# STUDIES IN TRANSLOCATION

## II. SUBMICROSCOPIC ANATOMY OF THE PHLOEM

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*Summary*

Electron-microscope studies have been made of the cells of the phloem and pericyclic ground parenchyma of the stem of *Cucurbita pepo* (Duchesne). The mature sieve element contains no cytoplasm, either on the side walls or at the sieve plate, such as occurs in other cells. The walls are lined with a parietal layer which encloses an aqueous solution in which are dispersed fibrils of slime. The parietal layer appears to be composed of from one to several membranes, with which are associated numbers of vesicles and a very few mitochondria. The parietal layer lines the sieve plate and extends as a lining to the open sieve pores. There is no closing layer or membrane across the sieve pores. Slime is dispersed more or less uniformly through the lumen, and is continuous through the pores. Dense connecting strands of slime are considered to be an artefact. The individual elements are continuous with each other via the open sieve pores, forming a conduit, the sieve tube. The absence of cytoplasm and organelles suggests that the sieve tube is metabolically inert.

The companion cells, in contrast, are rich in cytoplasm. They are packed with mitochondria and other inclusions, and appear capable of high levels of activity in several aspects of metabolism.

The phloem parenchyma cells are similar in appearance to the pericyclic parenchyma cells and are much less rich in cytoplasmic contents than the companion cells.

## I. INTRODUCTION

The nature of the angiosperm sieve-element protoplast has been a controversial matter since the time the phloem was first investigated, and currently two divergent viewpoints about its normal state exist. These opposing views are intimately related to the diverging hypotheses of the mechanism of translocation. The "mass flow" hypothesis assumes that the protoplast is "denatured" (i.e. disorganized, non-functional, and freely permeable), and the sieve elements are believed to form a continuous conduit through which a mass flow of translocate occurs. Alternatively, the various "active" hypotheses assume that the protoplast contains functional protoplasm with normal differential permeability, and that the translocate is transported from protoplast to protoplast via connecting strands of cytoplasm.

Most of the recent evidence, discussed in detail later, supports the view that the sieve element contains cytoplasm possibly organized in a complete, though enucleate, protoplast.

The current studies were undertaken to determine whether the sieve-element protoplast contains such structures as ground cytoplasm, plasmalemma and tonoplast, mitochondria, golgi bodies, and endoplasmic reticulum which are characteristic

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of the protoplast of other plant cells (Buvat 1958; Whaley, Kephart, and Mollenhauer 1959). The material used was the stem of *Cucurbita pepo* (Duchesne). Electron-microscope studies were made of the sieve element, companion cell, phloem parenchyma, and the pericyclic ground parenchyma. The results lead us to revise the recent views of the structure of the mature sieve-tube element.

## II. Methods

*C. pepo* plants were grown from seed in open plots containing a mixture of loam, sand, and dried manure. Approximately 15-cm lengths of stem were cut from the plant into buffered 0·2M sucrose solution as used by Currier, Esau, and Cheadle (1955). After 1–2 hr in this solution, 1-cm lengths of stem were cut with a sharp razor-blade from the centres of the 6th, 7th, and 8th expanded internodes from the tip. Vascular bundles of the inner ring, including a small amount of the surrounding parenchyma, were cut individually from the stem pieces while immersed in the solution. Examination with the light-microscope of sections of phloem cut from the material at this stage showed that the sieve-tube elements, except those at the cut ends, were apparently uninjured, and did not show slime plugs. The pieces of vascular bundles were transferred to one of the following fixatives:

(1) 2% osmic acid in acetate–veronal buffer at pH 7·3, adjusted to a concentration of 0·2M with sucrose. Fixation time was 5 hr.

(2) 1% potassium permanganate. The fixative was prepared by the method described in "Electron Microscopy" (Anon. 1957) for the osmic acid fixative, the osmic acid being replaced by potassium permanganate. Fixation time was 0·5–1 hr.

After fixation the material was washed several times and dehydrated with a series of ethanol solutions. It was then transferred by several stages to mono-methacrylate (1 part methyl to 6 parts butyl methacrylate) and left overnight before embedding in partially polymerized methacrylate. The polymerization was completed in an oven at 40–50°C, or in ultraviolet light. Some of the permanganate-fixed material was embedded in "Araldite" by the method described in "Electron Microscopy". The blocks were trimmed to include only the large-celled region of the phloem. Sections were cut with a Porter–Blum microtome, using a diamond knife, and examined with a Siemens Elmiskop I electron-microscope at 60 or 80 kV.

Droplets of exudate from freshly cut stems were allowed to coagulate and then fixed in 2% osmic acid fixative for 2 hr, and embedded in methacrylate as described for the vascular tissue. Exudate was also examined by the negative staining method of Brenner and Horne (1959). In this case the exudate was collected directly into an approximately equal volume of 4% ammonium acetate solution. The exudate remaining in solution in the buffer was sprayed on to a grid with an equal volume of 2% phosphotungstic acid.

## III. Results

The various cell types in the phloem are readily identified under the electron-microscope from a comparison with their appearance under the light-microscope (Plate 1, Fig. 1). The companion cells are small and contain a high proportion of

cytoplasm with numerous cytoplasmic organelles. The phloem parenchyma cells have less cytoplasm and fewer organelles, and frequently contain chloroplasts which do not occur in companion cells, and only very rarely in sieve tubes. Sieve tubes, in contrast, appear almost empty except for slime. The results for each cell type are described in detail below.

### (*a*) *Phloem Parenchyma*

These cells contain large vacuoles so that the cytoplasm forms a narrow layer around most of the periphery of the cell. Larger amounts of ground cytoplasm, however, are associated with the cytoplasmic inclusions, which usually occur in clumps. The cytoplasm is bounded externally by a plasmalemma and internally by a tonoplast. Within the ground cytoplasm the endoplasmic reticulum may be seen as double membranes and small vesicles (Plate 1, Fig. 2; Plate 8, Fig. 1).

The cells contain a number of mitochondria, about $0{\cdot}5\ \mu$ in width and from 1 to $2\ \mu$ in length. They are bounded by a membrane which in some areas can be seen to be double, and contain a number of cristae in the form of fine twisting tubules. The ground material is somewhat granular in appearance. Bodies are found which are similar to those which have been described as golgi bodies in other plant cells (Buvat 1958; Whaley, Kephart, and Mollenhauer 1959). They consist of bundles of flattened vesicles $0{\cdot}5\ \mu$ in length and $5\ \mu$ in thickness.

A number of chloroplasts are present, ranging from 1 to $3\ \mu$ in length. They are bounded by a membrane and contain lamellae and grana within a stroma. Small osmiophilic bodies are present, but these are not seen in material fixed in permanganate. Large starch grains are often present.

A nucleus with nucleoli is found in some sections.

Simple pits occur between the phloem parenchyma cells, but pits between phloem parenchyma cells and other cell types have not so far been observed. In the pits numbers of plasmodesmata occur.

### (*b*) *Companion Cells*

These cells (cf. Plates 2, 3, 4, and 5) are rich in cytoplasm, and the vacuole occupies a smaller proportion of the total cell volume than it does in the parenchyma cells. Because the cytoplasmic contents are electron-dense, both the plasmalemma and tonoplast are usually difficult to distinguish. The cytoplasm is packed with organelles, including mitochondria, plastids, and vesicles. The mitochondria are similar to those of the phloem parenchyma cells, but occur in much greater numbers.

The cytoplasm contains a large number of organelles, which vary in size and shape, are larger than mitochondria, contain a granular ground material, and are surrounded by a well-defined membrane. Some of these bodies contain a series of internal double membranes which extend from one part of the boundary membrane to another. Others contain a number of dark and non-staining bodies but only an occasional internal membrane. None of these organelles contains both a series of internal membranes and dark and non-staining bodies as well, suggesting that there may be at least two species of plastid.

A peculiar type of "vacuole" system, different from any yet described in plant cells, is occasionally found (Plate 2, Fig. 2; Plate 3). It consists of elongated vesicles swollen at the tips, somewhat resembling the golgi bodies of other cells. However, these vesicles are extraordinarily long, approximately 12 $\mu$ as compared with 0·5–1 $\mu$ typical of golgi bodies in plant cells. In addition the vesicles are swollen at intervals along their length and may be partly separated by ground cytoplasm. The ramifications of such a body in the cytoplasm are extensive. In addition to these large vesicle systems many small vesicles and elongated double membranes are found which resemble the endoplasmic reticulum of other cells. It is possible that the large vesicle systems form part of the endoplasmic reticulum which is more elaborate in these cells than in other plant cells.

As the companion cells are small, the nucleus sometimes occupies the major part of the area of the cell in transverse section. The endoplasmic reticulum is seen to be continuous with the outer membrane of the nuclear envelope (Plate 2, Fig. 3). In osmium-fixed material a number of patches of denser material are seen in the nucleus, often at its periphery. These are similar in appearance to what has been interpreted by other workers (E. H. Mercer, unpublished data) to be accumulations of ribonucleic acid (RNA) granules (or nucleoli). The cytoplasm also contains large numbers of granules generally taken to be RNA granules.

### (*c*) *Sieve Element*

On reaching anatomical maturity (defined as the stage at which the slime bodies have broken down and a sieve plate is present) the sieve element (cf. Plates 4–7) contains no cytoplasm, either on the side walls or at the sieve plate, such as is found in other plant cells (Buvat 1958; Whaley, Kephart, and Mollenhauer 1959). The side walls and sieve plate are lined by a parietal membranous layer, enclosing a matrix of slime (Plates 4–6). There appears to be no closing layer or membrane across the pores of the sieve plate. The parietal layer which lines the plate extends also as a peripheral lining to the pores so that it is continuous from one element to the next (Plate 6, Figs. 1, 2, and 3). Where the parietal layer is in contact with dense slime it is frequently difficult to distinguish as a separate layer. Elsewhere, however, it is usually seen as a conspicuous layer which appears to consist of one to several membranes separated by narrow, apparently empty, zones of variable thickness (Plates 4 and 5). The layer as a whole varies in thickness from 200 to about 1000Å.

A number of vesicles, 0·1–1·5 $\mu$ in diameter, are found, most of which are associated with the parietal layer while others are scattered, singly or in clumps, throughout the body of the cell.

The mature element contains a very small number of mitochondria, often in clumps interspersed with vesicles. In osmium-fixed material the sieve-element mitochondria differ from those of the companion cells and phloem parenchyma in that the cristae resemble rounded vesicles rather than fine tubules.

On rare occasions chloroplasts are found. These differ from those of the phloem parenchyma in that they lack grana. As is known from studies with the light-microscope (Esau 1950) the mature sieve tube is enucleate.

In the lumen of the mature sieve element is a somewhat granular, fibrous, or reticular substance. From comparative studies of mature and immature elements with light- and electron-microscopes, and from the absence of the inclusions normally found in cytoplasm, we conclude that this material is "slime".

The slime is distributed somewhat unevenly throughout the cell and sometimes, though not always (Plate 6, Fig. 2), tends to be concentrated at the plates. It is quite clear (Plate 6) that the substance filling the sieve pores and hence forming the "connecting strands", is not cytoplasm but is slime. Often the particles of slime appear to be orientated along lines of flow through the sieve pores (Plate 6), i.e. the sieve pores are open, and the slime matrix is continuous from element to element.

The wall of the sieve plate, as seen in transverse section, consists of three zones (Plate 6). These probably correspond with the middle lamella, primary cellulose, and callose layers as identified by light-microscopy.

Numbers of pits, such as described by Frey Wyssling and Müller (1957), occur on the walls between the companion cells and the sieve tubes. Plasmodesmata are present in the pits on the side of the wall towards the companion cell. On the side towards the sieve tube, however, there is a simple pore lined by the parietal layer of the sieve tube. Often pits may be seen close together and probably constitute part of a lateral sieve area.

### (*d*) *Phloem Exudate*

Phloem exudate fixed in osmic acid is identical in appearance with the contents of the sieve tubes (Plate 7, Fig. 2). It contains only a material resembling the slime seen in the sieve elements, and a few vesicles. The vesicles are similar in structure to those of the intact sieve element. The exudate contains no cytoplasmic organelles such as might be expected if the exudate were derived from a normal cytoplasm which is displaced as exudation occurs. The slime appears to consist of fibrillar particles (Plate 7, Fig. 3).

### (*e*) *Immature Elements*

As indicated from studies with the light-microscope, immature sieve elements contain cytoplasm similar to that of other cells (Plate 7, Fig. 1). They possess a ground cytoplasm with an endoplasmic reticulum, a plasmalemma and tonoplast, and contain inclusions such as mitochondria and golgi bodies found in other cell types. In addition the cells contain slime bodies. These have a dense granular structure. Both the number per cell and the size of the slime bodies is very variable, as has been observed with the light-microscope (Crafts 1932). As reported by Crafts, the slime bodies are "vacuolated", that is they contain less dense, or even apparently empty spaces within their matrix.

In the present studies no observations were made of intermediate stages of the breakdown of either slime bodies or cytoplasm. Hence it is not known whether the parietal layer of the mature sieve element is derived from one or more of the cytoplasmic membranes of the immature element, or whether it is formed as a new structure as the element matures.

### (*f*) *Ground Parenchyma*

The cytoplasm of the ground parenchyma (Plate 8, Fig. 2) is identical in appearance to that of the phloem parenchyma which has already been described. It would appear that the main difference between the two types of parenchyma is a difference in shape and volume which can be seen with the light-microscope. Owing to the larger volume of the cells of the ground parenchyma the cytoplasmic layer probably occupies a smaller proportion of the total cell volume in the ground parenchyma than in the phloem parenchyma.

## IV. Discussion

Electron-microscope studies have built up a well-documented picture of the structure of the protoplast of meristematic cells, parenchyma cells, and mesophyll cells of several species of angiosperms. In general, the nucleus, mitochondria, golgi bodies, and plastids are distributed in a ground cytoplasm which is bounded externally by the plasmalemma and, in vacuolated cells, internally by the tonoplast. The cytoplasm is permeated by an elaborate membrane system, the endoplasmic reticulum which is apparently continuous with the boundary and nuclear membranes. RNA granules occur scattered through the ground cytoplasm or are attached to the reticulum. Thus the plant cell protoplast is characterized by an elaborate submicroscopic structure (see Mercer 1960).

The observations described in this paper show that the structure of the protoplasts of the pericyclic cells, the phloem parenchyma, companion cells, and immature sieve elements of *C. pepo* are generally similar to those of other living plant cells. In contrast, the structure of the protoplast of the mature sieve element differs from that of other plant cells in several important aspects, including the absence of a ground cytoplasm, the scarcity of organelles, and the presence of slime.

### (*a*) *Ground Cytoplasm*

In the mature element the ground cytoplasm appears to be completely absent.

### (*b*) *Cytoplasmic Membrane Systems*

The only membrane systems in the mature sieve element are those which constitute the parietal layer and vesicles. These are most probably derived from one or more of the membrane systems (plasmalemma, tonoplast, and endoplasmic reticulum) of the immature element. The possibility remains, however, that they are formed as new structures as the element matures. Until their origin is clarified it is probably better to avoid such terms as tonoplast and plasmalemma to describe the structure of the lining layer. New terms may be needed if the layer is not derived directly from the plasmalemma or tonoplast, but until the necessary information is obtained we suggest describing the structure as the parietal layer.

### (*c*) *Organelles*

A small number of mitochondria and very rarely a few chloroplasts occur in the mature sieve tube. These organelles are associated with the parietal layer, being

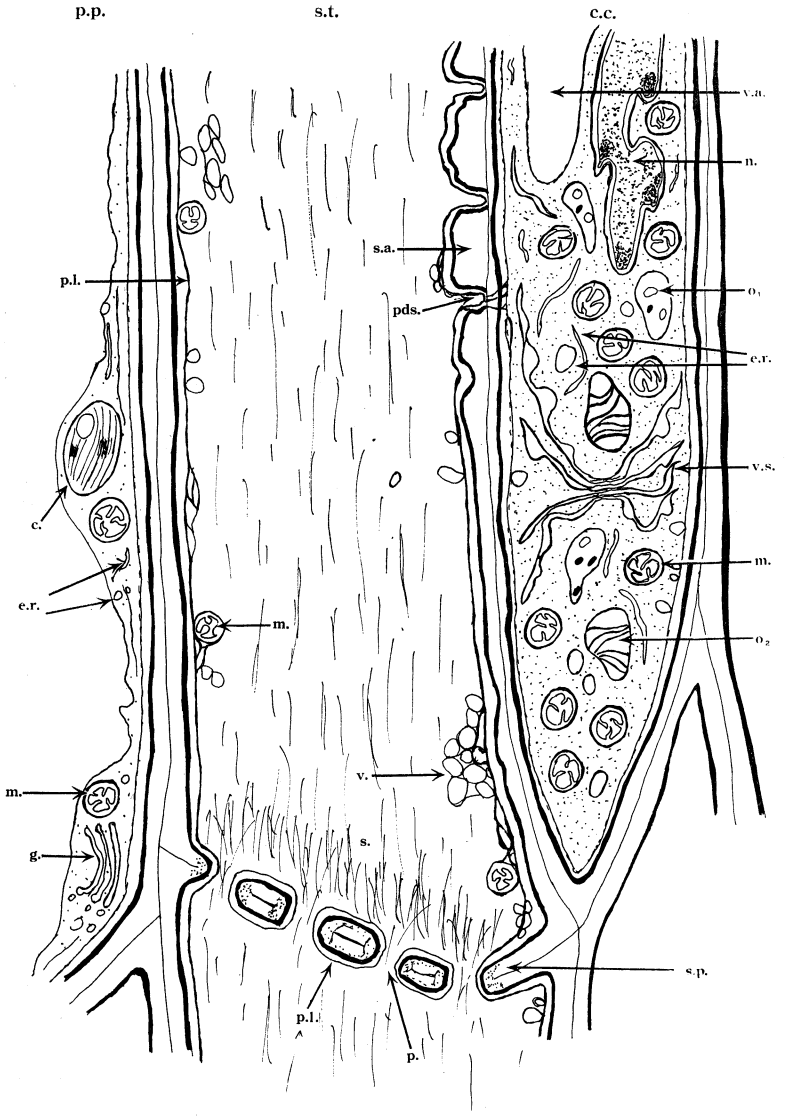


Fig. 1.—Diagram illustrating longitudinal section of phloem with portion of a phloem parenchyma cell (*p.p.*), sieve tube (*s.t.*), and companion cell (*c.c.*). Longitudinal section. Details include chloroplast (*c.*), endoplasmic reticulum (*e.r.*), mitochondria (*m.*), golgi body (*g.*), vacuoles (*va.*), nucleus (*n.*), unidentified organelles, possibly plastids, of companion cells ($o_1$, $o_2$), vacuole system (*v.s.*), parietal layer (*p.l.*) lining wall and sieve pores, drawn away from wall to show continuity of layer, vesicles (*v.*), slime (*s.*), sieve plate (*s.p.*), sieve pore (*p.*), sieve area (*s.a.*), and plasmodesmata (*pds.*).

frequently enmeshed in this membrane system. McGivern (1957) has demonstrated by histochemical means that apparently functional mitochondria occur in clumps in mature sieve tubes, suggesting that the clumping of mitochondria observed by us in the sieve tubes of *C. pepo* is not an artefact of fixation.

### (*d*) *Slime and the Continuity of the Sieve Elements*

The only material in the lumen of the sieve tubes is a granular or reticular substance which by virtue of its appearance and distribution we identify as slime. Frequently in electron-micrographs the slime is seen as a dense layer on the sieve plates and in the sieve pores. Unfortunately the exact distribution *in vivo* is not known. From studies with the light-microscope it is concluded that microscopic accumulations of slime on the plate are artefacts (Esau 1950). In the current studies light-microscope examination of the tissue showed that the contents remained intact during the period for which the tissue was equilibrating with the sucrose solution after cutting from the vine. However, after fixation a displacement of contents, of varying severity, was observed within the sieve elements. Under these conditions it seems likely that a movement of solution between sieve elements would occur, allowing the sieve plates to exert a "filtering" action on the slime, causing it to compact at the plate and in the pores. The lines of flow frequently seen in the slime in the vicinity of the plates is consistent with such an action (Plate 6, Fig. 2). The commonly occurring dense layer of slime at the plates and in the pores, therefore, may be an artefact of fixation. On the evidence so far available we consider it more probable that *in vivo* the slime is distributed more or less uniformly throughout the length of the element (Plate 6, Fig. 2) rather than compacted at the sieve pores, such as shown in Plate 6, Figure 3. In any case it would appear that it is slime rather than cytoplasm which is continuous from one element to the next through the open sieve pores. There is no closing layer or membrane across the sieve pores and the parietal layer which lines the side walls and sieve plate extends also as a lining layer to the pores. Hence both the parietal layer and the slime are continuous from element to element. The individual members of a mature sieve tube are thus much more intimately connected to one another than are most other plant cells. Furthermore the connections are not by strands of cytoplasm as in plasmodesmata, but by slime.

Presumably *in vivo* a solution containing the translocate is associated with the slime. This solution would be continuous from element to element throughout the length of the sieve tube, and since the boundary membranes cannot at the moment be regarded as homologous with a tonoplast, it is doubtful whether the term "vacuole" should be used to describe this solution phase in the sieve tubes.

The structure of the mature sieve element as deduced from the current studies may be summarized as follows: the sieve elements cannot be regarded as discrete entities, but must be thought of as segments of a conduit, the sieve tube, which contains a continuum of solution and slime enclosed by the membranous parietal layer (Fig. 1).

This view of the structure of the mature sieve element is at variance with the conclusion reached from recent investigations, namely that the lining layer and

connecting strands are composed of cytoplasm. This conclusion was based on three lines of evidence.

First, from electron-microscope observations Hepton, Preston, and Ripley (1955), Preston (1958), and Schumacher and Kollman (1959) concluded that the material of the parietal layer and connecting strands was composed of cytoplasm.

Secondly, the demonstration by Currier, Esau, and Cheadle (1955) of the plasmolysability of the sieve element indicated that the sieve-element protoplast is not "denatured" and freely permeable but does possess the property of differential permeability at least along the lateral walls.

Thirdly, the high respiration rates in the phloem calculated by Kursanov and Turkina (1952) and Kursanov, Turkina, and Dubenina (1953) have been taken to indicate the presence of a metabolically active protoplast in the sieve element (Preston 1958; Spanner 1958).

Each of these lines of evidence is discussed below together with the evidence of the current studies.

The indentification of substances with the electron-microscope is not always straightforward, and it is doubtful whether a shadowing technique such as was used by Hepton, Preston, and Ripley (1955) would reveal the distinction between slime and cytoplasm. As far as can be assessed from their published data the substance in the sieve elements more closely resembles the substance interpreted by us as slime. It has the granular, fibrillar appearance of slime rather than the complex structure of cytoplasm.

Similarly, the substance identified as cytoplasm by Preston (1958) and Schumacher and Kollman (1959) could be slime. Their electron-micrographs show that the substance is extremely electron-dense. It is not possible to identify the material from the published data, but there are no structural features to suggest that it is cytoplasm. The presence of a small number of organelles in the sieve element does not necessarily imply that a well-organized cytoplasm also exists, particularly when the organelles are swollen and possibly partially broken down, as they are in some of the published electron-micrographs.

As has been pointed out by Currier, Esau, and Cheadle (1955) the demonstration of reversible plasmolysis shows that the mature sieve-element protoplast is differentially permeable along its lateral walls, though not necessarily at the sieve plates. However, there is no reason to doubt that the membranous parietal layer could account for this lateral differential permeability, and that a layer of normal cytoplasm is not essential. The finding of the current studies of the open structure of the sieve pores obviates the problem raised by Currier, Esau, and Cheadle of the apparent anomaly of differential permeability in the sieve tubes and the longitudinal surging flow which can be induced in a series of sieve elements. The continuity of the elements through the pores also accounts for the plasmolysis forms found by Currier, Esau, and Cheadle, namely the sieve-element protoplasts being attached at the sieve plates and concave along the lateral walls.

Finally, from the evidence of the current studies it appears that the high respiratory activity which has been reported for the phloem can be accounted for

largely by the companion cells and phloem parenchyma, containing large numbers of mitochondria, rather than by the sieve elements as was previously suggested (Kursanov, Turkina, and Dubenina 1953). Indeed it seems that for *C. pepo* at least, the high respiration rates that are found in the phloem (Duloy 1960; Duloy and Mercer 1961) cannot be attributed to the sieve tubes, which show a marked scarcity of mitochondria.

The view of the structure of the sieve tube derived from the present study allows explanation of a number of the peculiar properties of the mature sieve element.

The phenomenon of exudation can be explained on the structure proposed here. Assuming a positive turgor exists in the sieve tubes, exudation would occur from the sieve elements following cutting because of the open structure of the pores and the high permeability of the sieve tubes in the longitudinal direction.

The difficulty of detecting the so-called "cytoplasmic layer" in mature elements, with the light-microscope (Currier, Esau and Cheadle 1955), the problem of an ill-defined "vacuole", the paucity of contents in the protoplast as seen under the light-microscope (Esau 1950), and the extreme sensitivity of the sieve-element protoplast to handling, can all be attributed to the extreme thinness of the parietal layer as revealed by electron-microscopy.

The absence of a ground cytoplasm would account for the fact that cytoplasmic streaming has never been observed in mature sieve elements. Again, the lack of ground cytoplasm and the scarcity of organelles might account for the weak tetrazolium reaction reported for mature sieve elements (Bauer 1953) and for the lack of affinity for "plasma" stains (Esau 1950). Similarly, the paucity of enzymes observed by Wanner (1953) in phloem exudate is consistent with the lack of cytoplasm and organelles in the sieve elements.

The absence of a ground cytoplasm may be the result of the absence of a nucleus in the mature element. If in these cells the maintenance of cytoplasmic proteins depends on the continued production of RNA, then it might be expected that the loss of the nucleus could be followed by a loss of the proteins of the ground cytoplasm.

### (*e*) *Comparison of Constituent Cells of the Phloem*

The scarcity of mitochondria and absence of ground cytoplasm indicates that the mature sieve element may be almost completely inert metabolically, and deficient in enzymes.

Again, the phloem parenchyma cells appear to be capable of levels of activity similar to those of the surrounding ground parenchyma, and lower than those likely to be found in the companion cells. The companion cells, in contrast to both sieve tubes and phloem parenchyma, are rich in cytoplasm and its inclusions. They contain large numbers of mitochondria indicating that the cells are capable of high levels of respiratory activity. The companion cells also appear to contain a large amount of RNA material, both in the nucleus and in the cytoplasm. Since the level of protein synthesis in a cell appears closely to parallel the amount of RNA material present (Brachet 1960), the implication is that the companion cells are capable of a high level

of protein synthesis. In addition these cells contain a large number of plastids, the functions of which are at the moment unknown, and an endoplasmic reticulum more elaborate than those found in other plant cells. This cytological evidence strengthens the view that the companion cells, with their close structural relationship with the sieve tubes, may be at least partly responsible for the maintenance of the mature sieve element. Whether or not a high level of metabolism in the companion cells is directly necessary for the process of translocation is still an open question.

### (*f*) *Conclusions*

Three main conclusions can be drawn from the current study about the phloem of *C. pepo*. First, from the distribution of cytoplasm and its inclusions in the cells of the phloem it seems that it is the companion cells rather than the sieve tubes that are responsible for the high levels of metabolic activity that have been found in the phloem. Secondly, there appears to be a reduction of the cytoplasmic contents of the sieve elements to an extremely thin parietal membranous layer, the remainder of the cell being occupied by a solution in which slime is in some way dispersed. Thirdly, the individual mature sieve elements are continuous with one another via the open sieve pores, so forming a conduit, the sieve tube.

## V. Acknowledgments

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## EXPLANATION OF PLATES 1–8

### PLATE 1

Fig. 1.—Transverse section of phloem showing parenchyma cells (*p.c.*), companion cell (*c.c.*), sieve tube (*s.t.*) with parietal layer (*p.l.*), vesicles (*v.*), and slime (*s.*). ×5000.

Fig. 2.—Portions of two phloem parenchyma cells. They contain a thin peripheral layer of cytoplasm bounded externally by a plasmalemma (*pl.*) and internally by a tonoplast (*t.*). Chloroplasts (*c.*), mitochondria (*m.*), and golgi bodies (*g.*) are seen. ×20,000.

### PLATE 2

Fig. 1.—Companion cell with part of neighbouring sieve element above it. The cytoplasm of the companion cell contains numerous mitochondria and plastids, and a few vesicles. The nucleus (*n.*), which occupies a large part of the area of the cell in this section, contains a nucleolus and several other areas of density greater than that of the nucleoplasm (possibly nucleolar material). The sieve element is bounded by a thin parietal layer (*p.l.*) in parts of which two membranes can be seen. ×10,000.

Fig. 2.—Companion cell, showing extensive system of cytoplasmic membranes and vacuoles. ×10,000.

Fig. 3.—Companion cell, showing nucleus (*n.*) at right, bounded by double membrane the outer layer of which is continuous with the endoplasmic reticulum (*e.r.*). The mitochondrion (*m.*) has fine tubular cristae, and an external double membrane which also appears to be continuous with the endoplasmic reticulum. Potassium permanganate–methacrylate. ×40,000.

### PLATE 3

Section of companion cell. The cytoplasmic membranes which ramify through the cytoplasm are closely packed, have a parallel arrangement, and enclose an extensive system of vacuoles. × *c.* 20,000.

### PLATE 4

Longitudinal section of sieve element and companion cell. The parietal layer (*p.l.*) of the sieve element is associated with numerous vesicles (*v.*), but no mitochondria are present in this section. Fibrous slime (*s.*) is dispersed throughout the lumen of the element. In contrast to the sieve element, the companion cell contains numerous mitochondria (*m.*) and plastids (*pt.*), and a nucleus (*n.*) with several dense areas (*d.a.*) taken to be nucleolar material. The wall between the sieve element and companion cell shows part of a sieve area, the side towards the sieve element being perforated by pores, while that towards the companion cell shows several plasmodesmata (*pds.*). ×15,000.

## Plate 5

Fig. 1.—Sieve element and companion cell. The companion cell at top shows granules in the cytoplasm, a plasmalemma (*pl.*), and tonoplast (*t.*), and mitochondrion (*m.*). In the sieve element the parietal layer (*p.l.*) can be seen to consist of from one to several membranes, separated at intervals to form vesicles. Part of a sieve area can be seen in the wall between the two cells, with plasmodesmata (*pds.*) on the side towards the companion cell. ×20,000.

Fig. 2.—Sieve element. A number of mitochondria (*m.*), small vesicles (*v.*), and membranes can be seen. This is the largest accumulation of cytoplasmic elements that was found in a mature sieve tube. Slime (*s.*) is dispersed in the lumen of the cell. ×10,000.

## Plate 6

Figs. 1–3.—Longitudinal sections of mature sieve elements in area of sieve plates. The parietal layer (*p.l.*) lines the side walls and sieve plate (*s.p.*) and in some places can be seen to extend as a lining to the sieve pores so that it is continuous from element to element. The parietal layer in the sieve pores is sometimes impossible to distinguish because of the deposits of slime. Slime (*s.*) is dispersed throughout most of the lumen of the element, and is continuous through the sieve pores. The slime sometimes, though not always (Fig. 2) tends to accumulate on one side of the plate. ×10,000.

## Plate 7

Fig. 1.—Immature sieve element, showing slime body (*s.b.*) with small "vacuoles". Mitochondria (*m.*) and golgi bodies (*g.*) can be seen in the cytoplasm. ×20,000.

Fig. 2.—Exudate fixed in osmic acid. ×20,000.

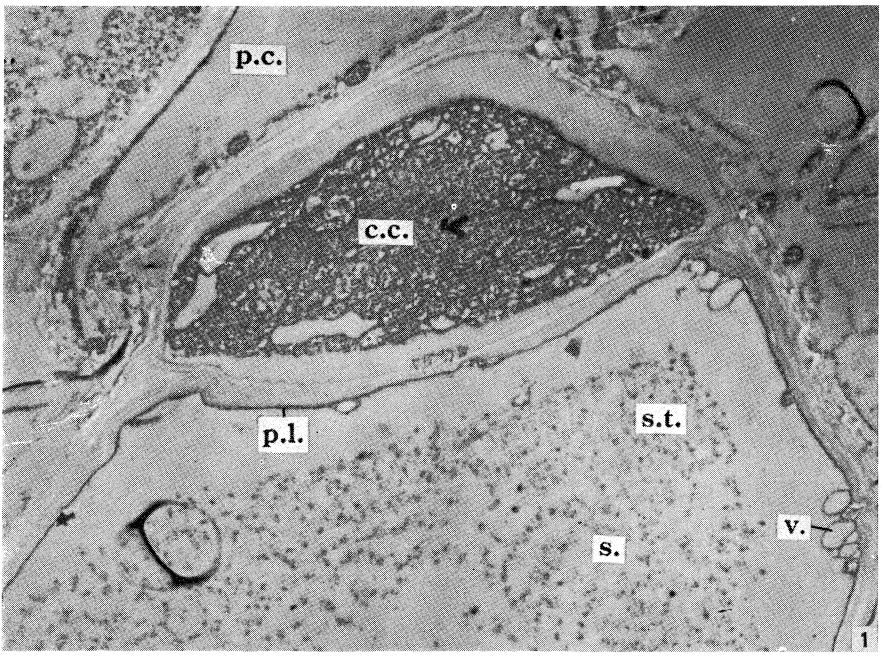
Fig. 3.—Exudate prepared by negative staining with phosphotungstic acid. ×80,000.

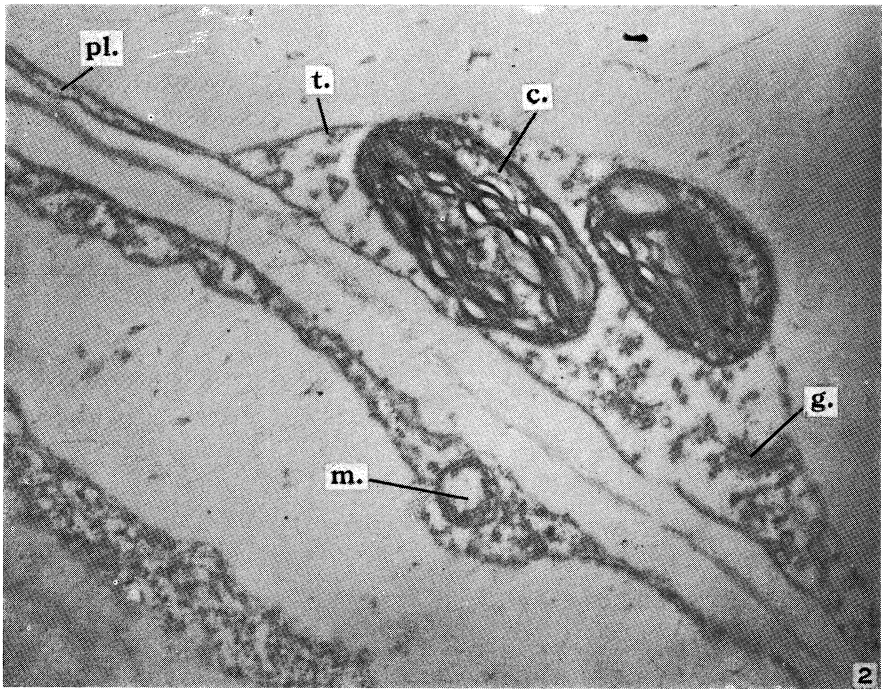
## Plate 8

Fig. 1.—Phloem parenchyma cell. A golgi body (*g.*), mitochondrion (*m.*), and chloroplast (*c.*) with starch grains (*s.g.*) can be seen in the cytoplasm, together with elongated membranes of the endoplasmic reticulum (*e.r.*). Part of the tonoplast (*t.*) can be seen towards the right-hand corner of the section (compare with Plate 1, Fig. 2). Potassium permanganate–"Araldite". ×20,000.

Fig. 2.—Ground parenchyma cells. A greater part of the cell has the appearance shown in the lower cell of this figure, where elongated membranes of the endoplasmic reticulum can be seen. At intervals, however, a clump of cytoplasm occurs such as shown in the upper cell, with golgi bodies (*g.*), mitochondria (*m.*), and chloroplasts (*c.*). The cytoplasm is bounded internally by a tonoplast (*t.*). Potassium permanganate–"Araldite". ×40,000.

STUDIES IN TRANSLOCATION. II



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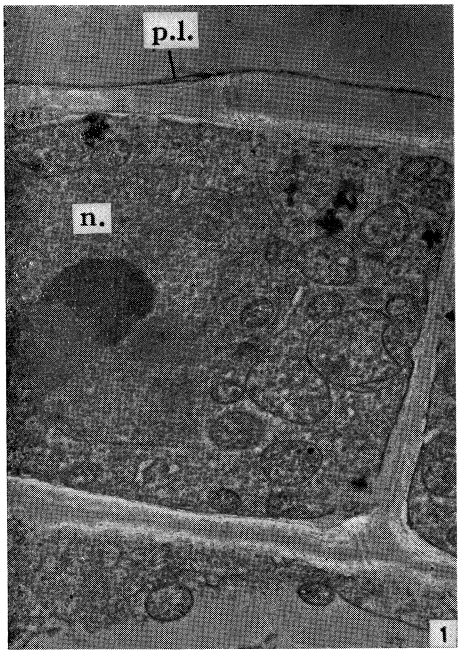



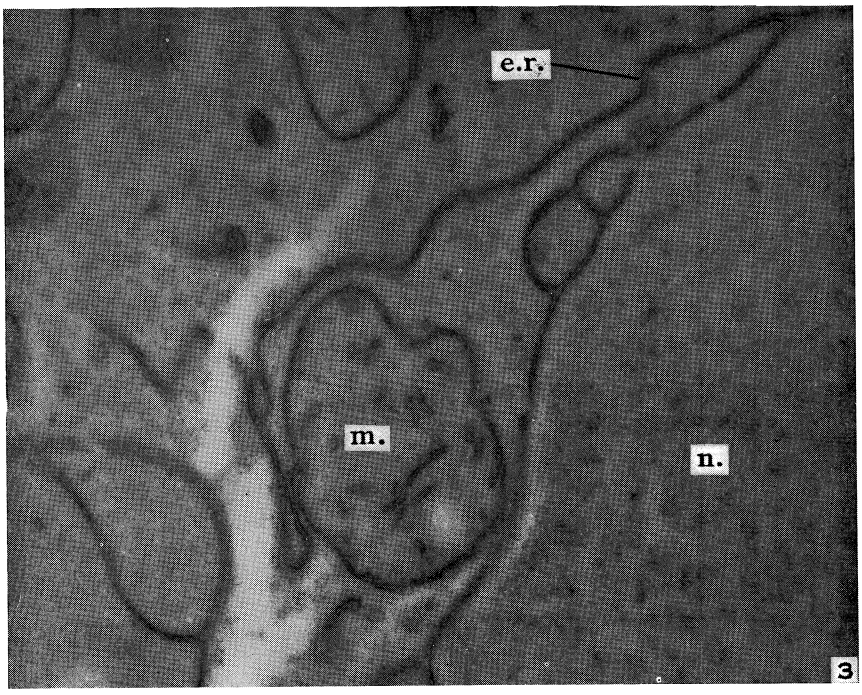

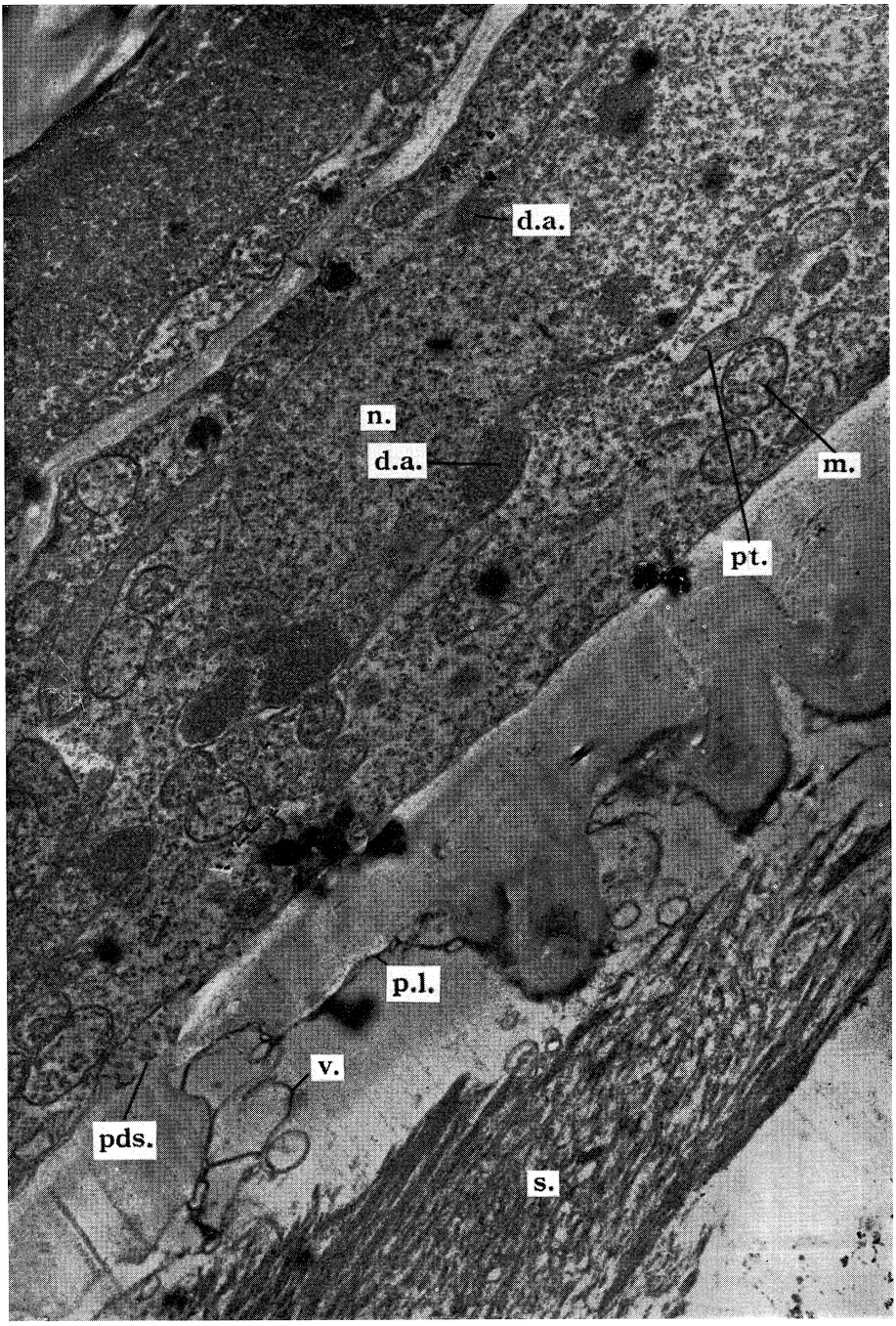
d.a.
n.
d.a.
m.
pt.
p.l.
v.
pds.
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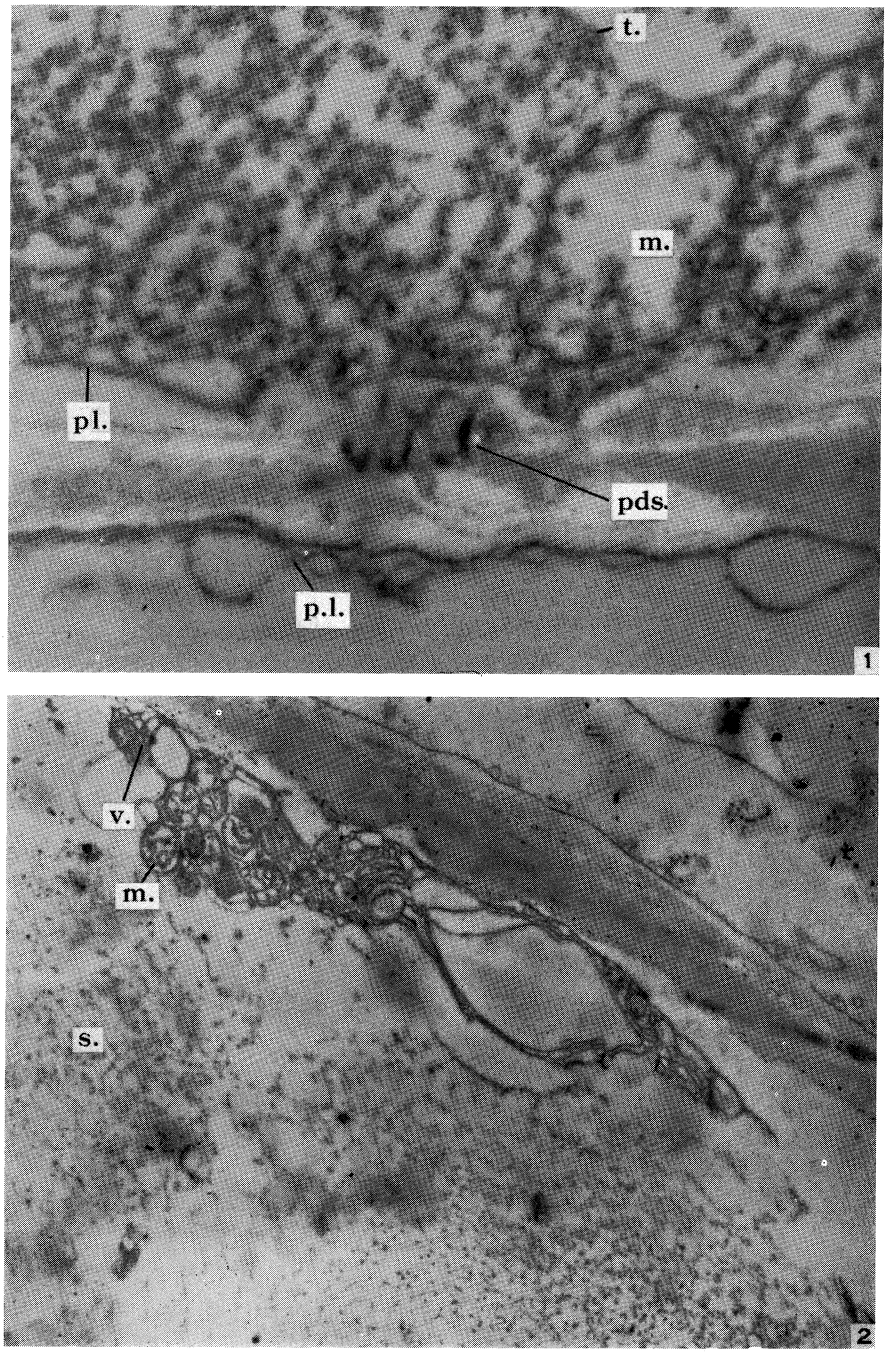

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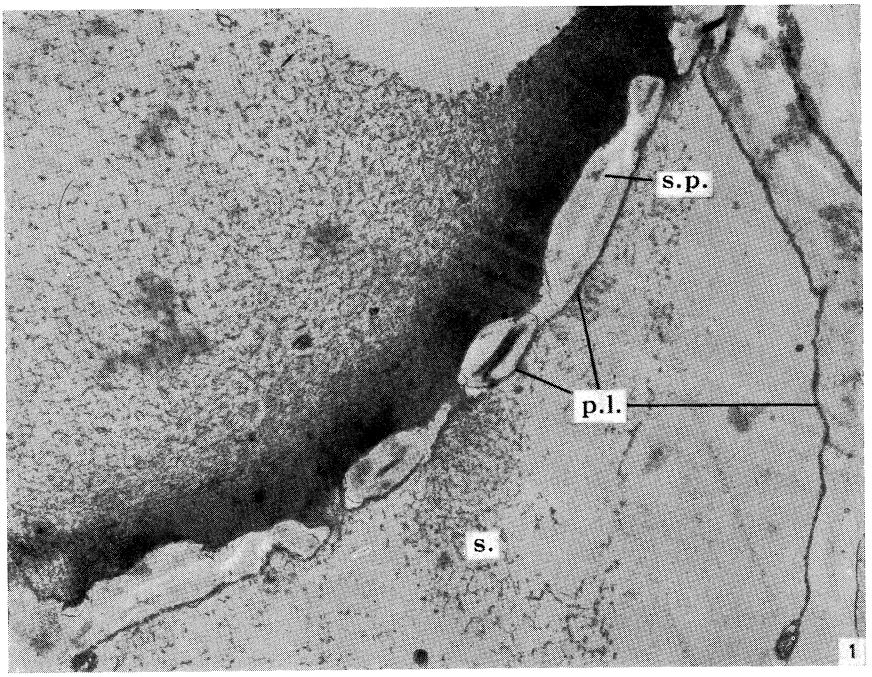


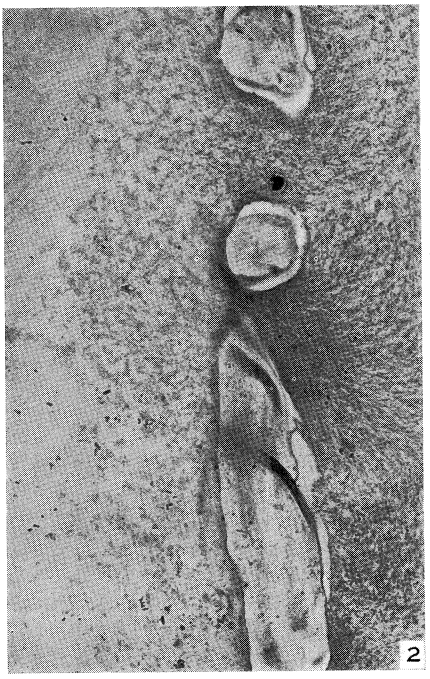


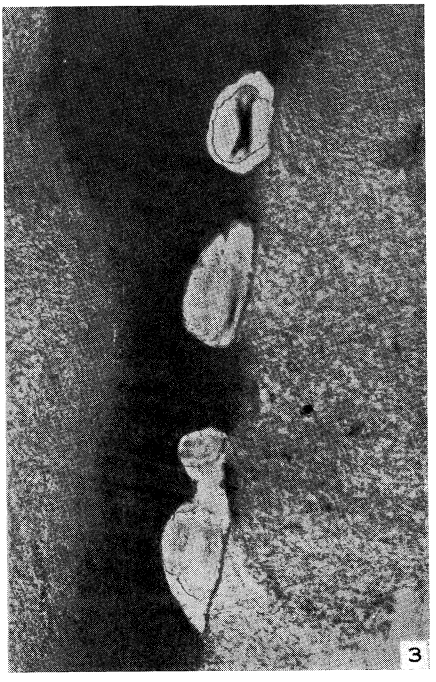

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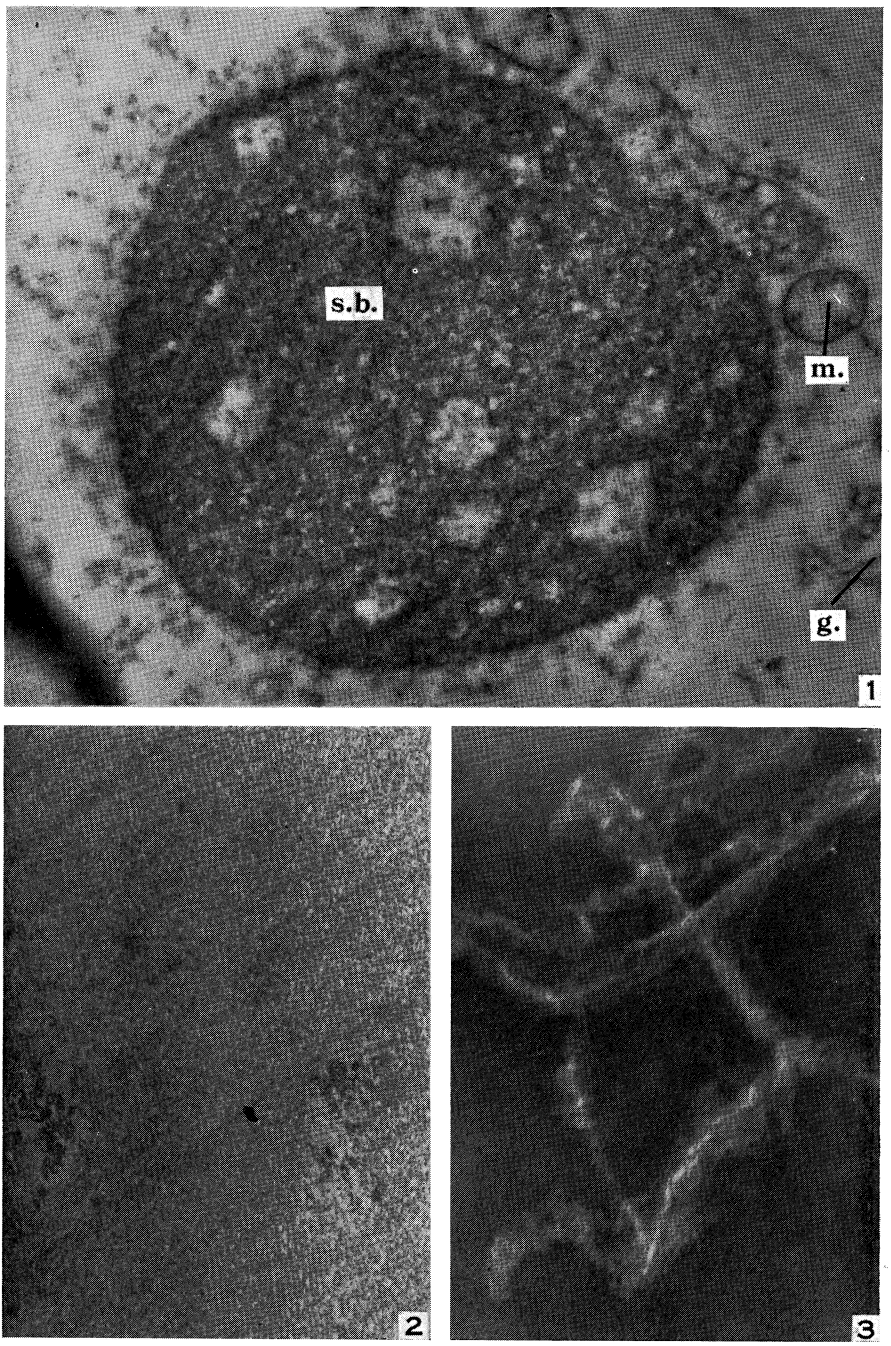

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