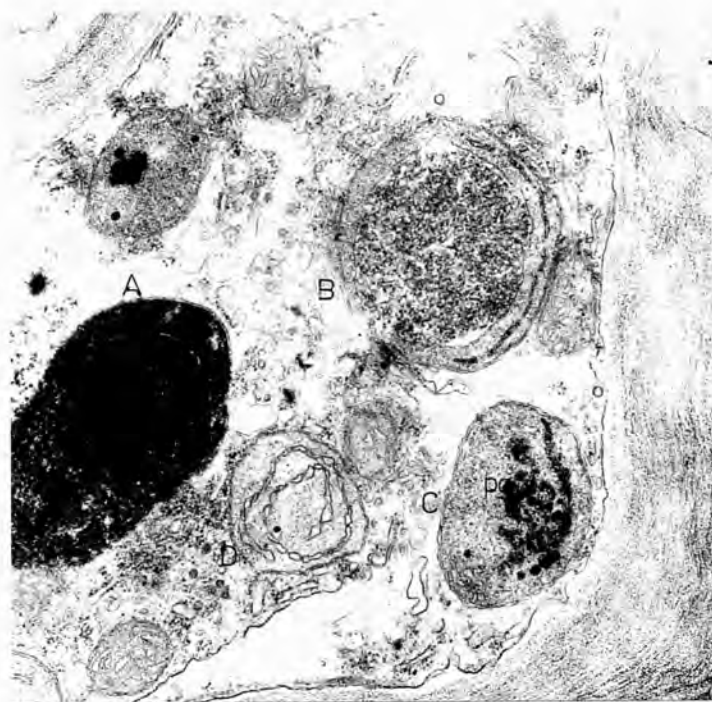


Fig. 2.15      Plastids with abundant plastoglobuli  
from a relatively mature companion cell  
as judged by the swollen mitochondria.  
From stem sampled in early October, 1976  
x 26.250

Fig. 2.16      'Peculiar body' found within a companion  
cell, possibly a plastid (note its double  
membrane). Contents are very electron  
dense. From TLS of stem sampled in late  
July 1976      x 26.250

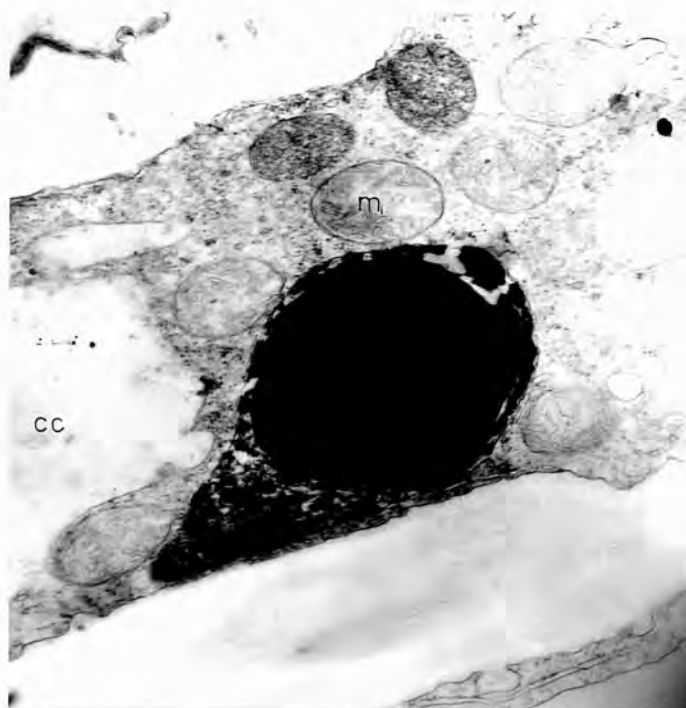
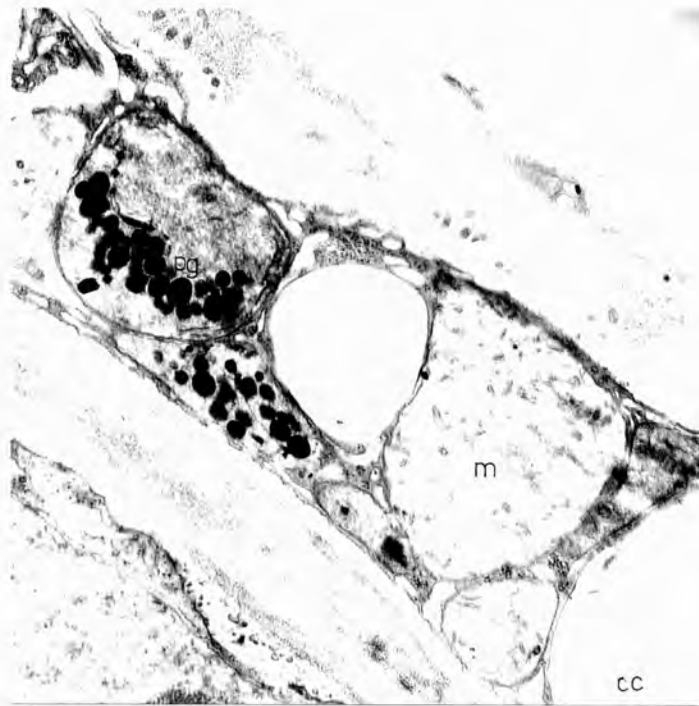


Fig. 2.17            The 'peculiar body' again but its membrane  
is ruptured and the contents have  
degenerated (cf 2.16). From TS of  
companion cell of stem sampled in mid-  
October, 1976    x 26,250

Fig. 2.18            Dumb-bell shaped mitochondrion in a  
companion cell. Note the multivesicular  
body near it. From TLS of stem sampled  
in late May, 1975    x 52,000

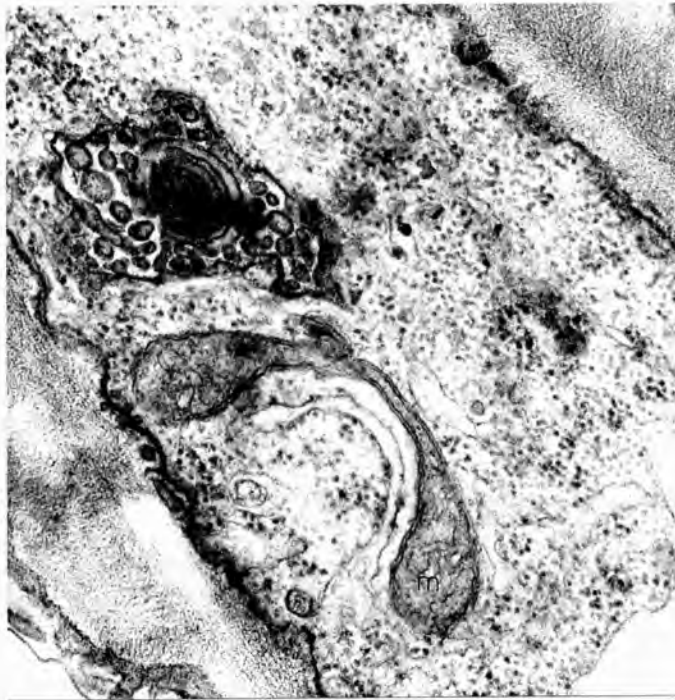
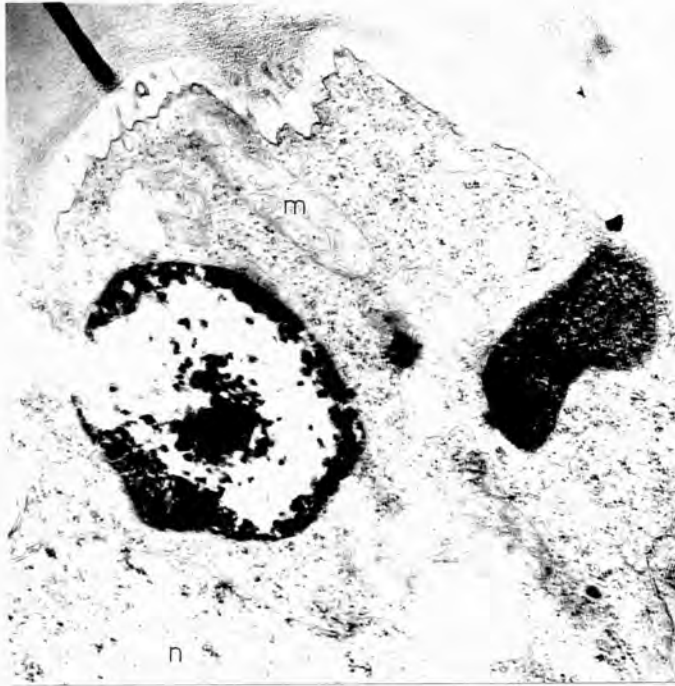


Fig. 2.19                      TLS of mitochondria in a companion cell.  
Cristae of mitochondrion on the lower  
side are partially disorganised and matrix  
has almost disappeared. ER cisternae also  
appear swollen. From stem sampled in early  
October, 1976      x 39,000

Fig. 2.20                      Plasmatic connection between a companion  
cell and a sieve element; note the feature  
which resembles a delta. Plasmalemma lines  
these channels throughout. ER conspicuous  
on the sieve element side especially.  
From stem sampled in late April, 1975  
x 52,000

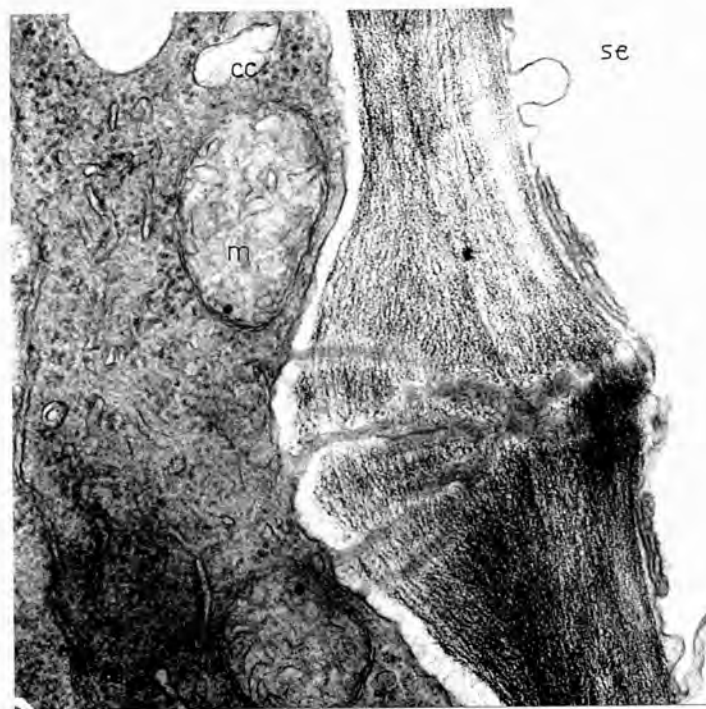
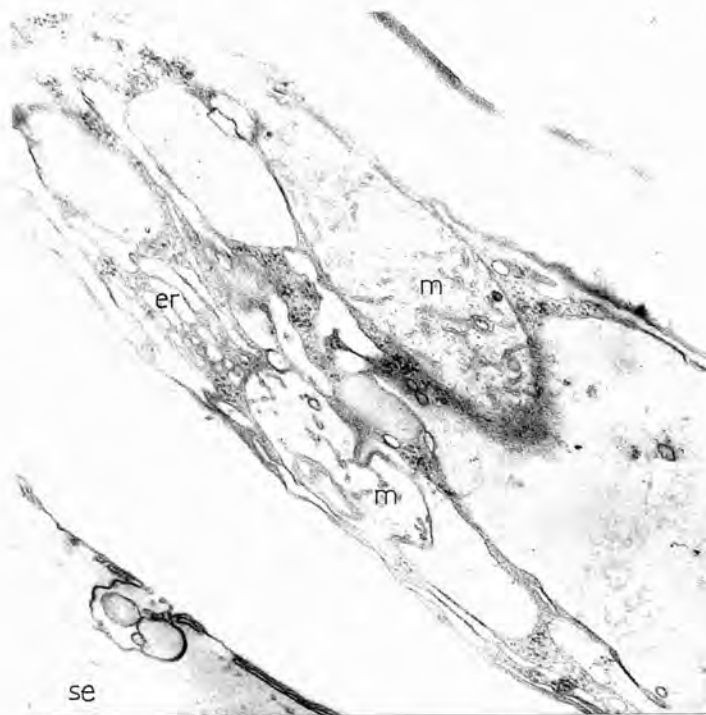




Fig. 2.21      TS of plasmodesmatal connection between mature sieve element and companion cell. Note median cavity at the level of the middle lamella. Plasmalemma lines the branches. Prominent multivesicular body present within the sieve element. From stem sampled in late April, 1976  
x 39,000

Fig. 2.22      Plasmatic connection between mature sieve element and companion cell. Note conspicuous callose (ca) on the sieve element side. From TS of stem sampled in mid-May, 1976    x 52,000

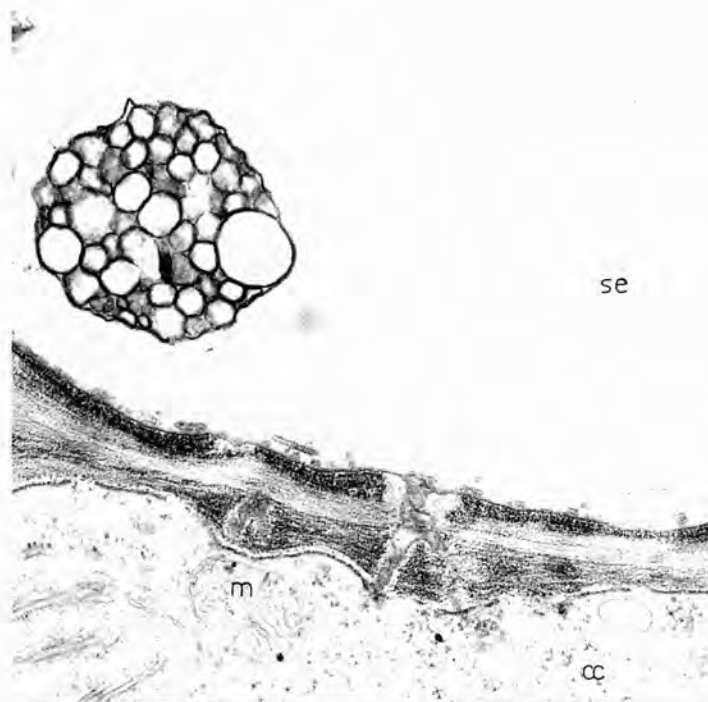


Fig. 2.23      TLS of plasmatic connections between  
companion cell and mature sieve element.  
Callose conspicuous on the sieve element  
side. From stem sampled in mid-May,  
1976      x 26,000

Fig. 2.24      TLS of a companion cell showing profusion  
of coiled ER. Notice the similarity to  
the ER stacks in mature sieve tubes,  
especially in the narrow, electron dense  
intercisternae. From stem sampled in  
early October, 1976      x 26,250

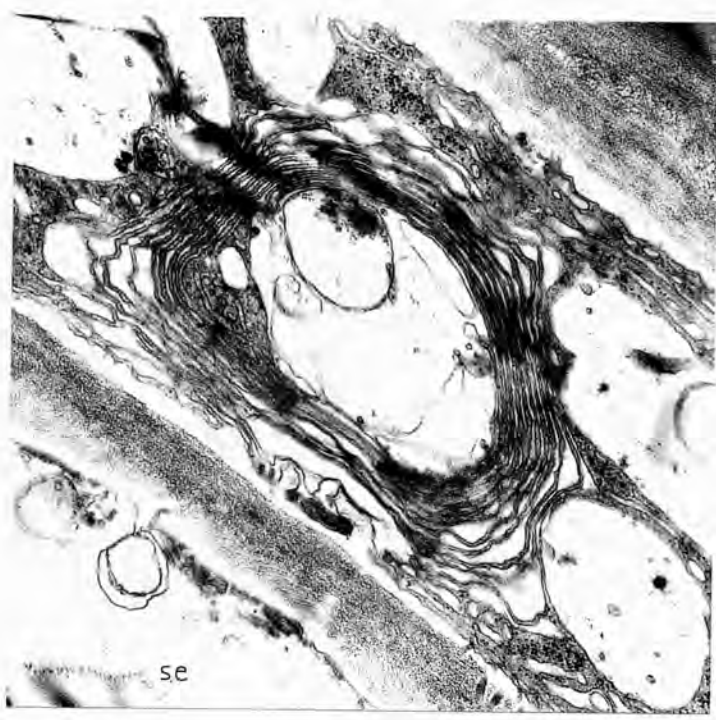
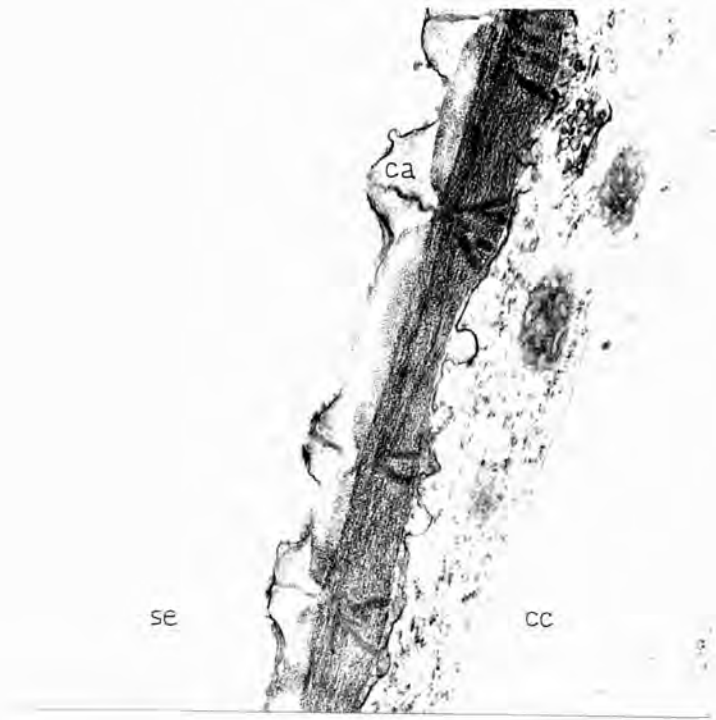


Fig. 2.25            TS of a companion cell with ER filling a corner of the cell. Note the dark groups of ribosomes and the electron dense intercisternal spaces. From stem sampled in mid-October, 1976    x 13,000

Fig. 2.26            TS of a companion cell showing plasmalemmal invaginations. Vesicles are numerous within these invaginations. Note the smaller vesicles and tubules almost within the cell wall itself. From stem sampled in mid-March, 1975    x 19,500

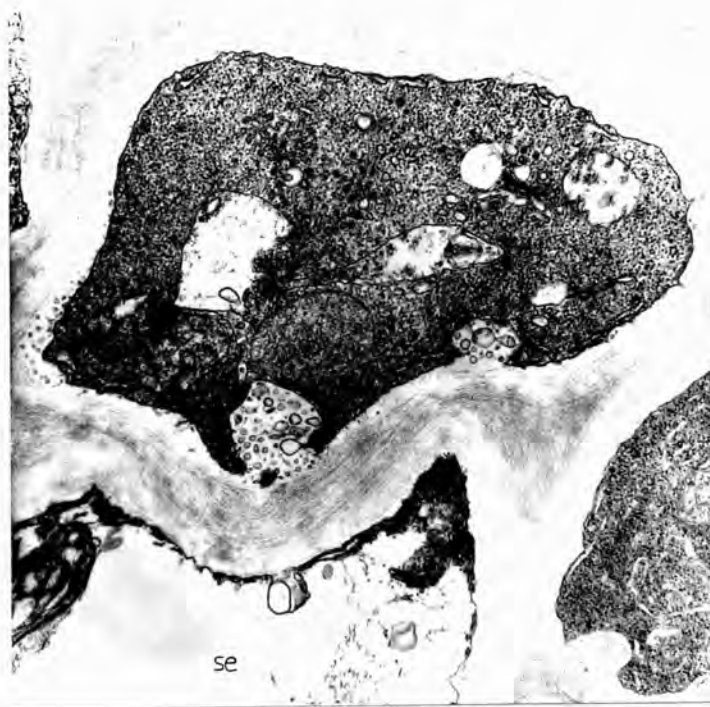
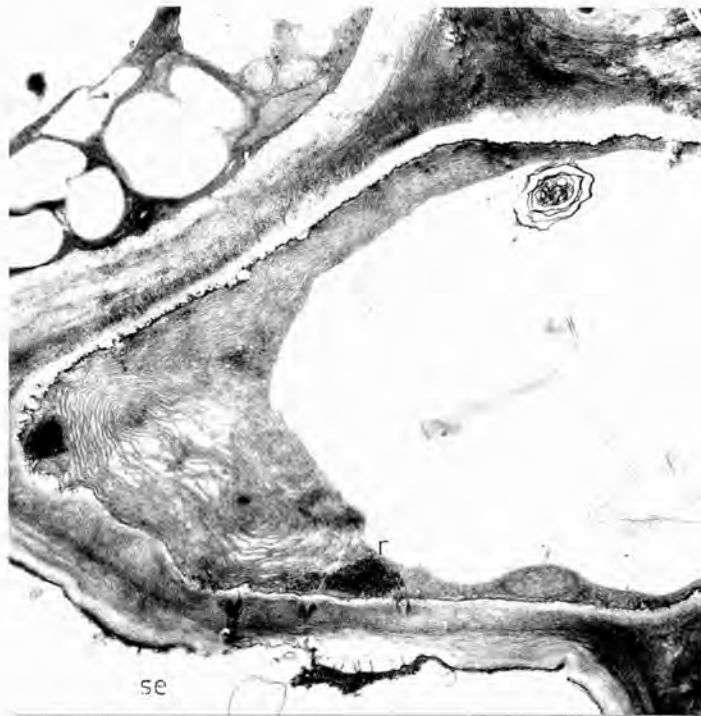


Fig. 2.27                      TLS of a companion cell showing multi-vesicular bodies. Membranes bounding them are single. From stem sampled in late May, 1975    x 26,000

Fig. 2.28                      TS of companion cell from stem sampled in late October, 1976. Cytoplasm thinly lines the periphery and there is only one large vacuole. Mitochondria appear swollen and plastid is disintegrating (p) x 19,500

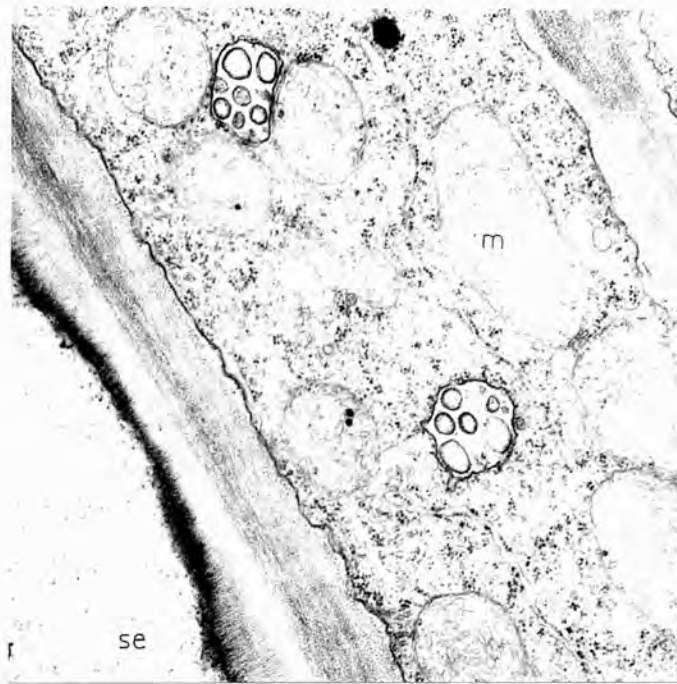




Fig. 2.29

TS of a companion cell. The state of the companion cell is comparatively similar to that of its sieve element. Cytoplasm thinly lines the periphery of the companion cell. From stem sampled in mid-May, 1976  
x 19,500

Fig. 2.30

TS of companion cell probably at the beginning of disintegration. The ER cisternae appear swollen and the plastid has disintegrated. Note that the neighbouring parenchyma cell (a) is well preserved. From stem sampled in late May, 1976 x 19,500

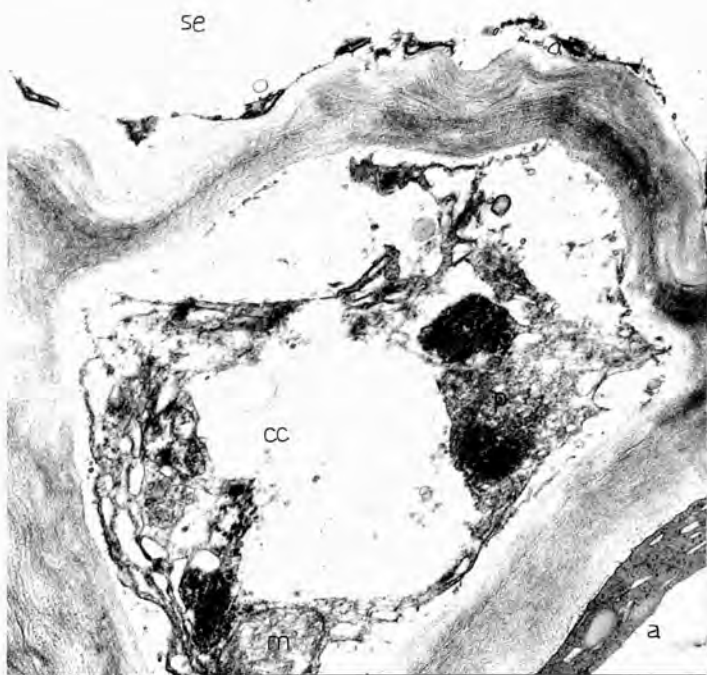
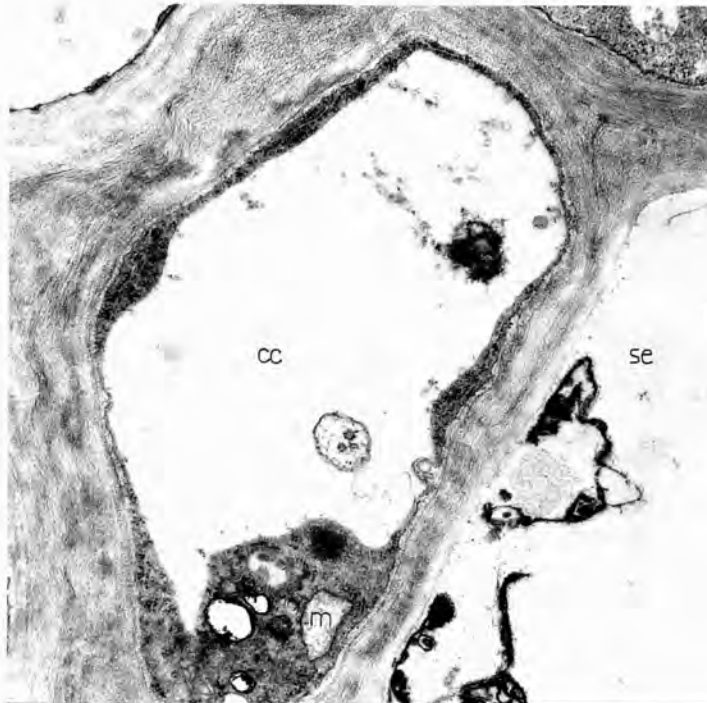
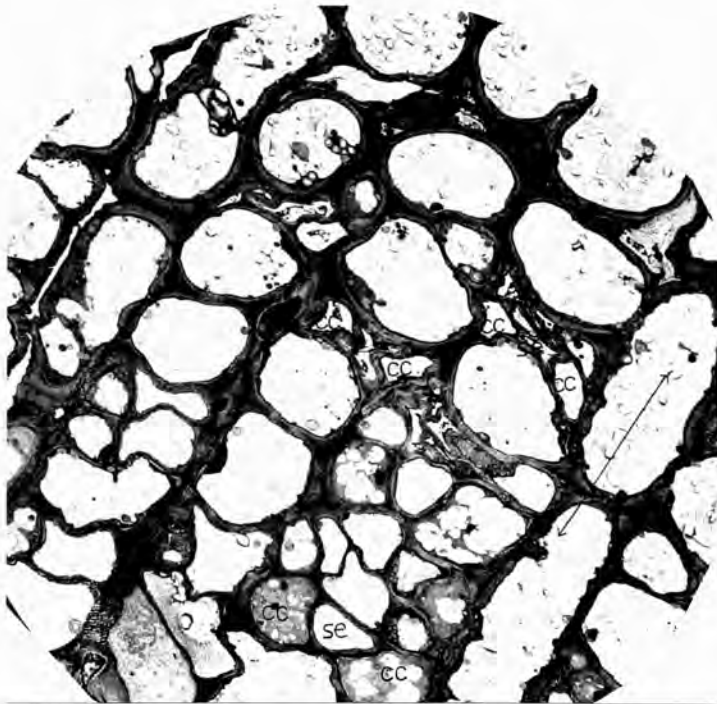
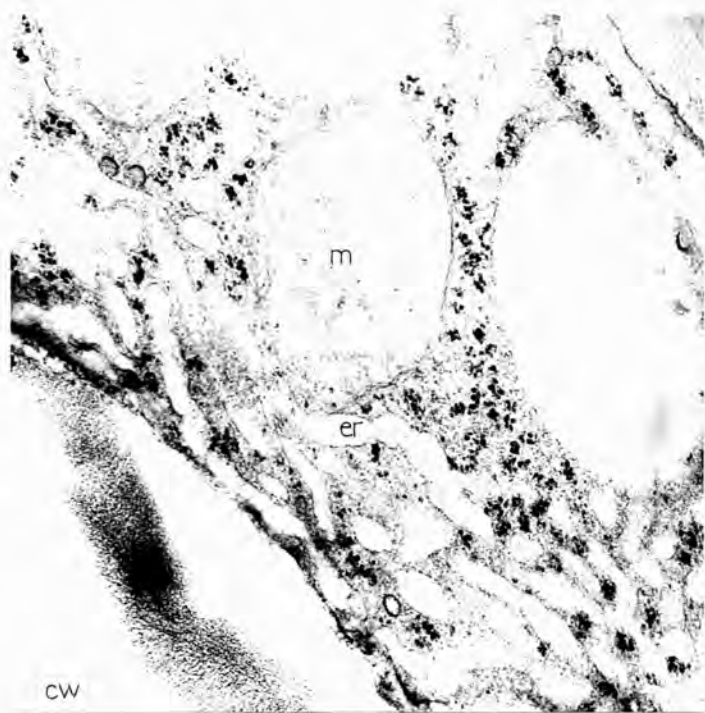


Fig. 2.31                      TLS of an ageing companion cell. ER  
cisternae become swollen and mitochondrial  
cristae fragment. From stem sampled in  
late October, 1976      x 43,750

Fig. 2.32                      TS of stem sampled in late June, 1976.  
Note the interdependence of the sieve  
elements and the companion cells,  
especially within arrowed region.  
x 1,300



### 3. PARENCHYMA CELLS

The parenchyma of the secondary phloem is organised in two systems - the axial and the ray (see Fig. 1.1). The axial parenchyma arises from fusiform initials (Fig. 31.) which are also the precursors of the sieve elements proper. The ray parenchyma originates from ray initials.

In the secondary phloem of Fraxinus, the axial parenchyma appears in tangential bands alternating with similar bands of sieve elements (see Fig. 1.3). In a longitudinal section (Fig. 3.2) the axial parenchyma cells form longitudinal strands, the individual cells are usually shorter than the sieve elements. The rays are either uniseriate or biseriate and are many cells in vertical extent (Fig. 3.2).

Modifications take place in both systems when the sieve elements collapse in the obsolete region of the phloem. The axial parenchyma cells become dilated (see Fig. 2.32) while the ray cells increase their size and number in the tangential direction (Fig. 3.3). By these means the phloem is adjusted to the increase in circumference of the axis resulting from secondary growth.

Ultrastructurally, parenchyma cells are of less interest when the structure of phloem is considered. Nevertheless, parenchyma cells contain all the cellular components characteristic of metabolically active plant cells.

Parenchyma cells in Fraxinus phloem are highly vacuolated, the vacuoles being either visually empty (Fig. 3.4) or containing tannin-like inclusions (Fig. 3.5), or crystals (Fig. 3.6). Tanniniferous cells do not occur in specific zones in Fraxinus. In Pinus strobus (Murmanis and Evert 1967) however, these are mostly stored in the mature axial parenchyma and younger ray cells.

Crystal formation occurs in both the parenchyma systems in Fraxinus (Fig. 3.6). Accumulations are more frequently found in cells contiguous with those undergoing sclerification and with sieve elements in the process of obliteration (see Fig. 2.32). In Pyrus malus (Evert 1963) the crystal containing cells occur in tangential bands and are commonly associated with fibre sclereids.

Holdheide (1951) who examined 77 species for crystal, categorised the masses in crystals in Fraxinus as "crystal sand". The shapes of the individual crystals vary from elongated to isodiametric (Fig. 3.6). When observed under the electron microscope, most of the crystals are visualised as the spaces vacated by them; presumably they have been removed during microtomy or preparation of the tissue. 80 % of these crystals were found to be calcium salts (Holdheide 1951).

The protoplast of a crystal-containing cell in Fraxinus however, is not lost as in Eucalyptus (Chattaway 1953) or eventually disorganised as in Pyrus communis and P. malus (Evert 1960 and 1963); it remains as a dark and very thin peripheral layer (see Fig. 3.6).

The nucleus in these parenchyma cells varies from spherical to elliptical (Fig. 3.7) with variously dispersed chromatin. It has also been observed that some ray cells contain crystals (presumably protein) often appearing rhomboidal or hexagonal included within the nucleus. The crystal often lies close to the enlarged nucleolus (Fig. 3.7).

Parenchyma cells also exhibit plastids with a variety of shapes ranging from ovoid (Fig. 3.8), elongated (Fig. 3.9) to dumb-bell (Fig. 3.10.). These plastids, as usual, are quite distinct from those of the sieve elements. They have more elaborate internal structures as well as a different type of starch grain.

Among the internal structures are electron dense bodies in the form which can be described as conglomerate (Fig. 3.10) or granular (Fig. 3.11). Membranous systems also occur single layered (Fig. 3.11) and stacked (Figs. 3.8 and 3.15). The stacks appear to be similar to the grana of chloroplasts. Chloroplasts in fact have been observed in ray cells (Fig. 3.12) with the usual grana and fret system. Ferritin is quite common in parenchyma cells (Figs. 3.8 and 3.9) as are plastoglobuli (Fig. 3.9). The structures described above are mostly found in plastids devoid of starch grains.

The presence of starch grains in plastids of parenchyma cells is interesting in that it shows seasonal fluctuation. In Fraxinus there is no very clear demarcation in the times when they appear and disappear, but they are particularly abundant during the inactive season. The reverse, however, happens in Pinus strobus (Murmanis and Evert 1967).

The size and number of starch grains per plastid varies too. They are normally large and numerous, almost filling the entire plastid during the inactive season. Fig. 3.13 was from tissue sampled in December. Starch is nearly absent in the growing season. Frequently electron dense structures appear to sheath these starch grains.

Lipid droplets (Figs. 3.4 and 3.9) too are common. Probably like the starch grains, they form the winter food reserve. Variation in their numbers with time of year was however not observed.

An organelle bounded by a single unit membrane and containing a fairly homogenous electron dense matrix; often with a crystalline inclusion has frequently been observed, in parenchyma cells. They would seem to be a type of microbody (Fig. 3.14).

Plasmodesmata are either distributed regularly or in primary pit-fields. Plasmodesmata in pit-fields are often branched (Fig. 3.15) in contrast to those distributed regularly. These branches meet at the median nodule (arrow) as in Fig. 3.15.

A scattered distribution of plasmodesmata is usually found in transverse and tangential walls of axial parenchyma; in radial walls the plasmodesmata are more frequent and are clustered in pit-fields (Fig. 3.16). Plasmatic connections between parenchyma cells and sieve elements however, have not been observed. The only connection between these cells may possibly be via the companion cells. Fig. 3.17 shows plasmodesmatal connection between a ray cell and a companion cell. There appears to be more branches on the companion cell side.



Fig. 3.1

TLS of vascular cambium sampled in late February, 1975. The fusiform initials (fi) give rise to the axial system of the phloem and xylem which also includes the axial parenchyma. Ray initials (ri) which are either uniseriate or biseriate give rise to the ray cells. Section (1µm thick) stained with toluidine blue. x 592

Fig. 3.2

TLS of the same specimen of bark (about 10µm away) as in Fig. 3.1. The ray cells (rc) may sometimes appear biseriate. The axial parenchyma cells (a) are rather shorter than the sieve elements, which are distinguished by their sieve plates, plastids and by the appearance of surge effects. x 384

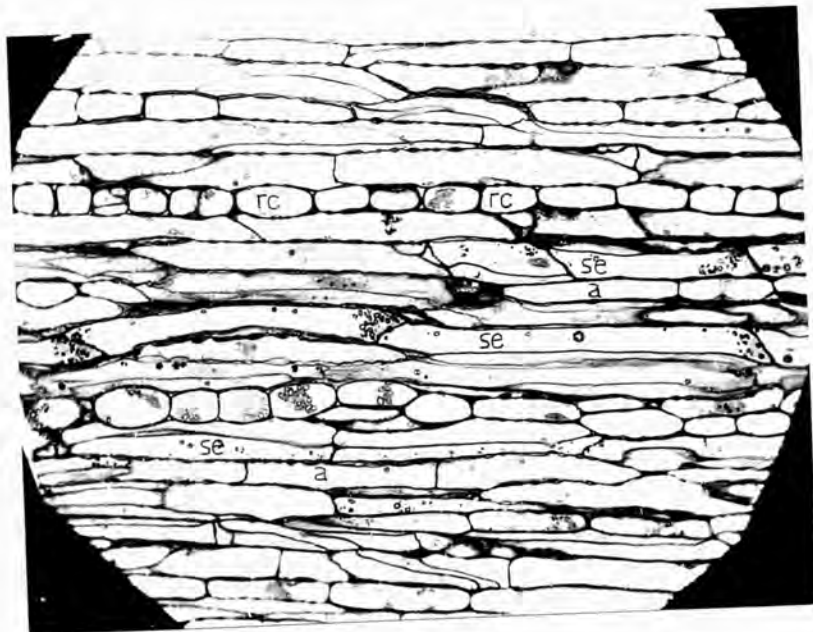
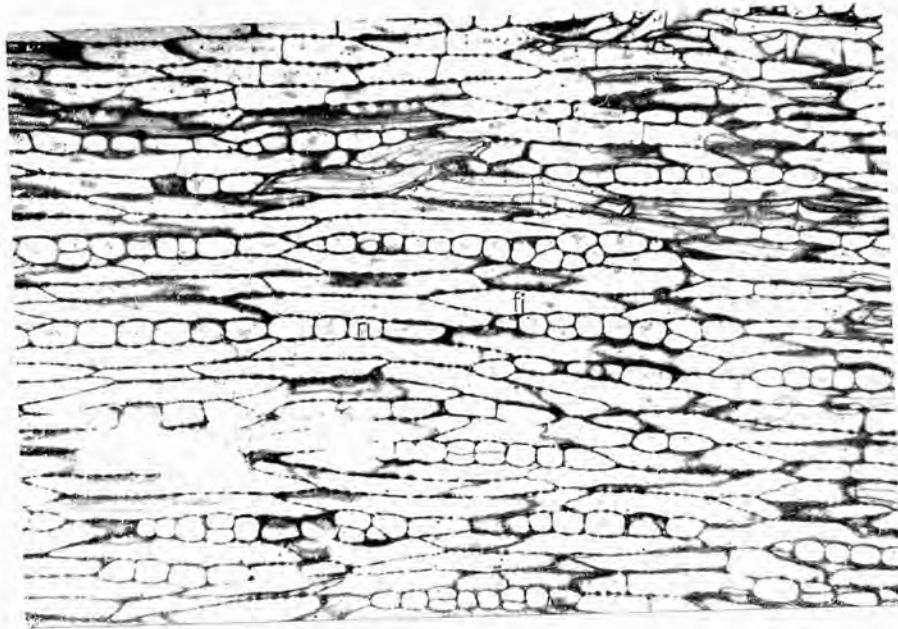


Fig. 3.3            TS of bark sampled in late February, 1975.  
Here, ray cells (rc) have increased in  
numbers and size in the tangential direction.  
The sieve elements do not yet seem to have been  
obliterated completely. Section (1mm thick)  
stained with toluidine blue.    x 266

Fig. 3.4            A typical axial parenchyma cell of  
secondary phloem from TS of bark sampled  
in late April, 1975. The cell is highly  
vacuolated. The protoplast contains the  
normal organelles: nucleus, plastids, lipid  
droplets (ld) and mitochondria. Note the  
obliterated sieve tube and companion cell  
to the right above.    x 6,500

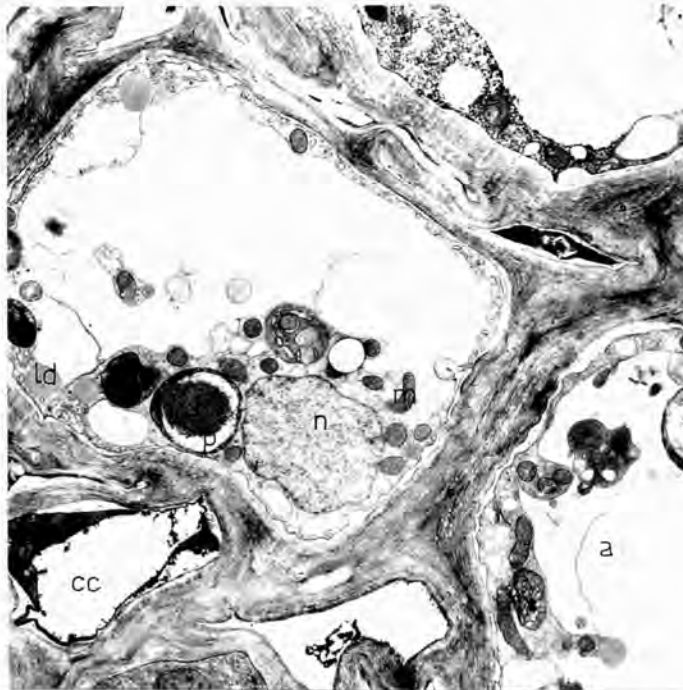
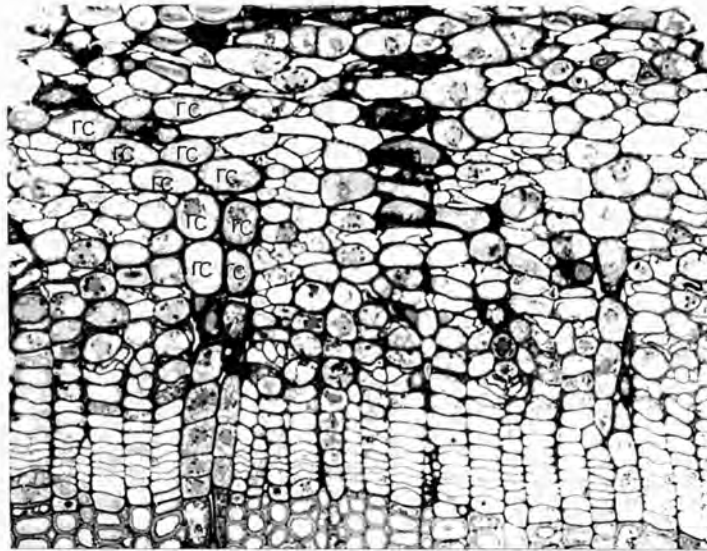


Fig. 3.5                      TS of bark sampled in late May, 1975.  
Note the presence of tannin-like  
inclusions in some of the axial  
parenchyma cells.    x 1,300

Fig. 3.6                      TS of bark sampled in mid-December, 1976.  
Both the parenchyma systems contain masses  
of crystal cavities. The crystals are  
reported to be mainly calcium salts, probably  
oxalate (Holdheide 1951). Note that protoplast  
remains as a very thin peripheral layer  
with visible organelles.    x 3,250

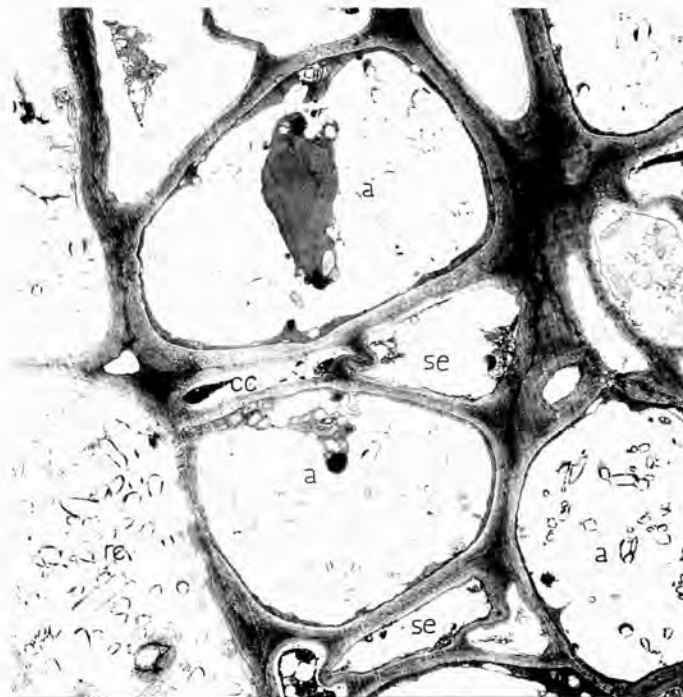
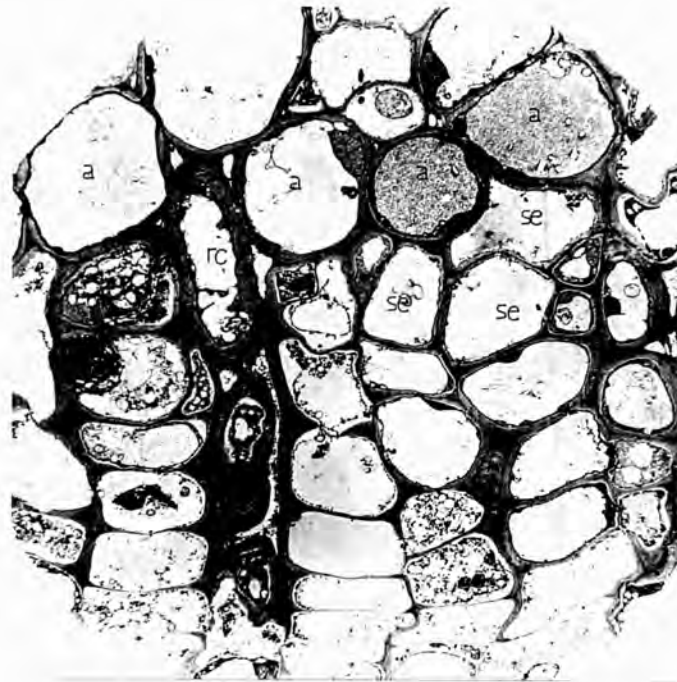


Fig. 3.7            Nucleus in ray cell (rc). Note the enlarged nucleolus and the crystal within the nucleus. Chromatin is evenly dispersed. From TLS of stem sampled in late May, 1975.  
x 13,000

Fig. 3.8            Ray parenchyma cell. Plastid is ovoid with electron dense stacked internal structure which probably represents grana. Note also the ferritin (f) within the plastid. Stroma is electron dense. From TLS of section of stem sampled in mid-July, 1975    x 52,000

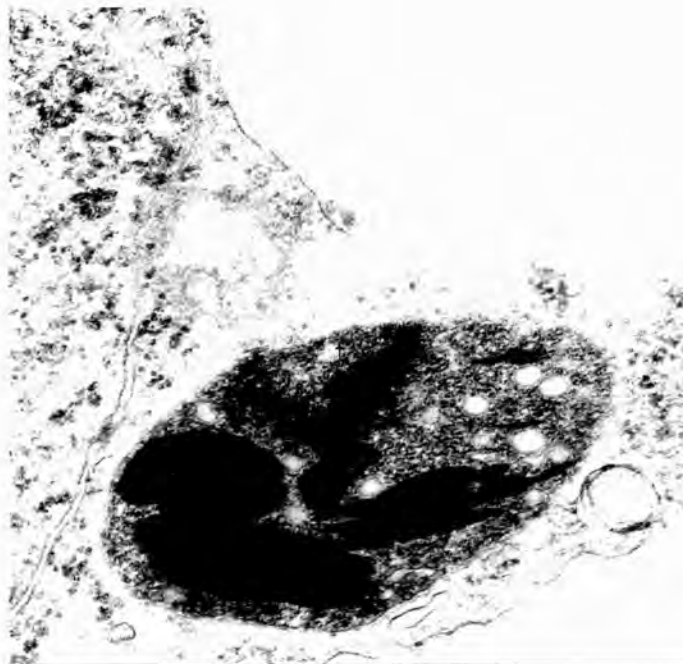
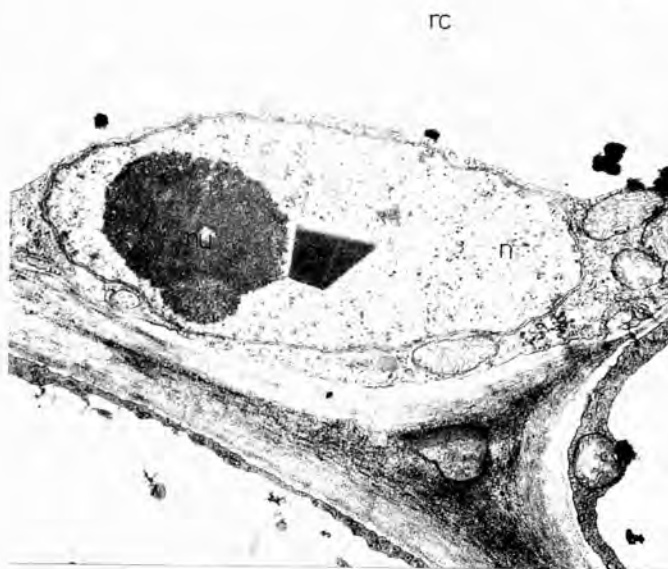




Fig. 3.9

Elongated plastid from axial parenchyma cell. Note the internal membrane system, ferritin (f) and plastoglobuli (pg). Lipid body (ld) is also present close to the plastid. From TLS of stem sampled in late April, 1975 x 39,000

Fig. 3.10

Dumb-bell plastid in ray cell (rc). Stroma is not so electron dense as those in the plastids of young sieve elements and companion cells. Internal membrane system forming part of an electron dense conglomerate. Note the plasmodesmata connecting the ray cell to the axial parenchyma cell (a). From TLS of stem sampled in late May, 1976 x 19,500

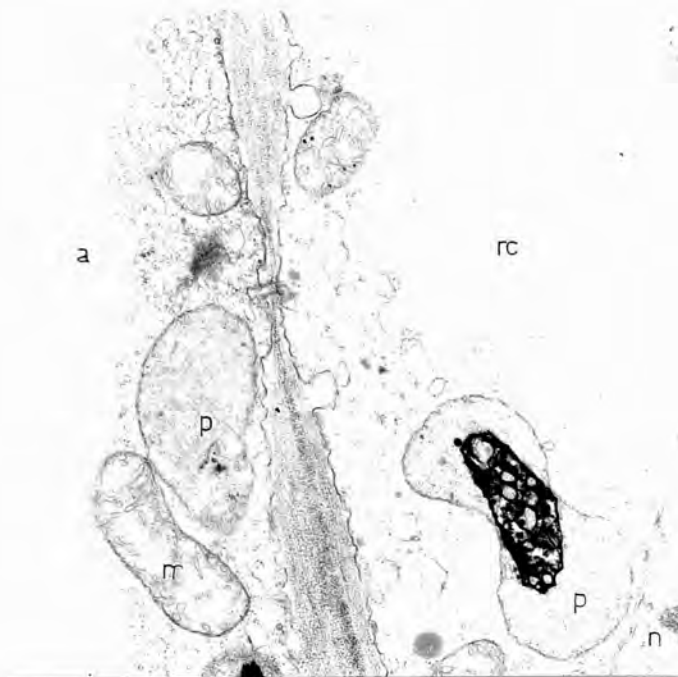
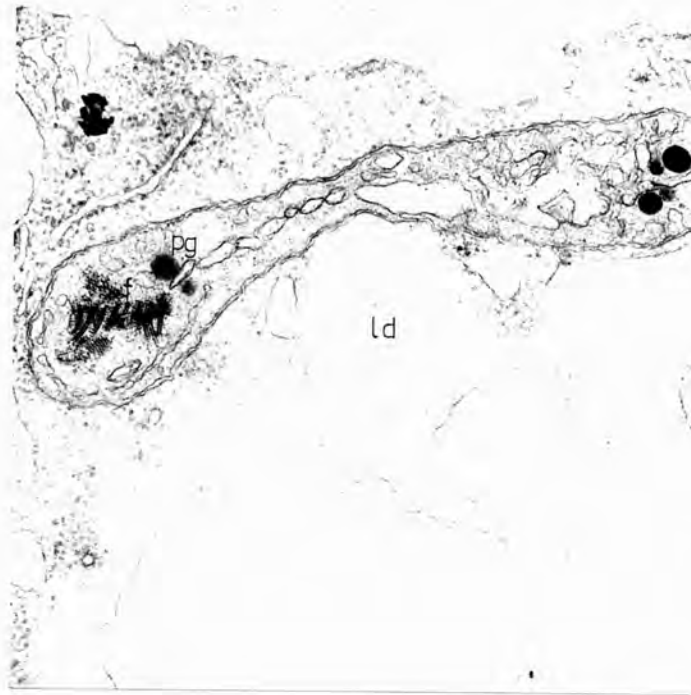


Fig. 3.11                      Plastid in axial parenchyma cell. Stroma is less dense than usual. Note the contents of the plastid: a granular mass, a small rounded body and an elongated membrane formation. From TS of stem sampled in mid-May, 1976      x 32,500

Fig. 3.12                      Chloroplasts in ray cell. Note the usual grana and fret system, some of the grana being seen in surface view. Starch grains are present in some of the chloroplasts. From TLS of stem sampled in late May, 1975      x 19,000

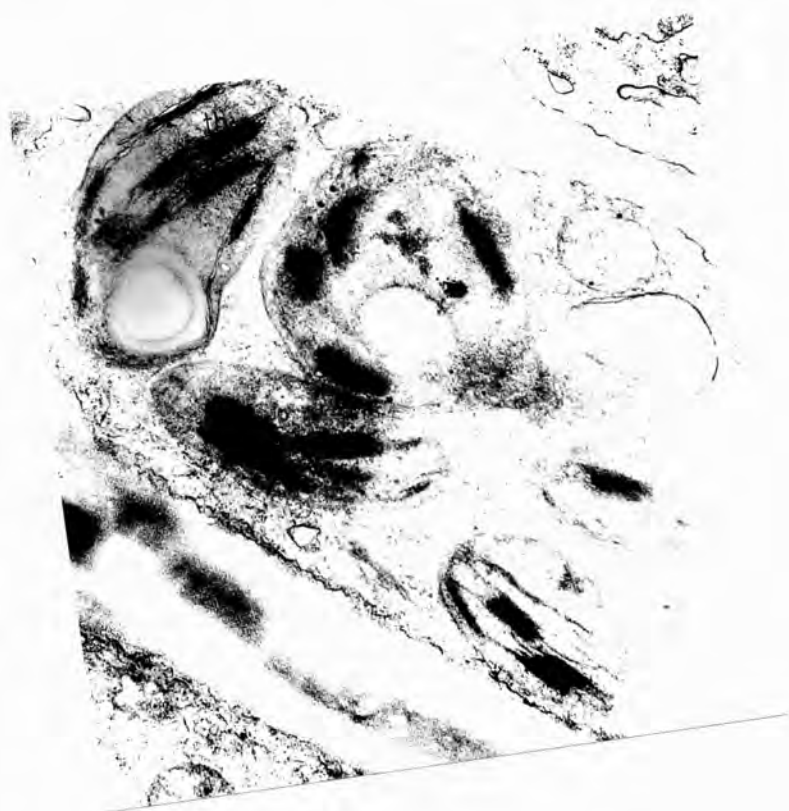


Fig. 3.13                      Plastid from axial parenchyma cell. Starch grains almost completely fill the plastid. A membrane appears to sheath some of these starch grains. From TS of stem sampled in mid-December, 1976    x 19,500

Fig. 3.14                      Microbodies with a dense granular matrix bounded by a single membrane, one containing a crystal. Note the presence of a lipid body (ld) in the vicinity. From TS of an axial parenchyma cell of stem sampled in late January, 1977    x 28,500

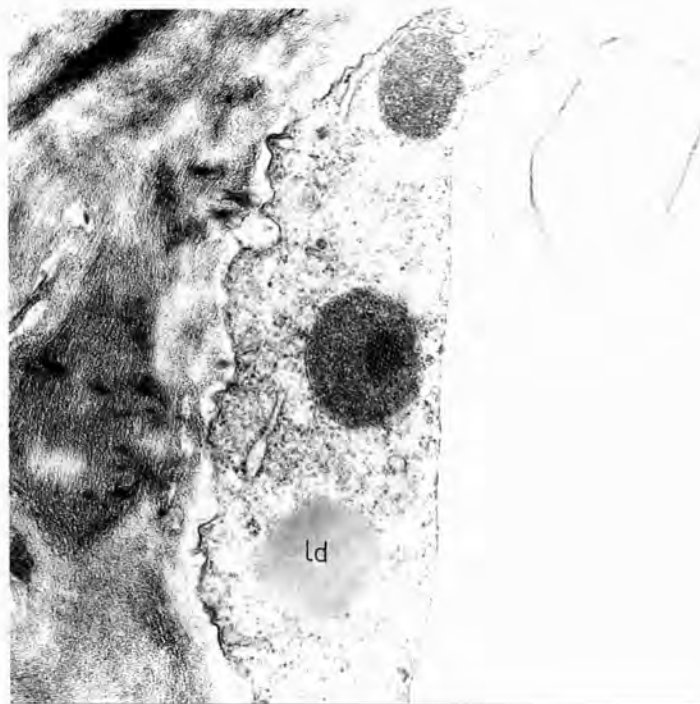
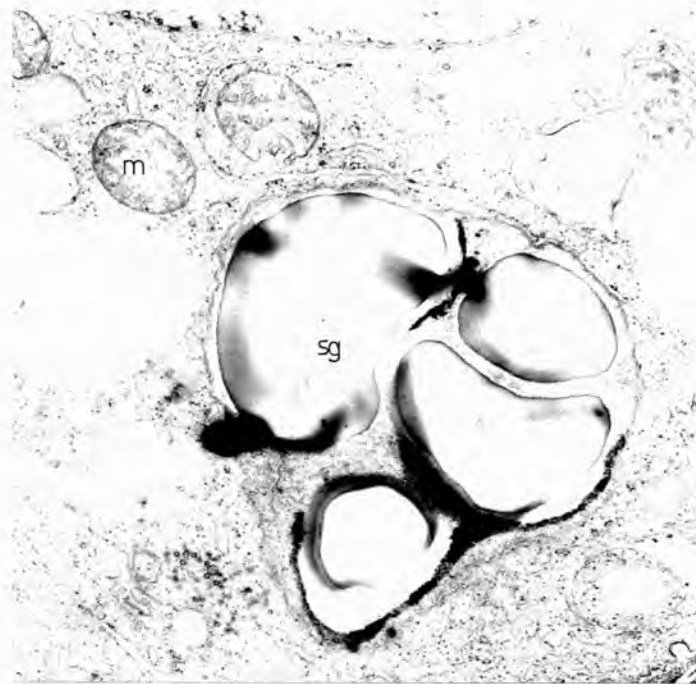


Fig. 3.15      Plasmodesmata in a primary pit-field between axial parenchyma and ray cells. Note the branching of the plasmodesmata at the small median nodule (arrow). Plastid contains a starch grain and electron dense stacked structure, probably grana. From TLS of stem sampled in late May    x 32,500

Fig. 3.16      TS of secondary phloem sampled in late May. Arrows indicate primary pit-fields which are mostly on the radial walls of the axial parenchyma cells. Note crystal cavities in the vacuoles of parenchyma cells.    x 2,850

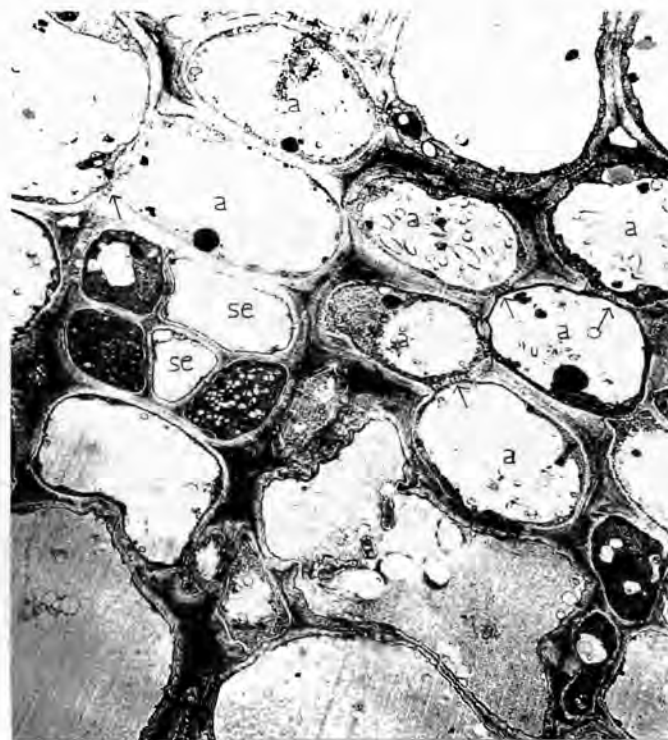
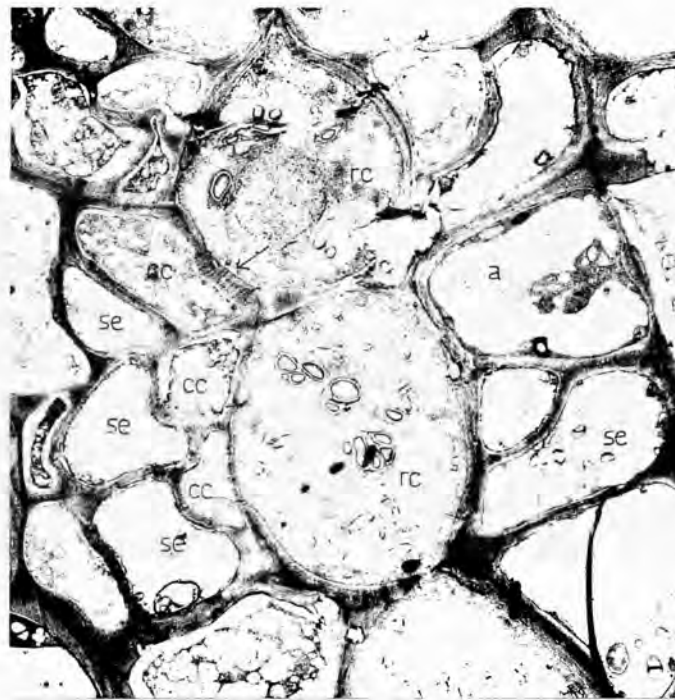




Fig. 3.17      Plasmatic connections between ray cell and companion cell (arrow). More branches appear on the companion cell side than on the ray cell side. Note that the ray cell is enlarged. TS of stem sampled in mid-December, 1976    x 3,250



CHAPTER IVDISCUSSION

The present investigation has managed to throw some light on the developmental sequences as well as on some of the features of the mature sieve tubes in Fraxinus excelsior.

There are no major differences in the differentiation of the sieve element when Fraxinus is compared with other species that have been investigated. The developmental sequence of the sieve-plate pores, the ultrastructure of the plasmodesmata, companion cells, parenchyma cells and the fate of the nucleus, tonoplast, plastids and ER when the sieve elements mature all follow the established trend.

Two unmistakable types of sieve elements are present: the wider type of the "summer phloem" and the narrower of the "autumn phloem". The difference in size is probably related to the intensity of translocation at the different times of the year. The ultrastructure of the sieve elements of the "autumn phloem" also suggests that they overwinter in a more or less functional state and remain so until new sieve elements are formed in early spring.

The P-protein originates somewhat similarly to that outlined by Behnke (1974). He was the first to reveal the close co-existence of ribosomes and P-protein during the early stages of the sieve element differentiation. Prior to this, there were only speculations but no direct evidence on the mode of formation of the first P-protein filaments.

From the micrographs presented, there is evidence to substantiate the view that polysomes are the source of the P-protein fibrils which may constitute the ontogenetically earliest P-protein material in Fraxinus. The micrographs which show the fibrils terminating on

polysomes are about the most cogent evidence. Polysomes exhibiting a helical appearance are numerous in most of the micrographs bearing on this point and this again is possibly suggestive of a relation with the helical P-protein fibrils. The previous origin of these polysomes themselves is uncertain but there is an indication that they may have been sloughed off from the nearby ER cisternae.

The granular P-protein masses also observed may be comparable with the early P-protein bodies seen in other species mentioned in Chapter III, section (ii). But granular masses surrounded by polysomes and the fibrillar material already discussed have been found to occur together, side by side. This probably means that the two types of elemental P-protein which may both have originated from polysomes have developed simultaneously along two different paths depending on the local density of the cytoplasm. Some of these early fibrils already showed the characteristic banded structure, also seen in other trees.

Fibrillar and tubular P-protein have both been observed from fixed sections and negative staining studies. There is also some kind of internal transformation of the fibrillar to the tubular form taking place in the well-developed larger P-protein bodies. As suggested by Parthasarathy and Muhlethaler (1969) the transformation probably involves the tightening of two helically-arranged filaments. In this study, two fibrils, loosely wound together in a helix, have been revealed in negatively stained phloem exudate.

Dispersal of the larger P-protein bodies also at first results in the usual tightly-packed flocculi which show the well-known semi crystalline banded structure (e.g. as in Ulmus) whose periodicity is made prominent by the lateral registration of the bands.

The differentiation of the sieve plate pore in Fraxinus, which is centred around a plasmodesmatal canal, is basically similar to other angiosperms already extensively investigated eg Cucurbita and Acer pseudoplatanus. However, differences exist in the deposition of the callose and the perforation of the pores.

Deposition of callose takes place quite early in the development of the sieve plates. As in Saxifraga (Deshpande 1974) and Cucurbita (Deshpande 1975) the wall underlying the callose persists until the final perforation. This disagrees with the earlier description of the process in Cucurbita by Esau et al (1962) who suggested that the two opposing callose platelets grow inwards in depth until they meet, dissolving the cellulose wall (middle lamellae) away as they do so. In both understandings of the process an open channel is formed which is lined completely (Esau) or partially (Deshpande) by callose from its inception.

In Acer, Northcote and Wooding (1966) had found it difficult to tell whether there is any lysis of wall material to make room for inwardly directed callose deposition, or whether there is a continuous outwardly directed increase in callose to keep pace with the general thickening of the cell wall. Perforation began by the proliferation of the ER in the middle lamella region forming a nodule, followed by erosion of the callose under the apposed ER. Thus a pore did not have to be lined completely by callose from its inception.

In this study, it is inferred (from the present micrographs) that once the callose is laid down, further growth of the cellulosic wall beneath the callose is stopped. Growth in thickness of the callose and intervening wall, however, takes place simultaneously, probably at rates which are out of step, for a pore site which appears sunken at

first (Fig. iii.3) later comes to protrude above the level of the intervening wall.

The process of widening of the pores is not fully documented here, especially whether it involves the removal of part or all of the callose cylinder.

ER cisternae were observed at the site of the sieve pore throughout its development. They were also found to be continuous with the plasmodesmata. They gave the impression of having to do with lysis of the callose and the middle lamella during the process of perforation, as suggested by Esau (1969) and Northcote and Wooding (1966).

A state of occlusion with P-protein seemed to be the natural one in the mature sieve plates of Fraxinus, judged by the many micrographs taken. This is an extremely controversial point; but in the present work the impression gained was that these were not artifacts due to the release of turgor pressure on excision. The appearance was too dissimilar from that of undoubted surge artifacts (see Anderson and Cronshaw 1969, Siddiqui and Spanner 1970, Eschrich et al 1971). However, other workers such as Anderson and Cronshaw (1969) take a different view. They believe that as a result of sudden release of pressure, the initially open pores became blocked by sieve element components such as P-protein and starch grains. Their sieve plates distant from the cut surface however showed occluded pores with peripheral plastids intact. The present writer believes that this is probably the natural state of affairs in a functioning sieve plate.

Many of the micrographs presented here show P-protein strands extending from the plate on its apparently downstream side and sometimes on its upstream side as well. Again this has a bearing on the nature

of the functioning sieve plates for an artifact due to sudden displacement would almost certainly produce P-protein dispositions very different on the two sides of the plate.

These extensions of the P-protein strands from the sieve plates are interpreted most readily as being consequences (rather than causes) of fluid streaming through the pores. This is in line with how Spanner (1975) envisages the initiation of the sieve tube movement in accordance with his electro-osmotic theory. However streamers long enough to extend to the next sieve plate have not been traced.

The semi-crystalline appearance of the P-protein fibrils within the sieve pores, also observed in other species (e.g. Acer and Salix) again suggests the likelihood of their being brought into position in a regulated fashion rather than being traumatically forced there, the electric potential created across the plate serving to align the P-protein fibrils along the electric line of force.

The compactness of the P-protein seen within the pores has probably been enhanced by the deposition of callose consequent on manipulation. The presence of callose here, is still of uncertain significance, for pores occluded with P-protein but with little callose have also been observed. Some even have densely compacted P-protein but little callose. Even though all the present micrographs were taken from tissues selected for uniformity and sampled by the same procedure, the possibility of the variable callose observed reflecting variations in the samples and treatment cannot be ruled out. Not all samples, for instance, may have been equally easily penetrated by the fixative.

In any case, it cannot be concluded that callose is only formed as a response to killing or injury (see Chapter III, section (iii)(c)).

for nearly all studies of sieve plate development have shown the presence of callose platelets early in ontogeny. Whether these callose platelets are removed completely during perforation and formed again on manipulation is still not settled.

The presence of broken plastids in most of the mature sieve tubes observed may be due to many causes. The obvious one is their sensitivity to the osmotic changes occurring on manipulation. Plastids are also often assumed to be rather unstable when they mature for nearly all the plastids encountered in immature sieve tubes remain intact though this may be a consequence of the closed nature of the immature sieve element itself. But quite often, intact plastids were found in mature sieve tubes especially in those whose sieve pores were somewhat occluded. To be found intact near unoccluded pores may suggest that the sieve tubes were not caught actively translocating and hence that their sugar content and osmotic pressure was relatively low so as to preclude the possibility of sudden changes.

Another interesting feature of the plastids in mature sieve tubes is that even though the peripheral plastids further inside the sieve element remain intact, those nearer the plates are much more likely to be ruptured. This is possibly to be attributed to some activity taking place in the vicinity of the sieve plates, but the point is very speculative.

The frequent occurrence of an empty space immediately above the sieve plates and enclosing many starch grains, is difficult to explain. It appears to be a sort of "barrier" preventing additional P-protein coming into closer contact with the already-occluded sieve-plates. In some instances, a fairly definite edge seems to bound a mass of released starch grains.



The spaces are also obvious in the montages present; nearly all the sequential sieve-plates in a file showing them. P-protein appears to pile up above these spaces while the sieve plates are occluded with unconnected P-protein. This certainly suggests that some translocation common to them all is taking place. They may represent a peculiar artifact. They have not been noticed before in the published work of others.

The nucleus as usual undergoes degeneration as the sieve element matures. In Fraxinus, no trace of the nucleus beyond the ruptured stage has been observed. It is probable that the nucleus is ultimately digested by enzymes which makes it difficult to detect any disintegrating nuclear fragments. In some species, nucleus has been found to be retained either in a normal or necrotic state in the mature sieve elements. Nothing corresponding to the so called "extruded nucleolus" as in Salix has been found during the course of the work.

The plastids in Fraxinus exhibit the S-type according to Behnkes' (1971) classification i.e. without proteinaceous inclusions instead of, or in addition to, the starch grains. The usual morphological changes like losing the turgidity and electron density of the matrix also take place when the plastids mature. The old starch grains too appear etched at the margins as observed in other species.

Fraxinus, in common with other species, both arborescent and herbaceous, shows the peculiar plasmalemmal 'blebs' whose nature and function is obscure. They appear as invaginations of the plasmalemmal membranes and differences exist in those found in the sieve elements and parenchyma/ray cells. From the micrographs presented, invaginations in the sieve elements appear to have developed in a continuous endocytic (pinocytotic) fashion as opposed to the exocytic direction which is

elsewhere probable; but interpretation here is not agreed and must be cautious. Pinocytosis is a mode of uptake of solutes and ions, and this may be a clue to its possible significance in the sieve tubes.

Companion cells being the distinguishing feature of angiosperm sieve elements show differences in size and numbers in the "summer and autumn phloem". "Summer phloem" has only one smaller companion cell and the "autumn phloem" normally two or more, equal in size to the sieve elements. They do not differ much from the other companion cells of other species ultrastructurally: the dense cytoplasm, enlarged nucleus, an assortment of plastids without any starch grains, abundant mitochondria and the usual 'delta' structure of the plasmatic connection to their sieve element.

Parenchyma cells are worth mentioning briefly for when sieve elements of Fraxinus are obliterated, the axial parenchyma becomes dilated and the ray cells increase in size and numbers to adjust to these changes. Their contents are typical and include tannins and crystals of calcium salt, probably oxalate.

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