STRUCTURE AND FUNCTION IN THE SIEVE TUBE - AN ELECTRON MICROSCOPIC STUDY.

By

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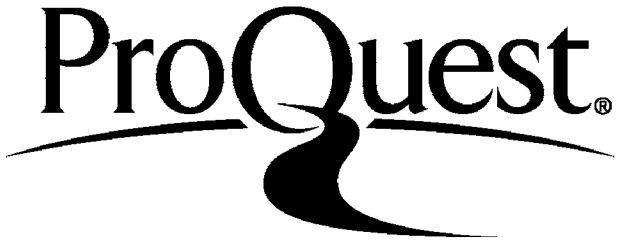

ABSTRACT

This thesis starts with a brief historical background to the modern study of translocation and discusses how different hypotheses of its mechanism have emerged and taken their present shape. This is followed by a review of the literature on the ultrastructure of the phloem tissue (especially the sieve tubes) down to the present year, with an emphasis on the state of the normal functioning sieve plate. At the moment a strong difference of opinion exists about this: The Californian School have argued the view that the sieve plate pores are normally 'open', while the contrary view has been maintained as energetically in London. The work reported consists of an investigation mainly of the sieve tube ultrastructure of Helianthus annuus hypocotyl and Saxifraga sarmentosa stolon. Material of these excised in 1 mm slices by twin cuts direct into fixative invariably showed the pores filled with slime fibrils. No open pores were ever encountered. The pores sometimes had callose cylinders and were sometimes without, but this seemed to make no difference to the degree of compaction. In a second approach the plant axes were plunged into boiling water. Subsequent excision, fixing and e.m. examination indicated that the pores were filled with coagulated slime. This indicates that the filling of the pores is not an enzymatic reaction artefact. It also discounts the view that it is due to turgor release. Finally material was wilted for several hours prior to surgery and fixation, contrary to the

findings of the Californian school, the pores still appeared filled.

The slime fibrils of both species have the typical banded structure and tend to 'crystallise' in the pores or when close together. Tubular material was rarely found, but membranous aggregates of several types were frequent.

Low power surveys embracing several sieve tubes at once revealed a similar polarisation state in all; and photo-montages of whole sieve tubes indicated the relationships of the centre and plate regions of functioning elements. On the whole, the work reported does not support the pressure-flow hypothesis, nor the tranvacuolar streaming theory.

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V O L U M E I.

I N T R O D U N T I O N

THE PROBLEM OF TRANSLOCATION

The long distance movement of water, minerals and metabolites within plants via the xylem and phloem was one of the earliest problems of plant physiology. Discovery of blood circulation by Harvey (1628) encouraged the study of translocation in plants. Malpighi (1679) by ringing plants demonstrated that nutrient sap flows both upward and downward in the stem perhaps through the vessels. Hartig (1837) described the sieve tube and found a clear distinction between the two vascular systems of the plant, the xylem and the phloem. Later on in 1858 Hanstein proposed that downward transfer of the organic assimilates takes place in the phloem. Because of the opposition it met from the eminent physiologist Sachs this important suggestion of the role of the phloem as the pathway for the movement of organic assimilates failed to receive the attention it deserved. Hanstein (1860) showed by ringing plants having collateral, bicollateral and scattered bundles that the phloem alone is probably responsible for conducting organic materials from leaves to the point of utilization.

The discovery of sieve tubes and the growing conviction that their role was conducting organic assimilates raised the question about a definite mechanism of translocation. Many hypotheses and theories which have a history of their own, have so far been put forward to answer this most interesting

and controversial problem of plant physiology.

Sachs (1863) distinguished between carbohydrate and nitrogenous compounds as to their channel of movement. He suggested that nitrogenous compounds move through the sieve tubes and carbohydrates move by diffusion alone through parenchyma tissues. De Vries (1885), on the other hand, realizing that diffusion was too slow to account for the rapid conduction of carbohydrates suggested that protoplasmic movement offered the best explanation. Birch-Hirschfeld (1919) found diffusion and protoplasmic streaming inadequate for translocation. She suggested the xylem as an alternative pathway for the backward movement. Dixon and Ball (1922) carried on their investigation. They measured the velocity of translocation as half a meter per hour, hence they rejected the sieve tube as a conduit of translocation and believed that the young tracheae of the xylem are the pathway of transport. As this suggestion does not have any real evidence in support it was short-lived. Mason and Maskell (1928), working on the cotton plant found strong reasons to believe that sugars travelled in the sieve tubes and that the rate of transport was proportional to the gradient of sugar concentration in the phloem. They found the sieve pores blocked with cytoplasm. They believed that the contents of the sieve tube would prevent a pressure flow and that the rate of streaming was too slow for translocation. They suggested that translocation occurs by means of "activated diffusion".

The conception of transport took a new shape when Ernst Münch (1927), after studying exudates from cuts into the bark

suggested a "mass flow" inside the sieve tubes. He described a physical system in which a maintained concentration gradient between two osmotic cells causes a mass flow of solution from the higher to a lower concentration through a connecting tube, water exchange taking place through the semipermeable walls of the cells. He applied this system to plants assuming that photosynthesis maintains the concentration difference between assimilating cells and storage or meristematic tissue, the sieve tubes acting as the connecting elements.

There are three basic requirements to make this mass flow theory work :-

(a) Impermeability of the cytoplasm along the sieve element walls to solutes;

(b) Vacuoles continuous through the sieve plate pores or at least cytoplasm on the plates with high permeability to the translocated solution;

(c) The turgor pressure gradient must be positive (i.e. negative in the strict mathematical sense) in the direction of flow.

General observation of the sieve plate does not usually show the required vacuolar continuity through the sieve plate pores. Moreover Münch's is virtually a "non-physiological" theory, whilst the phloem tissues themselves seem to have a fairly high physiological activity. Both of these objections have been met by Spanner (1958) in his electro-osmotic theory.

According to the electro-osmotic theory flow of water

accompanied by solutes through the sieve pores is brought about by polarisation of the sieve plates due to active uptake of an ion (probably potassium) on one side of the plate and release on the other side. This uptake could be effected by the adjacent phloem companion cells or by the sieve tube membrane. This unequal absorption and release of a charged ion would create an electrical gradient which would produce a unidirectional flow through the sieve pores as a consequence of the movement down the gradient of the ions. According to Spanner (1958) there are three critical requirements for electro-osmosis :-

(a) A charged membrane;
(b) a membrane potential, that is, a difference of potential between its two sides;
(c) a porous structure in the membrane coarse enough to allow the passage of highly hydrated ions without being so coarse as to allow them to pass out of range of the electric field of its fixed charges.

Spanner suggested that in functioning sieve tubes the sieve plate itself constitutes such a membrane, the electro-osmotic forces being developed in the interstices between the fibrils of the dense "slime" which apparently fills the sieve pores. Bowling (1968) working on excised Vitis vinifera, demonstrated that electrical potential differences in the same direction exist across successive sieve plates in the primary phloem. The potential difference was measured by insertion of micro-electrodes in the sieve tubes. The values observed which

ranged from 4 to 48 mV, were calculated to be of the right order of magnitude to maintain an electro-osmotic flow as postulated.

The protoplasmic streaming hypothesis which was first propounded by De Vries (1885), was strongly supported by Curtis (1935). Later on Curtis and Clark (1955) renewed it by proposing that strands or layers of protoplasmic substance might be moving from cell to cell through comparatively large protoplasmic connexions across sieve plates.

Cyclosis is commonly observed in different kinds of plant cells and it would be a suitable mechanism for carrying sugar. The very first objection to this hypothesis was that the energy requirements for mass transport by cyclosis are very high (Mason, Maskell & Phillis, 1936).

Thaine has fairly recently extended the protoplasmic streaming hypothesis by means of his transcellular streaming concept. He suggested that the lumina of sieve elements are commonly crossed by cytoplasmic strands which penetrate the end walls. Thaine (1964) further suggested that food particles move within transcellular strands and the particles within a single strand move always in one direction. Nevertheless, the direction may be both upwards and downward in the same sieve tube element in different strands. Thaine believed that the energy required for the movements might be provided in the form of ATP by the mitochondria-like particles which he believed were associated with trancellular strands.

Esau, Engleman & Bisalputra (1963) have maintained that the

strands which Thaine has seen in thick hand-cut sections are nothing but lines caused by diffraction of light from walls out of focus. These lines are visible in dead cells also. Thaine however, has controverted this.

Some more hypotheses which have little or no support at the present time include the proposal of Van den Honert (1932) who suggested a mechanism for rapid interfacial movement along a diffusion gradient. In support he demonstrated a model by placing two layers of immiscible liquids and introducing a surface active solute. A velocity was measured by the movement of coloured regions 68,000 times greater than of simple diffusion. He suggested a vacuole-cytoplasm interface as the possible site of such movement. He believed that solutes such as sucrose absorbed at the interface would lower the surface tension and the motive power would be maintained by the removal of solute molecules at the lower end of the interfacial path.

The objection to this theory was that the phase boundaries of typical phloem cells would provide insufficient surface area to account for the known quantities which are translocated, and also that sugars and other assimilates are not surface active.

The "active diffusion" theory which was originally proposed by Mason and Maskell (1928) has been adopted and modified by Kursanov (1956a, 1956b) who in two consecutive review articles described the work done in Russia on translocation. He proposed that movement occured as "an active transfer of molecules themselves due to peculiar biochemical activity of the conductive cells". He was unable to elaborate in detail

any mechanism operating on these principles; consequently it is difficult to criticise his conception. He believed that translocation was based on the principle of "push" whereby substances start moving throughout the length of the moving path. He suggested that conducting tissues of plants are capable of oxidation and reduction reactions of carbohydrate phosphorus metabolism, This he thought ensured the mobilization of energy required. In all important respects the whole process seems quite far away from simple diffusion.

Different theories so far have been brought forward, and all of them have some evidence in support; but it is still difficult to say which one is adequate to take the responsibility of explaining this complicated and highly controversial problem of plant physiology. Various techniques of research have been applied by different workers. Old methods of ringing, treatment in oxygen-free atmosphere, use of inhibitors etc., now joined by such modern techniques as analysis of sieve tube sap obtained as an exudate from cut phloem and phloem punctured by mouth parts of insects, labelled tracers, autoradiography and electron microscopy. These modern methods have made fundamental contributions to our knowledge about structure, development and functioning of sieve tubes.

One of the most desirable requirements for any theory on mechanism of translocation is to throw reciprocal light on the changes involved in the differentiation of the sieve tube, but the very unusual sensitiveness of the protoplast and the fragile nature of cytoplasmic strands and other sieve element inclusions make it difficult to say if appearances in the sieve

elements are artificial or if they represent natural forms of degeneration during the ontogeny of the sieve tube.

It is quite possible that electron microscopy can often lead to misleading results owing to poor techniques. It is not necessary to suppose that all fixatives act alike. Thus, $KMnO_4$ distorts many protein structures (Johnson 1966) and crystals forming in vitreous ice disrupt the fine structure, (Mishra and Spanner 1970). The controversy is consequently still continuing about the structure of the sieve element protoplast. Hence the following questions posed by Weatherley and Johnson (1968) are still to be answered in a final sense :-

(i) At what stages during their ontogeny do sieve tubes translocate most effectively ?

(ii) Do undisturbed functioning sieve elements contain cytoplasm or other material within the pores of the sieve plates, or is material which is often seen in the pores blown into them while they are being prepared for electron microscopy ?

(iii) Does such material continue across the lumina of functioning sieve elements as transcellular strands ?

(iv) What is the structure and what are the dimensions of such materials - before it has been fixed, dehydrated and embedded for electron microscopy ? (If these dimensions were known then the resistance offered to any movement of substances through the sieve tubes might be calculated).

(v) Does protoplasmic streaming or other movement

which depends on locally expended energy of metabolism occur in sieve elements ?

In the present work the attempt has been made to answer the first three of the above mentioned questions, working mainly on two species of unrelated families, Helianthus annuus and Saxifraga sarmentosa. The evidence collected is discussed mainly in relation to the electro-osmotic theory of translocation. In the light of this theory this work emphasises the relation between ultrastructure and function of the sieve tube and omits many of the physiological suggestions on translocation which seems to contribute little new insight regarding the detailed mechanism. Before the results of the present work are presented, however, a brief review is given of the findings of other workers in the field of phloem ultrastructure over the past fifteen years.

RECENT LITERATURE ON PHLOEM ULTRASTRUCTURE

The use of electron microscopy in research on sieve element structure has revealed details that increase greatly our understanding of some of the peculiarities of these highly specialized cells. Most of this work has been carried out in the last fifteen years; before that whatever had been shown had added little information to previous knowledge gained with conventional microscopy. Huber & Kolbe (1948) were the first to attempt to examine sieve tubes with electron microscopy. This was before the invention of the glass knife and the use of plastic embedding media. Volz (1952), on the other hand used macerated material of Betula spp.

The first real advance was in the year 1955, when Hepton, Preston and Ripley together studied the ultrastructure of the sieve plate pores. On the basis of the results of their work and presuming that the pressure gradient required would be too high, they rejected the pressure flow hypothesis of Munch. They also questioned the cytoplasmic streaming and diffusion-at-an_interface theories. They emphasised vital processes that might be playing an important part in translocation. Beer (1959) studied the fine structure of Cucurbita and described the mature sieve tube pores which appeared to be open with no indication of membranes across them. He reported on slime bodies, which were bounded with a double membrane, plasmodesmata between sieve tubes and companion cells, and companion cells densely packed with cytoplasmic particles, mitochondria and nucleus. He could not see any mitochondria or endoplasmic reticulum in mature sieve elements. Schumacher

& Kollmann (1959) working on Passiflora coerulea observed the sieve tube protoplast which, they reported, contained mitochondria, Golgi-bodies, plastids, starch grains and a tonoplast. In their opinion the sieve tube pores were plugged homogeneously with microtubular cytoplasm.

Buvat (1960) studied the ontogeny of sieve tubes in Cucurbita pepo. He did not find any vacuolar continuity between sieve tube elements. The densely-packed pore fibrils which range from 80 to 150 A in diameter led him to discard the pressure flow theory. Hepton & Preston (1960) studied and compared various species of angiosperm and gymnosperm. They confirmed that sieve plate pores were filled with cytoplasm and there was no vacuolar continuity between sieve tube elements. They believe in some form of active transport. They had fixed their specimens after releasing turgor pressure by pre-immersion in sucrose solution.

Kollmann (1960) further studied phloem in Passiflora coerula and described the sieve plate pores as plugged densely with"cytoplasmic threads". He further described the plastids as having a simple structure with two outer membranes and starch grains. The mitochondria were smaller in size and fewer in number than elsewhere. He did not agree with the idea of a passive mechanism of translocation. Ziegler (1960) prepared his specimen of Heracleum Mantegazzianum by first freezing in liquid air before fixation and concluded that sieve pores are filled with solid cytoplasm, there were no intact mitochondria nor is a tonoplast present. The cytoplasm which exhibited a specific structure of lamellar form was continuous into the sieve pores and corresponded to

endoplasmic reticulum.

Duloy, Mercer & Rathgeber (1961) working together on Cucurbita pepo described sieve tubes as having a thin parietal layer of cytoplasm composed of one to several membranes, dispersed fibrillar slime throughout the lumen and scarcity of mitochondria and plastids. Sieve plate pores they presumed were open with a thin cytoplasmic lining. They were of the opinion that sieve elements are metabolically inert and vacuolar continuity forms an open conduit. Esau & Cheadle (1961) working on Cucurbita maxima got some variable results for the structure of the sieve plate pores. "Some views" they declared, "suggest intervacuolar connection between sieve elements.....; others show solid connecting strands". They emphasised that slime in the pores may be an artifact and it may be either vacuolar or cytoplasmic in nature. Kollmann & Schumacher (1961) continuing their work on Metasequoia glypt ostroboides, claimed that the dormant phloem (January) has dense cytoplasm with numerous tubules. In a rather different context, Parker, Johnson & Philpott (1961) observed in the brown alga Macrocystis pyrifera, "sieve plate" pores blocked with dense material resembling endoplasmic reticulum. Esau, Cheadle & Risley (1962) described the development of pores in the sieve plates of certain dicotyledonous young sieve elements, after the disaggregation of the slime body. At an early stage of development, the site of the future sieve pore is covered with cytoplasmic material and is marked by opposing deposits of callose in the form of platelets, one on either side of the sieve plate. Endoplasmic reticulum becomes applied to the pores site and is associated with the callose. Perforation

occurs when the callose platelets fuse and break through the fused part. Approximately at the same time the nucleus disappears and slime bodies disaggregate. They fixed their material with potassium permanganate and embedded it in methacrylate.

In further papers Kollmann and Schmacher (1962), described their results after fixing Metasequoia glyptostroboides at various times of the year. They observed that the cytoplasmic threads in sieve areas were five to six times broader than the plasmodesmata in pitfields but had the same structure, i.e. they are lined with plasmalemma and traversed by numerous tubules of endoplasmic reticulum. The phloem tissue which they fixed in spring appeared to have a loosened protoplast in its cells. There were remarkable changes in endoplasmic reticulum whose thin tubules become dilated, branched and expanded and later disaggregated into vesicles. Mehta and Spanner (1962) studied the fine structure of the sieve tubes of the water plant Nymphoides peltatum. They described the prominent nacreous wall, specialised endoplasmic reticulum, oriented spirally and becoming finer near the sieve plate. Sieve plate pores, they declared were filled with solid, densely staining material that appeared to involve endoplasmic reticulum tubules.

Buvat (1963 a, b, c & d) worked further on Cucurbita pepo and published his results in four consecutive papers covering different aspects. He described the tonoplast as disappearing on maturation of the sieve tube and the perforation of the sieve plates as happening after disintegration of the nucleus. He claimed that there was RNA present in the slime bodies

which resembled the nucleolus; hence the slime bodies should be regarded as cytoplasmic. He described the sieve plate pores as filled with cytoplasmic substances. He suggested that movement occured within a cytoplasmic phase and that sieve tubes possessed polarity even prior to perforation of the plates. He did not favour the passive pressure-flow mechanism for translocation.

Englemann (1963) studied Impatiens sultanii and observed the sieve plate pores containing fibrils which were 100-120 A in diameter while in the lumen their diameter was 170-250 A. Some pores contained 35-125 A diameter light regions and in some places fibrils were degraded into an amorphous material ($KMnO_4$ fixation?). He concluded that dense pore-filling substance in the sieve plates was fibrillar rather than membranous and the absence of enzymes and membranes made him believe that the slime was not cytoplasmic but vacuolar.

Eschrich (1963) (a) studied the callose in the primary phloem of Cucurbita ficifolia. He reported questionably that hexose phosphate is abundant in the sieve tube sap; slime bodies appeared fibrillar or microtubular (tubules up to 170 A in diameter). He concluded that material in the pores contained lipoproteins and reticulum and was continuous with similar material in the lumen. Again Kollmann & Schumacher (1963) studied the fine structure of connecting strands in Metasequoia glyptostroboides at different stages of growth between March and October. They confirmed their previous results (1962a). They remarked that connecting strands differ in size and probably in fine structure from some highly specialized angiosperms sieve tubes. Falk (1964)

reported that in Tetragonia expansa, plastids contained a dense ring-shaped inclusion. He remarked that sometimes plastids broke down to release these inclusions which disintegrated into fibrillar slime. Kollmann & Schumacher (1964) continuing their work on Metasequoia, studied the changes in sieve cells during the period March to November. They declared that during differentiation a remarkable increase in endoplasmic reticulum occured. At the maximum extension of endoplasmic reticulum, the tonoplast and dictyosomes disintegrated. They further declared the sieve cells functioning at their earliest stage of development. Kollmann (1964) again in a review on sieve cells of species of conifers explained that there were "essential differences" between coniferous sieve areas and sieve plates of highly specialized angiosperms. He was uncertain if the fine structure of cytoplasm in sieve plate pores was tubular or fibrillar.

Behnke (1965 a, b & c) studied the conducting cells in the Monocotyledonous genus Dioscorea. He reported a network of fibrils which were about 100A in diameter sometimes accompanied by tubules of the ER, and which extended over the entire lumen and also through the sieve plate pores. Present also in differentiated sieve tube elements was a lattice-like body resembling the Heitz-Leyon crystals of young plastids. These he further reported on in 1968. The same year (1965) Bouck & Cronshaw contributed a study on the fine structure of differentiating sieve elements of Pisum sativum. They described the sieve plate pores as plugged with a finely fibrous material, which was believed to be slime possibly synthesised by ER. What seems a significant observation in

the light of later suggestions of Spanner & Jones (1970) is that the sieve tube reticulum is composed of flattened cisternae "usually attached by their lateral margins perpendicularly to the surface of the wall". The plasmalemma of the sieve tube was correspondingly rarely demonstrated convincingly. In young sieve tubes the layers of the wall adjacent to the cytoplasm showed up markedly darker than the corresponding layers of the companion cell wall.

Engleman (1965 a & b) continued his studies on Impatiens sultanii, with particular reference to the callose reaction and to sieve tube ontogeny. His findings relevant to the present study are that the mictoplasm (the result of the disintegration of the tonoplast and mixing of the cytoplasm and vacuolar contents) is continuous through the pores, with the plasmalemma. He emphasised that in well preserved sections the starch grains should be enclosed in their plastids, the latter congregating in a lateral position at both ends of the elements. His sieve plate pores were plugged sometimes densely, sometimes hardly at all. This may be related to the use of $KMnO_4$ as fixative, alone or with formaldehyde. Also in 1965, Esau reported that the plastids in the sieve elements of Beta vulgaris contained a ring of proteinaceous fibrils which were not visible when the specimen was fixed in $KMnO_4$-- fixation with glutaraldehyde and Osmium gave satisfactory identification. The same year, after studying thirty species of Vitis, she gave a comprehensive account of the anatomy and cytology of its phloem. She declared that during dormancy the sieve plates were occluded by massive deposition of callose which during Spring was

removed by an enzymatic process, or possibly connected with the proteinaceous slime. Esau & Cheadle (1965) collaborated in a major paper the same year and published their results on various species of unrelated genera, principally Cucurbita. Unfortunately nearly all of their material was fixed in potassium permanganate; nevertheless the sieve plate pores very frequently showed some degree of plugging. The authors give an account of the development of the sieve plate pores but do not venture an opinion on the question of mechanism. They seem, however, to favour a mass-flow concept.

Evert & Murmanis (1965) the same year studied Summer and Winter samples of secondary phloem of Tilia Americana. They described mature enucleate sieve elements as lacking endoplasmic reticulum, tonoplast and other organelles except for a few mitochondria. They confirmed that connecting strands of sieve areas differ structurally from plasmodesmata; the sieve plate pores are traversed by slime strands composed of fine fibrillar material (when fixed by gluteraldehyde and osmium). This material was organised into coarser strands which crossed the lumen and connected sieve areas; possibly it has an enzymatic function connected with translocation. Certainly, their work revealed sieve plate pores in this species to be densely plugged.

Continuing the work of 1961 on Macrocystis pyrifera, Parker & Huber (1965) described the fine structure of sieve tubes of this alga with improved techniques. Fixation was in various fixatives including osmium and glutaraldehyde, with or without previous freezing in liquid nitrogen. They

concluded that sieve tubes are possibly metabolically independent as they lack companion cells or any other such connected cells and possess numerous organelles such as mitochondria and plastids with internal lamellae. Their findings on sieve plate pores were not clear cut; they decided that they were occupied with fibrillar material but scarcely more densely than the lumens. Callose was present.

Wark & Chambers the same year (1965) reported on the secondary phloem of Pisum sativum. Material was fixed by introducing fixative into the pith cavity; an osmium-dichromate fixative was used. The study was ontogenetic, and no firm comments were made on the state of the sieve plate pores in "mature" elements since the authors decided that the functional stages were probably earlier ones. Their diagrams, however, indicate close plugging in the "mature" stage. Endoplasmic reticulum is prominent and of a characteristic form. The crystalline protein mass characteristic of Phaseolus and other Leguminosae they referred to as a "slime body"; "fibrotubular bodies" were also present. Nothing corresponding to Thaine's transcellular strands was seen. Trabeculae were reported as prominent ingrowths of the companion cell wall, apparently rich in membranous material.

Two papers by Wooding & Northcote (1965 a & b) noted a sheathing of the plastids by endoplasmic reticulum, temporary in sieve tubes but permanent in companion cells. The plasmodesmata between sieve element and companion cell has a characteristic form, much branched on companion cell side.

In a short but valuable review by Esau the following year (1966) she accepted that a satisfactory interpretation was not yet available for translocation of organic materials in the phloem. Although she believed that physiological data supported the interpretation of mass flow, she agreed that anatomical evidence was rather against it. In a mainly ontogenetic study of Cucurbita primary phloem (Evert, Murmanis & Sachs, 1966) the authors noted that the mature sieve element was lined with a "plasmalemma one or more cisterna-like layers of E.R. and a membrane which apparently delimits the parietial layer of cytoplasm from a large central cavity". This was apparently however, not the tonoplast. The abundance of membrane material here is noteworthy. Numerous fibrous strands, derived from the slime-bodies run from cell to cell through the pores of the sieve plate, which are "lined with cytoplasm". Many pores are traversed by slime early in their development, although the state of the mature functioning sieve plate pores was not enlarged on micrographs seem to suggest that it was one of only moderately-dense plugging. Slime substance was found in the young companion cells.

Published work on phloem anatomy was by now becoming quite a flood. Johnson (1966) noted the unreliability of $KMnO_4$ fixation; it gave a precipitate with sucrose. LaFleche (1966) reported on the flagellate body of the sieve tubes of Phaseolus. She found that this finely-striated crystalline protein body disintegrates into fine fibrils as the elements mature, and she suggested a reserve food function. Murmanis & Evert (1966) investigated the ultra-structure of a gymnosperm, Pinus strobus. They reported that

here also, as in Cucurbita, the slime bodies were bounded by twin membranes. The mature sieve cell, is lined by plasmalemma and E.R. and the sieve plate pores were also traversed by numerous endoplasmic membranes. On the whole, however, fixation and sectioning were poor.

Better results were presented by Northcote and Wooding (1966) for Acer. In an able ontogenetic study they described in detail the relationship of the E.R. to the development of the sieve plate, and the origin of the fibrillar slime from granular bodies. Very relevant to the present work was their finding of pores densely plugged with fibrillar material continuous with fibrils in the lumen. The function of the latter "remains obscure". If longitudinally oriented it would offer little resistance to flow, and could form a passive skeleton "designed to maintain the plastids and mitochondria" in position. Alternatively, the material could be "capable of sol-gel interconversion", mass flow occuring in the sol form. Callose formation, they suggest, compresses the fibrils in the sieve plate pores and so gives a wrong impression. Lamellar stacks were figured.

Several other papers appeared in 1966. Srivastava and O'Brien reported some work on the phloem of the gymnosperm Pinus strobus. The cytoplasm of albuminous cells is "extraordinarily rich in mitochondria and rough E.R." The strands connecting two sieve cells are complex. The pore is lined with a membrane, and the interior is composed of a dense mat containing sieve tube reticulum (occasionally seen) and other dark staining materials. A good deal of E.R. is present in the sieve cells. Tamulevich & Evert investigated

Primula obconica, the species in which transcellular streaming had been reported by Thaine. Besides the usual findings they noted "longitudinally-oriented slime tubules" in the central cavity of the sieve tubes. These they believed formed strands which connected the sieve plates and penetrated them, but they were quite unlike Thaine's. Slime synthesis apparently continues during perforation of the plates. Lamellar stacks are conspicuous, and the lamellae often curve round to meet the wall perpendicularly. No very definite conclusions appear about the nature of the sieve plate pores; they seem to contain fibrils and reticulum.

Another study on gymnosperms appeared this year; Wooding on Pinus pinea. As the sieve element matures the "sparse rough E.R. is succeeded by a complex meshwork of smooth E.R., and these in turn by aggregations of membranous material, which persist into the mature sieve element". This is interesting in view of the recent suggestions about the sieve element wall. Poor fixation, they suggest, leads to large vesicular material in the sieve element lumen; perhaps this accounts for Kollmann's results with Metasequoia, published before the advent of glutaraldehyde. Wooding remarks that "cross-sections of the sieve area pores shows an empty lumen surrounded by callose under a unit membrane". However, the figure to which he refers shows an electron-dense plug. Presumably he means "empty of vesicular traces". Certainly his best micrograph seems to show densely-plugged pores.

Research papers on phloem structure continued to appear during 1967. Behnke and Dorr described the development of what they re-named "plasmatic filaments" in Dioscorea, and

their spreading over the whole lumen. In the young sieve elements they noted that the ground plasm was extremely rich in ribosomes. They found that the filament in cross-section appeared tubular. It had an overall diameter of 120-150 A. No "banded" appearance seems to have been noticed, except perhaps in a longitudinal section of a plugged pore of Cucumis, (fixed in osmium and embedded in methacrylate, an obsolete procedure). Cronshaw and Esau in an important paper on Nicotiana introduced the term P-protein for slime material in sieve elements. This was regarded as proteinaceous on the basis of cytochemical studies on other species; the authors confirmed it for Nicotiana with mercuric bromophenol blue, (apparently not a very reliable indicator). They distinguished tubular P1-protein (diameter $321 \pm 2{\cdot}5$ A) and fibrillar P2-protein ($149 \pm 4{\cdot}5$ A) into which the former became reorganised. The P2-protein showed the banded structure first encountered apparently by Northcote & Wooding (1966). The sieve plate pores in Nicotiana were densely plugged with P2 fibrils, which the authors suggest, on the analogy of their virus work, represent part of the moving stream. They do not note the objection that this cannot really meet the problem of resistance in the pore, since the protein cannot be moving nearly as fast as the solution. Endoplasmic reticulum was again evident.

Virus behavious in sieve tubes was reported on in two papers by Esau, Cronshaw and Hoefert (1967a & b) on Beta and Nicotiana. Yellow virus particles occur in mature sieve elements scattered throughout the lumen (in parenchyma cells they are confined outside the tonoplast). The particles penetrated the sieve plate pores and also plasmodesmata;

their movement was compatible with a mass flow in the phloem. Tobacco mosaic virus also occurs in sieve tubes as well as other cells, where it penetrates the vacuole as well as the plastids, nucleus and plasmalemma. The evidence was compatible with the view that it could travel in the phloem, though it may do so in the xylem also.

A different topic was provided by the report by Newcombe (1967) of spiny vesicles in slime-producing cells bordering the protophloem elements of the procambial region, though not in the sieve elements themselves. They may accordingly represent another mechanism for the production of P-protein. Parthasarathy and Tomlinson raised a new topic too in their paper on metaphloem in palms. They reported that in these long-lived elements there was an absence of slime. The pore contents stained deeply with haematoxylin; there was no evidence of any transcellular strands; but plastids with typical sieve-tube starch were present.

Wooding published two papers on Acer during the year. In the first on the development of the fibrillar contents of the sieve tubes he supported the view that at maturity the fibrils were dispersed throughout the lumen, rather than being aggregated as strands, as suggested by Thaine. "The reproducibility and definite structure of the fibrils argues against their being purely a precipitation or coagulation artefact produced by the fixative". His micrographs show dense plugs in the sieve plate pores, with very extensive lateral registration of the dark bands. He did not however remark on this, though it is good evidence against the

'turgor release artefact' nature of the plugs. He suggested that the fibrils might play an active role similar to that of fibrillar elements found in streaming cytoplasm of e.g. slime moulds. His second paper reported that curvilinear membrane aggregates formed from E.R. in developing sieve elements. These strongly recall some features of the lamellar formations in mature tubes (e.g. Behnke 1965). He suggested that the formations represent a sequestering of the membranous material in an inactive form. It was apparent from Wooding's micrographs that a definite thickening of the membranes occured during their development.

Considerable research on sieve tube ultrastructure was published in 1968. Behnke reported further on the "lattice-like membrane structures" of Dioscorea following his paper of 1965. At the end of sieve tube ontogeny the E.R. cisternae aggregate to small stacks, the extra"plasmatic space" being continuously reduced. Finally the cisternae become tubuli (220-250 A diameter) twined like helices, forming the subunits of the lattices. The internal diameter of the tubuli is about 80-100 A, and the space is continuous with the intracisternal spaces of adjacent E.R. Plasmatic filaments are associated with these structures, or at least form similar lattice-like condensations. Other rather coarser forms of curvilinear membrane aggregates also occur. Some micrographs seem to show a connection with the cell wall.

Crafts contributed a short paper on the problem of sieve tube slime. It is interesting as coming from a convinced supporter of the Münch hypothesis. He believes that "recent work with the electron microscope indicates that the

pores of functioning phloem are normally open". He distinguishes between the "filamentous reticulum" of the sieve tube and the slime. The slime is dead material due to break down, pass out in the assimilate stream, and be metabolised. Apparently Esau's P1-protein is in this category. Her P2-protein, the banded fibrils, is not slime, but forms a "stationary, living, filamentous reticulum that persists throughout the functioning life of the sieve element". It plugs the sieve pores of old elements "long after the slime has been washed out by the assimilate stream". There are thus three mechanisms bringing about sieve-plate plugging: (1) Callose formation, reversible in early stages; (11) slime plugging which occurs rapidly in young newly differentiated sieve tubes; (111) filament plugging, responsible in the older functioning plates. Further, filament plugging can occur by three methods: (1) turgor release may cause an elastic contraction of pores; (11) callose may be rapidly laid down, constricting the pores, (111) there may be a mechanism for agglutination of the filaments so that they hydrate and swell. He seems to agree that the plugging with banded fibrils is not caused by turgor release "tearing the meshwork free from the parietal cytoplasm."

In two further papers Cronshaw & Esau (1968 a & b) elaborated their views on P-protein. The first dealt with the development of P-protein bodies. In <u>Cucurbita maxima</u> two distinct types of P-protein bodies are formed; a larger composed of fine fibrils and a smaller of 242 A diameter tubules. This latter appears identical to the P1 material

of Nicotiana, and the tubules can be arranged in a regular square array. The other type of body consists of aggregates of fine fibrils and has been designated P3. It can pass into a tubular form (179 A diameter) designated P4. The slime bodies have no membrane, and do not seem to be associated with ribosomes. The second paper carried the observations as far as the mature sieve elements. Normally the slime bodies disperse, but in extrafascicular sieve elements they frequently fail to. The appearance of the mature plate reflects this; where the bodies disperse the sieve pores usually have considerable callose and are filled with P-protein, whereas where the bodies do not disperse they show little or no callose and are essentially open. The micrographs presented show numerous plates with varying degrees of plugging of the pores.

Currier and Shih (1968) gave a report early in the year of the phloem of Elodea leaves. The conclusions were not startling, and evidence for or against normal blocking of the sieve pores was inconclusive. Two papers by Eschrich and Steiner (1968 a & b) concerned the conducting tissue of the Moss Polytrichum. Assimilates had previously been shown to move in this at a high rate (32 cm per hr.). The conducting cells (leptoids) have end walls which show strong fluorescence with callose stains (aniline blue). The young leptoids contain long E.R. cylinders extending between the end walls, which have numerous plasmodesmata. Later these elements lose their organisation, the E.R. and other structures become deformed or dissolved, and the plasmodesmata are constricted by callose. "Thus assimilates moving upward

may penetrate the whole lumen at lower levels, but they are restricted to the cisternae of the E.R. at higher levels..". There does not appear to be any mention of slime.

Three further papers from 1968 remain to be reviewed. An early one by Esau & Cronshaw gave an account of the sieve tube E.R. of Cucurbita. This exists in two forms; one is a network closely applied to the plasma membrane and is possibly continuous through the sieve plate pores and associated with the plasmodesmata; the other, less abundant, consists of stacked membranes. These sometimes, apparently, are continuous with dark lines in the cell wall, and the authors limit themselves to only a "tentative interpretation" of the stacks as E.R. A paper by Johnson (1968) presented the first account of freeze-etching applied to sieve elements. The technique is difficult, and the introduction of glycerol to reduce crystal structure apparently caused plasmolysis and perhaps additional callose. Banded fibrils do, however, appear in the pores, but there is no suggestion of any strands as suggested by Thaine. In chemically-fixed sections tubular material (240 A diameter) appears to fray out into the banded fibrils; pores in sieve plates were sparsely filled. Finally, an interesting paper by Wooding described the phloem of Pinus pinea callus tissues. This can be considered along side a following paper (1969) on Nicotiana callus. In both the tissue is very similar to that in the intact stem. In Pinus callus little callose is formed, and no trace of any fibrils similar to those common in angiosperms elements. In Nicotiana P1 protein microtubules (220-240 A diameter) are prominent and may be formed within rough E.R. The mature sieve pores contain P1 tubules but never P2 fibrils, which occur only rarely. Colchicine treatment does not affect the P1 tubules, although it greatly reduces the number of microtubules; the two seem therefore to be different. The P1 tubules may have lateral connections to one another and to the plasmalemma. The presence of P1 tubules rather than P2 fibrils

in the pores may be correlated with the low level of translocatory activity. In so far as the author favours a mechanism of translocation it is that of Crafts (1968).

Further work was reported in 1969. A paper on the sieve plate pores of Nicotiana was contributed by Cronshaw and Anderson (1969) which concluded that the functioning pores were probably open. Fixation was by various aldehydes, and by three methods, one of which involved freezing of the whole plant in liquid nitrogen. Glutaraldehyde and glutaraldehyde-formaldehyde fixation showed filled pores. Acrolein-fixed material showed filled pores usually, with sparsely filled pores occasionally. After rapid freezing, by contrast, many pores were open or only loosely filled, but fixation certainly appeared inferior. There was less callose. Inconsistently it seems the authors decided that their results provided "evidence in favour of open pores in functional sieve elements". A second paper of theirs (1969) on the effects of pressure release came to the same conclusion. The effects of turgor pressure release was vividly shown in the formation of slime plugs, the rupturing of plastids, and the blocking of the pores by starch grains. Again, it seems that the authors have misinterpreted their data and that their evidence indicates that functioning pores should be regarded as plugged with slime fibrils.

Three papers by Behnke (1969a, b & c) appeared during the year on phloem structure. The first dealt with the sieve

tube plastids of monocotyledons. Twenty four species from twenty one families (except one) all possessed plastids with cuneate proteinaceous bodies. Many monocots also had starch grains in the plastids. Sieve tube plastids of Nuphar and Nymphaea contained starch only. The second was more relevant to the present study and dealt with sieve-tube differentiation in monocots. The sequence of events is fairly similar to that in dicots. Behnke notes that the uniform rough surfaced E.R. in young elements changes to a tubular or vesicular form and partly fuses to a lattice-like array. Polar dictyosomes produce coated vesicles besides smooth Golgi vesicles. Neither dictyosomes nor ribosomes are seen in differentiated sieve tubes. There is no evidence on mature sieve plate structure. The third paper discusses the "plasmatic filaments" and the sieve plate structure of some monocotyledons and Nuphar. In Musa the first filaments seem to condense out of finer ones which become arrayed in bundles. Following disintegration of the tonoplast, these change from 160-200 A diameter to others of 80-120 A in the mature tubes. Tamus also contains striated filaments whereas Nuphar still has tubular ones (150-180 A diameter) at this stage. Sieve plate formation seems fairly normal; "open pores of Musa, Nuphar, Tamus and Tinantia are crossed by plasmatic filaments that are equally distributed in carefully fixed pores without callose". Some of the micrographs shown reveal pores only sparsely plugged; others are tightly plugged.

Johnson (1969) reported some interesting observations on

Nymphoides phloem. Fibrils about 0·1 micrometres diameter were found to be composed of parallel filaments and in transverse section seemed to consist of a lattice of parallel tubules. Some of the fibrils were attached to complexes of membranes which recalled the lattice-like structures of Dioscorea (Behnke 1965, 1968) and Acer (Wooding 1969). Although he did not draw attention to it the sieve tube wall shows marked radial striations in his micrographs. He shows numerous lamellar stacks. The banded fibrils seem to fray out from the tubules, recalling Esau and Cronshaw's P1 to P2 transition. Sieve plates are not illustrated.

Shih and Currier (1969) published a short paper on cotton phloem, and their conclusion was that "when special precautions were taken to permit killing and fixation of sieve elements before they were cut sieve pores were found to be open". This conclusion seems veryquestionable, since it is not given any statistical support and is evident apparently only with very special treatment - longitudinal slits in the continuous hypocotyl to permit lateral entry of fixative into the tubes, and acrolein-glutaraldehyde fixation. If glutaraldehyde alone was used, the pores were slime-filled. So were they when fixation was by either fixative on 3 mm lengths excised out by two simultaneous cuts. "Thus both cutting and slow killing seem to contribute to the formation of slime in the sieve pore". This leads to the unlikely suggestion that the plugging is due to an enzyme reaction.

An elegant paper of Steer and Newcomb (1969) concerned the P-protein in Coleus. The earliest appearance of the

tubular slime is associated with spiny vesicles in phloem parenchyma cells. These are similar to those reported in Phaseolus (Newcombe 1967) and are believed to contribute to slime formation. The tubules become associated into a single spindle-shaped body, sometimes showing a highly-organised hexagonal structure. Subsequently the tubules (200 A diameter) are converted to 70 A banded fibrils sometimes with their bands very regularly aligned. Later these become dispersed through the cell lumen. The authors do not assign P-numbers to the various forms, perhaps interconvertible, as they consider this premature. They think that the tubules are not converted to fibrils in one stage; the process seems to be via tubules of intermediate diameters and progressively thinner walls.

This brings the review to the present year. Of papers published this year three from this laboratory (Mishra and Spanner, 1970; Siddiqui and Spanner, 1970; and Spanner and Jones, 1970) have argued strongly for the normal state of sieve-tube pores being the plugged one. Another, from California (Anderson & Cronshaw, 1970) has argued equally strongly for the opposite state of affairs.

MATERIALS & METHODS

MATERIALS

The present work is based on the study of ultrastructure of the sieve tubes of the following four plants :-

1. Helianthus annuus
2. Saxifraga sarmentosa
3. Phaseolus vulgaris
4. Heracleum Mantegazzianum.

In the beginning work was started on Heracleum but experience proved that this was not an easy plant for a beginner in electron microscopy to commence with. It involved three main difficulties. Firstly, owing to the sheer size of the axes the vascular strands had to be pulled out from the ground tissue and then placed in the fixative. Although a middle part of such strands was used for study, the tearing out is liable to cause some damage to the delicate phloem tissue. This is avoided where the tissue can be fixed without such forcible isolation from its neighbouring tissues.

Secondly, tough phloem fibers apparently made it difficult to dehydrate and embed it in a satisfactory way leading to breaking up of sections when the blocks were cut. Later it was found that a harder plastic embedding mixture and a diamond knife are more suitable for handling material from this plant. The third difficulty was in the seasonal nature,

limiting work to the Summer only. Consequently attention was diverted to a more suitable plant, Helianthus annuus, easy to grow and not used as a subject before for electron microscopy. Seeds of Helianthus were soaked in water for twenty four hours and then sown in pots of vermiculite. The pots were placed in a constant temperature room (25°C) where light was given for 16 hours out of the day. Pots were watered on a regular schedule, and the plants used for fixation when 10-20 days old and 4-10 inches high.

Subsequently attention was given to Saxifrage sarmentosa. This attracted interest because of its thin stolons which were easy to handle and cut for fixing. A slice of stolon less than 1 mm long was easily removed by two simultaneous end cuts and the small overall size gave the fixative minimal distances to penetrate. Plants were grown in pots in the greenhouse and watered on a regular schedule. They were used when the stolons were reasonably long (about 12 inches) but before the development of any roots on the daughter plant, Segments were excised direct into fixative from a point about 4" distant from the parent plant. Samples were fixed at about 11 a.m., when the plants had been in daylight for at least three hours and photosynthesis and presumably translocation were active.

The work was mainly done on these two plants but Phaseolus vulgaris was also used to confirm the results of boiling the tissue prior to fixation on the appearance of the sieve plate. Phaseolus was grown in the same way as Helianthus.

METHODS FOR ELECTRON MICROSCOPY.

METHOD OF EXCISION

Various fixatives have been used successfully to preserve animal and plant tissue, but workers on the phloem have found it a difficult task to choose a suitable procedure for fixation so as to cause minimal damage to the delicate sieve tube protoplast. The main problem has been the high turgor pressure and the low resistance to longitudinal movement of the contents. To avoid surge damage Ziegler (1960) froze intact phloem bundles of Heracleum with liquid air, then cut them from the plants, freeze dried them, and fixed them in 1 % osmium tetroxide. Mehta & Spanner (1962) amongst others tried to reduce turgor pressure in sieve elements of Nymphoides peltatum by a method similar to that of the plasmolytic studies of Currier, Esau & Cheadle (1955). They placed 12 cm long petiole segments in 0·2 M sucrose buffered with 0·01 M phosphate at pH7 for two hours. This was followed by a similar one hour treatment on the central 5 cm length in 0·25 M sucrose. While in this, 0·5-1 mm thick sections were cut from the middle of the length and fixed in osmium tetroxide containing 0·3 M sucrose.

Other methods too have been used in attempts to avoid damage due to turgor pressure release in sieve elements. Esau & Cheadle (1961) injected potassium permanganate as a fixative into hollow stems of Cucurbita maxima to fix sieve elements before excising tissue. Buvat (1963) on the other hand removed the epidermis from the stem of Cucurbita and

placed drops of fixative on the exposed surface. He also injected fixative into some plants. Kollmann & Schumacher (1964) surrounded stems of Metasequoia glyptrostroboides with a glass trough full of fixative before cutting them. Johnson (1968) used the freeze etching technique and reported that cells had been badly damaged by ice crystals. Mishra & Spanner (1970) to reduce turgor in Salix caprea kept the tissue for an hour in 0•25 M mannitol in M/15 phosphate buffer at pH7.

These methods are typical of those used by many workers. In an effort to overcome the surge problem two prior treatments were tried in the present work. The first was to boil the stem briskly for three minutes in water before fixation. Hypocotyls of Helianthus and Phaseolus and stolons of Saxifraga were given this treatment. The specimen was plunged horizontally into a beaker full of boiling water and held in that way for three minutes. From the middle of the treated part a 1-2 mm segment was then cut directly into fixative and finally embedded.

The second treatment was to wilt plants at room temperature for 2 - 4 hours before fixation. Helianthus plants were pulled up from the pot without injuring the roots and Saxifraga stolons were cut near the base and laid on filter paper at room temperature for four hours, then fixed in 2% glutaraldehyde for four hours.

Apart from these special treatments, Helianthus was fixed

by first making a longitudinal incision in the hypocotyl with a sharp razor blade, exposing the inner tissues over a diametral plane. This was done when immersed in glutaraldehyde to allow fixative to penetrate radially and so cause turgor release in the radial direction. Then it was cut transversely by two simultaneous cuts 1 mm apart to give a thin slice while still immersed in fixative.

A slightly modified procedure was applied to Saxifraga. The uncut stolon internode was immersed in 2% glutaraldehyde and as quickly as possible two simultaneous cuts made with razor blades fixed about 1 mm apart.

FIXATIVE

The primary fixative used was glutaraldehyde, obtained either from L. Light & Co., or TAAB Laboratories as a 25% solution. All fixatives were buffered with 0·075 - 0·1 M phosphate buffer at pH values of 6·8, 7 or 7·4.

Three fixation procedures were used :-

(1) The specimens were fixed for six hours in 4% glutaraldehyde buffered to pH7·4 and containing 0·25 molar mannitol. They were then washed well in plain mannitol buffer and post-fixed for one hour in 1% osmium tetroxide buffered to pH7·4 and again containing 0·25 molar mannitol.

(2) Specimens were fixed for twenty hours in 2% glutaraldehyde buffered to pH 6·8 and with 0·25 molar mannitol, washed and

post-fixed as before.

(3) Specimens were fixed for four hours in 2% glutaraldehyde buffered to pH 7 and after washing post-fixed in 1% osmium tetroxide for one hour.

1% osmium tetroxide was prepared in buffer of required pH from 2% solution supplied by Messrs. B.D.H. in 10 ml vials.

Heracleum was fixed by the first procedure only. With Helianthus the third method was found to be the most suitable and therefore it was used for all later work.

All fixations were carried out at room temperature, except that after fixing in glutaraldehyde and first washing the tissue was left overnight in plain or mannitol buffer at 4°C in the refrigerator.

DEHYDRATION

After treatment with osmic acid the specimens were washed in distilled water for 10 - 15 minutes then passed through grades of acetone as follows :-

30% acetone ..... 30 minutes
50% acetone ..... 30 minutes
75% acetone ..... 30 minutes
95% acetone ..... 30 minutes
100% acetone ..... one hour (with two changes)
1:1, acetone:propylene oxide ..... 15 minutes

100% propylene oxide ..... one hour (with two changes)

10:1 propylene oxide resin ..... one hour

1:1 propylene oxide resin ..... 24 hours

100% resin ..... 48 hours (with two changes)

They were then placed in resin in polythene capsules and polymerised at 60 C for 48 hours.

EMBEDDING MEDIA

(1) At the start of this work several embedding media were tried. The Glycolmethacrylate procedure proposed by Leduc Bernhard (1967) was one of the first mixtures used in this work. The specimens were infiltrated with 80% and then 97% glycolmethacrylate (G.M.C.) and embedded in resin of G.M.C. and Methacrylate, with 1% "Luperco" as catalyst, and polymerised under ultraviolet light. This method was found to give poor contrast in the specimen, also shrinkage occurred as the resin hardened. Bubbles of gas sometimes appeared round the specimen and made it difficult to trim for sectioning.

(2) Araldite resin was then tried according to the following formula taken from Kay (1965) :-

Araldite CY 212 ..... 10 ml

Dodecenyl succinic anhydride (DDSA) ..... 10 ml
(as curing agent)

Dibutyl phthalate ..... 1 ml

tri dimethylamino methyl phenol (DMP 30) ... 0.5 ml
(as an accelerator)

The ingredients were mixed into a polythene bottle. Before adding accelerator DMP 30, the mixture was thoroughly stirred

and then "tumbled" at a slow speed for an hour or so. Then the accelerator was added and the mix tumbled for one hour more. Polymerization was carried out at 60° C for 48 hours.

Section cutting in araldite was satisfactory but it was found later, sections gave less contrast in the specimen than with Epon (Epikote 812); and it was necessary to double stain the grids for a long time in lead citrate, uranyl acetate and then lead citrate again. Araldite was used for all work on Heracleum and some of that on Helianthus.

Epikote 812 which was later used was found to be much better for cutting and it gave a good contrast too. A ten minutes staining of lead citrate and ten minutes staining of uranyl acetate was sufficient to give very bright images under the microscope.

Epon 812, an aliphatic epoxy resin which is less viscous than Araldite, penetrates more easily into the specimen. If Epon 812 is mixed directly with the hardner DDSA, blocks are too soft and so a small proportion of another anhydride Methyl nadic anhydride (MNA) is added. The resins used were in the following proportion :-

Epikote 812 (Epon)	50 ml or 60·5 g
DDSA	54 ml or 49 g
MNA	36 ml or 43·2 g
DMP 30	2·7 ml or 2·7 g

After using Epon satisfactorily for some time, attention was diverted towards a new embedding resin introduced by TAAB Laboratories, that is a developed epoxy resin without any published composition. The resin is claimed to have the following valuable properties :

1. Relatively low viscosity
2. good cutting characteristics
3. can be used with uncoated grids.

A wide range of hardness could be obtained by using different proportions of the hardeners, DDSA & MNA . DMP 30 is used as the accelerator. For most of the work it was used according to the following schedule :-

TAAB resin ..... 50 ml
DDSA ..... 25 ml
MNA ..... 25 ml
DMP 30 ..... 2 ml

Sectioning was satisfactory and a ten minutes staining with lead citrate gave a very good contrast. It was used for all embeddings of Saxifraga and for some of Helianthus.

SECTIONING

Sections were cut on a Huxley microtome. Glass knives used were made on an LKB knife maker. Sections were floated on distilled water. They were white to golden in colour and were picked up on 100 & 150 mesh copper grids, coated with

formvar film on which a thin layer of carbon had been evaporated.

STAINING

Stains used were Reynolds lead citrate (1963) and a saturated solution of uranyl acetate in distilled water. Staining procedure differed with each embedding medium ——

1. Sections cut from Araldite embedded specimens were stained for five minutes in lead citrate, washed with distilled water and stained in lead citrate again for ten minutes.
2. Sections cut from Epon embedded specimens were placed in lead citrate for ten minutes, washed with distilled water, stained in uranyl acetate for ten minutes and then washed.
3. Specimens embedded in TAAB resin were stained for ten minutes in lead citrate only.

All staining was carried out by floating grids on drops of stain placed on a denture wax slab, kept in a covered petri dish whose floor was covered with filter paper soaked in saturated solution of sodium hydroxide.

Sections were examined in an AEI - EM6B electron microscope, normally at 60 KV. Photographs were taken on Ilford N50 plates and Ilford line N5·50 film processed in Ilford Bromophen and Hypam

O B S E R V A T I O N S .

PRELIMINARY OPTICAL SURVEY.

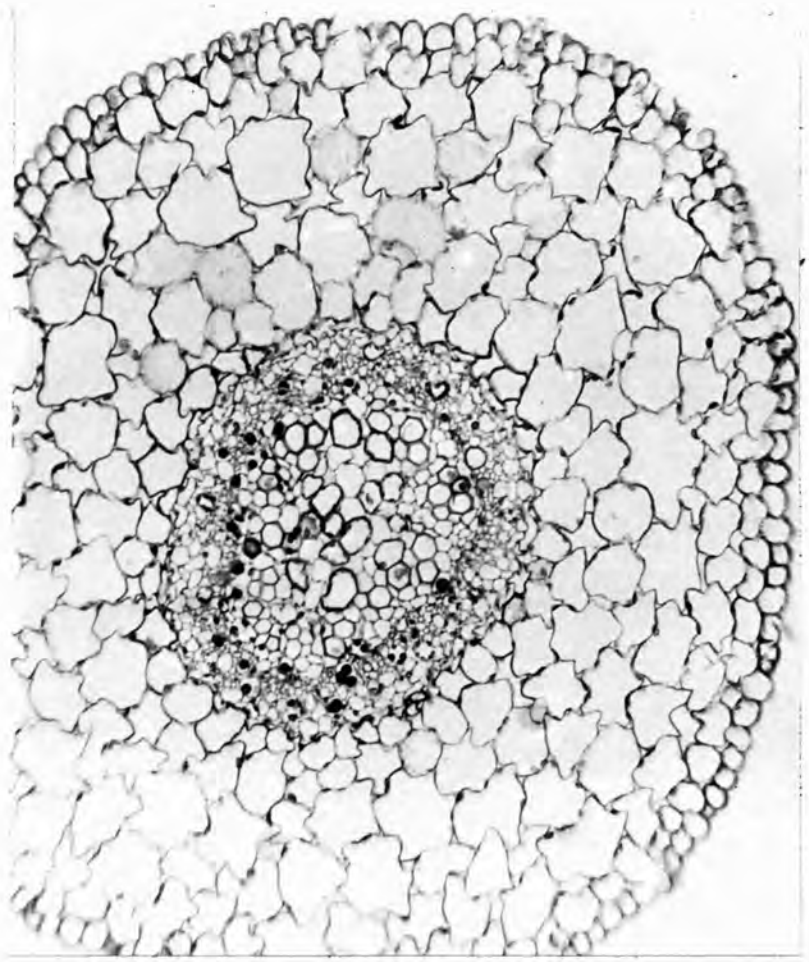
As a background to the electron microscopical work to be described, material of Helianthus and Saxifraga was embedded in paraffin wax and sectioned for optical microscopy. It was stained by Sharman's method. Plates A and B show the results, and a few brief comments follow.

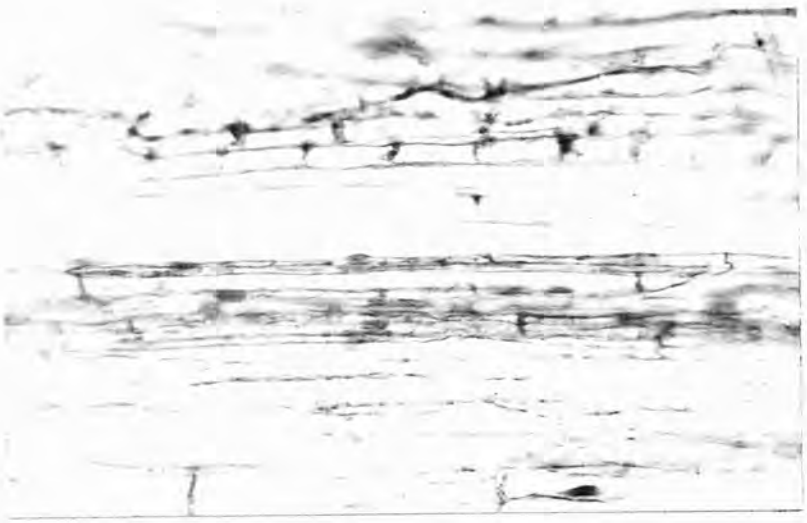
In Saxifraga stolon (plates A1, A2, A3, A4 and A5) the structure appears all primary. The sieve tubes are numerous and very small, typical dimensions falling in the range 3 - 5 μm in diameter and 100 - 300 μm in length for individual elements. Tannin globules are conspicuous in the phloem parenchyma, and it is mainly for this reason that smaller but similar material in the sieve tubes (see electron micrographs) is regarded as tannin also.

In Helianthus (plates B1, B2, B3, B4, and B5) the structure seems to be mainly primary. The sieve tubes are rather wider (7 μm) and longer (120 μm). Tannin and other special constituents are not conspicuous.

Plate A1 Saxifraga sarmentosa: T S stolon. Stained in Sharman's combination. Note tannin globules in phloem parenchyma and small size of tubes. X 150.

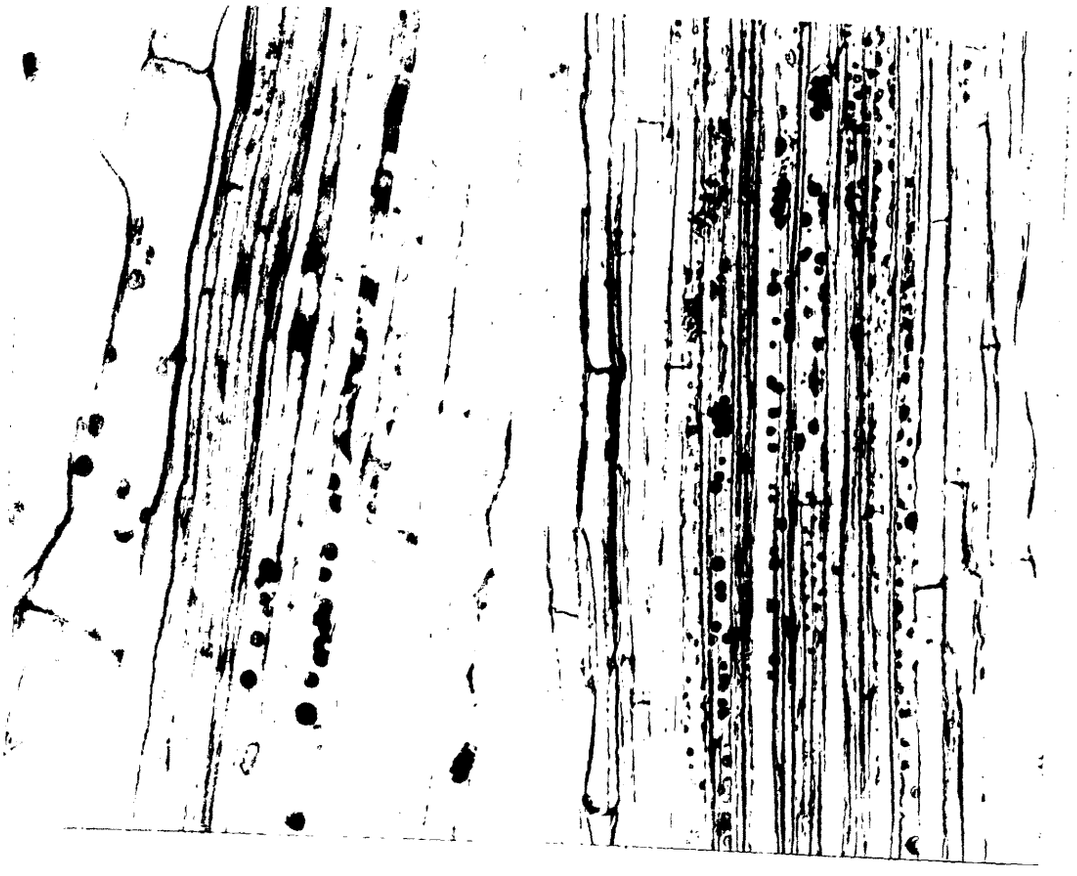
Plate A2 Ditto: Longitudinal section. The sieve tubes are the narrow dark cells just below the centre. X 350.





Plates A3 & A4 — <u>Saxifraga sarmentosa:</u> L S stolon. Note tannin globules. The sieve tubes are narrower and appear darker than the others.
X 100.

Plate A5 — Ditto: Enlarged T S of stele.
X 400.



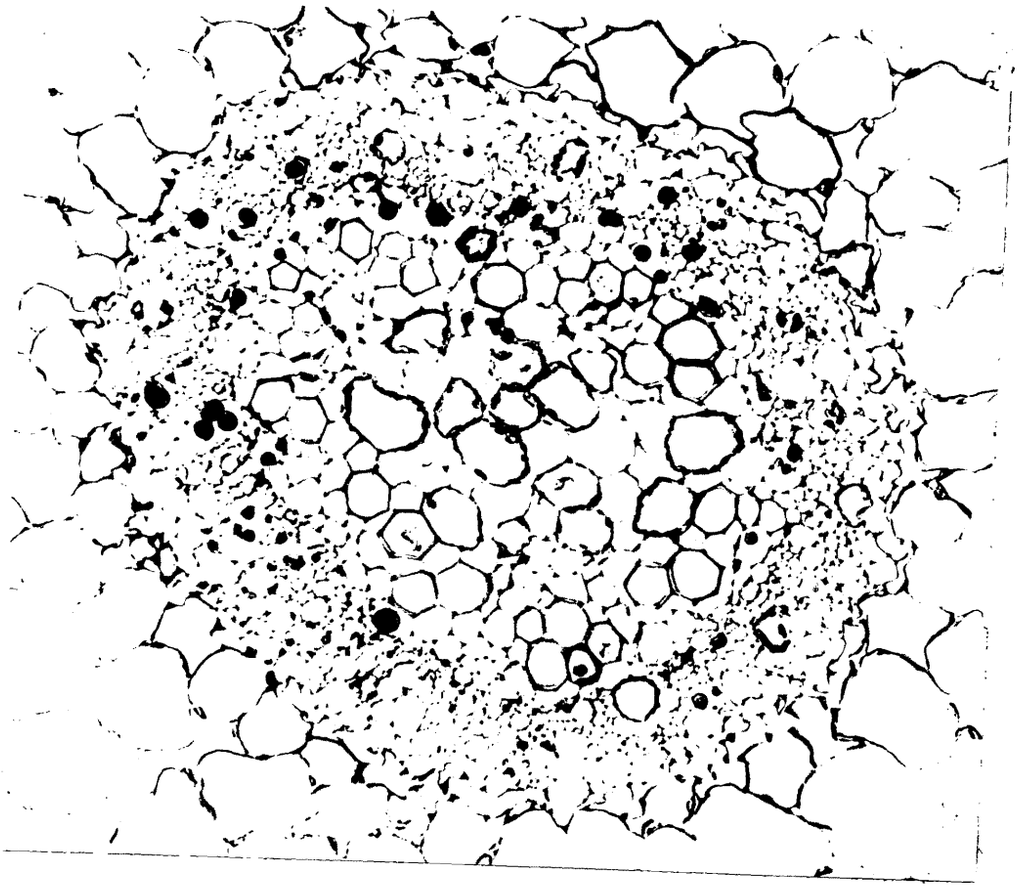
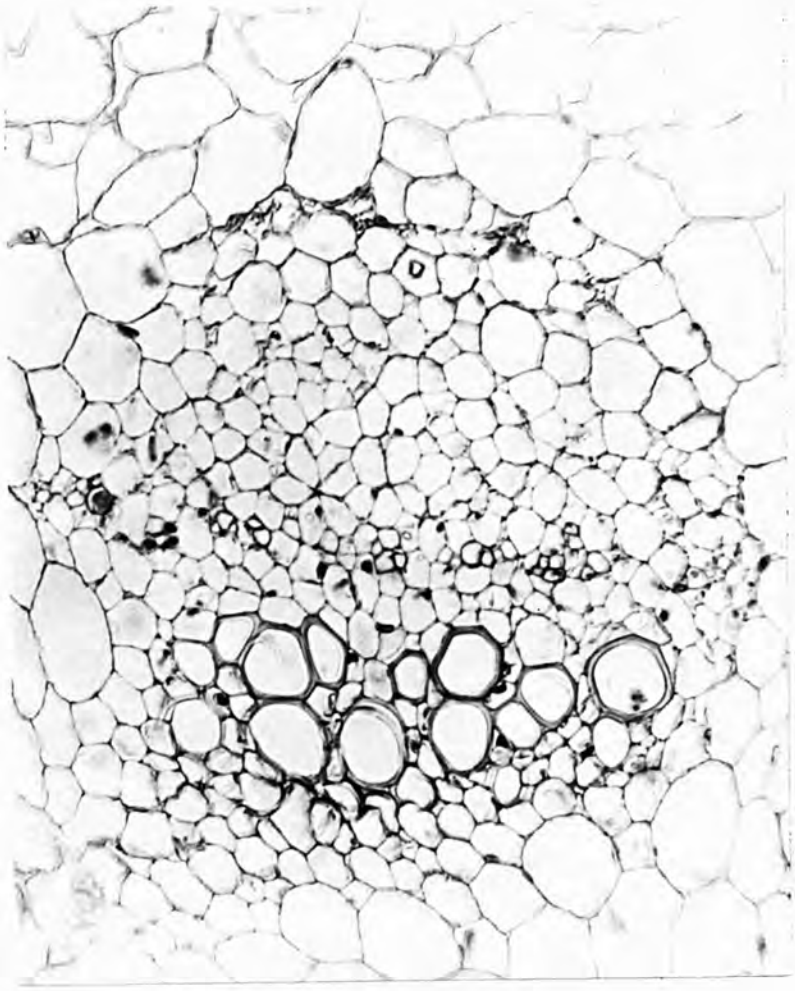


Plate B1 Helianthus annuus: T S hypocotyl bundle. Stained in Sharman's combination. X 400.

Plate B2 Ditto: L S Hypocotyl bundle. The sieve tubes appear just above the xylem vessels. X 400.



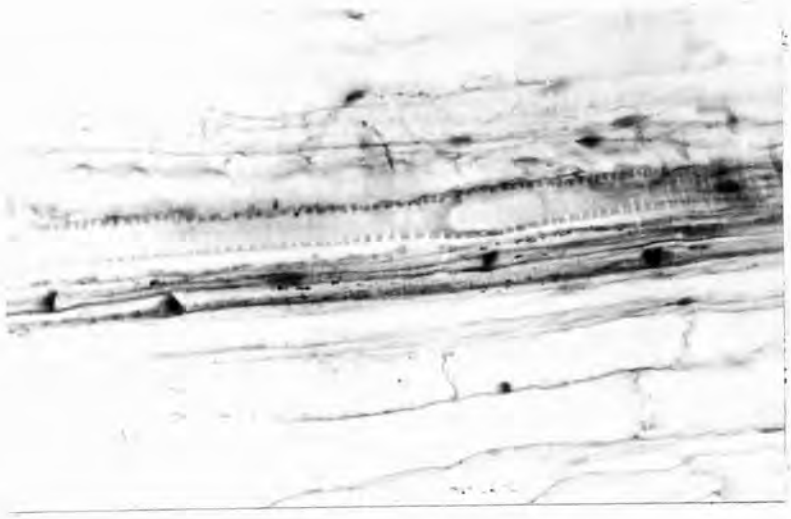
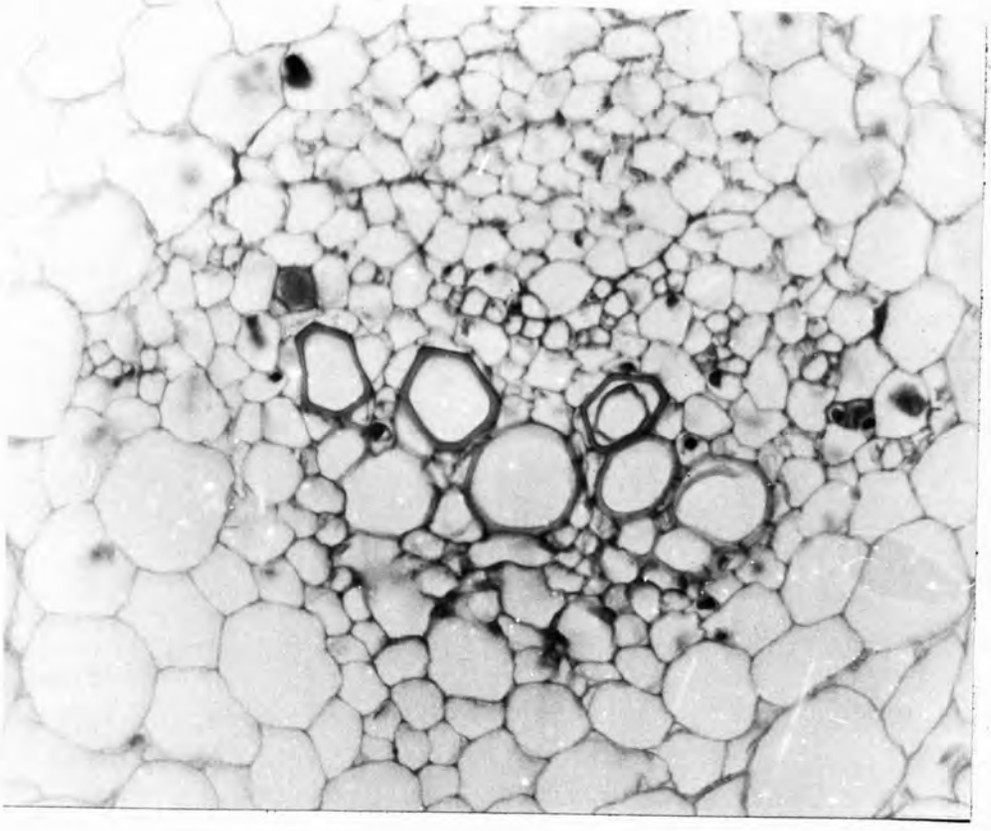


Plate B3 Helianthus hypocotyl: L S bundle showing sieve tubes.
X 500.

Plate B4 Ditto: sieve plate under oil immersion.
X 2,000.

Plate B5 Ditto: T S vas cular bundle.
X 450.



ELECTRON MICROSCOPE RESULTS

The Sieve Plate

The first structure that merits attention in connection with the functioning of the sieve tubes is the sieve plate. This study has embraced a very large number of sieve plates of four species: Saxifraga sarmentosa, Helianthus annuus, Heracleum Mantegazzianum and Phaseolus vulgaris (principally the first two). In addition the preparation for electron microscopy has involved several different physiological treatments or manipulative techniques such as wilting prior to fixation, fixation after a lengthened period of darkness, or pre-treatment of the translocating axis in boiling water. The fundamental observation that has emerged from all this work is that the pores of the sieve plates are almost invariably plugged with material (usually clearly fibrous in nature) which stains deeply with electron stains. Very rarely, if ever, were plates found which had the pores more or less open as illustrated (on occasion) by Shih & Currier (1969) Cronshaw & Anderson (1969) Johnson (1968 Esau 1966 Cronshaw & Esau (1968) and others. This is a striking fact and leads the author to conclude that the normal state of the functioning sieve plates is to have their pores plugged fairly compactly with slime fibrils. A selection of some of the micrographs obtained follows, with comments on points of interest.

(I) Saxifraga sarmentosa - (normal preparation)

The phloem of this species was obtained from the stolons of potted greenhouse plants. They were cut and fixed as described earlier. Treatment was usually carried out at

about 11 am after the plants had been in the light since 7 am. Thus translocation should have been in active progress. Plates 1, 2, 3, 4, 5 & 6 show typical examples of the image in longitudinal section. Sometimes the pores are free, or almost free from callose (plates 1 & 4); sometimes the callose cylinder is fairly thick (plates 3 & 6); and sometimes it is uneven or of medium thickness (plates 2 & 5). It is not easy to see the reason for these differences, but it may be related to the age of the elements and to a lesser extent to their relationship to the cut ends of the section to which they belong. In all case the pores appear dens ly plugged with slime fibrils (P2 protein of Cronshaw & Esau 1967); and the density of the latter seems to have little to do with presence of a callose cylinder, a circumstance which lends support to the idea that the compactness of the material in the pore is not just an artifact due to the rapid development of wound callose, as has often been suggested (Engleman 1965/a). The sieve plate in most cases appears polarised. On one side the slime fibrils often tend to run transversely and on the other longitudinally (plates 1, 2 & 3): In such cases the former side may show freed starch grains while the latter has none, though these two appearances may be due in part at least to a turgor release effect. To anticipate, within the limits of the four species examined it was noted that the plastids containing the starch grains are of different degrees of robustness. In Phaseolus it is rare to find grains still enclosed in intact plastids in the sieve tubes; in Helianthus it is the other way round. Saxifraga seems to be of intermediate fragility,

and plate 1 indeed shows both free-and plastid-enclosed grains. On the other hand some of the sieve plates of Saxifraga show little evidence of polarisation; plates 4 & 5 illustrate two such cases. Free starch grains appear on both sides of the plate. Finally, in some plates polarisation while not obvious in the immediate neighbourhood of the plate is clear enough in a larger view (plate 6, central sieve tube).

Not many transverse sections of the sieve plate pores were obtained. Of the few which were plate 7 is from an oblique section of the stolon and plate 8 from a transverse one. The rather stellate shape of the lumen is striking in the former. The pore contents appear too dense to discern any fine structure.

Before leaving this section it is worth mentioning a few impressions which have been gained about the incidence of artefacts in the micrographs presented. Starch grains free from their plastids, such as those above the sieve plate in plates 1 & 2, are believed to be unnatural. The normal state is probably represented by the lower plastid in the first plate. The effect is due, very likely, to a sudden change of turgor; though since the axis was severed by two simultaneous transverse cuts not more than about 1 mm apart there is hardly much scope for longitudinal surges. The sieve plate in fig. 1 was, indidentally, the second in from one of the cut surfaces and this emphasises the foregoing point. Nevertheless that some longitudinal surge occured is

suggested by the position of the starch grains and by the way the slime fibrils are compacted into a V at the upper entrance to the pores. This latter feature, shown also in plates 2 & 3 is believed to represent a mild artefact (compare with the serious artefact of plate 20 from a wilted plant).

(2) Helianthus annuus - normal preparation

The phloem from Helianthus was obtained from the hypocotyl of young seedlings about 2 - 3 weeks old and 10 inches high. These were grown in vermiculite in pots in the greenhouse, and the stem was excised into fixative similar to Saxifraga, by two simultaneous cuts with razor blades fixed about 1 mm apart. Plates 9, 10, 11, 12 & 80 show some typical results, the last three illustrating sections which are more ($1^{0}7$) or less (80) at right angles to the pores. These micrographs reveal the same features as those of Saxifraga; the presence of callose cylinders either thick (plates 9, 11, 12, 80 & 107) or more rarely thin (plate 10) but in each case occupied centrally by a densely-staining plug of fibrillar material. Again, some degree of polarisation is usually evident in the distribution of the fibrillar material (9 & 10). A significant point is that lateral sieve areas (11) can show quite typical plugging. With the method of preparation employed it is difficult to envisage this as an artefact due to turgor pressure release, for the adjacent tubes would be opened almost simultaneously on cutting. This is an objection to the interpretation as artefact independent of any depending on the configuration of the fibril mass in the pores.

The callose cylinders again reveal the stellate structure seen with Saxifraga suggesting rapid callose deposition with perhaps a resistance of the plasmalemma to contraction as the pore diameter decreased.

(3) Heracleum Mantegazzianum

This was the first subject tried in the present work, Zeigler (1960) having already done some work on it and the phloem being readily separable. For some reason it was found difficult to embed and cut, the sections breaking up very readily. Plates 13, 14 & 15 show some typical sieve plates comparable with the foregoing; plates 16 & 17 show examples with some of the pores more or less open. The reason for this is not at once obvious, but it may be an artefact or the plate may have been obsolescent. The pores in question are fairly large (about 0·5 μm clear diameter) compared with those of Helianthus and Saxifraga (0·2 μm). This would make it easier for the contents to be displaced out of the pore by turgor release, but there seems to be no indication that this has occured - i.e., the displaced material cannot be identified anywhere, either in these micrographs or in plate 18 from which 16 is taken. Callose is sparse in these latter examples, as in the transverse section shown in plate 19; it is more abundant in plates 13 & 14.

In the early stages of the work before techniques had been perfected longitudinal surges due to turgor release were undoubtedly sometimes present. Plates 21 & 117 show

turgor release effects of this sort, and are of value in showing how such artefacts may be recognised. In one of these plates (117) it seems obvious that the material was about to move clear and leave an empty pore, a pointer to the possible significance of such empty pores. Plate 20 shows a turgor release artefact in Saxifraga.

Effect of prior boiling

In an effort to meet the objection that the material in the pores was an artefact of preparation due either to turgor pressure release or to enzyme action, the effect of two prior treatments on the e.m. picture were tried. The first was boiling of the axis before fixation; the second, previous wilting.

The effect of boiling was tried on the stolon of Saxifraga, the hypocotyl of Helianthus and the young stem of Phaseolus. The procedure was to plunge the axis, held horizontally, into briskly boiling water and to keep it there for about three minutes. This was done before any other sort of manipulative treatment had been given. At the end of three minutes a section of a centimetre or two in length was cut out, fixed as usual in glutaraldehyde and carried up into epoxy resin in the normal way. It was then sectioned and examined in the microscope.

Plates 24, 25, 26, 27, 28, 29, 30 & 31 represent the entire spectrum of results obtained for Saxifraga. With a few exceptions (conclusive neither way) all have been included to avoid unconscious bias, and it can be seen that the

evidence is unanimous that the pores are occupied with darkly-staining material. This shows no suggestion of having hastily assumed the pore position as a result of pressure release; and needless to say the idea that it was deposited enzymatically is ruled out. It constitutes strong evidence therefore that in this species, at least, the functioning sieve plates have occluded pores. Incidentally, it appears at least in some of these that there is a fair amount of callose present (e.g. 24, 27 & 28) though it is not easy to be sure of the outlines of the latter owing to the coagulation and possible shrinkage of the slime strands.

Plates 32, 33, 34, 35, 36 & 37 represent the results for Helianthus. These are not perhaps quite so conclusive. The absence of clear strands in some pores (e.g. 32, 35 & 37) seems to be due to the fact that the section has gone very close to the edge of the pore, showing the middle lamella as a continuous strip across it. In conjunction with shrinkage of the slime material (see 34) this leads to the result found. None of the pores appears indubitably open (with the possible exceptions of one in (32) and one in (33) while plates 34 & 36 are particularly conclusive.

Plates 38, 39, 40, 41, 42 & 43 show the results for Phaseolus. The same remarks as for Helianthus seem to apply, and the results are interpreted as supporting the conclusion put forward. A peculiarity of Phaseolus among the present species is the large amount of membranous material commonly

present at the sieve plate, and even in the pores. This might well react differently from protein to the boiling treatment, and might be less effectively fixed in situ, allowing the development of artefacts. This might account for the less conclusive results, though they seem adequate. The membranous material, incidentally, seems to withstand the temperature fairly well, and remains prominent in Saxifraga (25), Helianthus (32 & 33) and Phaseolus (41 & 43), even in the form of lamellar stacks (44). This is perhaps surprising when one considers its considerable lipid content.

Effect of prior wilting

In a recent paper Anderson & Cronshaw (1970) have reported the results of wilting plants of tobacco and bean and then fixing them whole before excising any of the phloem. With this treatment a proportion (not apparently very large) of the plates appear with no compacted slime plugs. It was decided to try this treatment with Saxifraga and Helianthus. In the case of Saxifraga the stolons were cut off at their origin and set aside to wilt for two to four hours. Thin sections from the middle of the length (about 1 mm thick) were then excised by simultaneous cuts into glutaraldehyde as usual. In the case of Helianthus the seedlings were carefully uprooted, allowed to wilt for two to four hours, and then thin sections excised as above from the hypocotyl.

A selection of the results of these manipulations for Saxifraga are shown in plates 46, 47, 48, 49, 50 & 51. All the micrographs taken gave the impression of considerable

damage, those shown having rather less than most. In no case however were empty pores found. The micrographs presented all show dense fibrillar material on the plates usually very asymmetrically arranged and compacted on one side, presumably the upstream. There seems to be evidence that the slime substance can become compacted into a form which stains very much more deeply (see plates 48 & 51) where this intergrades with a lighter form). In some cases darker material globular in outline appears with the slime substance on the plate and seems rather more distinct from it (plates 46 & 49), though in view of the appearance of the slime substance in other cases (plate 47) it is arguable that this globular form is merely the locally compacted fibrillar slime. Similar dark globular bodies appear in material treated in the ordinary way (plates 4 & 52) and appear to show some internal structure. Further in some cases material not given the wilting treatment shows a curious difference in the appearance of the slime fibrils on the two sides of the plate (53). The dark globular material is discussed further below.

The results of the wilting of Helianthus are presented in plates 54, 55, 56 & 108. These represent a fair selection of a much larger number of micrographs, in not one of which was there any suggestion of the pores being other than compactly plugged. All of them on the other hand showed a fair development of callose, and some, obvious displacement artefacts (for instance 56). Plastids are often present and apparently intact, some indication of satisfactory technique. In general, the appearance of the plants in Helianthus was judged to show less severe artefacts

than in Saxifraga; this may have been due to the fact that the plants were wilted whole.

Sieve Plate Callose

Before leaving the subject of the Sieve Plate there are a few further remarks to make on the subject of callose. This has already been shown to be variable in amount in both Saxifraga (compare 4 & 3) and Helianthus (compare 9 & 10). In boiled Saxifraga it is present (24) but not quite so easy to distinguish owing to the possible coagulation and contraction of the pore contents. In boiled Helianthus it is difficult to tell if any callose is present, though plates 35 & 36 may be taken as indicating its presence. One needs to interpret these findings with caution however, as callose may be solubilised by brief boiling. The starch grains in Saxifraga certainly give the impression of being less solid after this treatment. Callose does not always form equally on areas fairly close together; plate 57 shows a case in point, the lateral sieve area being almost occluded. In this case however unlike the terminal plate, it connects to an element of possibly different age. The ontogeny of the sieve plates will be discussed more fully later. Here it need only be added that as has been reported for other species (Esau, Cheadle & Risley 1962, Northcote & Wooding - 1966) opposed callose plates form on either side of the middle lamella and deepen towards each other until they join up. During this process they are covered by membranes of the endoplasmic reticulum. Plate 58 shows a stage in this process for Helianthus. The callose platelets are very

conspicuous, and in one place they have begun to be penetrated and dissolved in the median position. Plate 59 shows an early stage for Heracleum.

The Slime Substance

In what follows it is assumed that the slime substance is the same as the fibrillar material which is so prominent in the lumen and especially on the plate in the mature elements. To anticipate, it can be said that this substance proved to have the same fine banded-fibrillar structure as has been so frequently reported in the past for other species (Wooding 1967, Cronshaw & Esau 1967, Johnson 1968). Plates 3, 5, 6, 61, 63, 64 & 65 show some of its aspects in Saxifraga. In 63 & 65 it is very evident that the fibrils have a beautiful banded structure, with a period of about 10nm and a diameter of 12nm. It is not so evident in micrograph 65 that the bands are laterally aligned at the entrance to the pore, as has been reported elsewhere (Northcote & Wooding 1966, Johnson - 1968); this may be due to some forcible displacement on turgor release, as seems to be evidenced by the loose starch grains and the V - depression under one of them. Plate 64. where there seems to be less indication of turgor displacement also seems to indicate better lateral alignment of the bands; plate 63 among many others indicates the same, with a suggestion that there is a spirality about the fibrils. The slime substance in Saxifraga can be fairly abundant, as is obvious from many of the foregoing micrographs (cf. also plate 67). In younger tubes it is present in the body of the element (plates 66, 122 & 123) but not unnaturally seems

to move to the end when the tube is functioning (67). It is capable of being drawn out into coarse strands (plates 68 & 69). It sometimes appears much coarser than at others in fixed preparations; occasionally both appearances are apparent in a single micrograph (plates 70 & 71). Other workers have given evidence that in its ontogeny the slime material may exist first in the form of bundles of fine tubules, often in paracrystalline array (Steer & Newcombe 1969) and of diameter about 20 nm. No such tubules were indubitably found in the present material, though some micrographs show material rather remotely resembling them especially near sieve plates (61, 62 & 70), and the "much coarser" slime previously mentioned (see especially 70) may in fact be the diffused tubular form, though why it should change to the fibrillar form as it passes through the plate is not at once obvious. Undispersed slime bodies of fibrillar material are sometimes found (72 & 73).

In Helianthus the slime substance is rather similar. Mature elements reveal the banded structure clearly in plates 74, 75, 76 & 77. In the mature lumen it is seen in plates 78 & 79, and in a developing sieve tube in 81 & 82, the latter being before dispersal of the slime bodies. Tubular forms, so frequently reported in the literature, (Steer & Newcombe 1969, Esau & Cronshaw - 1967) were not seen. Coarse strands formed from the fibres were sometimes met (74), but nowhere did they correspond to the membrane-bound transwellular strands of Thaine.

Heracleum also possesses banded fibrils in the pores (14),

but enough work was not done on this species to find other stages.

Membranous formations

Membranous formations of various sorts are fairly conspicuous in the sieve tubes of many species (Bouck & Cronshaw - 1965, Tamulevich & Evert - 1966, Behnke - 1968 and Johnson - 1969) and can take many different forms. A number of these forms were encountered in the present work and are described below.

Stacks of parallel lamellae were fairly frequent in Saxifraga. Sometimes these were arranged perpendicularly or nearly perpendicularly to the wall (69, 84, 85, 118 & 119); at others they lay flat on the wall (99a & 99b), and frequently both orientations occured close together (86, 87, 88, 89, 90, 91, 92 & 93). Sometimes curvilinear formations very similar to those found by Johnson (1969), Behnke (1967) Wooding (1967) and others were found (83 & 121), and sometimes the two intergraded (84).

In Helianthus the occurance of lamellar stacks was the same (45, 78, 94, 95, 96 & 97). Unfortunately enough work was not done on Heracleum and Phaseolus to confirm that in these species too membranous stacks are frequent, though plate 44 shows a formation in Phaseolus subjected to prior boiling.

A second type of formation was found fairly frequently in

Helianthus. This consisted of curious concentrically or spirally - arranged layers, usually revealing very clearly their unit membrane structure. Plates 98, 100, 101a & 101b show examples in increasing order of size. It is not easy to decide what these represent. They may be composed of the ruptured membranes of plastids, or of the degenerated tonoplast, or obsolete endoplasmic reticulum. There is a suggestion of parallel striation on the membrane surface in places (100) and it is interesting to recall something remotely similar in the sieve element plastids of Beta (Esau, 1965a). Similar bodies were also found in Saxifraga. Plate 116 gives a rather poor example.

Multivesicular bodies lying apparently outside the plasmalemma were also frequent in Helianthus. Plates 101a, 101b & 102 show examples. There is a distinct suggestion that they are associated with plasmodesmata communicating with the companion cells, and in view of the fact that the contents of the inner vesicles stain more darkly it may be surmised that they are connected with the movement of some component of fairly high molecular weight from the companion cell into the sieve tube. (Plates 12, 104 & 105 interestingly show similar bodies within companion cells and parenchyma cells. Plate 103 shows what is apparently a similar body near a plasmodesma but lacking the internal vesicles. It seems possible that the multivesicular bodies are connected somehow with the concentric bodies noted earlier, plates 98 & 103 representing intermediate stages. Plate 120 seems to show a similar structure in Saxifraga.

Besides these fairly well-defined membrane structures there is a good deal of apparent debris of a membranous character. This probably represents an artefact of preparation, the material being derived from plastids ruptured during fixation, or from plasmalemma, tonoplast or endoplasmic reticulum remains. Plates 2, 12 & 85 show examples of this.

Plastids and Mitochondria

Plastids and mitochondria are well-known as organelles present im sieve tubes. Both were found in the species examined. Plastids contained typical carbohydrate reserve particles, usually several to a plastid. In both Saxifraga and Helianthus the particles (usually referred to as "starch grains" although their reactions indicate that they are not typical starch, see Palevitz & Newcombe 1970), have very rough outlines, especially in Saxifraga. Plates 57, 61, 65 & 106 show plastids and starch grains for Saxifraga; plates 76, 80, 105 & 107 for Helianthus, and plate 109 for Heracleum. It can be seen that the rough surface of the grain takes electron stains rather heavily, but where the section cuts through the grain the centre appears quite unstained. Plastids show the usual double envelope and often internal membranes. Grana are never present.

Mitochondria are shown in plates 110, 111 & 118 for Saxifraga, these being the only good examples found in the sieve tubes of this species. By contrast, mitochondria were reasonably abundant in Helianthus though not nearly so

frequent as plastids. Plates 45, 96, 101, 107, 113 & 114 show examples in sieve tubes; with these may be compared others in companion cells (plates 12, 102 & 103). Generally the latter are larger and fuller. It has been noticed by R.L. Jones (unpublished observation) that in Nymphoides membranous aggregates may be formed from what is presumably the plasmalemma and which appear very like mitochondria. He has traced a series of stages in the formation of these which appear to support this interpretation. In the present work plates 78, 79 & 118 show something of the same kind. The first two are probably from the same sieve tube, and are very likely serial sections.

Miscellaneous inclusions

Of miscellaneous inclusions the most notable in the present work were the tannin drops in Saxifraga, seen for instance in plates 4, 8, 46 & 52. Tannin is present in the phloem parenchyma in fairly large masses (2) and it is on this ground mainly that the sieve tube globules are so identified. Plate 114 shows two bodies bounded by what appear to be E.R. cisternae in Helianthus, but further identification is difficult. A similar body appears near the sieve plate in 10 and may possib y be a plastid.

Sieve tube ontogeny

Although the present work was not directed at ontogeny, a number of stages in the development of the sieve tube were encountered and are briefly described. Plates 124 & 127 show the formation of the sieve plate in Saxifraga. As reported

by earlier workers (Esau, Cheadle & Risey - 1962, Northcote & Wooding 1966) the young sieve plate develops paired platelets of callose which thicken inwards apparently at the expense of the cellulose. In the centre a pore develops, swelling in the process to form a median nodule at the intersection with the middle lamella (124). The callose platelets appear to be covered with E.R. At this early stage the nucleus is present but becoming less stainable (126), plastids are as usual clustered at the ends of the elements (compare 124 with 126) and the groundplasm is fairly rich in ribosomes, endoplasmic reticulum and other organelles, though noticeably less so than the companion cells (124 & 126). Plates 122 & 123 show two stages in the dispersal of the fibrillar slime. In 122 it is already distinctly banded. As mentioned earlier, the tubular form (P1 of Esau & Cronshaw) was not found.

In Helianthus the evidence was similar. Plate 115 shows microtubules near the wall of an immature sieve element still containing numerous ribosomes. Plate 82 shows an undispersed slime body of P2 form (Esau & Cronshaw) and 58 an early stage in the perforation of the sieve plate. Endoplasmic reticulum lies over the callose platelets, and the developing pore enlarges in the median position, as with Saxifraga. Plate 59 shows a comparable stage with Heracleum.

The Sieve tube Wall

The sieve tube wall was examined fairly carefully to see if the micrographs could supply any evidence for or against

the suggestions of Spanner and Jones (1970). Not a great deal of evidence was found. The radial striations so conspicuous in Phaseolus and Nymphoides were indeed present, and can be seen in such micrographs as 10, 13, 59, 61, 65, 71, 91, 92, 113, 114 &118, though in none of these was it striking. This is possibly due to the fact that the sections were not thin enough, Srivastava (1969) having noted that the herringbone pattern he found in the cellulose fibrils of the secondary sieve-element wall of Pinus strobus only show up in very thin sections. Many of the present micrographs do show a rather more deeply staining inner layer to the wall (13, 65 & 69); and one (13) has a faint suggestion that the cellulose fibrils may form a herringbone pattern as suggested by Srivastava.

THE WHOLE SIEVE TUBE

Two lines of attack in assessing the condition of the phloem tissue have been rather neglected by electron microscopists; Firstly, the recording electron micrographically of entire sieve tube elements, so that the final longitudinal distribution of their contents could be understood; and secondly, the recording in low power survey photographs of fields of sieve tubes. This would have an obvious bearing on the question of whether two-directional movement in the tissue (a fairly well established phenomenon) took place in single tubes as a unitary happening, or whether it had to be regarded as occuring in parallel strands, with movement in each strand in one direction only.

Accordingly, attempts were made to obtain photomontages of whole elements, and of sieve tube fields. Some difficulty was experienced in this, as the blocks naturally had to be aligned very exactly so that the plane of section remained within a single tube; and further the tubes had to be reasonably straight or the foregoing condition could not be satisfied. The EM 6B instrument is not perhaps ideal for this purpose. Without a goniometer stage the tubes could not be aligned with the mechanical movements. Further, the pin cushion distortion at low magnifications made fitting the micrographs not always very exact. However, some useful results were obtained and are briefly discussed below.

(I) Single tubes

Plates 128, 129, 130 & 131 show some results, all of

Helianthus. The first three are of young sieve tube elements, the last of a more mature element. These will be considered in turn.

In plate 128 the section only catches a lens shaped region of the companion cell, identified by its position, the nature of the wall between it and the sieve element (contrast with the opposite wall), and the density of its contents compared with the parenchyma cells above and below. The sieve element shows plates in process of developing pores. Ribosomes are still abundant in the cytoplasm, the nucleus is intact but lacking in density, and an undispersed slime body is present (fibrilar in texture rather than tubular). There are a number of large vacuoles, and like the starch-containing plastids these are especially noticeable near the ends. This position for the plastids, already apparent in these immature elements, needs to be recalled when deciding on the intervention of turgor release artefacts in mature tubes; it cannot be assumed that plastids or starch grains on the plates are evidence of surges operating over any appreciable distances. Mitochondria are also present in the element.

Plate 129 shows rather more than half of an element at about the same stage as the previous. The nucleus is perhaps slightly denser. There is a large and a small fibrillar slime body, the former with a cavity or an invagination, containing ribosomes (perhaps throwing light on Buvat's (1963) report of a positive reaction of the slime

bodies of Cucurbita to pyronine, an RNA stain). Plastids with starch grains are again prominent near the plate, to which the large vacuole makes a close approach.

Plate 130 shows what is, in fact, an entire element (the right hand sieve plate is hidden under a fold in the section). Two lines of companion cells are present, and it is not clear which is associated most closely with the central tube. Both possess abundant mitochondria. The sieve element is rather more mature than the previous two; the nucleus while still intact, has retained little power to attract stain. The slime has largely dispersed, the sieve plates show a greater development of callose, though the section has missed the pores. Vacuoles if present, are not obvious.

The last plate, (No. 131) shows an apparently mature tube, with its associated file of two companion cells. It is not quite obvious whether this tube is about to reach its functioning life, or has passed it. The plate appears too heavily callesed for a tube about to commence conducting, and the companion cells appear aged. However, the plastids and mitochondria appear normal, as does the distribution of fibrillar slime, and this argues against an obsolete tube. It may be that the heavy callosing is an artefact. Normally the technique of rapidly cutting very thin slices would be thought to allow the loss by diffusion of the soluble carbohydrates too quickly to permit extensive callose formation. However, in the present case some factor may have operated to prevent this. The lowest cell in the micrograph appears to

be a second sieve element, though the plastids in the centre are unusual. The wall has been cut at grazing incidence at each end; the appearance especially at the left hand end (note the longitudinal contour marks) is rather typical of the walls of sieve elements, and seems to suggest a layering of the wall.

The upper sieve tube seems to contain well dispersed slime concentrated rather towards the ends, but hardly showing any polarisation over the plates. The pores of the plates would seem, if anything, to be slime filled. It is difficult however, to press the evidence further than this.

Sieve tube fields

For the low-power examination of sieve tube fields Saxifraga stolon was chosen as the material since it was essential to have the material straight and the sieve tubes numerous and small. The sections were cut slightly thicker than usual to prevent fragmentation but the blocks were fixed and embedded as usual. In the photomontages presented (plates 132, 133, 134, 135, 136 & 137) the sieve tubes are picked out in red to enable their courses to be followed more easily.

In general it can be said that the evidence often indicates that parallel tubes are polarised in the same direction as judged by the location of the starch grains and in a less obvious way, the fibrillar slime (compare similarly-lettered sieve plates in 133 & 134). Sometimes however, the

observations are inconclusive (135 & 136). The same may be said of the polarisation of the two ends of a single element. On the upstream side of the sieve plate the fibrils will tend to set themselves transversely, and on the downstream side they will tend to adopt the longitudinal or trailing position. Thus the evidence in 132 for the two ends of the sieve tube shown is consistent. Since most of the elements are about 200 /um long and the section was only a little longer than this (say 1 mm) surve effects consequent on the twin simultaneous cuts would often be expected to be in opposite directions; hence the polarisation shown in 132 is probably that corresponding to natural translocation. Apart from the released starch grains visible near one plate there is little evidence of artefact damage, and the presence of an intact plastid may indicate that the free grains are natural. Plate 135 is interesting in that the transverse orientation of the fibrils to the right of sieve plate a seem to indicate flow to the left. Sieve plate b with its trailing strands suggests the same. On the other hand sieve plate c (note the disposition of starch grains) suggests the opposite, though not conclusively. Sieve plate d is perhaps most naturally interpreted as indicating movement to the left, possibly violent; but it is difficult to see how, in a thin section, this could have occured as a cutting artefact. Plates 136 & 137 call for no special comment. Some further remarks are presented in the legends.

D I S C U S S I O N

The present investigation set out to contribute towards answering three questions (page 8) :

(i) At what stages during their ontogeny do sieve tubes translocate most effectively ?

(ii) Do undisturbed functioning sieve elements contain cytoplasm or other material within the pores of the sieve plates, or is material which is often seen in the pores blown into them while they are being prepared for electron microscopy ?

(iii) Does the material continue across the lumina of functioning sieve elements as trans-cellular strands ?

It remains to draw together the threads of the present work and discuss in what respects light has been thrown on these problems.

The first point that can be made concerns particularly Saxifraga sarmentosa stolon. Work currently in progress in this laboratory by F. Qureshi shows that the stolon of this plant, at the stage in which it was electron microscopically examined, constitutes a strongly transporting organ. The transport, as judged by tracers applied at mid-length, is in one direction only, i.e. the conducting tissues are polarised uniformly

Further, the organ while it can be encouraged to grow in length by darkening the extremity does not enlarge in diameter. These observations suggest strongly that the sieve elements appearing fairly empty in the lumen but with fibrous slime collected around and through the sieve plate (see low power views - 132, 133, 134, 135, 136 & 137) constitute the conducting stage of the conduits. This conclusion follows because there are in fact very few sieve tube elements in any other ontological state present (there is of course no secondary thickening); and because elements at this stage typically show polarisation, lacking in younger stages. Thus if one believes that it is sieve tubes which conduct (rather than parenchyma) it would seem to follow that this is conducting stage. Certainly this conclusion is in line with the microautoradiographic work of numerous authors who have employed sugars labelled with $C-^{14}$ and tritium (see for example two fine recent papers, Schmitz, 1970 and Fritz & Eschrich, 1970). These have shown radioactive material confined more or less to the sieve tubes, and to sieve tubes in what has usually been considered the mature condition, i.e. the stage described above. The present evidence is thus consistent with the usual view.

Thus with regard to the state of the functioning pores the most obvious fact from the present work is that in almost all cases the electron micrographs show plugged pores. Sometimes surge effect seems to have been superimposed on the natural condition, but even when this is so the natural condition seems to have been a "plugged" one. In Saxifraga in spite

of the favourable nature of the material (i.e. its small diameter) and the length of the sieve tubes (100 - 300μm) relative to the thickness of size cut (1 mm), no unplugged pores were ever encountered. In Helianthus it was the same. Only in Heracleum were more or less empty pores sometimes encountered and these were always accompanied by filled ones in nearby positions on the same sieve plate (see 17 & 18).

The second observation concerns the detailed appearance of the pore contents. This differed markedly from that of pores subjected to undoubted surge effects. The latter showed uneven and sac-like penetration of the fibrillar mass through the plates, very dissimilar from the 'lines of force' configuration shown by the best undisturbed material (contrast 4 & 9 with 20 & 21).

Thirdly it was often noticed that the fibrils in the pores were arranged in such a way that their dark and light regions become laterally aligned (see 63 & 64). This agrees with the observations of many other workers with different species, and it argues strongly against violent forcing of the material into the pores on turgor release, i.e. it must be assumed to be naturally present.

Fourthly, the appearance of material boiled or wilted prior to excision and fixing supports the same conclusion.

Temperature travels in an aqueous medium at about two hundred times the speed at which a molecule like glucose moves in open solution[1]; hence the sieve tube membranes of the thin stolon would be denatured by heat and the cell turgor released sideways probably much too quickly for longitudinal surges to take place. The coagulated slime which is apparent in the pores must therefore be assumed natural in its position. It cannot, of course, have been laid down enzymatically as an artefact with this treatment. While the evidence from prior wilting lacks the same force it is consistent with the other evidence, and in no case were any open pores found. This is in contrast to the work of Anderson & Cronshaw (1970) on wilted bean and tobacco. They reported that in "two tobacco plants" pores were mostly plugged only to the density in the lumen, while "in many of the wilted plants" dense plugs were present; but they nevertheless conclude that the open condition is the natural one. Such a conclusion is not only contrary to the present work but seems very questionable on the basis of their own.

The foregoing remarks relate this work principally to the requirements of the Münch hypothesis. The evidence presented however is equally relevant to the transcellular strand

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1 Dr. Spanner has provided this figure based on a comparison of the thermometric conductivity ($K/_{\delta e}s$) for water and the diffusion coefficient (D) for glucose in open solution. Penetration through cell walls and denatured membranes would very greatly increase this ratio.

δ = density

s= specific heat

K= thermal conductivity

hypothesis of Thaine. There is a marked absence revealed of any structures which could sustain this mechanism; and conversely, plenty of evidence of structures which would oppose it (e.g. the dense fibrillar material in the pores). Further coarse stranded material has been found in the lumens which might conceivably represent the structures seen by Thaine without supporting his mechanism, since in no case has it been found to be membrane-bound.

The observations from low power survey work were not so conclusive as had been hoped. Novertheless they do illustrate several points: the absence of sieve tubes in developmental stages other than the one here considered as the functioning one (leading to the conclusion that this must be the conducting stage); the similar polarisation of the two ends of the elements and of adjacent tubes; and the relative emptiness of the lumen. The conclusion about polarisation is however uncertain to the extent that it does not clearly distinguish between polarisation related to the natural function, and polarisation imposed by the technique of preparation. It is to be hoped that better methods, such as freeze-substitution, will elucidate this point further.

As a final conclusion on the physiological level it may be stated therefore that the present work provides further evidence discounting the Münch pressure flow hypothesis (which can hardly accomodate the observation that slime fibrils block the sieve plate pores), and the transcellular

strand hypothesis of Thaine. While it does not provide much unambiguous and direct evidence for the electro osmotic theory, this theory does seem the best able of those available to accommodate its findings, in particular the occluded nature of the pores, the physico-chemical nature of the occluding material, and the abundance of membrane material in various forms in the sieve tubes. To this extent therefore the work embodied in the present thesis may be said to support the electro-osmotic theory.

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STRUCTURE AND FUNCTION IN THE SIEVE TUBE - AN ELECTRON MICROSCOPIC STUDY.

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1971.

Plate 1 Saxifraga stolon: L S of sieve tubes.
The released starch grains against the sieve plate are an evidence of a surge artefact, probably mild (note intact plastid to left). The slime fibrils are very clearly banded. Lamellar stacks and membranous vesicles are prominent near the wall. Normal treatment, stained with lead citrate X 20,000.

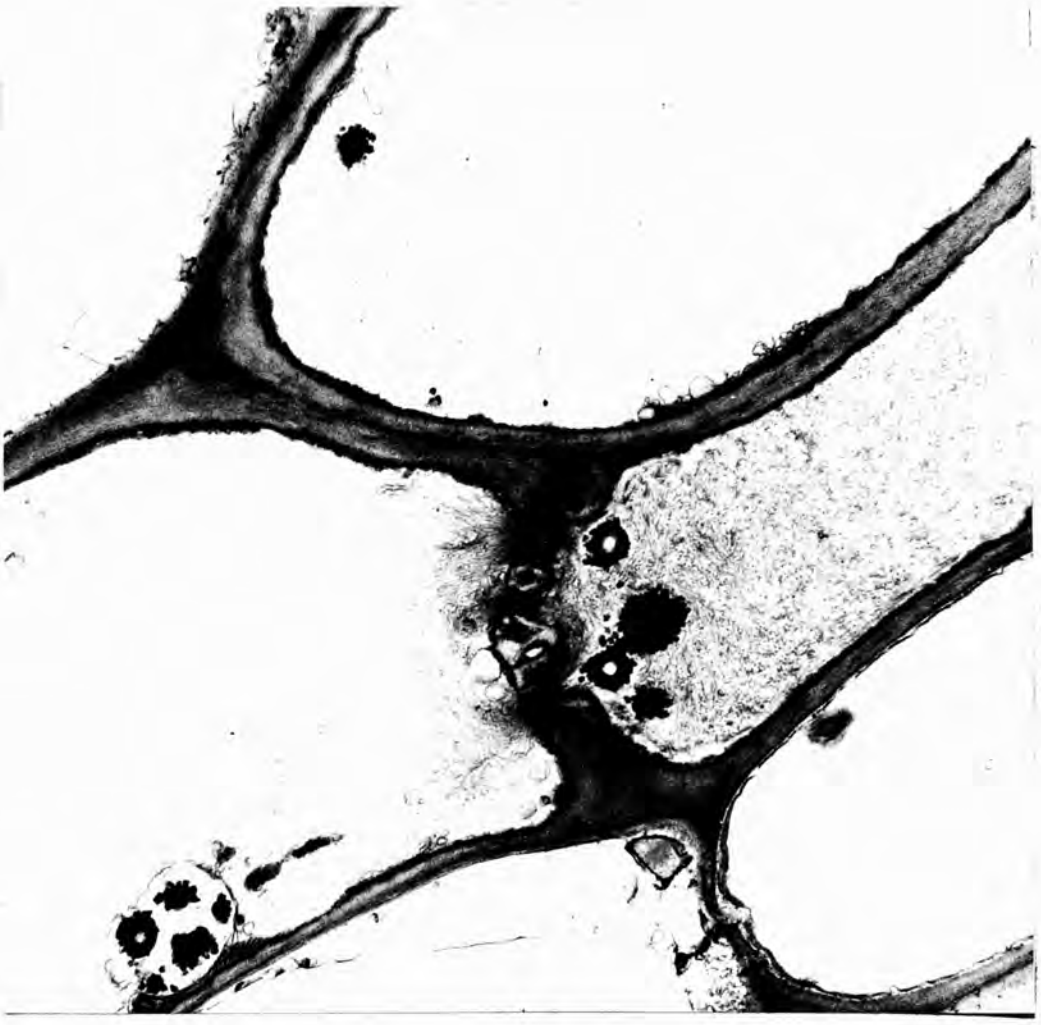
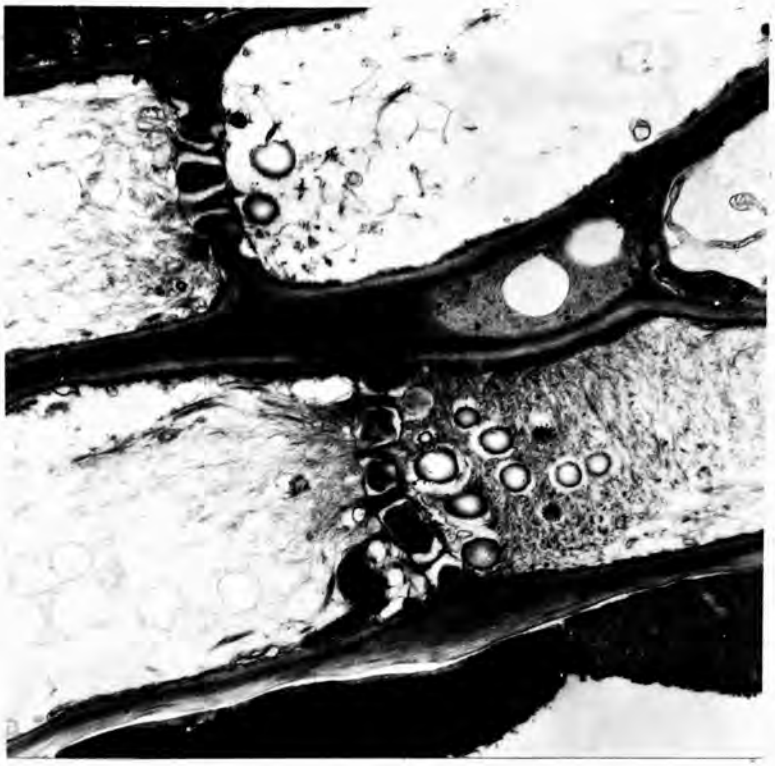


Plate 2 Saxifraga stolon: L S showing two adja cent sieve tubes. Normal treatment. A surge artefact is probably indicated by the released starch grains piled against one side of the plate with membranous fragments (plastid envelopes ?) on the other. The darker mass in the lower parenchyma cell is probably tannin. Such inclusions are common. Note the sieve plates are only 3 µm in diameter. X 11,250.

Plate 3 Ditto. A surge artefact, if present is much less. The disposition of the fibrillar material in the lumens argues against that in the pores being artificial (compare plate 65). X 22,500.



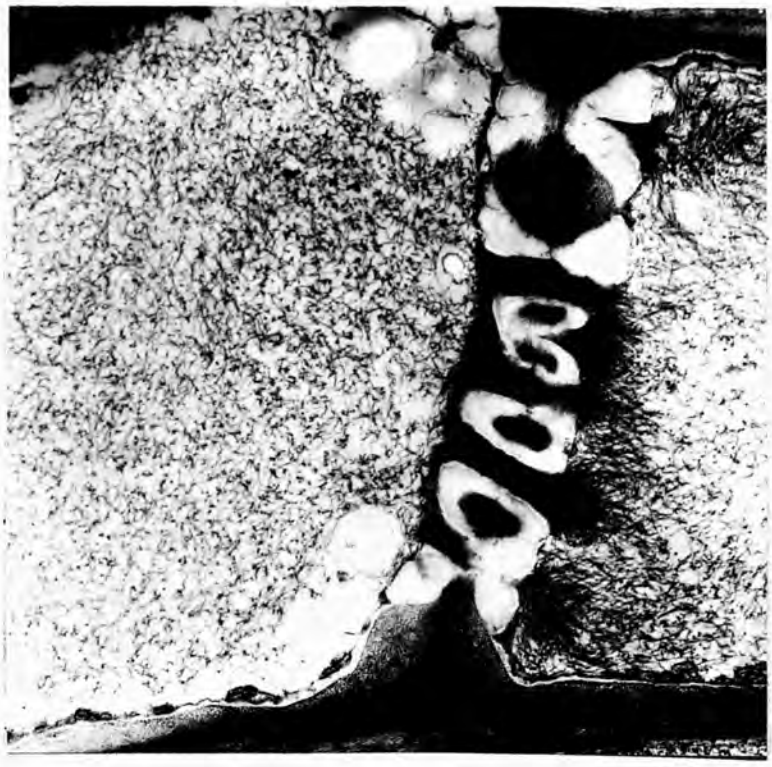


Plate 4 Saxifraga stolon: L S showing sieve plate, normal treatment. The dark spherical bodies may be tannin or osmophilic granules of Janine Salmon. Note the absence of callose. Stained with lead citrate. X 45,000.

Plate 5 Ditto. Note displaced (?) mitochondrion on plate. X 22,500.

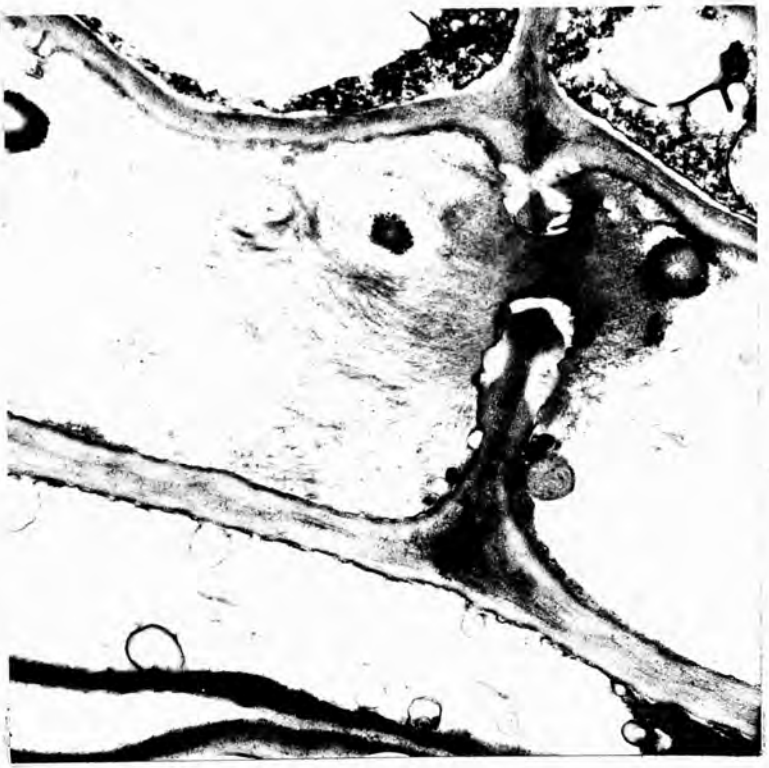


Plate 6 *Saxifraga* stolon: L S of sieve tubes. Slime fibrils are concentrated on same side of the plate in both tubes. Note thick callose cylinders. Normal treatment; stained with lead citrate. X 15,000.

Plate 7 Ditto. Oblique L S of a lateral sieve area. Note the thick callose cylinders and plugged pores. X 22,500.

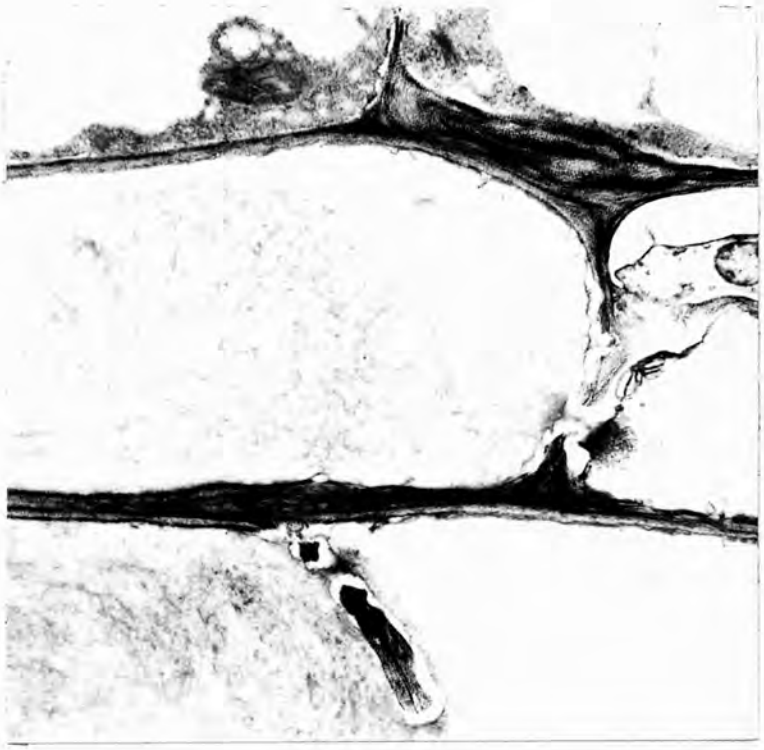


Plate 8 Saxifraga stolon: T S showing pores of sieve plates densely plugged with stained fibrous material. Note the thick callose cylinder and globular material of tannin or osmophilic granules of Janine Salmon where section is cut just above or below the pores. A lamellar structure is visible in the third sieve tube which appears almost empty. Sieve tubes are about 3 5 μm in diameter. Normal treatment; stained with lead citrate. X 30,000.

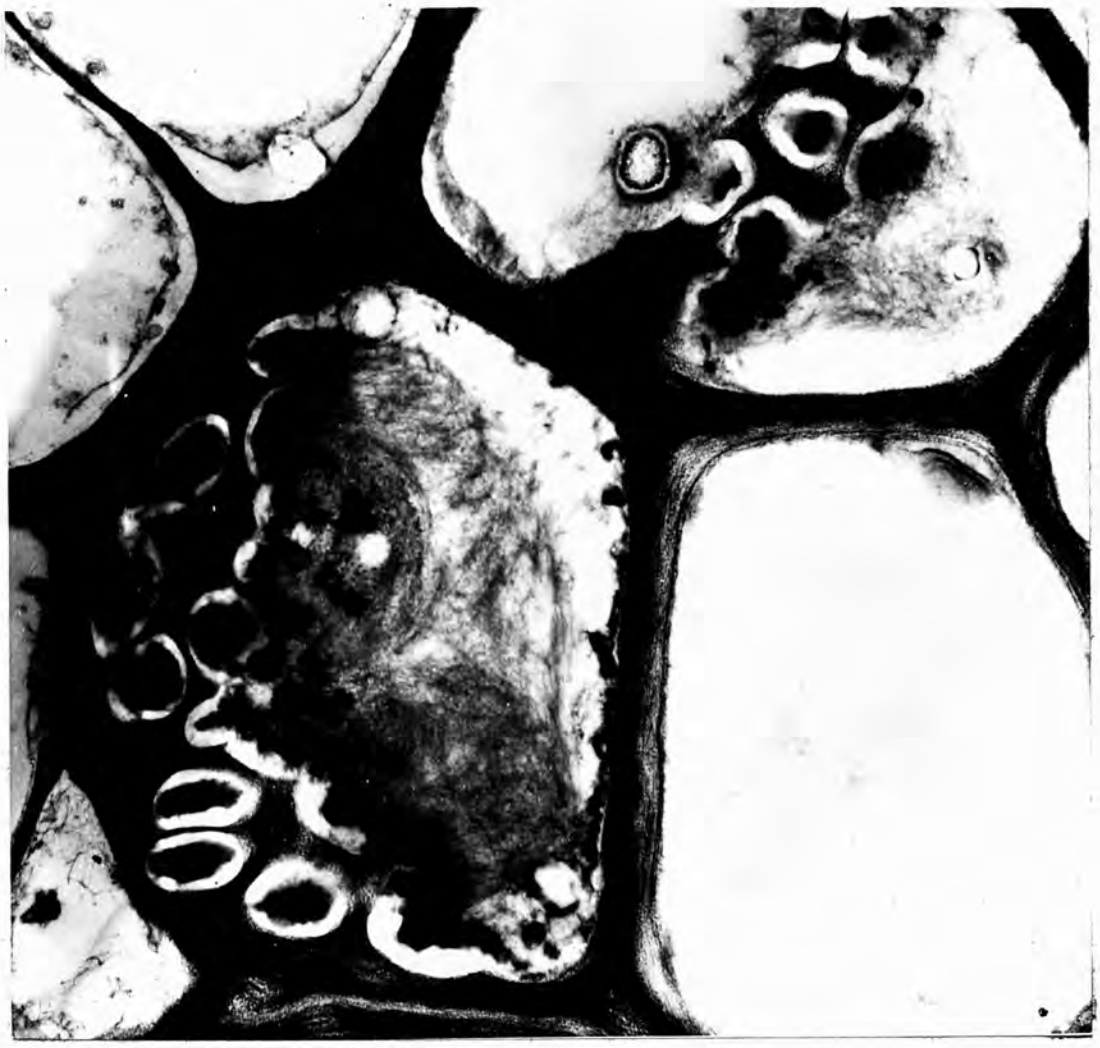


Plate 9 Helianthus annuus: L S of sieve plate in hypocotyl. Densely stained fibrous material is present in the pores. A degree of polarisation is evident in the distribution of fibrils on either side of the plate. Note the thick callose cylinder. Normal treatment; stained with lead citrate and uranyl acetate. X 20,000.

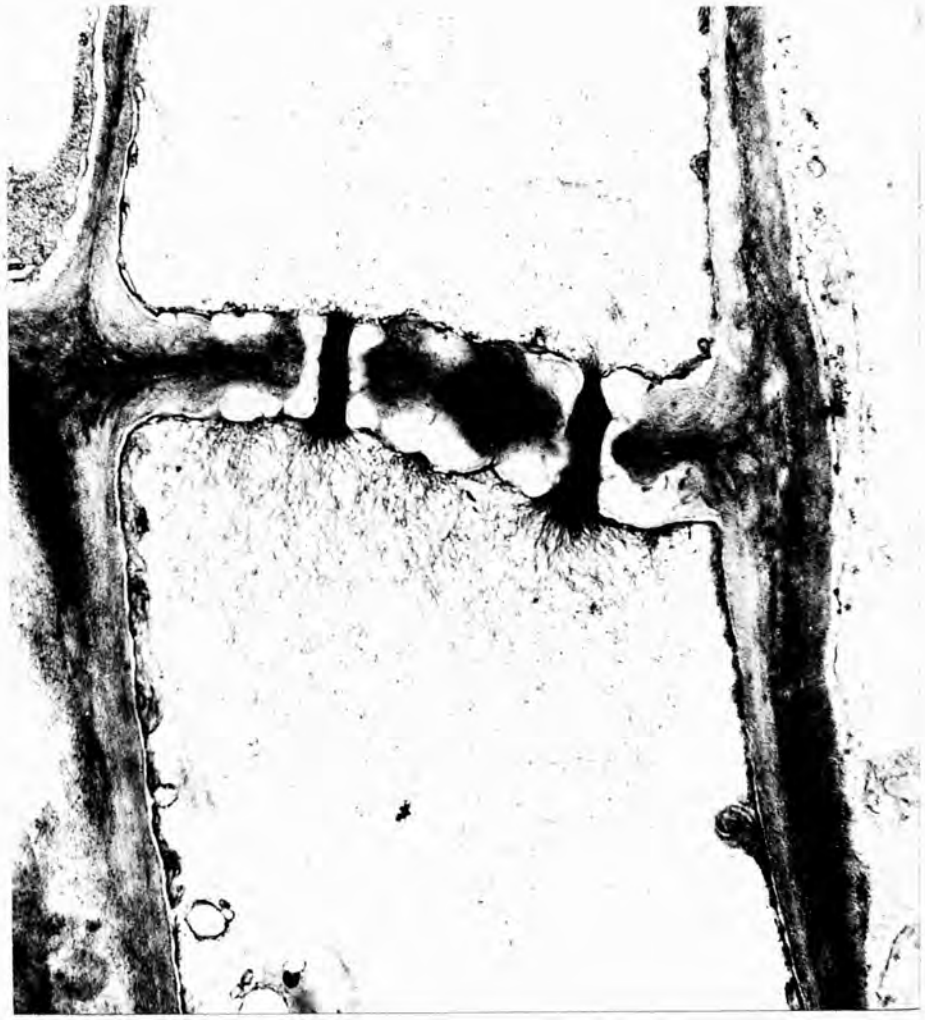


Plate 10 Helianthus hypocotyl: L S of sieve tube. Pores are plugged with densely staining material and there is little callose. A plastid bearing a starch grain (?) is very close to the sieve plate. Note the radial striations in the sieve tube wall. Normal treatment; stained with lead citrate and uranyl acetate. X 15,000.

Plate 11 Ditto. L S of sieve tubes. A lateral sieve area shows typical plugging not apparently polarised, and hardly caused by sudden release of turgor. Note thick callose cylinders around the pores. X 7,500.

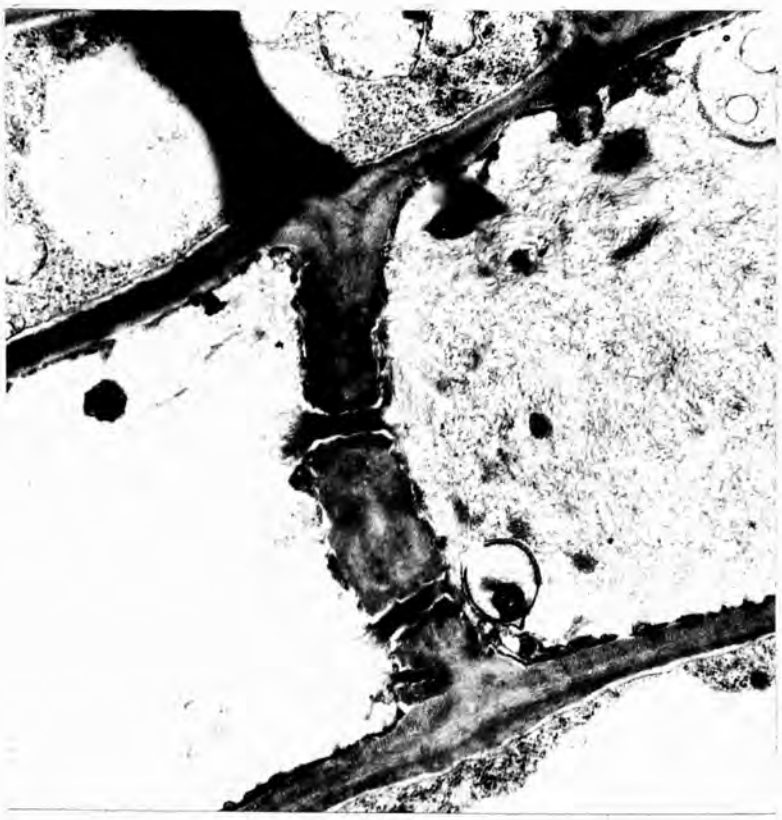
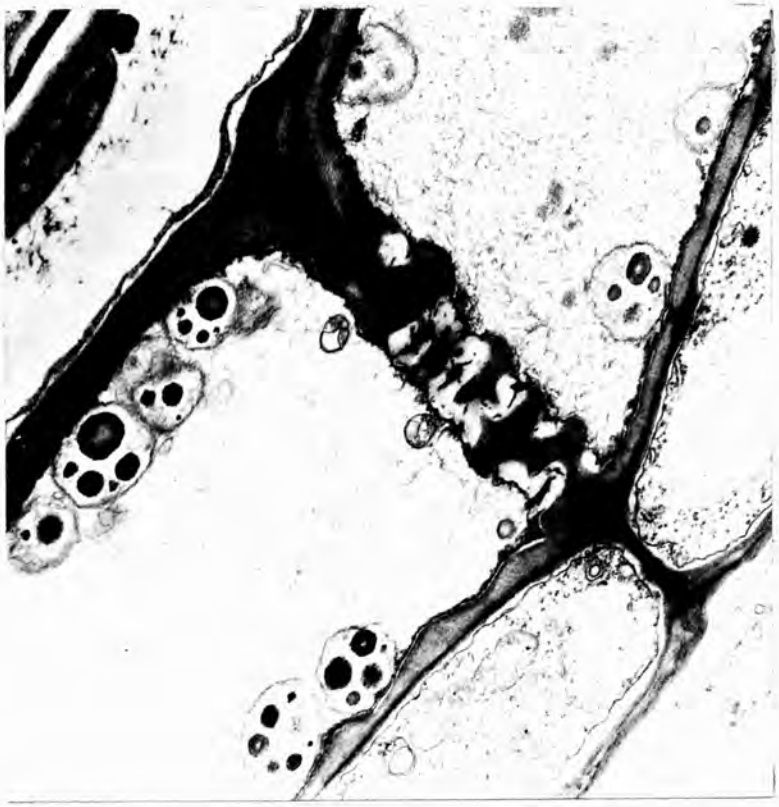


Plate 12 Helianthus hypocotyl: L S of sieve tube with obliquely cut sieve plate. Intact plastids and well dispersed slime fibrils suggest an undisturbed condition of the sieve tube. Note the companion cells to the lower right. Normal treatment. X 15,000.

Plate 13 Heracleum internode: L S of sieve tube. A densely plugged pore with thick callose cylinder is present. Note the radial striation of the cell wall with suggestion of a herringbone pattern of cellulose fibrils. Normal treatment; stained with lead citrate, uranyl acetate and lead citrate. X 11,200.



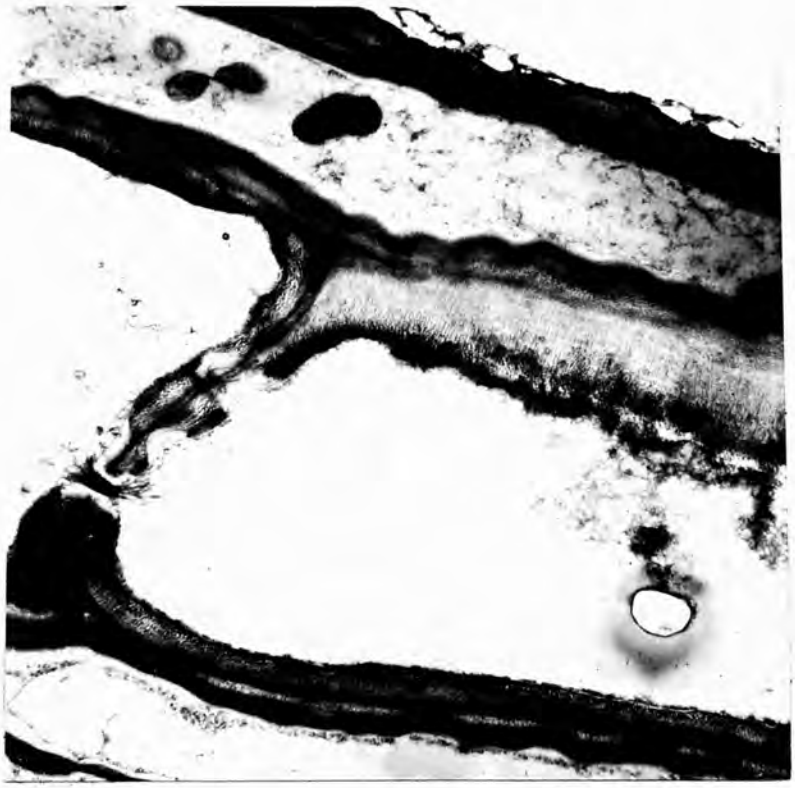


Plate 14 Heracleum internode: L S of sieve plate. Pores are plugged with fibrils 10 nm in diameter with little to suggest an artefact. Note the thick callose cylinder. Normal treatment; stained with lead citrate, uranyl acetate and lead citrate. X 30,000.

Plate 15 Ditto. Showing a plugged pore with its internal fibrillar structure visible. The fibrils are banded. The plasmalemma on the plate is visible on the lower left. X 60,000.

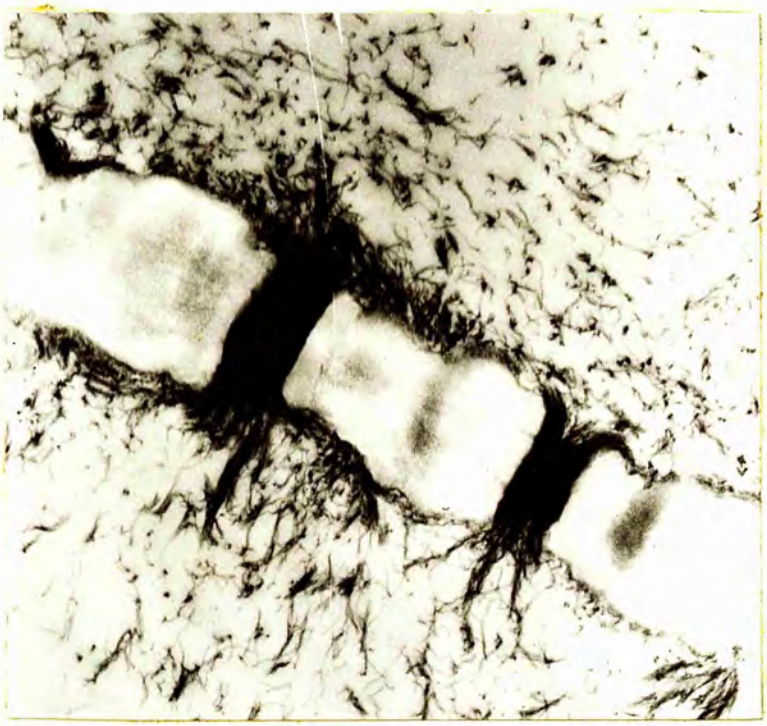


Plate 16 Heracleum internode: A magnified part of plate 18. An unplugged or loosely plugged pore is adjacent to others which are densely plugged. Note that the unplugged pore is the largest (0 3/µm in diameter). There is little callose. Normal treatment; stained with lead citrate, uranyl acetate and lead citrate. X 30,000.

Plate 17 Ditto. L S of sieve plate. Open and loosely plugged pores are present. Note the absence of callose. The largest pore is unplugged. X 30,000.

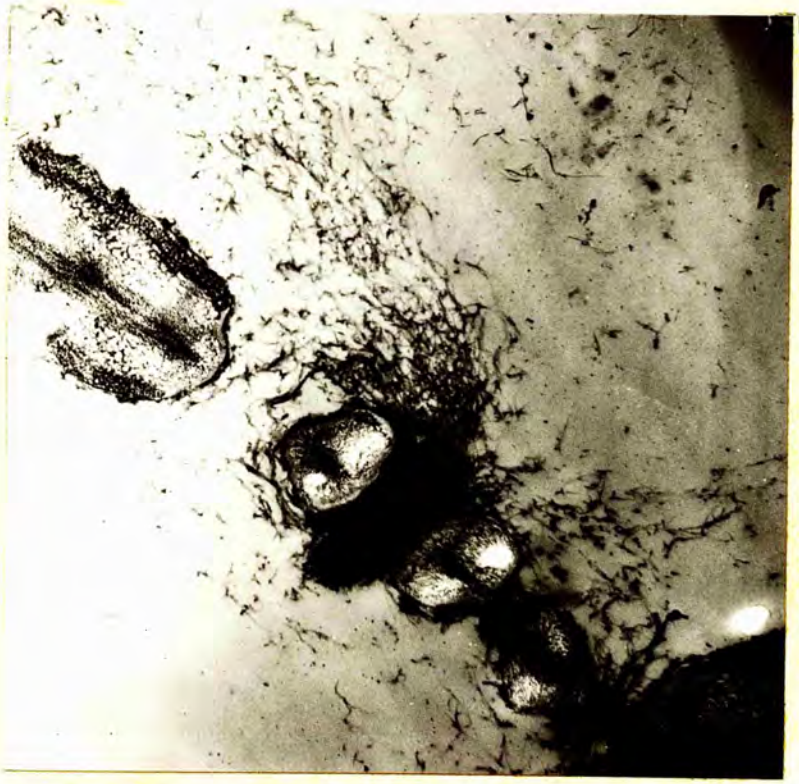
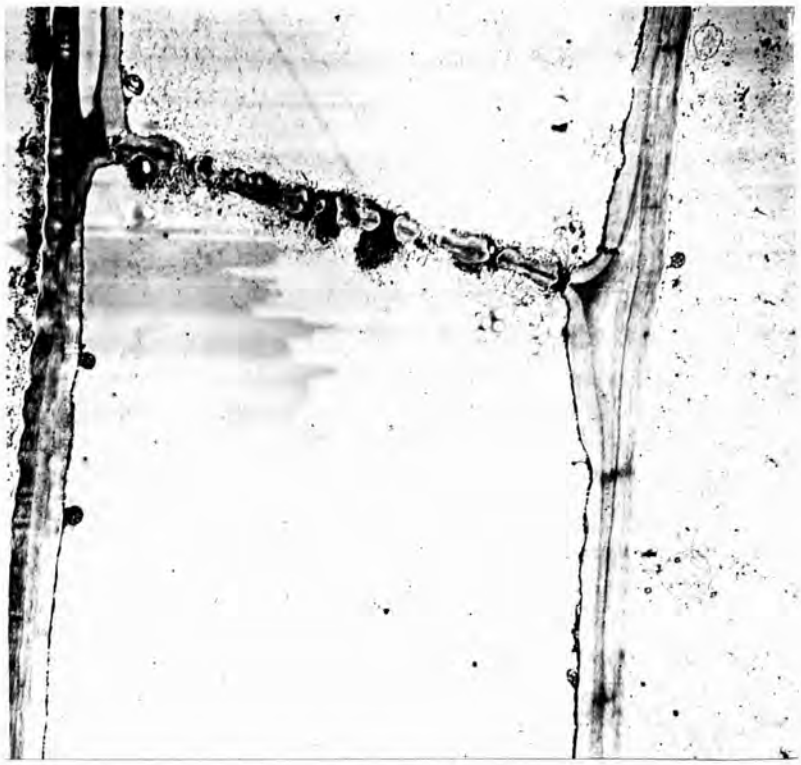


Plate 18 Heracleum internode: L S of sieve tube. Various conditions of sieve plate pores are visible. Note that the very wide sieve tube (20μm in diameter) is almost empty, (see also plate 16). Normal treatment; stained with uranyl acetate and lead citrate. X 3,750.

Plate 19 Ditto. An oblique L S of a sieve plate showing plugged pores with little callose around them. X 15,000.



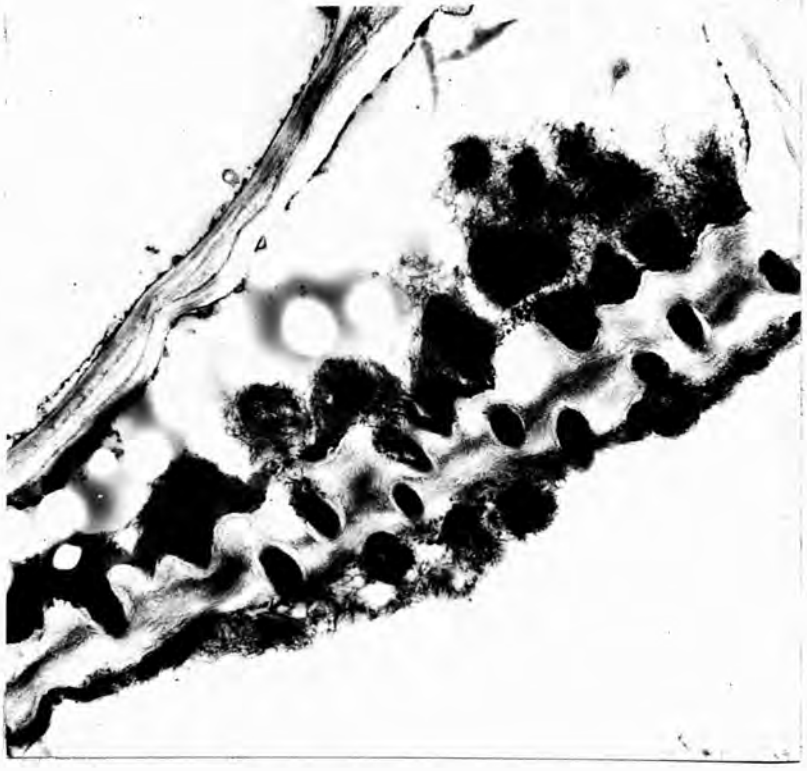
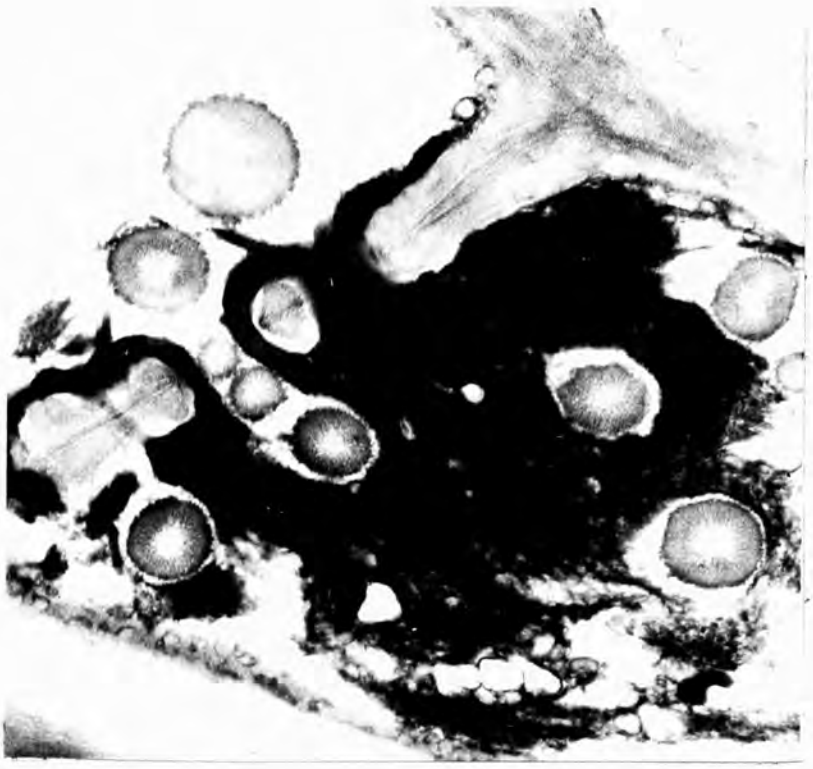


Plate 20 Saxifraga stolon: L S of sieve plate showing an undoubted artefact of turgor release. The starch grains have been released from the plastids and violently displaced. There is little callose. X 30,000.

Plate 21 Heracleum internode: L S of sieve plate. Longitudinal surge due to turgor release is clearly recognisable. Note the absence of callose. Normal treatment; stained with lead citrate, uranyl acetate and lead citrate. X 22,500.



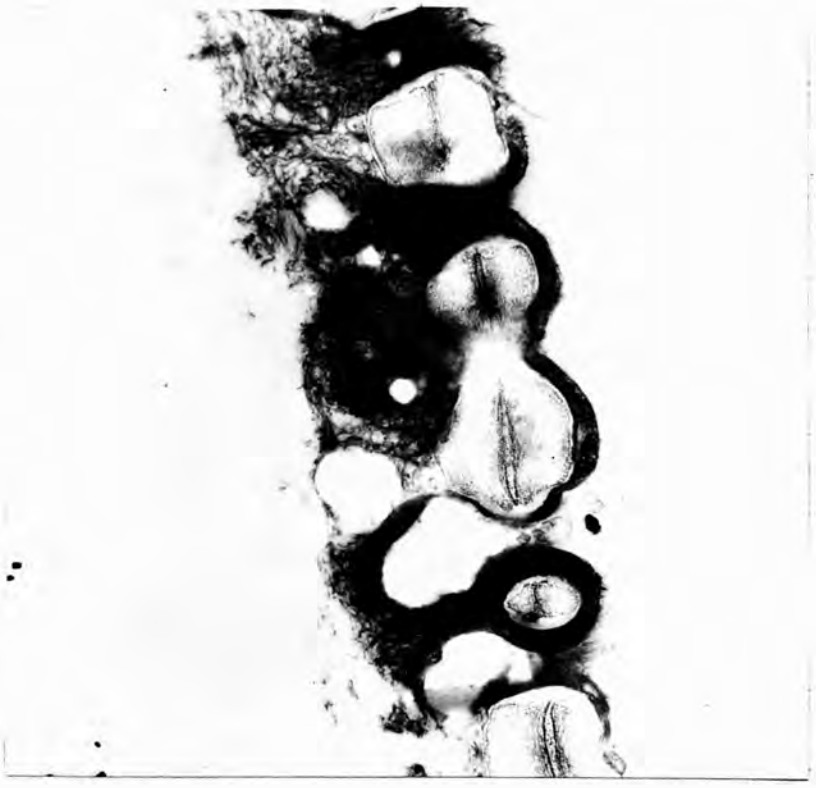
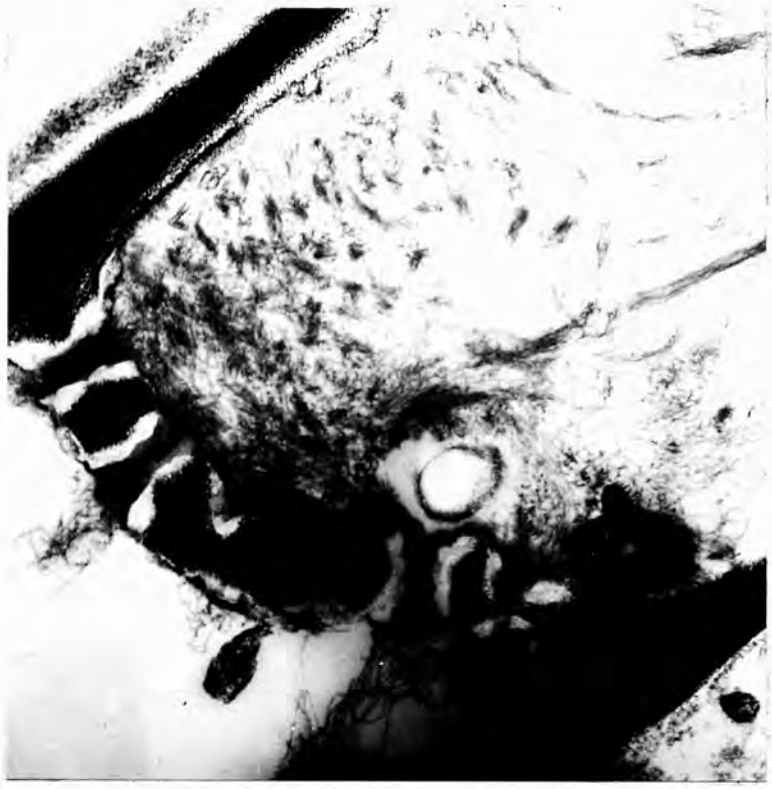


Plate 22 Saxifraga stolon: L S of sieve plate, showing sieve plate pores typically plugged with slime. Strands of slime fibrils are seen in the lumen, some transversely orientated. Note thick callose cylinders. Normal treatment; stained with lead citrate. X 37,500.

Plate 23 Saxifraga stolon: L S of sieve plate. Pores are densely plugged with slime which seems coarser than usual. Compare plates 53 & 70 (to right). X 15,000.



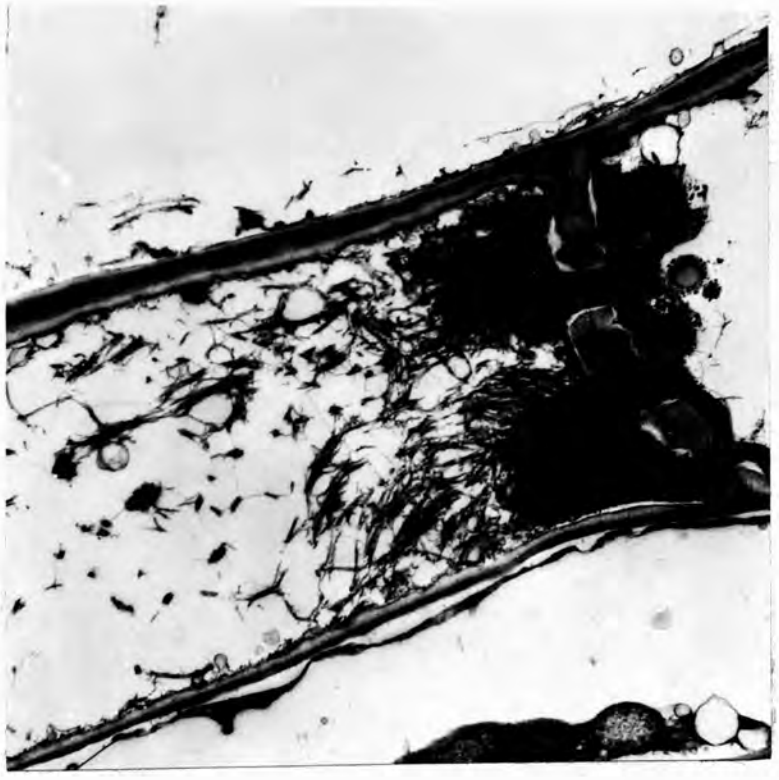


Plate 24 Saxifraga stolon: L S of sieve tube boiled before fixing. The contents of the tube have been denatured but the pores are as usual. Note the membrane (plastid envelope ?) relatively unaffected by heat. Stained with lead citrate and uranyl acetate. X 20,000.

Plate 25 Ditto. Showing an oblique sieve plate. Pores are plugged. X 10,000.

Plate 26 Ditto, Showing a lateral sieve area. Pores are plugged and there is a thick mass of coagulated material around the plate. X 10,000.

Plate 27 Ditto. Similar to 24. Callose cylinders may be present. X 15,000.

Plate 28 Saxifraga stolon: L S of sieve tube with pores plugged with coagulated slime. Boiled before fixing. X 10,000.

Plate 29 Ditto. X 20,000.

Plate 30 Ditto. X 20,000.

Plate 31 Ditto. Note the coagulated slime on both sides of the plate. X 20,000.

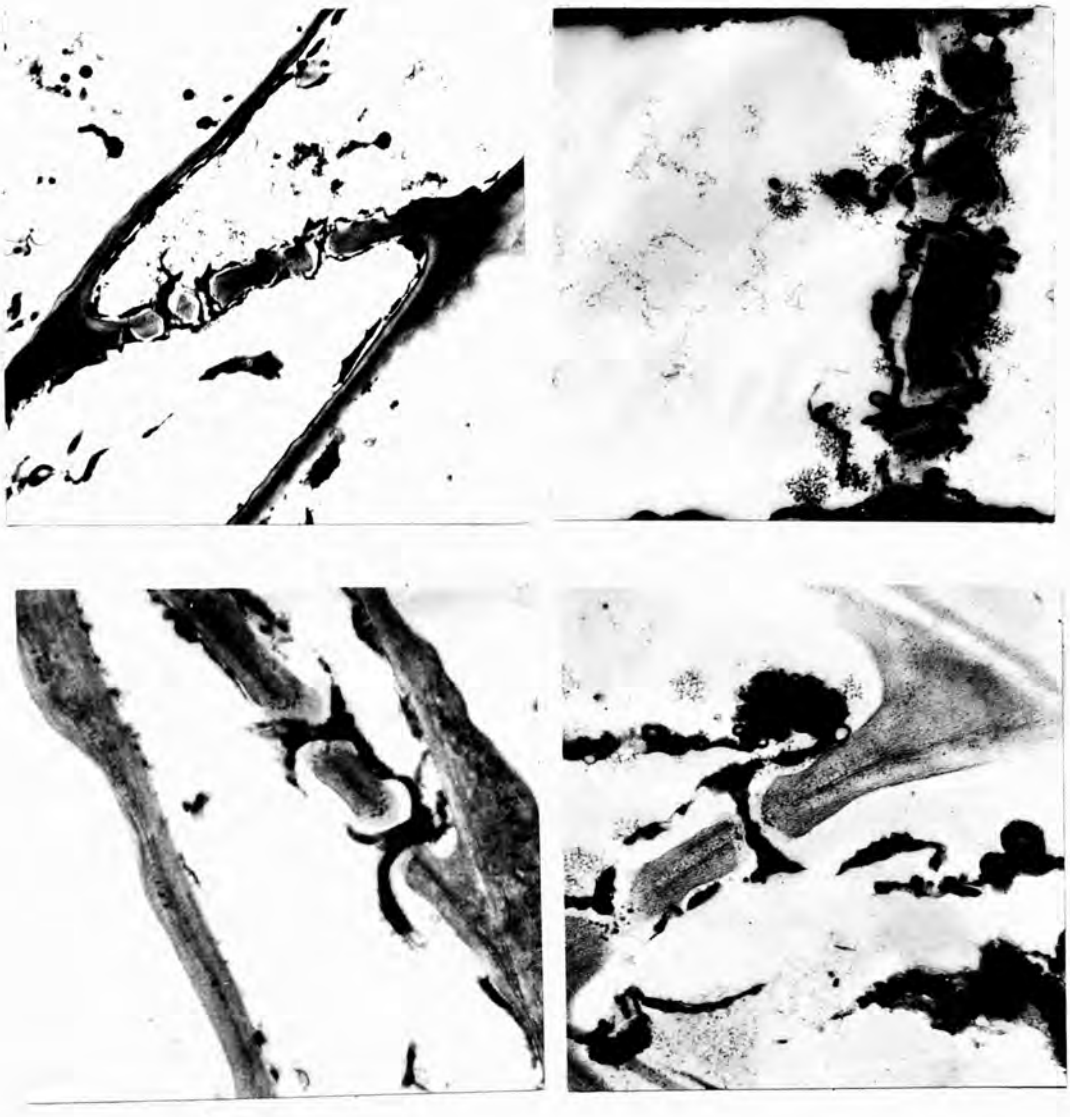


Plate 32 Helianthus stem boiled before fixing: L S of sieve plate. The section seems to miss the centres of the pores visible. Note the lamellar stacks on the lower side. Stained with lead citrate and uranyl acetate. X 15,000.

Plate 33 Ditto. Sieve plate pores are sparsely filled. Note membraneous material. X 15,000.

Plate 34 Ditto. Coagulated slime blocks the sieve plate pores. X 7,500.

Plate 35 Ditto. Section passes close to the edge of the pores. One pore blocked with slime material can be seen. X 10,000.

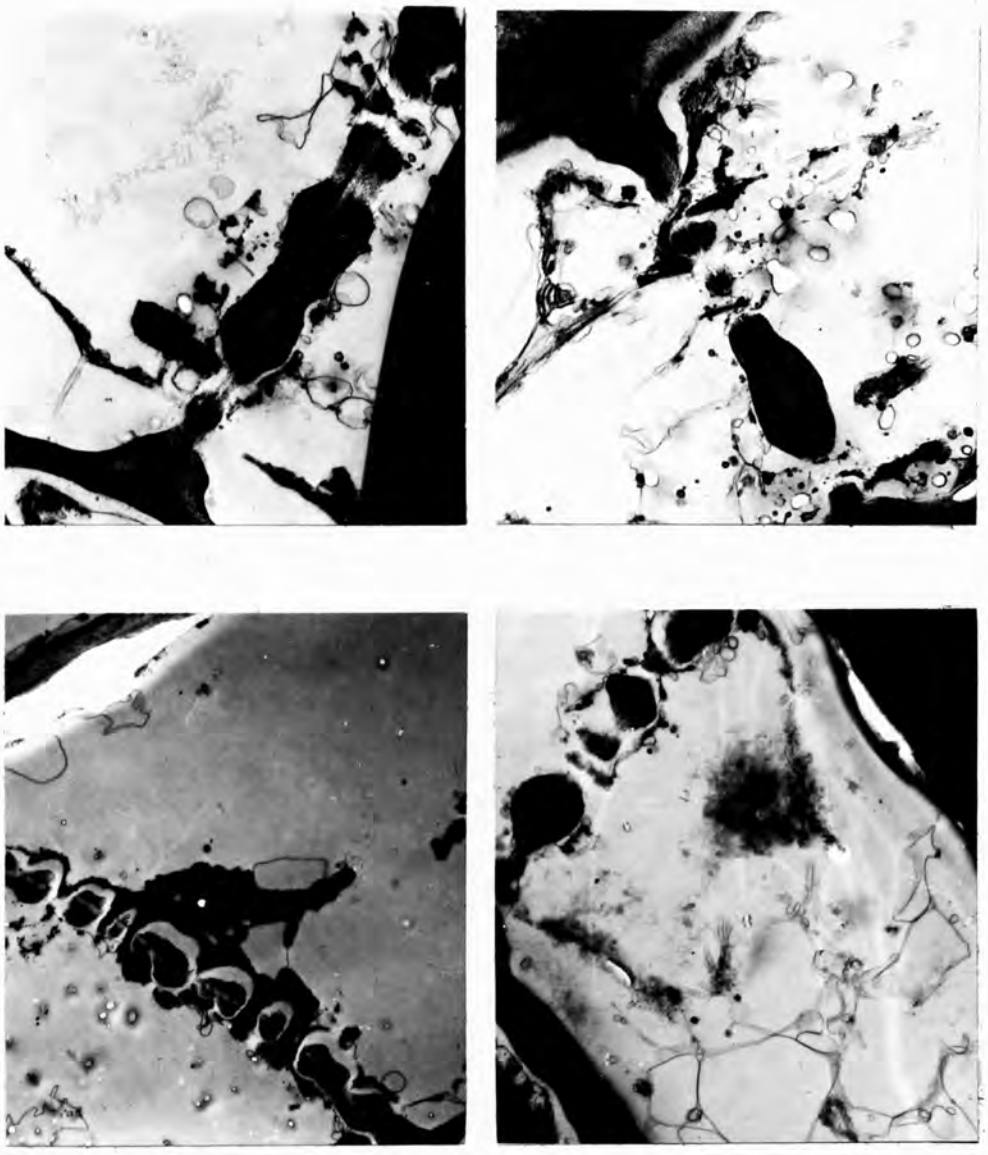


Plate 36 Helianthus stem boiled before fixing: L S of sieve tube showing a sieve plate cut obliquely. Pores are filled with densely staining material. X 15,000.

Plate 37 Ditto. Section passes near the edge of two sieve plate pores and middle lamella seems to be continuous across them. One sparsely filled pore is visible. X 15,000.

Plate 38 Phaseolus stem boiled before fixing: L S of sieve tube. Sieve plate pores appear to be plugged. Note the membranous material around the plate. X 10,000.

Plate 39 Ditto. Sieve plate is surrounded by amorphous substance which also fills the pores. X 10,000.

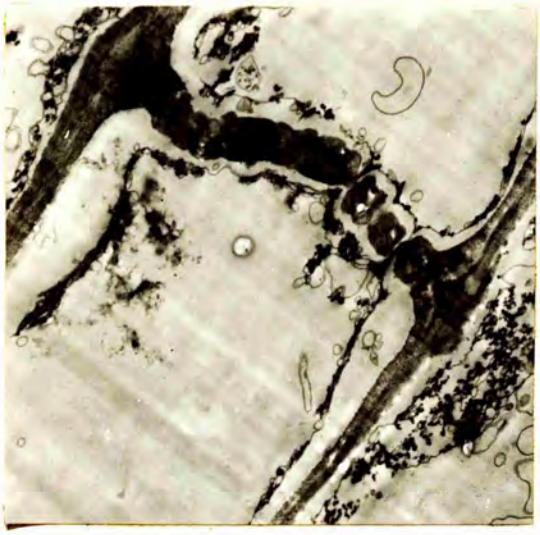
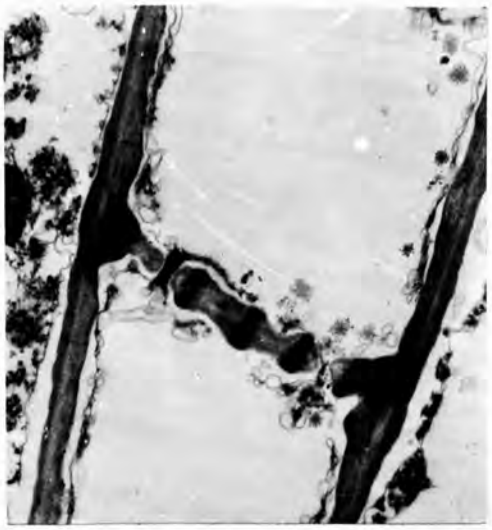


Plate 40 Phaseolus stem boiled before fixing; L S of sieve tube. One pore in the plate seems to be plugged while the other appears only loosely blocked with slime substance. Stained with lead citrate and uranyl acetate. X 10,000.

Plate 41 Ditto. Sieve plate pores seem to be loosely plugged where the section is central enough. Note the shrunk mass of clearly membranous material below the plate (see 44). X 10,000.

Plate 42 Ditto. Two pores are clearly plugged with coagulated material; the third is inconclusive. X 10,000.

Plate 43 Ditto. One obviously plugged pore is present. Note the membranous material possibly derived from plastids. X 10,000.



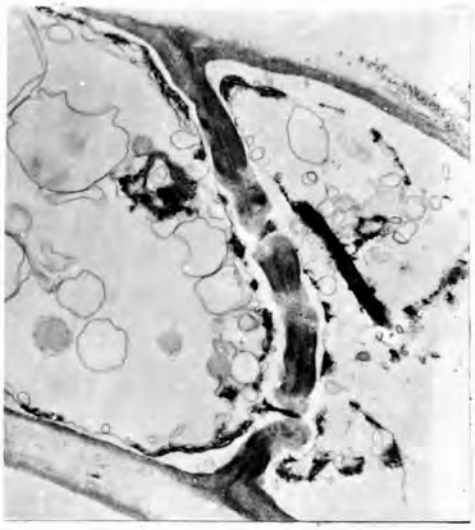
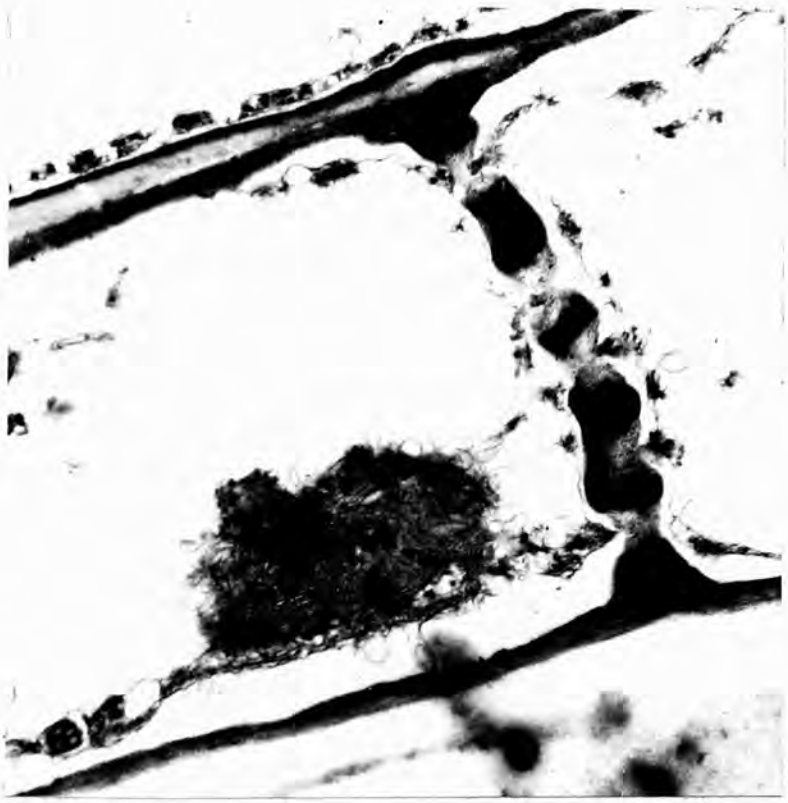


Plate 44 Phaseolus stem boiled before fixing: magnified part of plate 41. Note the lamellar stacks in the coagulated material. X 15,000.

Plate 45 Helianthus hypocotyl: L S of sieve tube. Fibrous material is uniformly spread in the lumen of narrow sieve tube (2·2 μm in diameter), and plastids near the plate have lost their envelopes. Compare the size of mitochondrion in the sieve tube with that of companion cell. Note the lamellar stacks close to the sieve tube wall and thick callose cylinders around the pores. Normal treatment; stained with lead citrate and uranyl acetate. X 22,500.



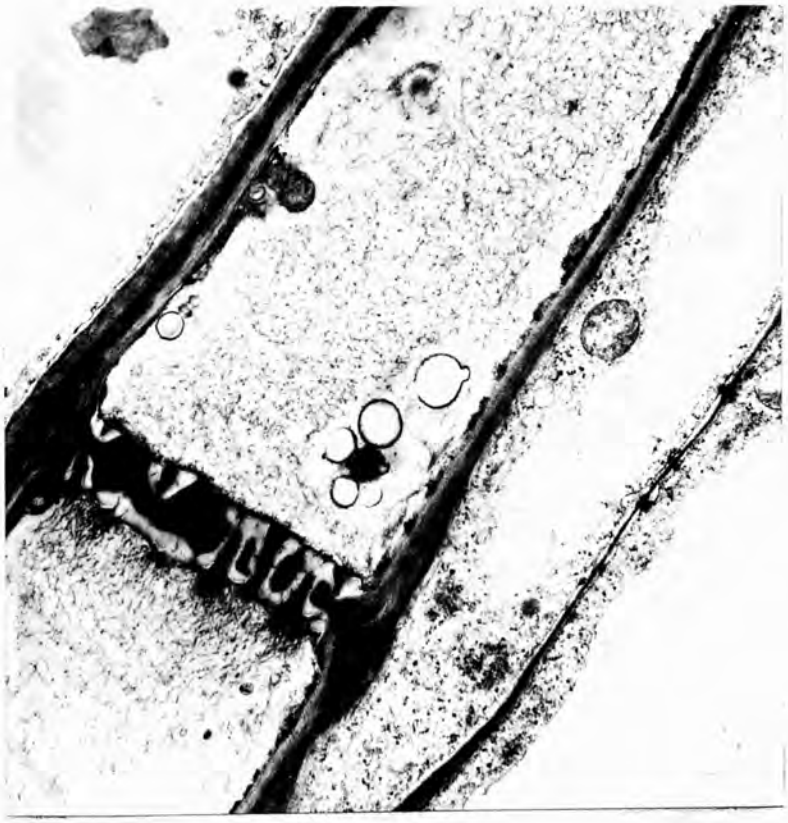
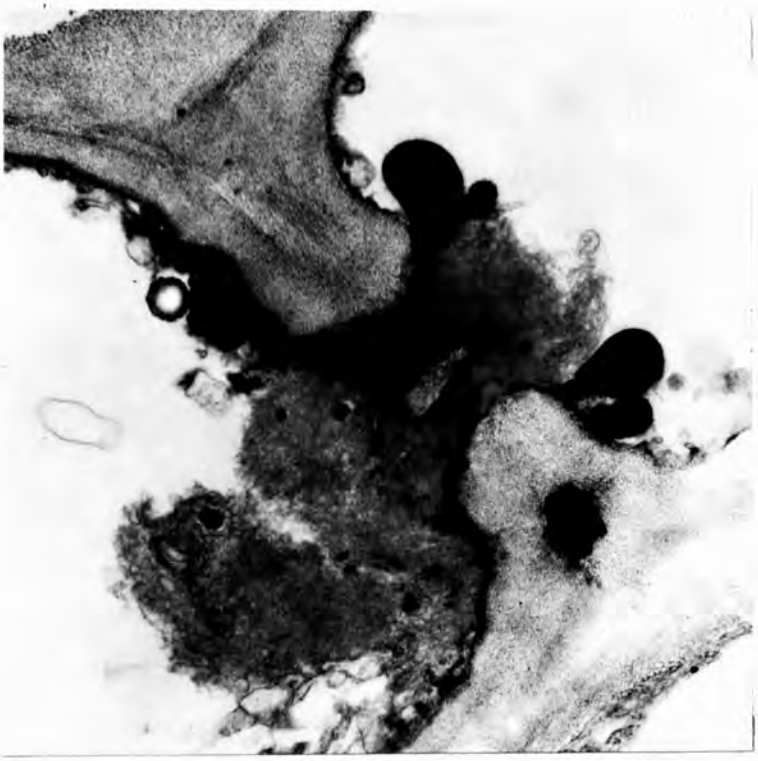


Plate 46 Saxifraga stolon wilted before fixing: L S of sieve plate. Pores are plugged with slime substance. Note the dark globular bodies. These may be tannin drops or similar to the osmophilic granules of Janine Salmon. Callose is absent. Stained with lead citrate. X 30,000.

Plate 47 Ditto. Pores are plugged with densely stained compact material. Callose is absent. Note released starch grains and apparent surge effect. X 22,500.



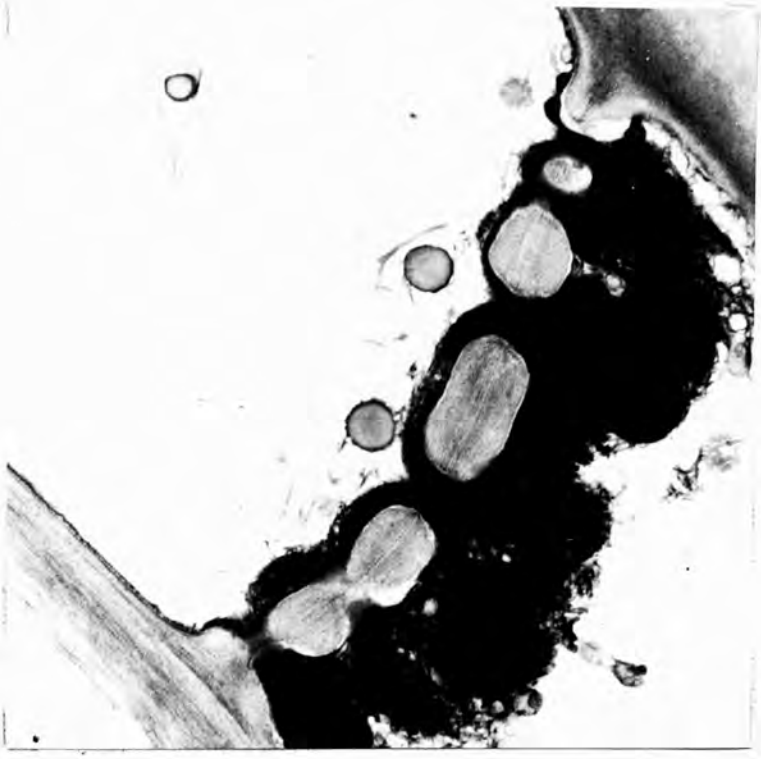
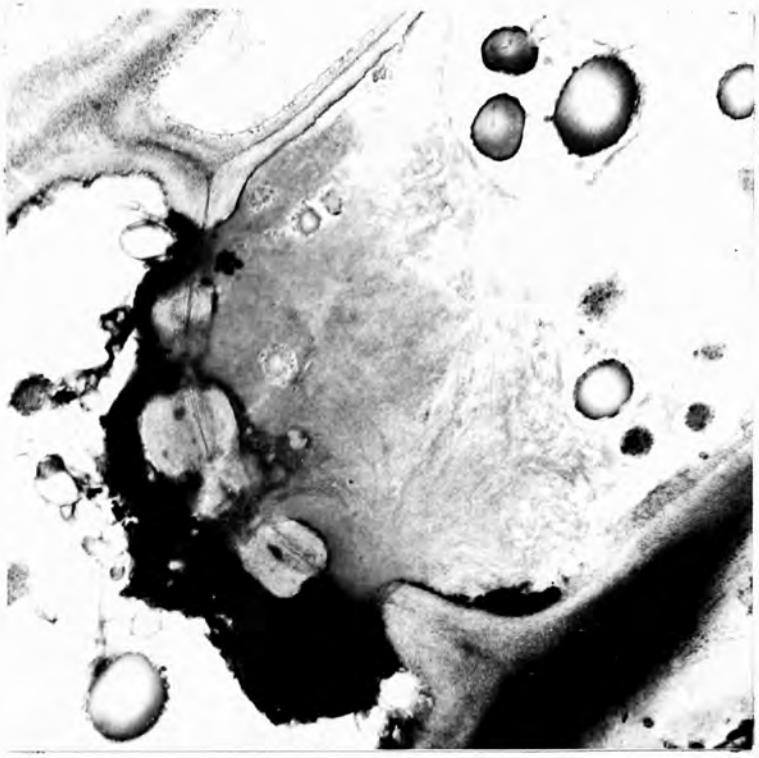


Plate 48 Saxifraga stolon wilted before fixing: L S of sieve plate. Note the difference in appearance of the slime substance on the two sides of the plate. There appears to be an intact plastid at the top right. Surge damage is probable, and apparently the direction seems to be from left to right. Note that callose is absent. Stained with lead citrate. X 22,500.

Plate 49 Ditto. Pores are filled with material rather amorphous in appearance. Darker material (compare 46) can also be identified. Callose is absent. X 30,000.



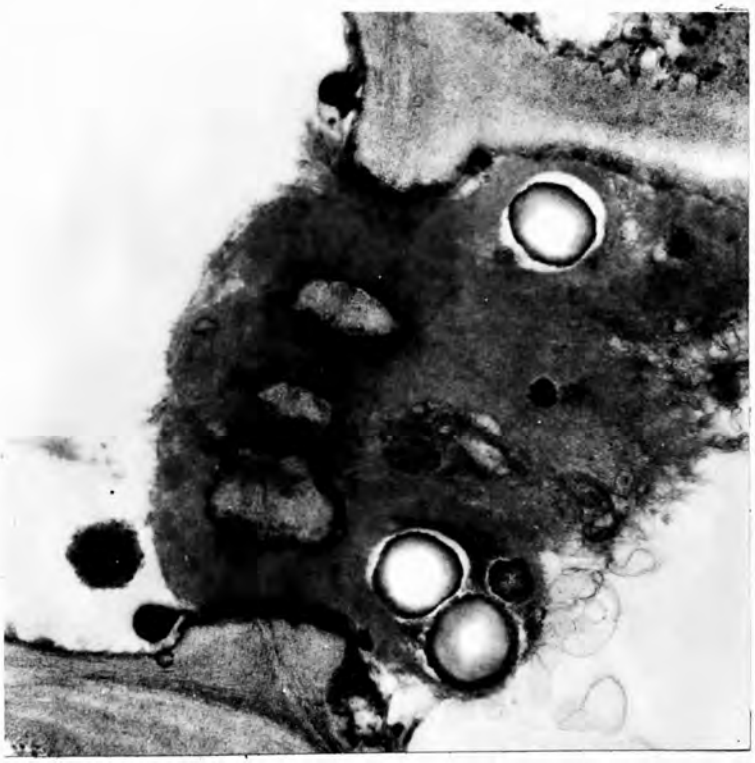
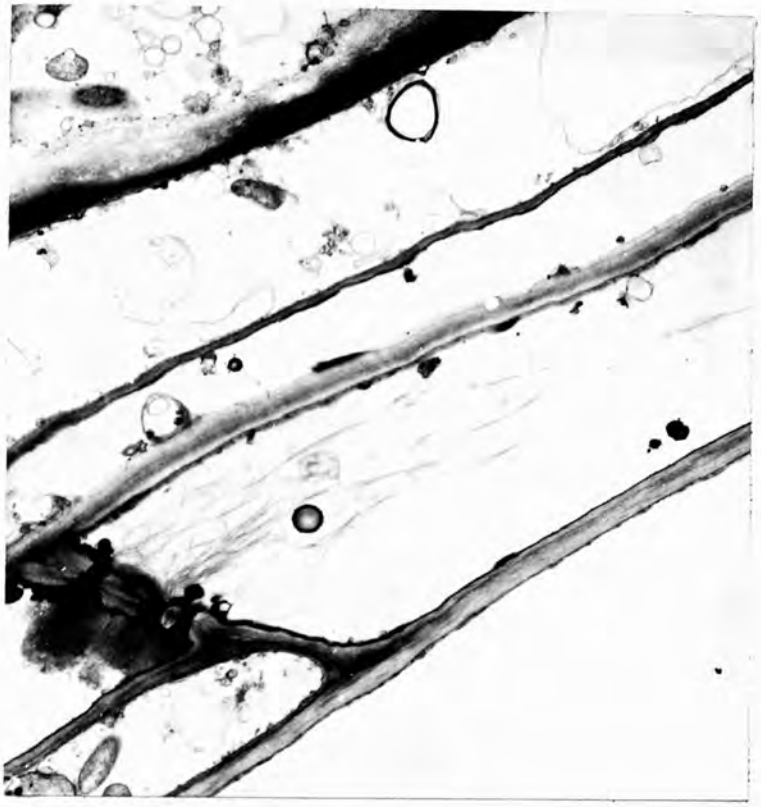


Plate 50 Saxifraga stolon wilted before fixing: L S of sieve tubes. Long strands of slime fibres are dispersed in the lumen. Dark globular material is around the pores; callose is absent. Direction of flow appears to be from right to left but is arguable. Stained with lead citrate. X 7,500.

Plate 51 Ditto. A rather oblique section showing a sieve plate. Sieve plate pores are blocked with slime. A cluster of membranous material is visible on the right hand side of the plate. Movement appears to be from left to right. X 15,000.



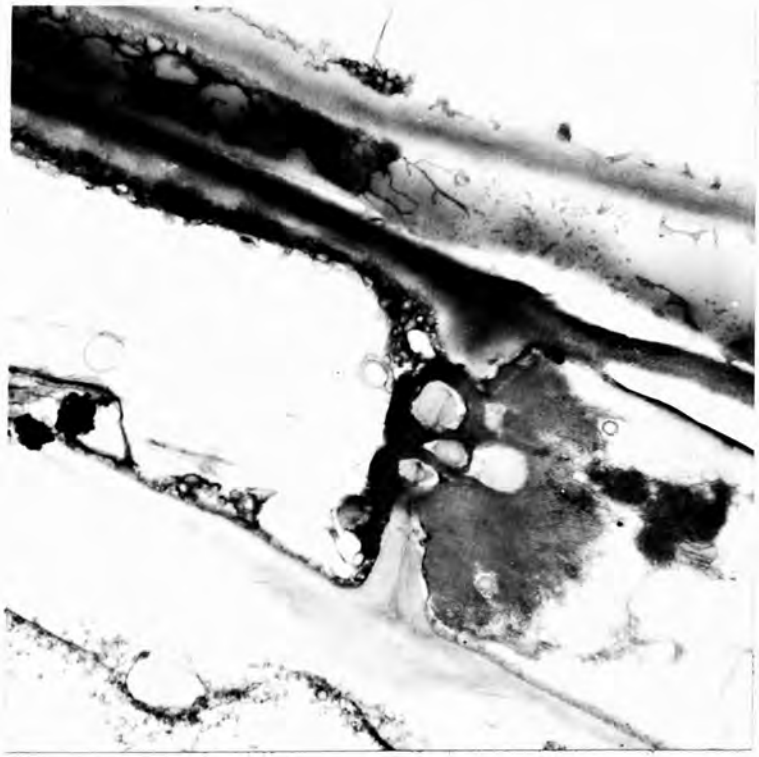


Plate 52 Saxifraga stolon: L S of sieve plate. Pores are plugged with slime and surmounted by dark globular bodies (tannin or osmophilic granules ?) which appear to show some internal structure. Note the plasmodesma cut obliquely at sieve tube wall. Callose is moderate. Normal treatment, stained with lead citrate. X 30,000.

Plate 53 Ditto: The difference in the appearance of the slime fibrils on the two sides of the plate is noteworthy. A large cavity in the cell wall shows some internal structure. The direction of flow appears to be from right to left. Callose is almost absent. X 22,500.

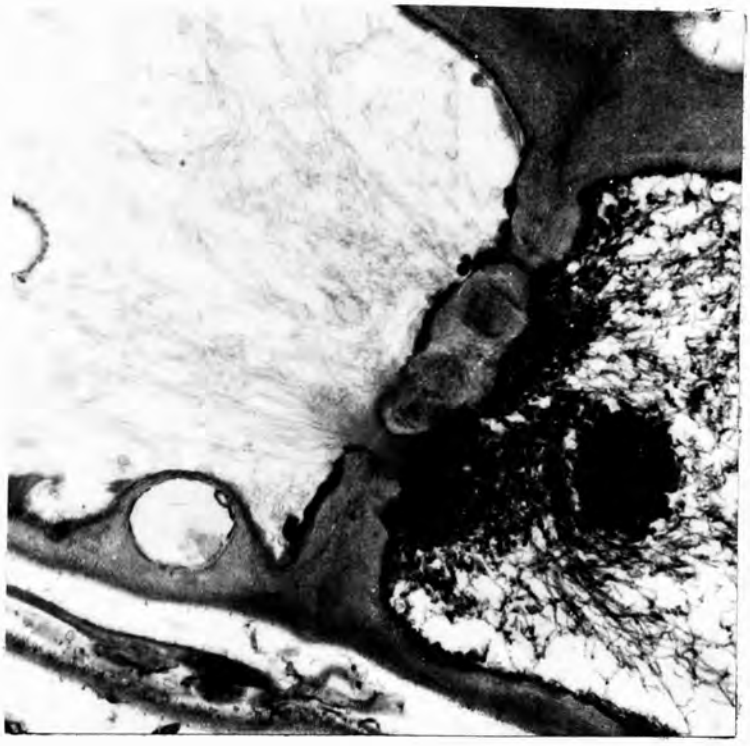


Plate 54 Helianthus hypocotyl wilted before fixing: L S of sieve plate. Slime fibrils are dispersed uniformly on the upper side of the plate while they are scarce on the lower side. Callose is abundant. Intact plastids can be seen on both sides of the wide plate (9 µm in diameter), discounting the likelihood of a violent surge on cutting, yet all pores are densely filled. Stained with lead citrate and uranyl acetate. X 11,250.

Plate 55 Ditto. Pores are plugged with slime. Direction of flow seems to be from right to left. Callose is fairly abundant. Note that were the pores filled by a surge artefact the slime would in all likelihood be piled up on one side of the plate (e.g. plate 56). X 30,000.

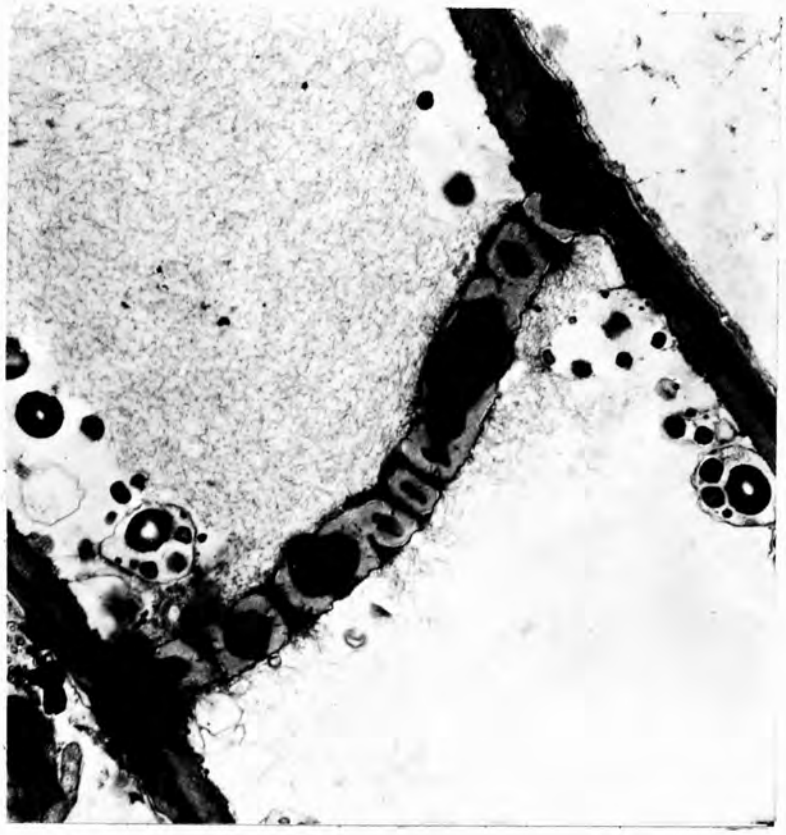


Plate 56 Helianthus hypocotyl wilted before fixing; L S of sieve plate. Pores are plugged with slime which seems to have been forced through in 'sacs' by a surge artefact. Note also the presence of liberated starch grains very close to the plate, and the piling up of the slime substance on one side. Contrast plate 55. Stained with lead citrate and uranyl acetate. X 15,000.

Plate 57 Ditto. A sieve plate and lateral sieve area, both heavily callosed. The liberated starch grains and general appearance indicate surge damage. X 7,500.

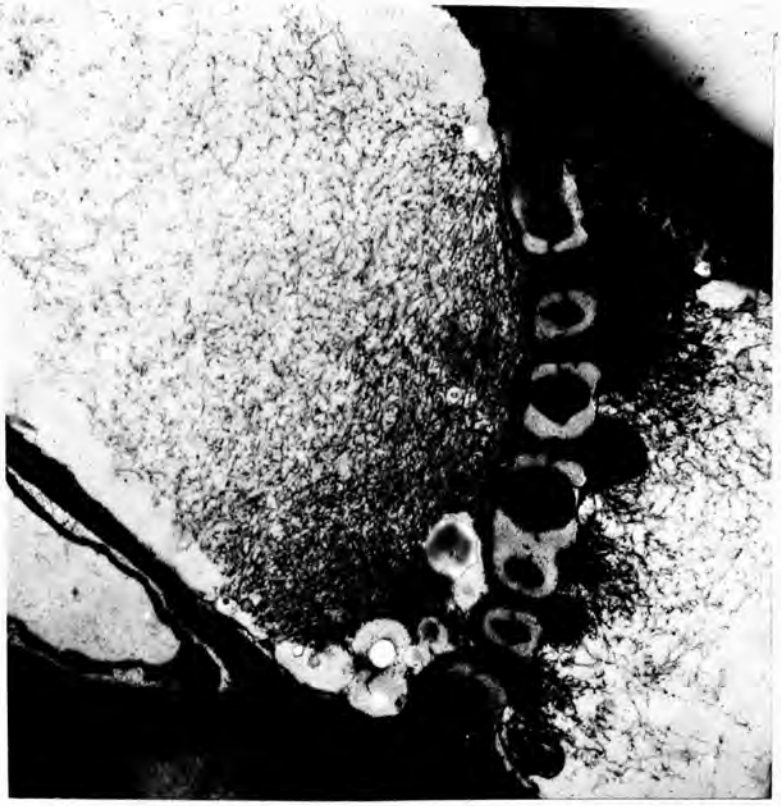
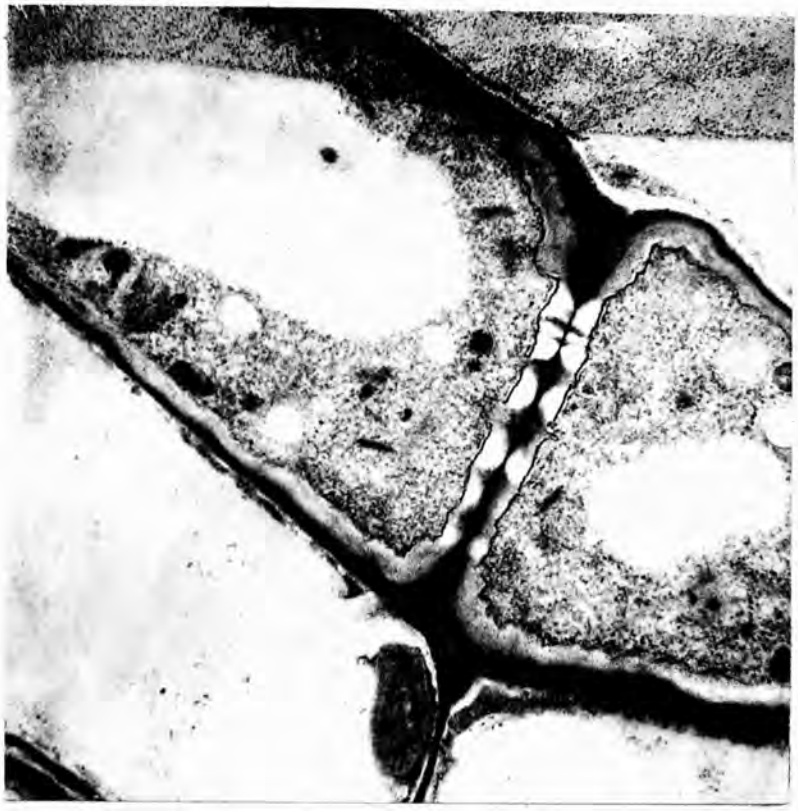


Plate 58 Helianthus hypocotyl: L S of young sieve tube showing the development of the sieve plate. Note paired callose Platelets. At one place pore formation has clearly started by the breakdown of the middle lamella and the development of a'median nodule'. The cytoplasm appears rich with ribosomes, and other organelles. Normal treatment, stained with lead citrate and uranyl acetate. X 11,250.

Plate 59 Heracleum internode: L S of young sieve plate. Callose platelets on the future pore sites are thinner than the cell wall (compare with plates 58, 124 and 127). Ribosomes are present in the cytoplasm. Normal treatment, stained with uranyl acetate and lead citrate. X 15,000.



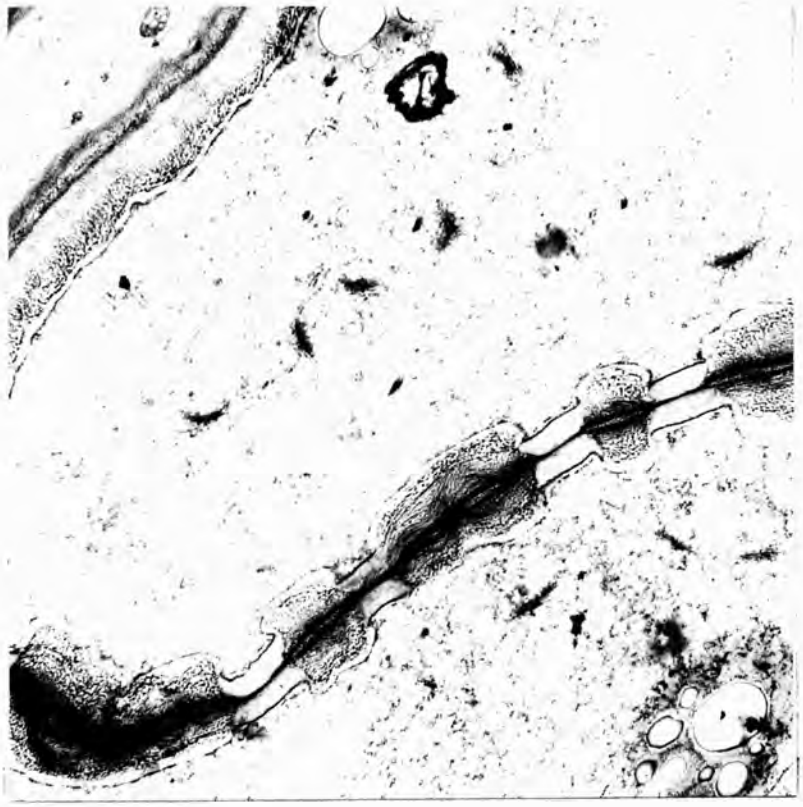


Plate 60 Helianthus hypocotyl: T S of phloem. Three sieve tubes can be identified in the middle by their thick walls and contents. The lowest one still has a nucleus whose content appears hardly denser than the lumen; probably it is near to disintegration. A plasmodesma runs between the tube and its companion cell below. The sieve tubes above show slime fibrils and some lamellar stacks. Note the distinct plasmalemma layer bounding the sieve tube contents. Normal treatment, stained with lead citrate and uranyl acetate. X 7,500.

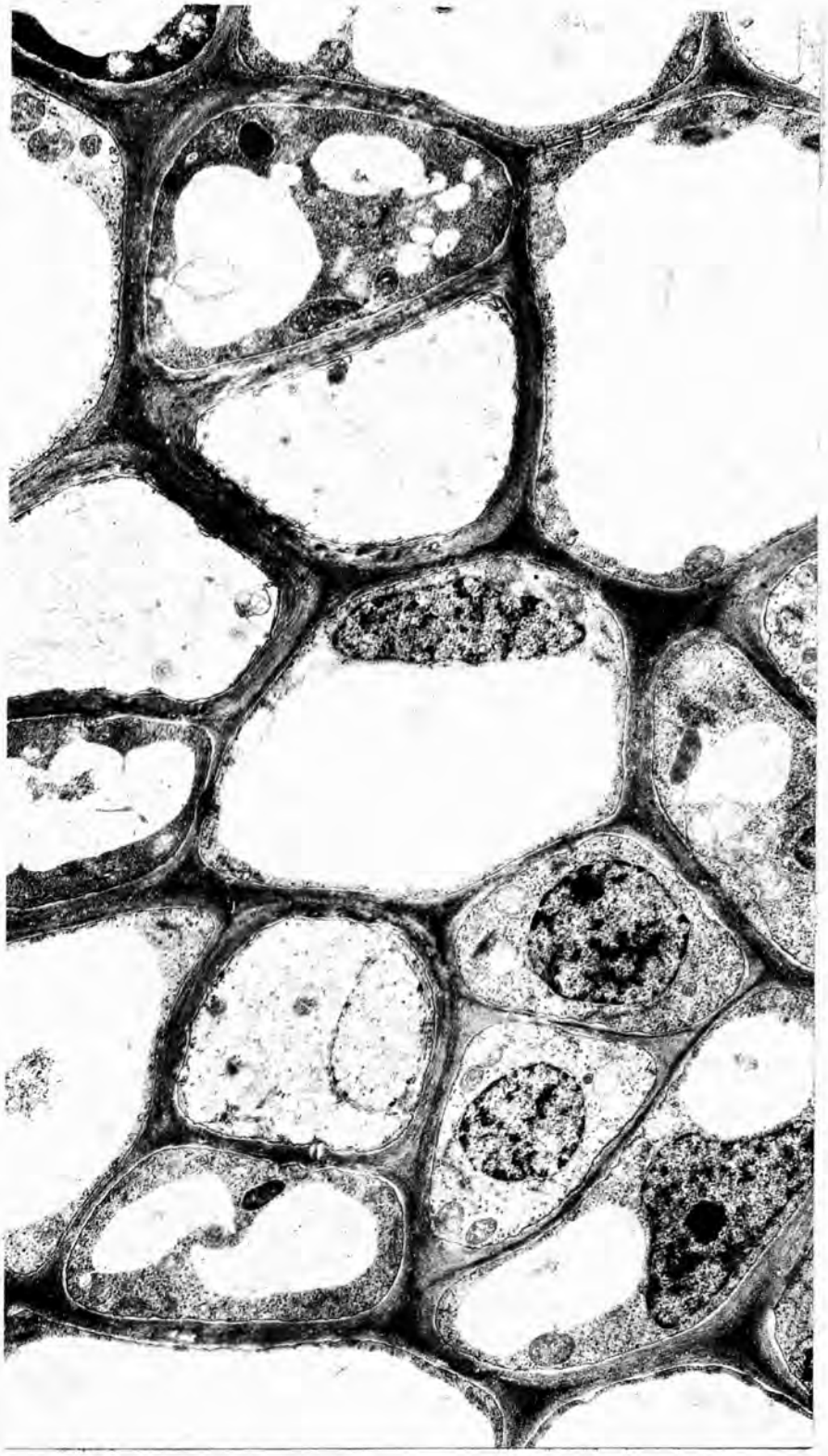


Plate 61 Saxifraga stolon: L S of sieve plate. Slime fibrils which are fraying out from the pores are clearly banded and their transverse alignment is wide (e.g. left opening of upper pore). Slime substance near the plate at the bottom left is tubular in form and seems to show a haxagonal pattern when it is cut transversely. Liberated starch grains and plastid envelopes can be seen on the right. Normal treatment, stained with lead citrate. X 45,000.

Plate 62 Ditto: A magnified part of plate 69. Note the formation of a coarser strand from the slime fibrils. X 60,000.

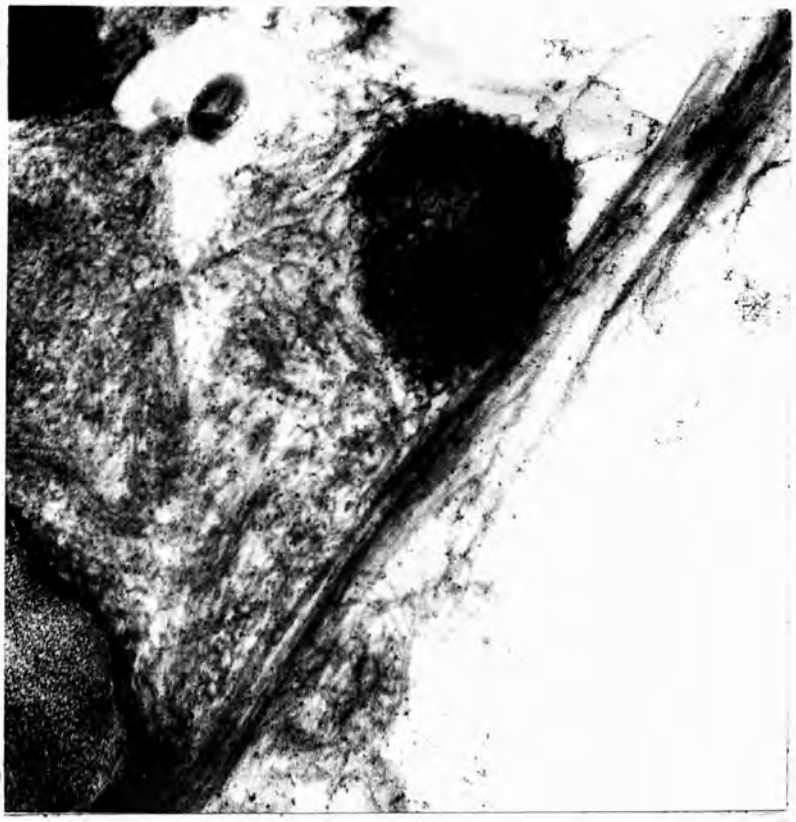


Plate 63 Saxifraga stolon: L S of sieve plate showing a plugged pore. The fibrils fraying out of the pores are banded with a period of 10 nm and a diameter of 12 nm. Note the extent of transverse alignment. This would argue against the pore contents being simply a surge artefact, even though liberated starch grains are visible. Normal treatment, stained with lead citrate. X 60,000.

Plate 64 Ditto. A pore plugged with slime fibrils again showing extensive lateral alignment of the bands. Callose is absent and there is little evidence of surge artefact. X 90,000.

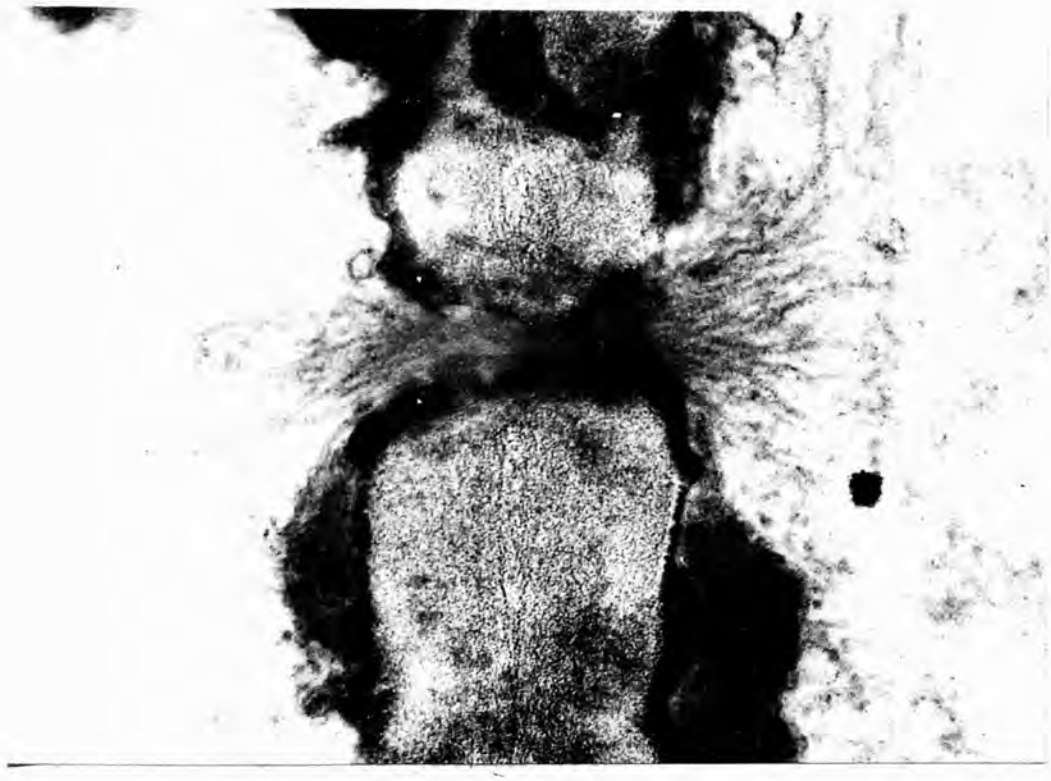


Plate 65 Saxifraga stolon: L S of sieve tube showing the banded structure of fibrils. A displacement on turgor release is suggested by the presence of liberated starch grains near the pores. However these grains show an 'aged' appearance and may have been naturally released. Compare the same plate at lower magnification (No. 1). X 40,000.

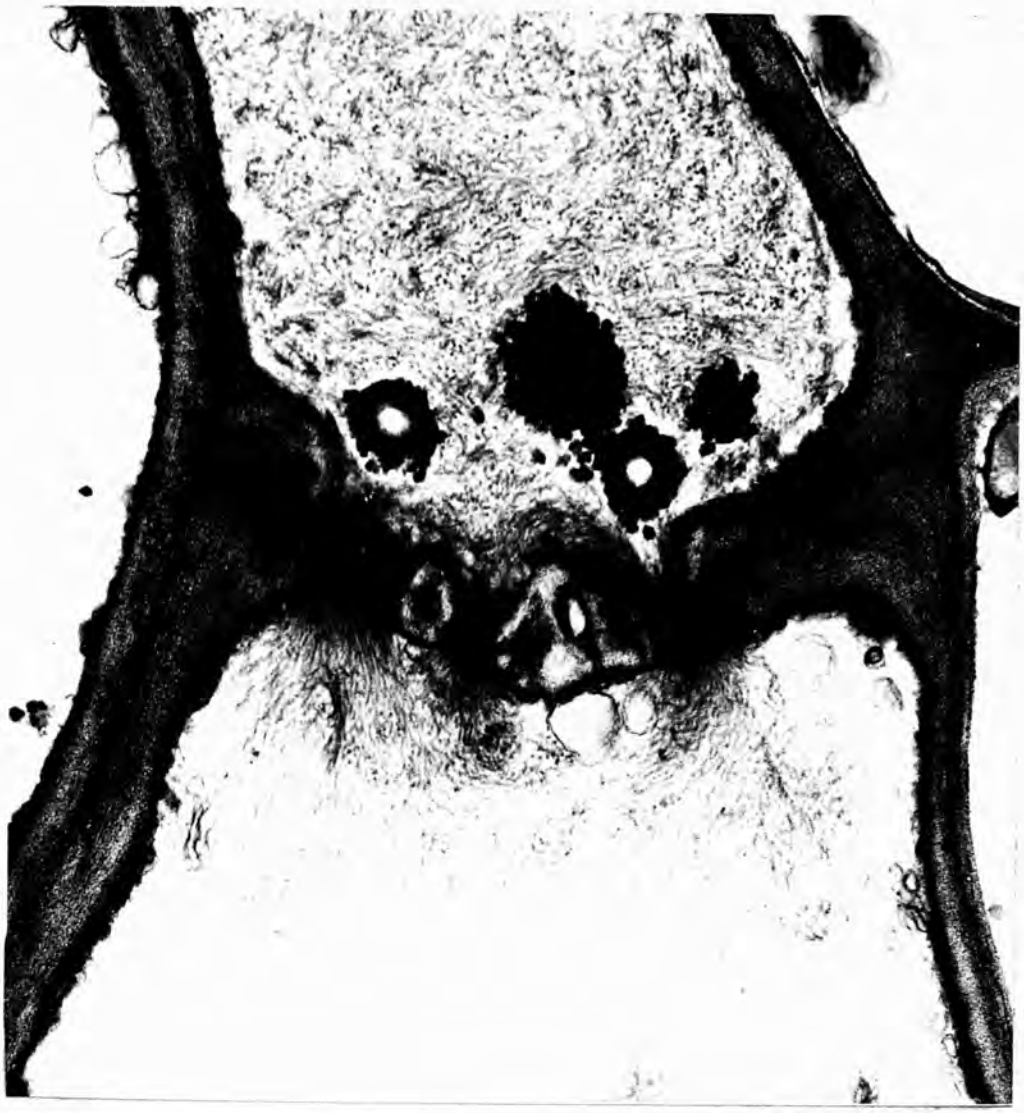
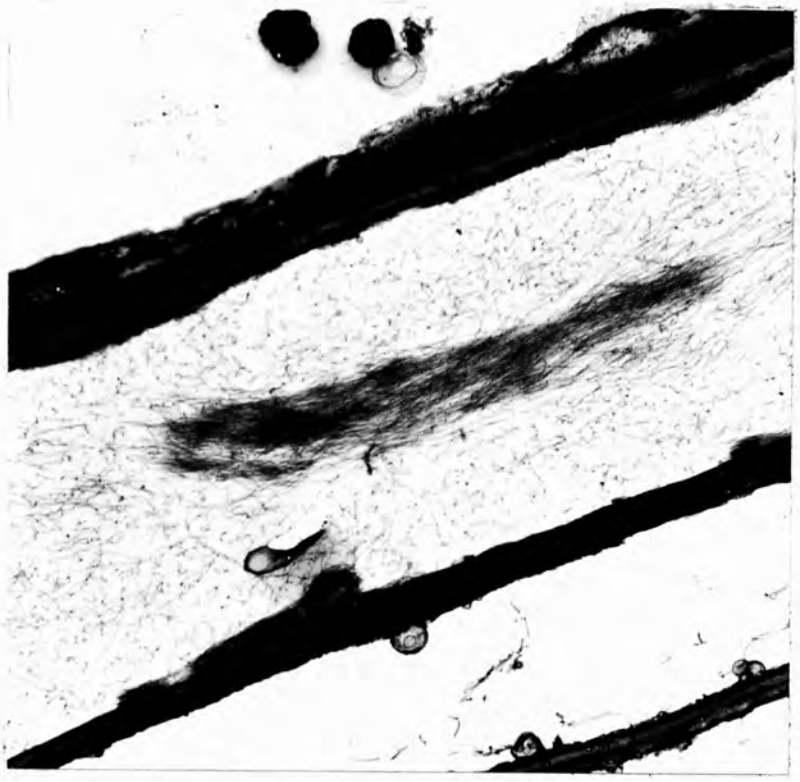


Plate 6 Saxifraga stolon: L S of sieve tube showing a disintegrating slime body. There is no indication of tubules. Normal treatment, stained with lead citrate. X 15,000.

Plate 67 Ditto. Showing three sieve tubes, in one of which is a large mass of slime fibrils. Note the transverse orientation of most of the fibrils in the mass, indication of a flow effect (not necessarily an artefact). X 11,000.



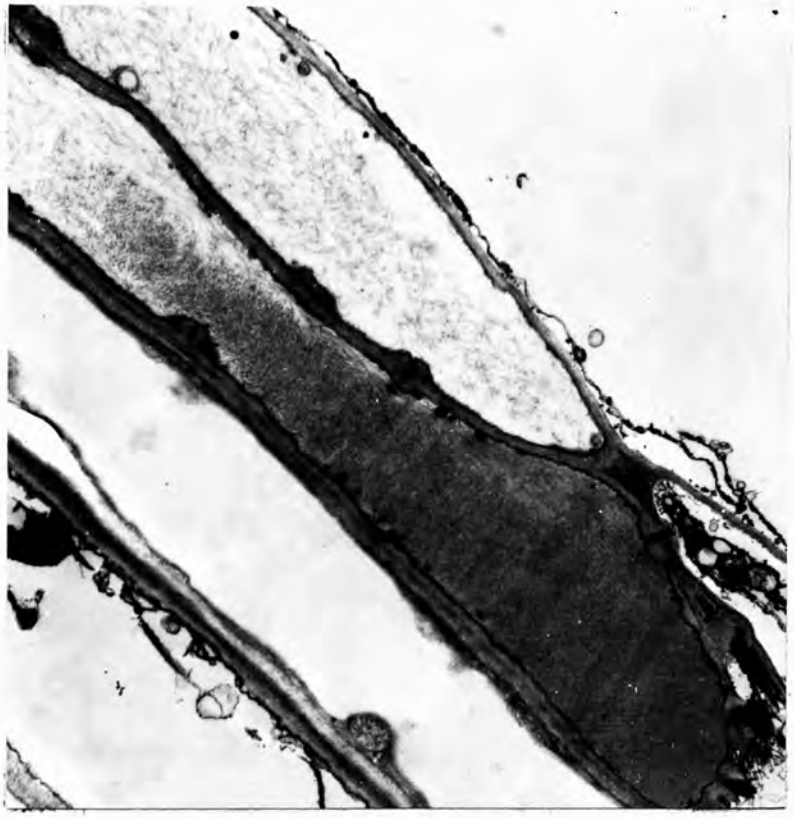


Plate 68 Saxifraga stolon: L S of sieve tubes; at least four tubes can be identified. In the upper tube a long strand of slime extends from sieve plate into the lumen. Intact plastids and dispersed slime can be seen in the tube below (lower centre). Normal treatment, stained with lead citrate. X 3,750.

Plate 69 Ditto. A tubular structure and thick coarse strand of slime are prominent. Note the lamellar stacks perpendicular to the wall. X 30,000.

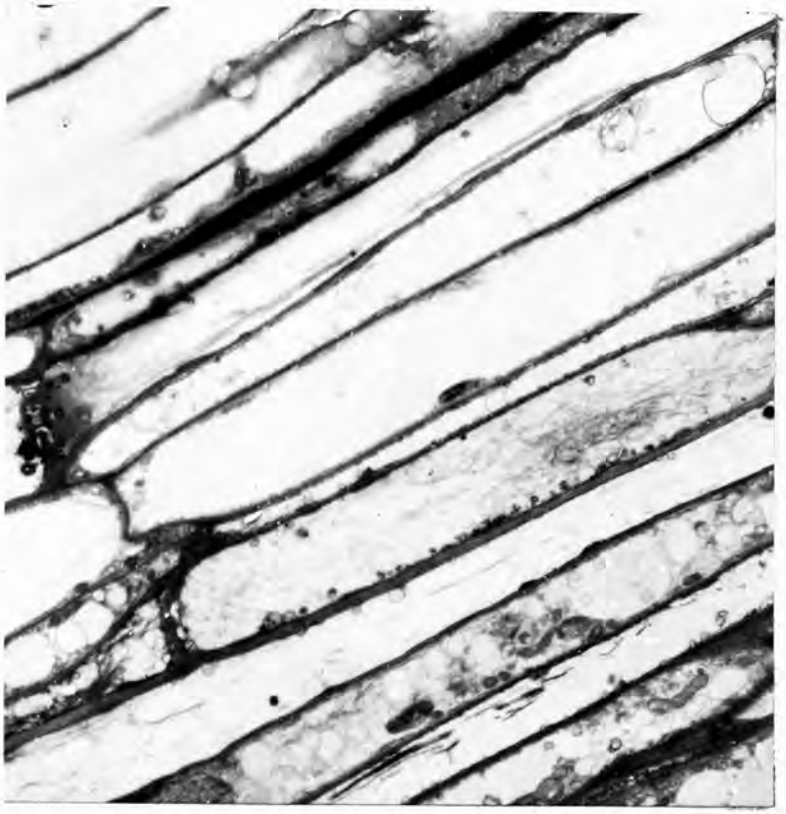


Plate 70 Saxifraga stolon: L S of sieve plate. Note the tubular aggregate, and the dissimilar appearance of the slime material on the two sides of the plate. There is little suggestion of any surge effect. Normal treatment, stained with lead citrate. X 45,000.

Plate 71 Ditto. The tubular body appears on the reverse side of the plate. Note the suggestion of wall structure at the bottom. X 45,000.

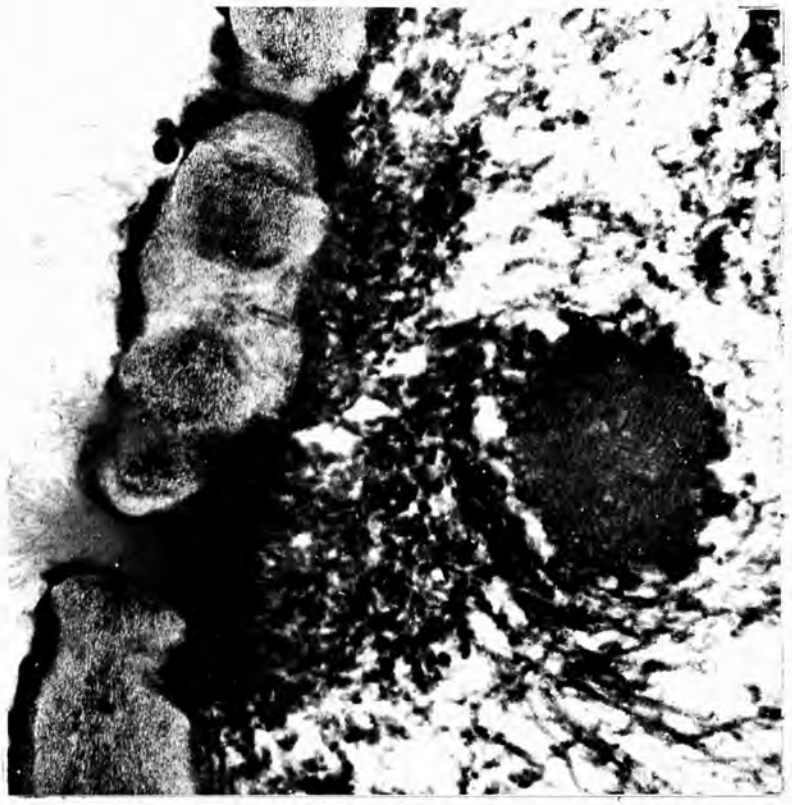
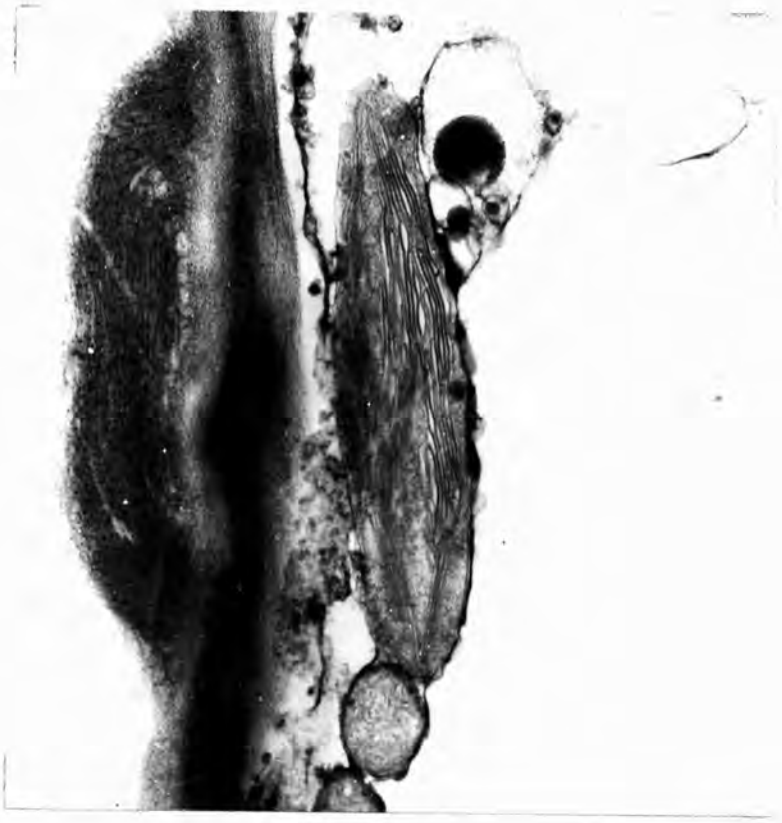


Plate 72 Saxifraga stolon: L S of sieve tube. A lamellar stack (or undispersed slime body ?) is attached very intimately to the sieve tube wall, (see especially at the ends). Note the chloroplast in the adjacent parenchymatous cell for comparison. Normal treatment; stained with lead citrate. X 37,500.

Plate 73 Ditto. A body similar to that in 72. At the upper end the material seems tubular. X 30,000.



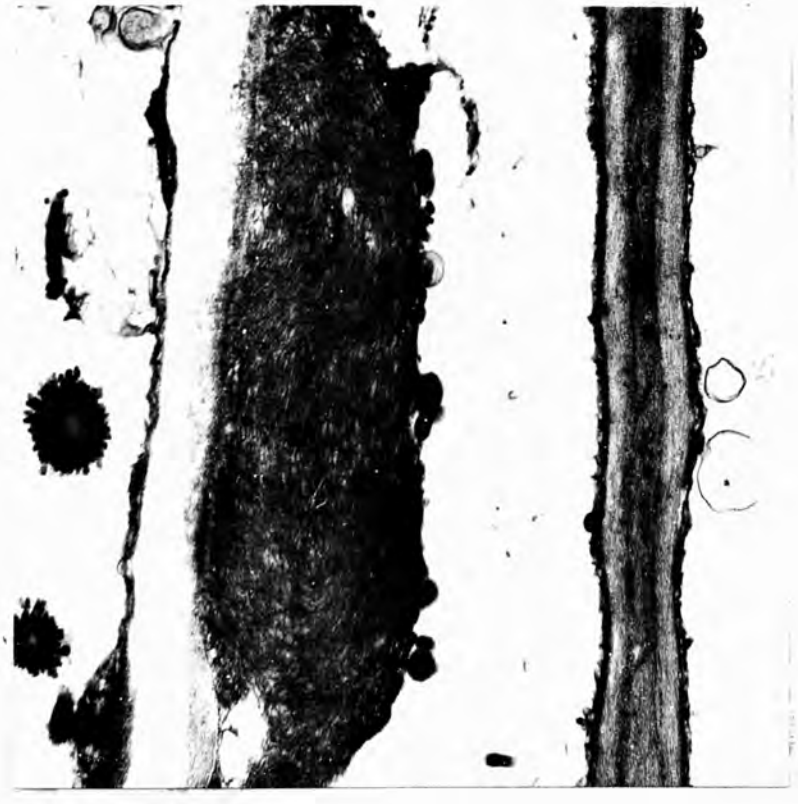
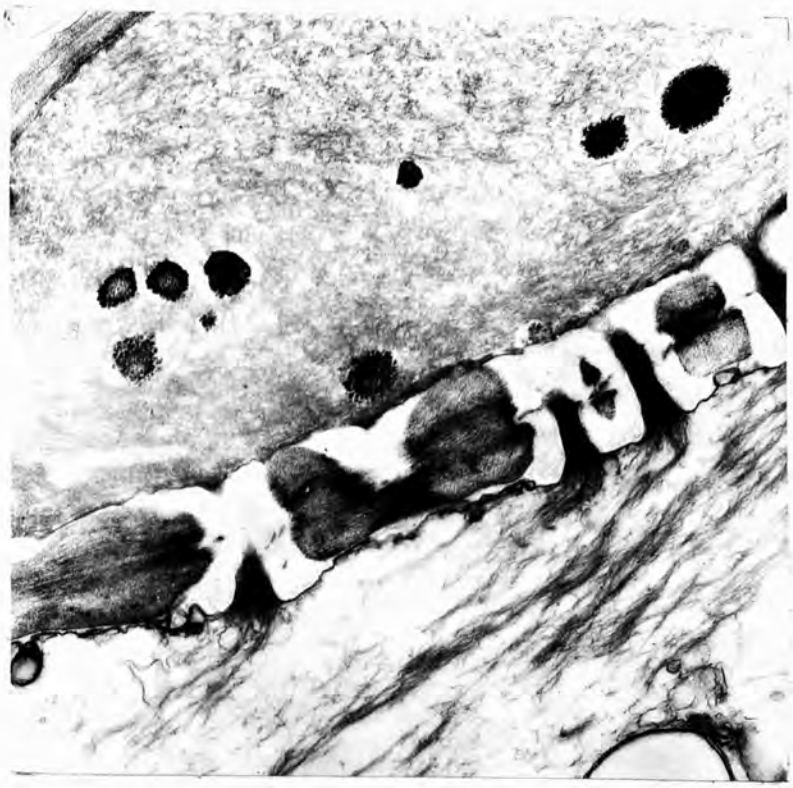


Plate 74 Helianthus hypocotyl: L S of sieve plate. An oblique section shows the plugged pores and strands formed by slime fibres. Plasmalemma seems to be continuous through the pores. Note the thick callose cylinders. Released starch grains suggest some degree of damage. Stained with uranyl acetate and lead citrate. X 22,500.

Plate 75 Ditto. Sieve plate pores are plugged with slime although there is no evidence of a surge artefact. An intact plastid is present near the plate. Fibrils are typically banded. X 22,500.



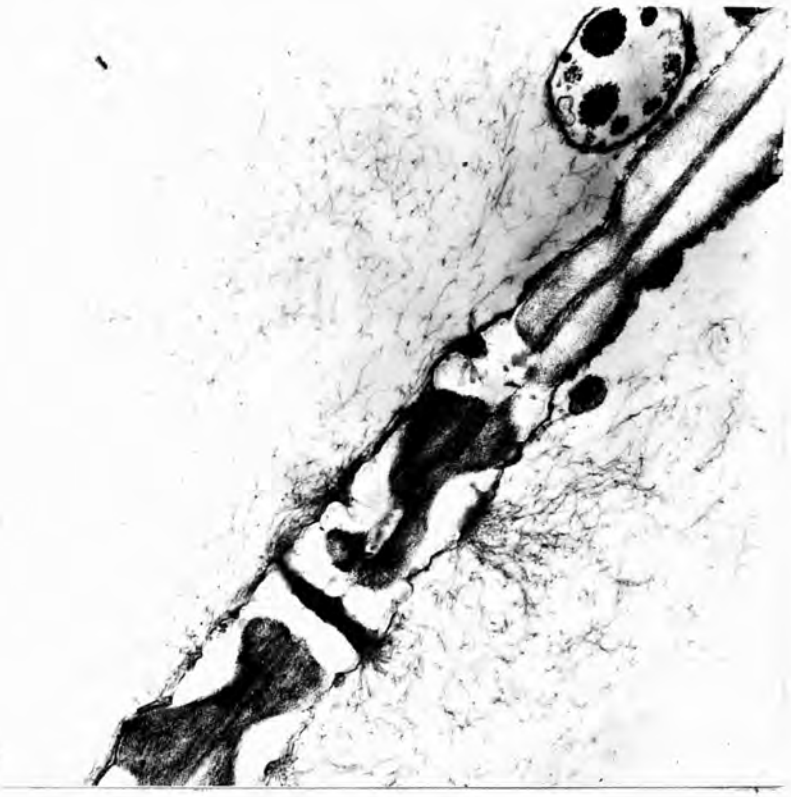
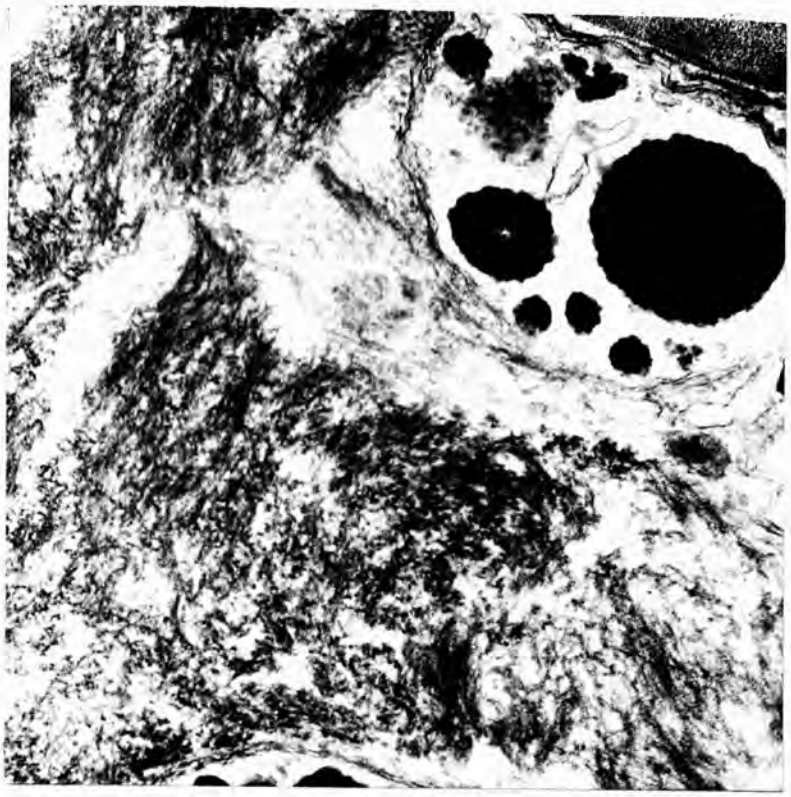


Plate 76 Helianthus hypocotyl: L S of sieve tube showing plastids and dispersed slime fibrils which are clearly banded. Normal treatment, stained with uranyl acetate and lead citrate. X 60,000.

Plate 77 Ditto. Showing sieve plate pores; note the banded fibrils and thick callose cylinder. The plasmalemma is clearly visible above the callose. X 80,000.



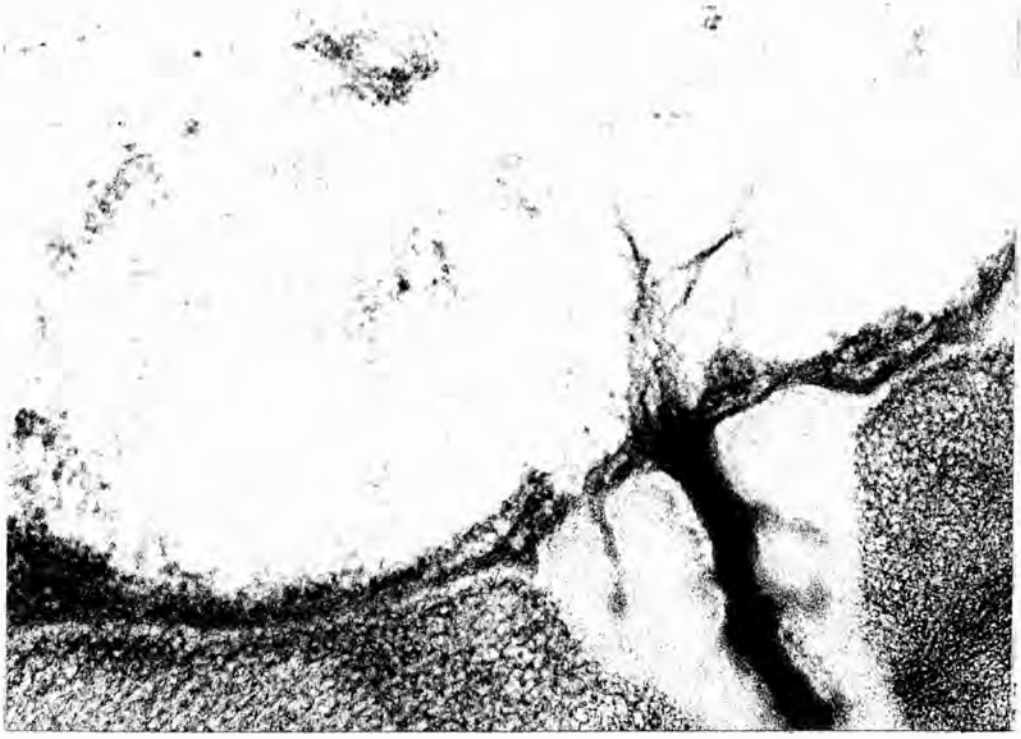


Plate 78 Helianthus hypocotyl: L S of sieve tube showing the slime fibrils in the lumen, the fibrils are typically banded. Note the membranous structure near the wall, simulating a mitochondrion. Normal treatment, stained with uranyl acetate and lead citrate. X 20,000.

Plate 79 Ditto. Note the membranous structures at lower right; a ruptured plastid is shown on upper side of the plate. X 30,000.

Plate 80 Helianthus hypocotyl: L S of sieve tubes showing a large oblique sieve plate. All pores are densely plugged and surrounded by callose. Intact plastids are present. Normal treatment, stained with uranyl acetate and lead citrate. X 7,500.

Plate 81 Ditto. T S of sieve tube and companion cell. Slime fibrils are scattered in the cavity and run mainly transversely. Lamellar stacks and a mitochondrion (?) are present against the sieve tube wall. X 25,000.

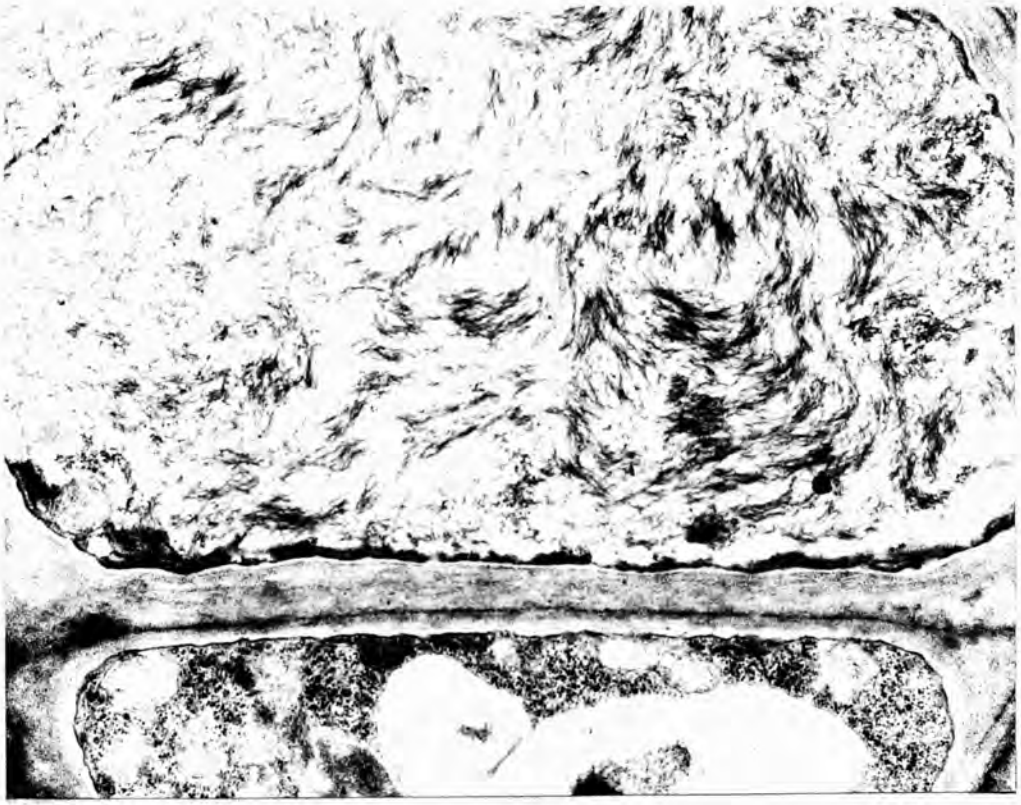


Plate 82 *Helianthus* hypocotyl: L S of a young slime body, apparently of fibrils (Esau and Cronshaw's P2 form). Note the ribosomes and the plasmodesma. Normal treatment, stained with uranyl acetate and lead citrate. X 15,000.

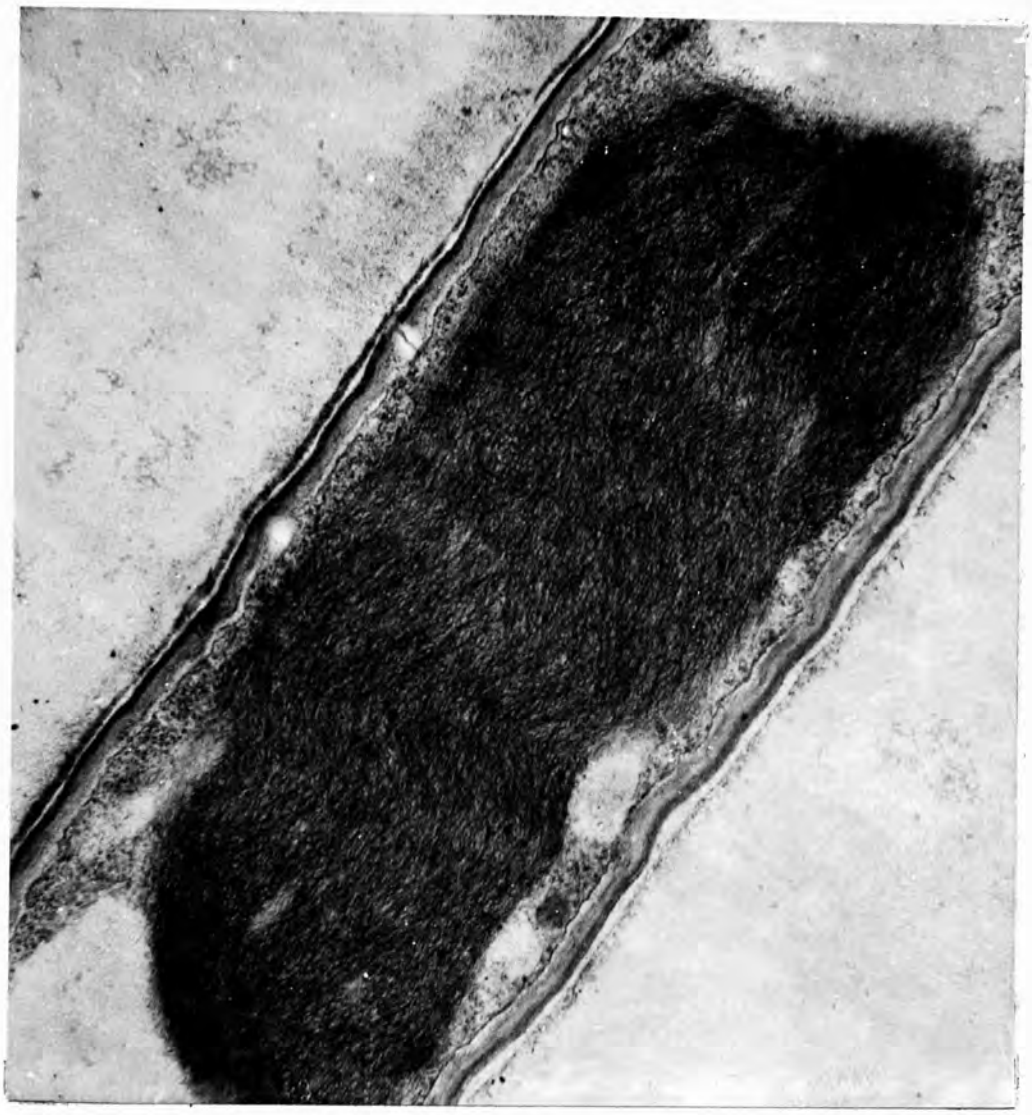
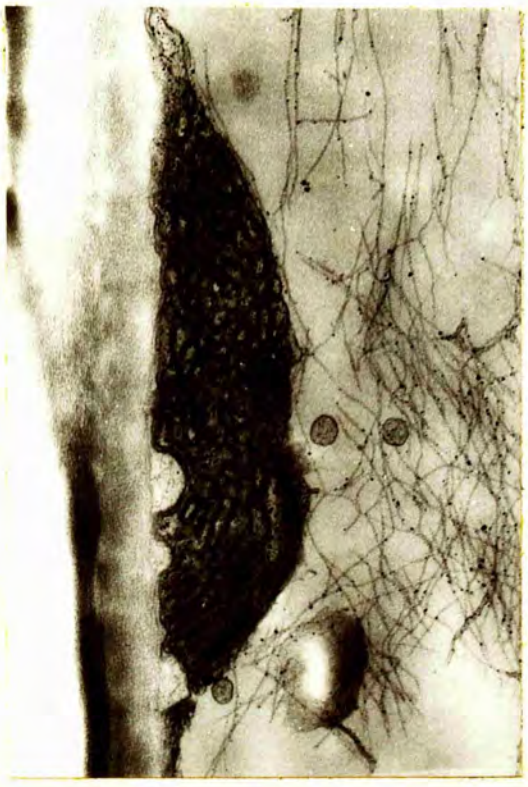


Plate 83 Saxifraga stolon: L S of mature sieve tube containing a curvilinear form of membranous aggregate, closely appressed to wall. Note fine fibrillar material in spaces between aggregate and wall. Normal banded fibrils are present in the lumen. Normal treatment, stained with lead citrate. X 45,000.

Plate 84 Ditto: Showing lamellar stacks attached perpendicularly to the wall and intergrading with a curvilinear aggregate. Normal slime fibrils are present. X 80,000.



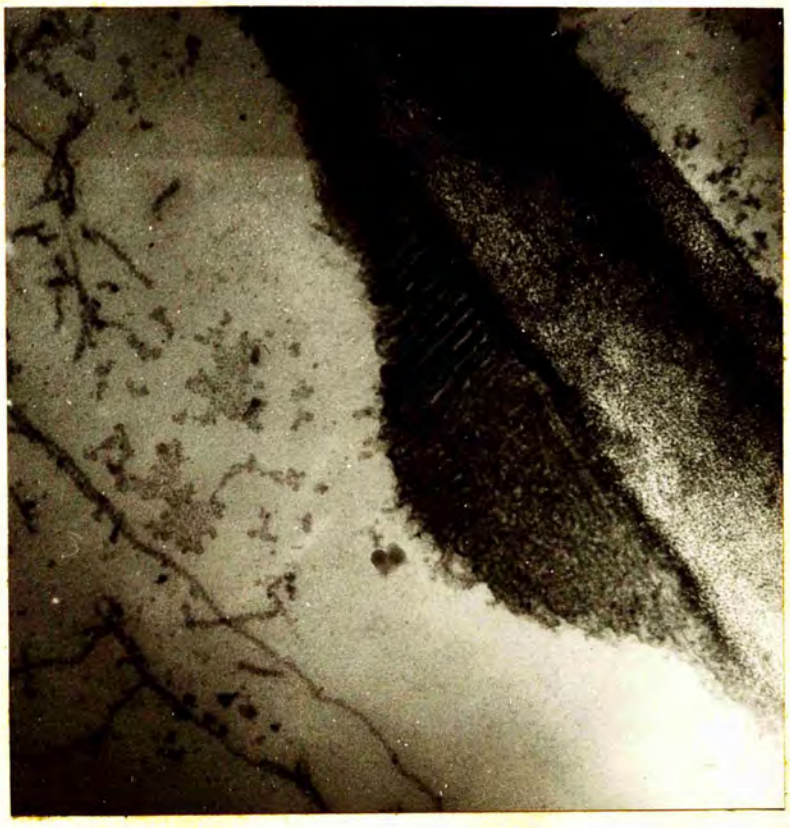


Plate 85a Saxifraga stolon: L S of sieve plate. Pores are densely plugged with slime; lamellar stacks are anchored on the tube wall. Note the membranes around the plate. Some evidence of damage is present. Normal treatment, stained with lead citrate. X 22,500.

Plate 85b Ditto. Showing the plugged pores and lamellar stacks at magnification X 45,000.

Plate 86 Saxifraga stolon: L S of sieve tube. Two types of lamellar stacks are seen, one perpendicular to the wall and the other laying parallel to it (of plate 72). Normal treatment, stained with lead citrate. X 37,500.

Plate 87 Ditto. L S of sieve plate, a magnified part of plate 88. The fibrils fraying out from the pores are clearly banded and show a wide extent of transverse alignment of dark bands to right of plate. Lamellar and tubular stacks are oriented in different ways and show a close association with the wall. X 45,000.

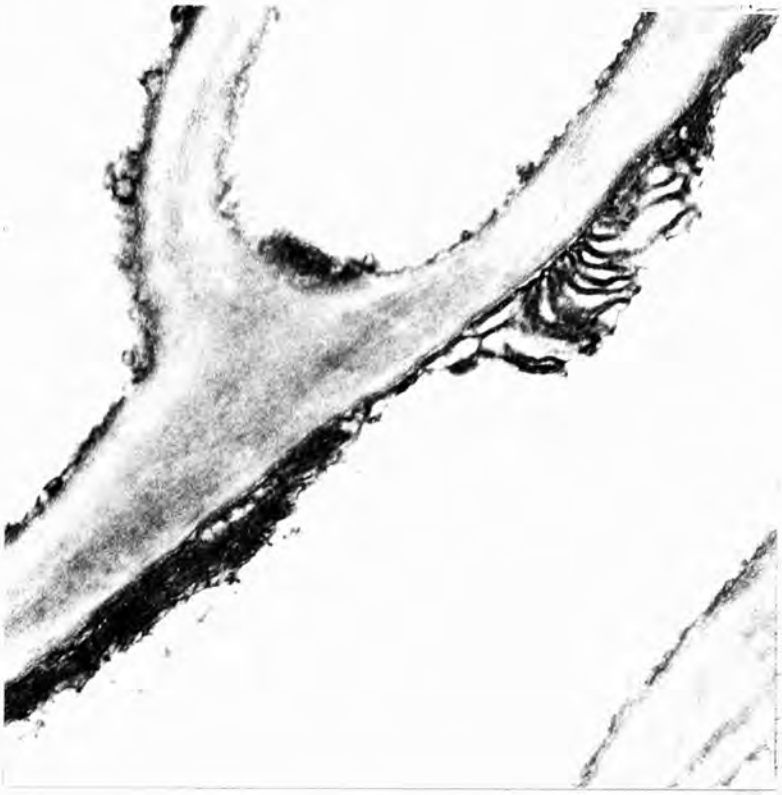
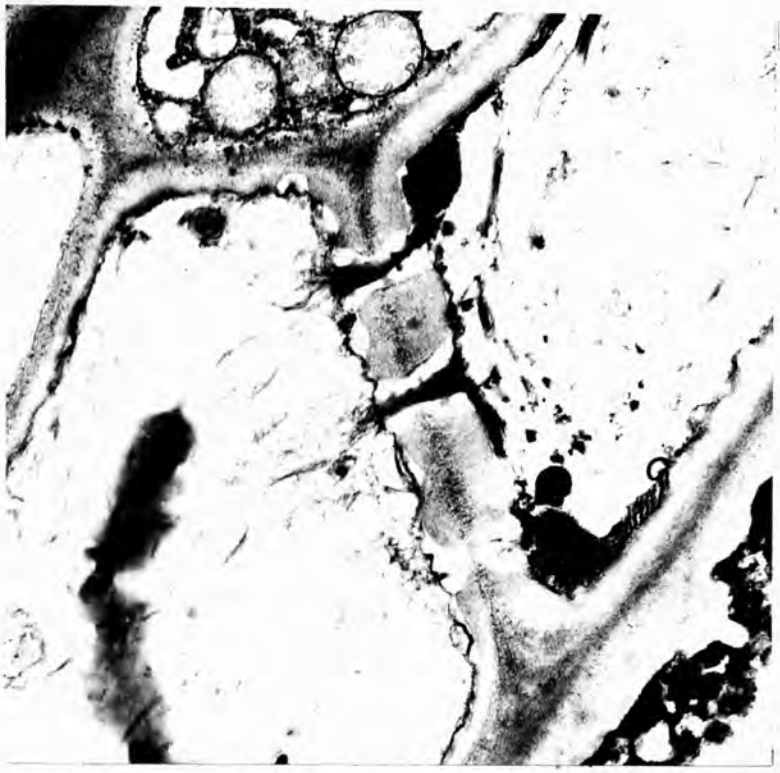


Plate 88 Saxifraga stolon: L S of sieve plate. The pores are plugged but there is little evidence of this being an artefact. Note the membranous material on two corners of the plate. Magnified parts of this plate are shown in plates 87 and 89. Normal treatment, stained with lead citrate. X 22,500.

Plate 89 An enlargement of part of 88. Note the membranous material near the plate in close association with the wall, and also the "pitting" within the callose cylinders. X 45,000.



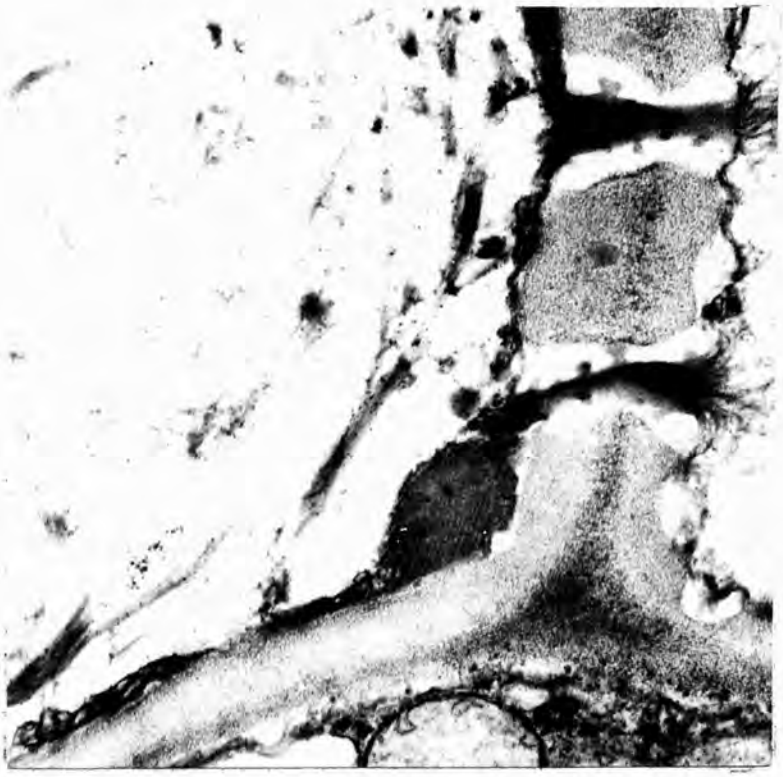


Plate 90 Saxifraga stolon: L S of sieve plate showing various forms of membranous stacks which are close to the cell wall. Plates 91, 92 and 93 are magnified parts of this plate. Normal treatment, stained with lead citrate. X 22,500.

Plate 91 Ditto. Note the lamellar stack parallel to the sieve tube wall and the appearance of the other side of this wall facing into an adjacent tube. The slime fibrils are coarse (e.g. plates 53 & 70). X 45,000.

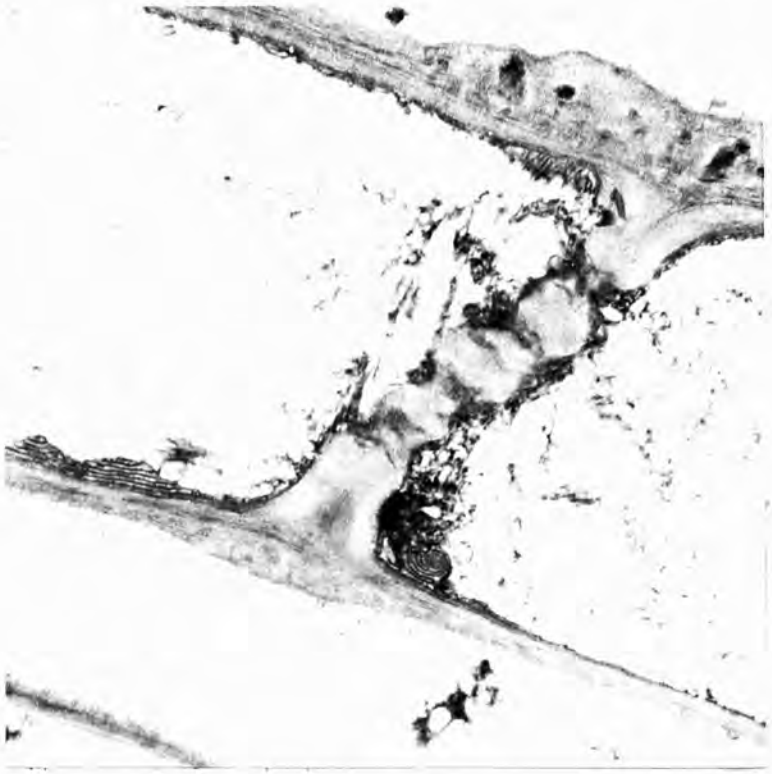


Plate 92 Saxifraga stolon: L S of sieve plate.
Note the membranous material deposited
near the sieve plate in myelin form.
a magnified part of plate 90.
X 45,000.

Plate 93 Ditto. A magnified part of plate 90.
Note lamellar stacks anchored on the wall.
X 45,000.

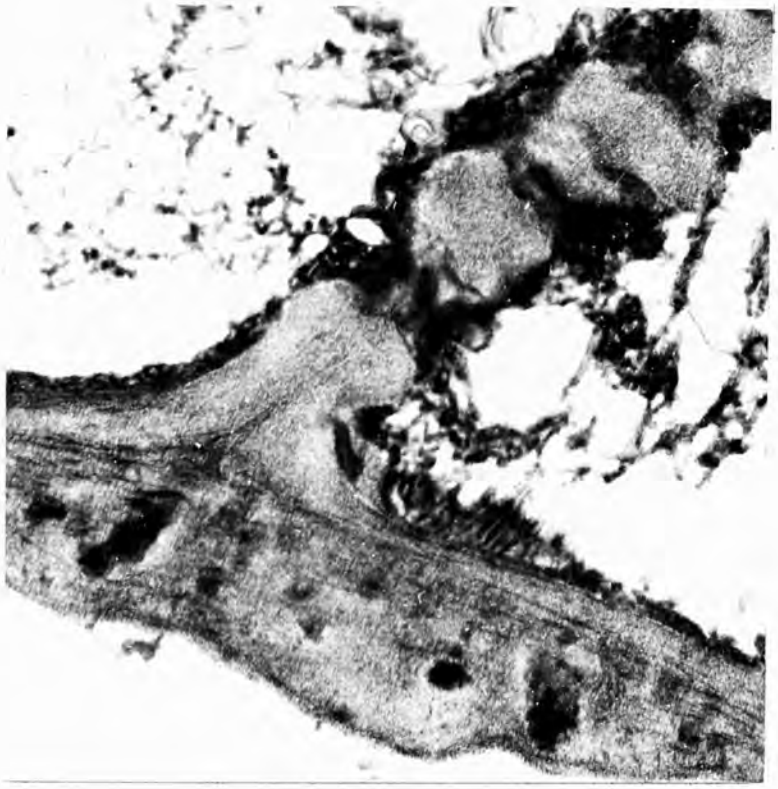


Plate 94 Helianthus hypocotyl: T S of phloem tissue showing at least four sieve tubes (thick walled) with companion cells. Part of a young sieve tube can be seen on the bottom left of the plate. Another tube, more mature, is in the bottom right with two dense companion cells. This tube contains some slime fibrils and several mitochondria. A large mature sieve tube with a companion cell can be seen in the middle of the plate. The tube contains some lamellar structures. A fourth tube on the top right shows a vesicle which seems an invagination of the plasmalemma. Normal treatment, stained with uranyl acetate and lead citrate. X 5,000.

Plate 95 Ditto. L S of sieve plate cut slightly obliquely. Pores are plugged with dense slime and there is a little evidence of damage. Note the lamellar stacks laying on the sieve plate and the indication of wall structure at the bottom. X 22,500.

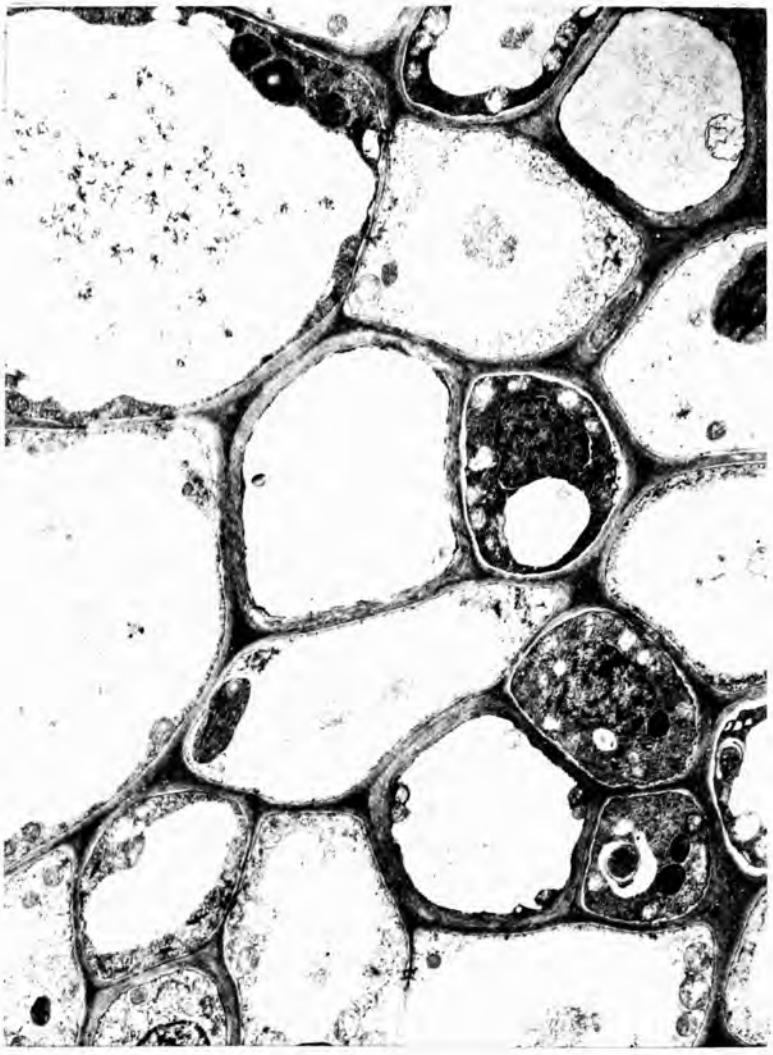
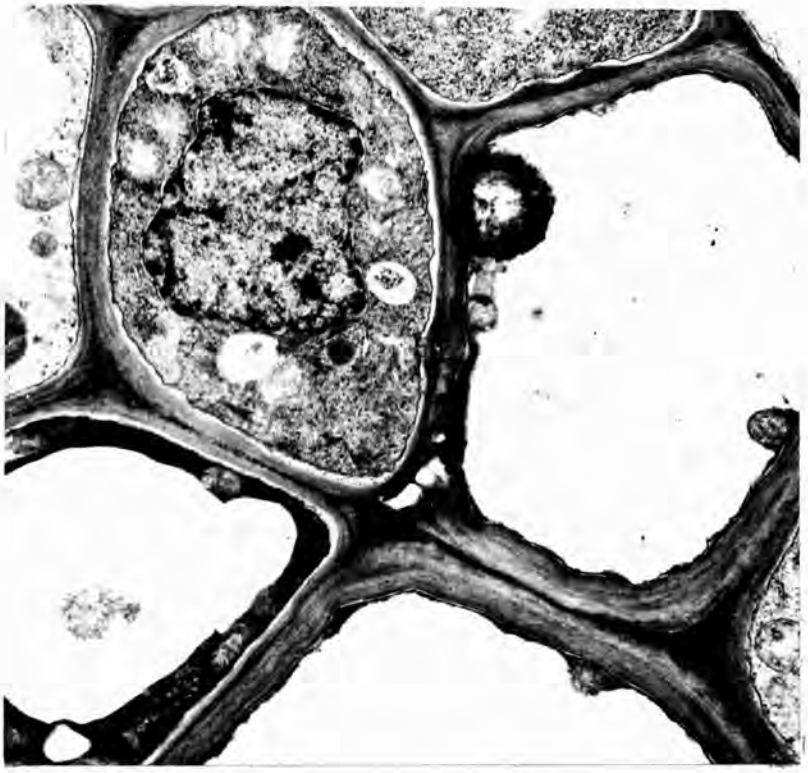


Plate 96 Helianthus hypocotyl: T S of phloem showing two sieve tubes, two companion cells and parenchymatous cells. Slime fibrils, mitochondria and lamellar stacks can be seen in the sieve tubes. Note the plasmodesma in the centre branched towards the companion cell side. Normal treatment, stained with uranyl acetate and lead citrate. X 11,500.

Plate 97 Ditto. An oblique section showing lamellar stacks very close to the wall separated by a continuous white region. X 45,000.



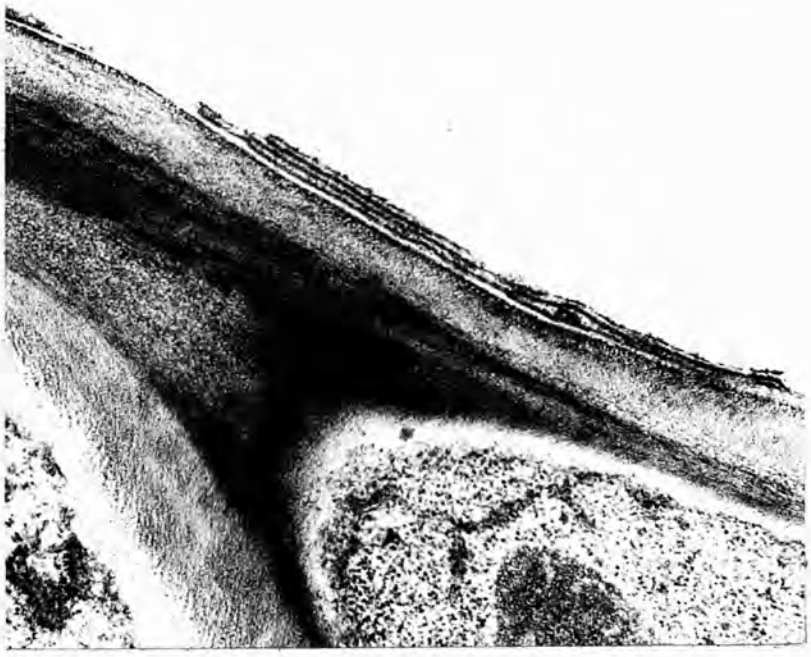


Plate 98 Helianthus hypocotyl: L S of sieve tube, showing a myelin-like membranous structure. Companion cell to right. Normal treatment, stained with uranyl acetate and lead citrate. X 60,000.

Plate 99a Saxifraga stolon: L S of sieve tube, showing parallel lamellar stacks. Normal treatment, stained with lead citrate. X 45,000.

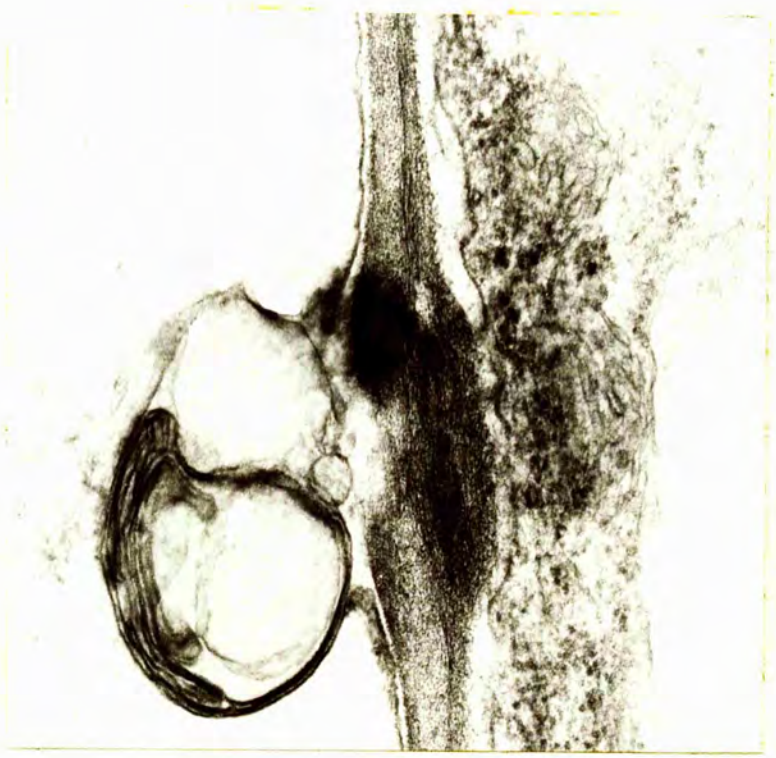


Plate 99b Saxifrage stolon: L S of sieve tube showing membranous structure. Note the parallel lamellar stacks with suggestion of tubules very closely associated with the wall. Normal treatment, stained with lead citrate. X 60,000.

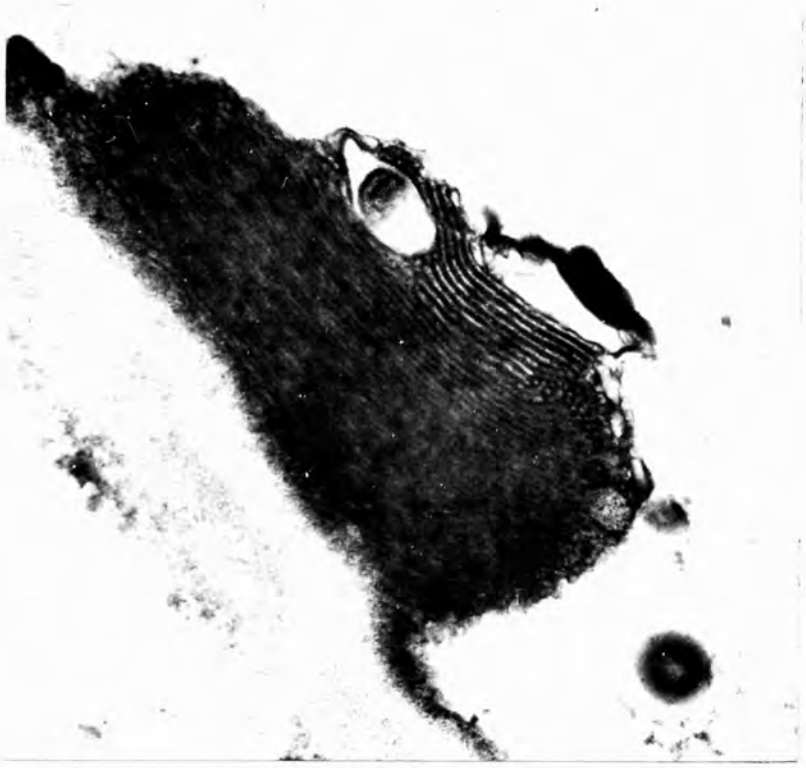


Plate 100 Helianthus hypocotyl: L S of sieve tube showing a myelin-like structure with probably spirally arranged layers. Companion cell to left. Normal treatment, stained with lead citrate and uranyl acetate. X 37,500.

Plate 101a Ditto. Showing a myelin-like structure, a mitochondrion and a multivesicular body. Note the plasmalemma seems discontinued where the multivesicular body is in contact with the wall at a thickened region. Companion cell to left. X 37,500.

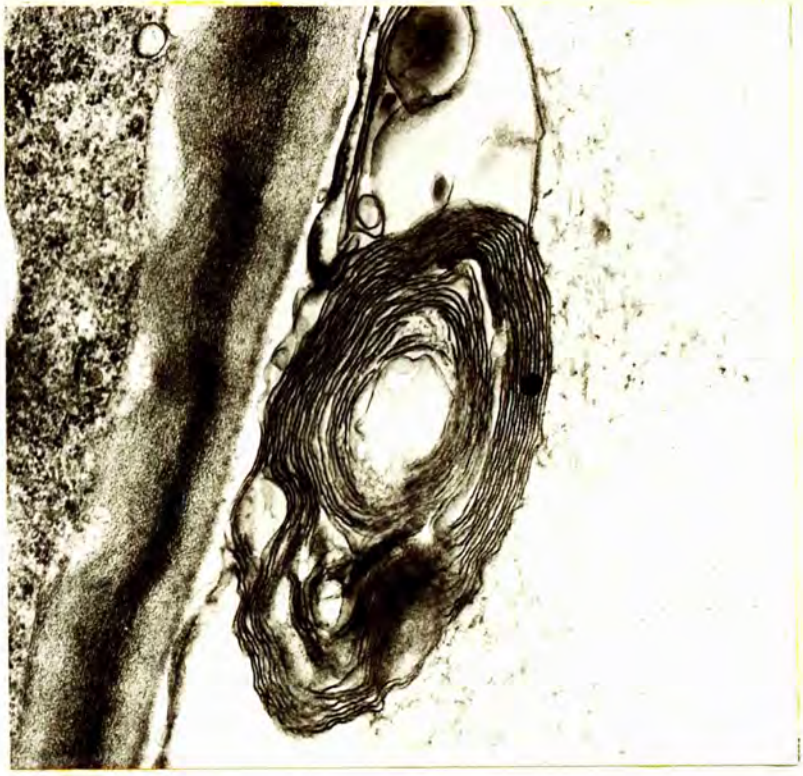


Plate 101b *Helianthus* hypocotyl: L S of sieve tube showing membranous structure. Note a multivesicular body near the wall (covered by plasmalemma ?) Normal treatment, stained with lead citrate and uranyl acetate. X 100,000.

Plate 102 Helianthus hypocotyl: L S of sieve tube. Note the association of mutivesicular bodies with plasmodesmata at thickened regions of the wall. Companion cell to left. Normal treatment, stained with uranyl acetate and lead citrate. X 37,500.

Plate 103 Ditto. showing a membranous structure. Note its position near the plasmodesma. Companion cell to right. X 37,500.

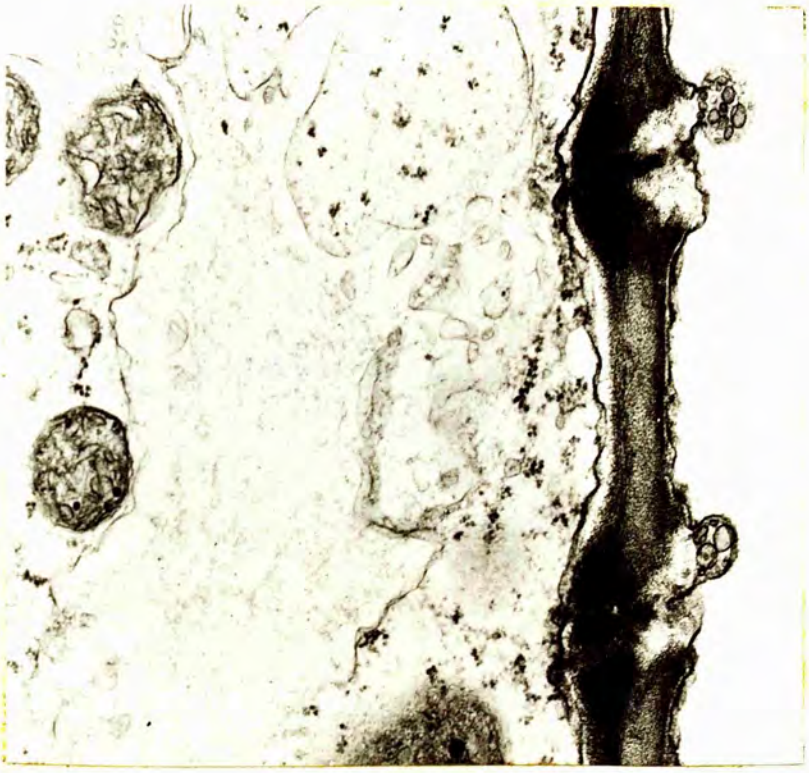


Plate 104 Helianthus hypocotyl: T S of sieve tube and companion cell. Slime fibrils are dispersed and a membranous structure is seen in the bottom of the plate. Plasmodesma is branched towards companion cell. Nucleus in companion cell contains a big nucleolus. Note multivesicular bodies in companion cell near plasmodesma. Normal treatment, stained with uranyl acetate and lead citrate. X 30,000.

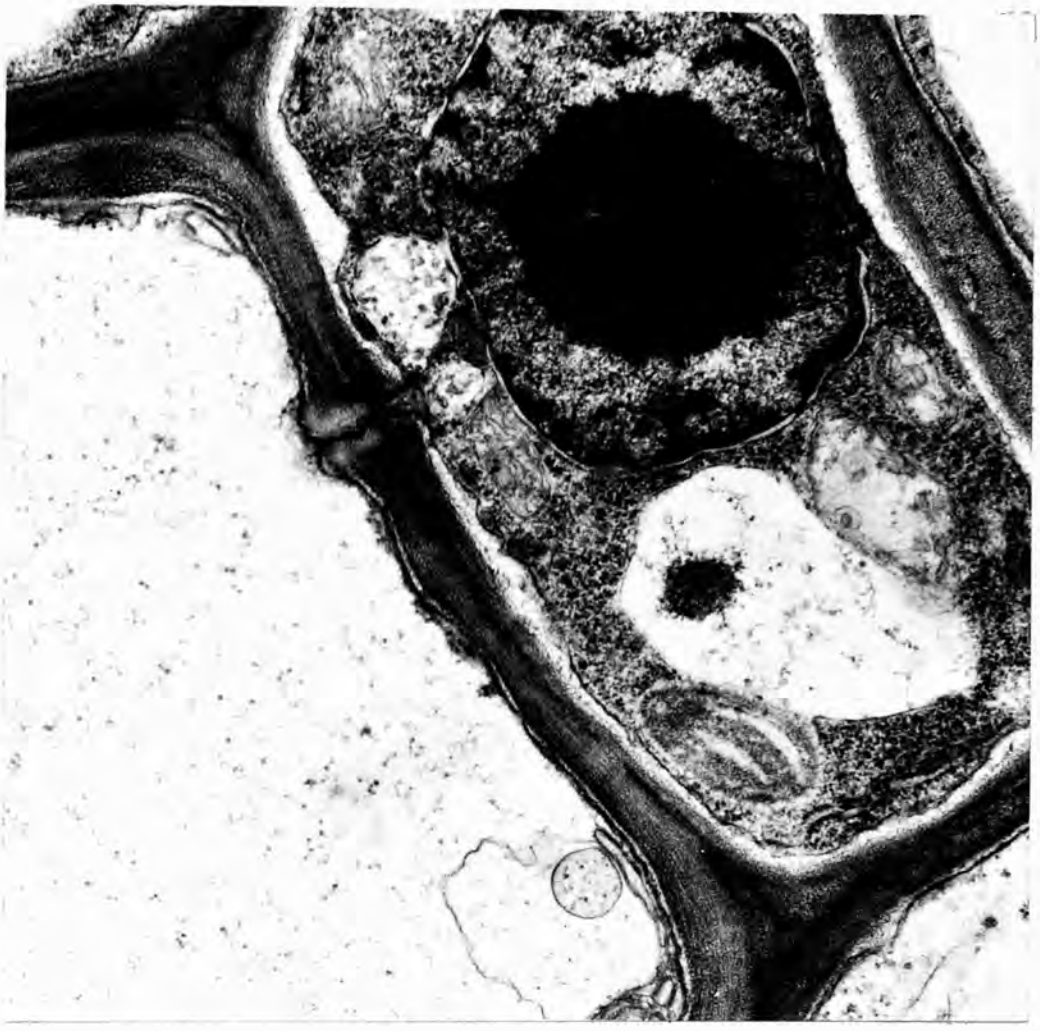
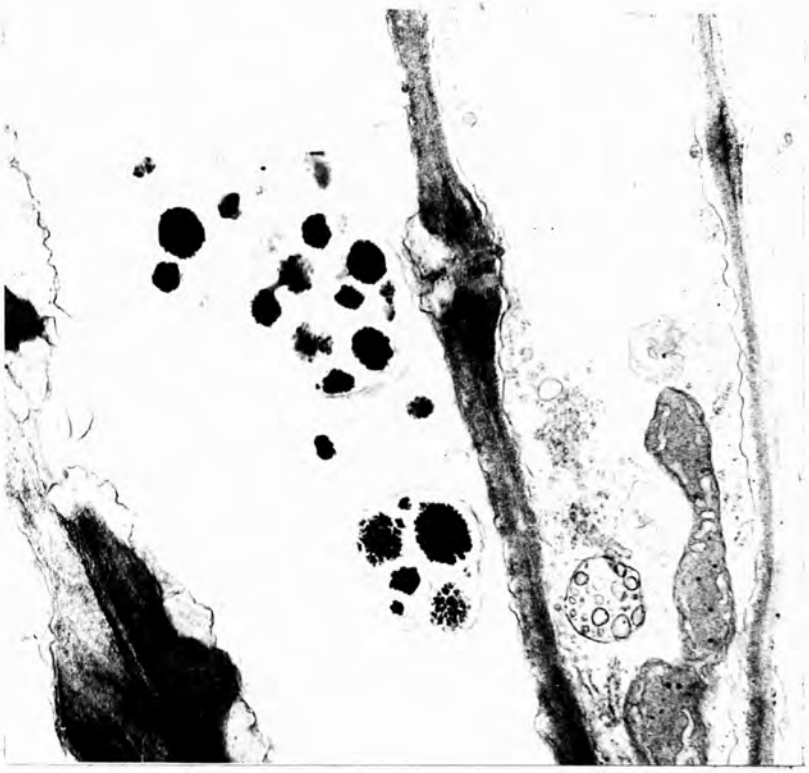


Plate 105 *Helianthus* hypocotyl: L S of sieve tube showing intact plastids, plasmodesma and radial structure in cell wall. Note the multivesicular bodies and mitochondria in the companion cell. Normal treatment, stained with uranyl acetate and lead citrate. X 22,000.

Plate 106 *Saxifraga* stolon: L S of sieve tube showing a plastid. Direction of envelope process is towards sieve plate, which is fairly close. Normal treatment, stained with lead citrate. X 45,000.



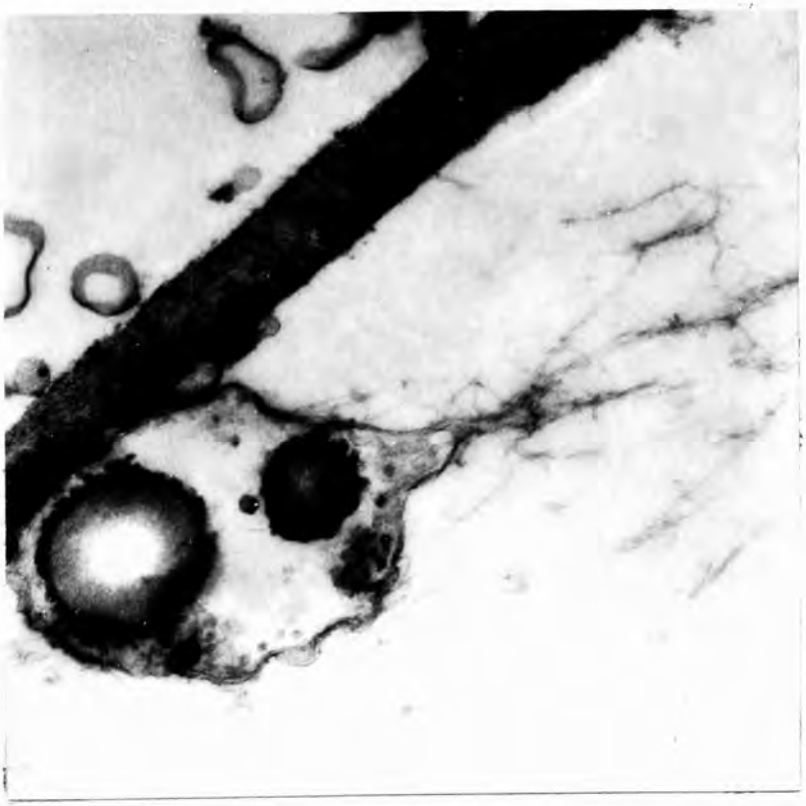


Plate 107 Helianthus hypocotyl: L S of sieve tube showing an oblique sieve plate densely plugged sieve pores which are surrounded by thick cylinders of callose. Intact plastids and mitochondria can be seen on either side of the plate. There is no evidence of a surge artefact. Normal treatment, stained with uranyl acetate and lead citrate. X 22,500.

Plate 108 Ditto. An oblique sieve plate showing plugged pores. Note a strand of slime whose ends are immersed in two different pores. X 11,250.

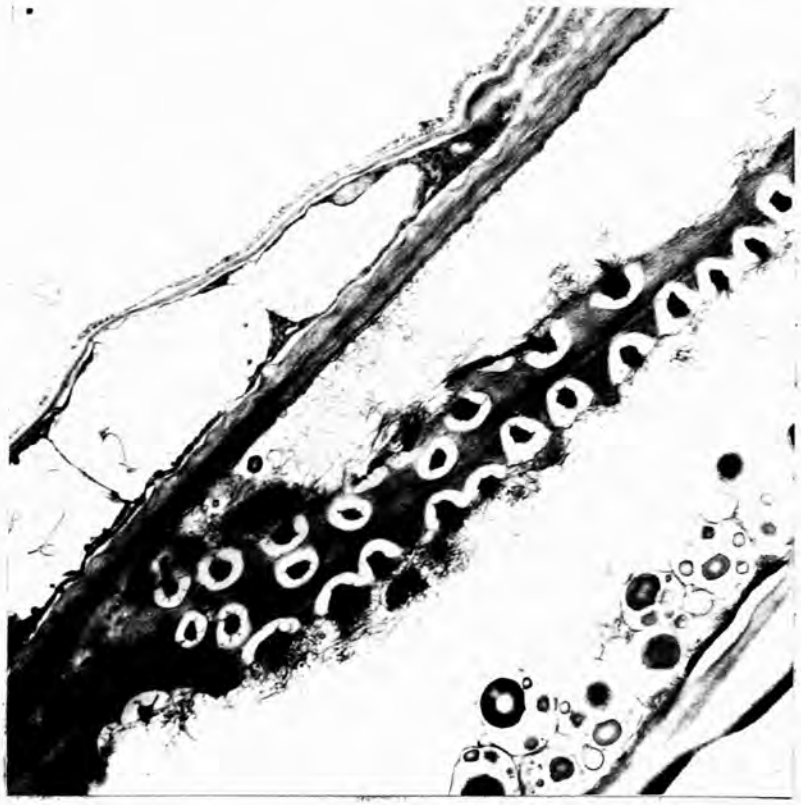


Plate 109 Heracleum internode: T S of sieve tubes showing long slime fibrils fraying from the pore. Normal treatment, stained with lead citrate and uranyl acetate. X 38,000.

Plate 110 Saxifraga stolon: L S of sieve plate showing a mitochondrion near a plugged pore. Presence of starch grains and mitochondrion near the plate seems to be a displacement artefact due to release of turgor. Note the plasmalemma is continuous through the pore. Normal treatment, stained with lead citrate. X 60,000.

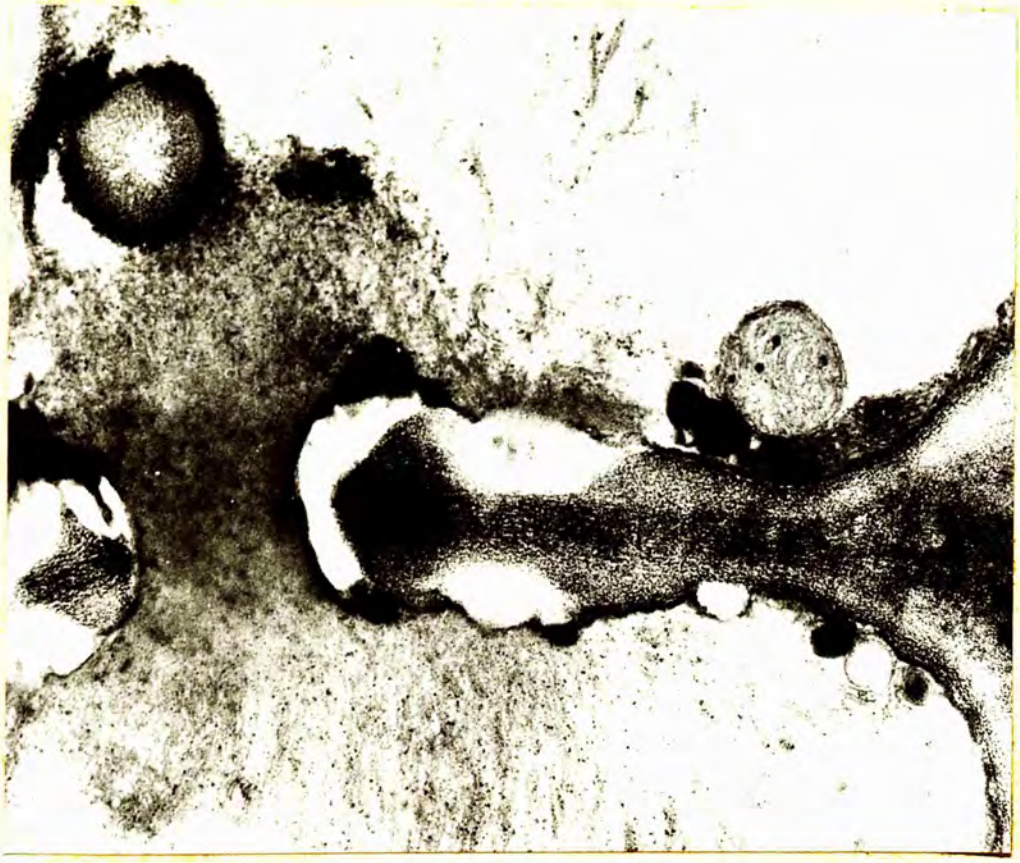


Plate 111 *Helianthus* hypocotyl: L S of sieve tubes. An intact plastid can be seen below and a mitochondrion above in the central tube. Note the membrane and vesicles around the plate which is covered with a thick layer of callose. Normal treatment, stained with uranyl acetate and lead citrate. X 7,500.

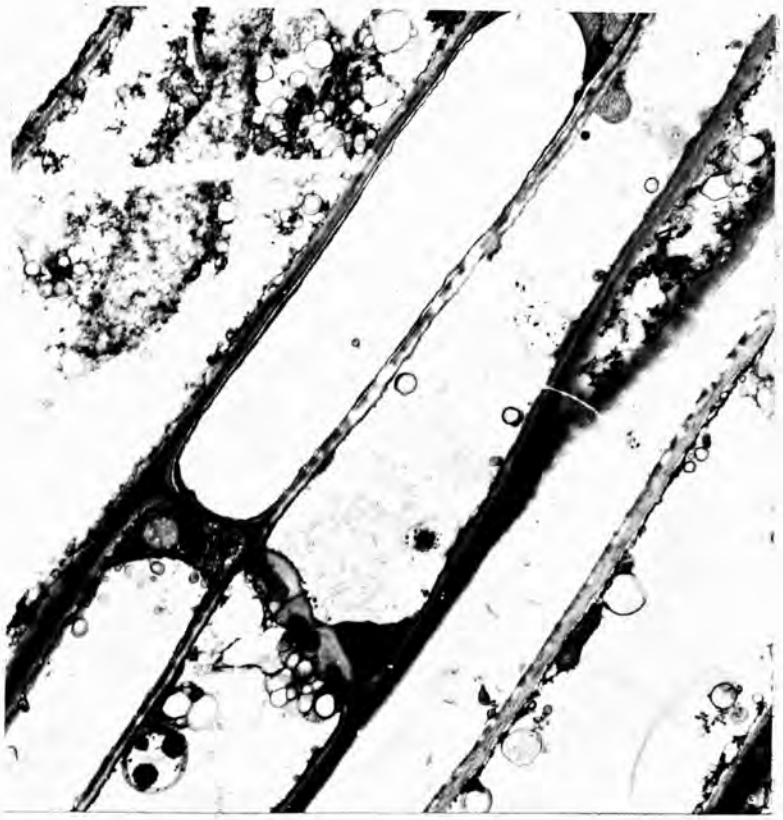
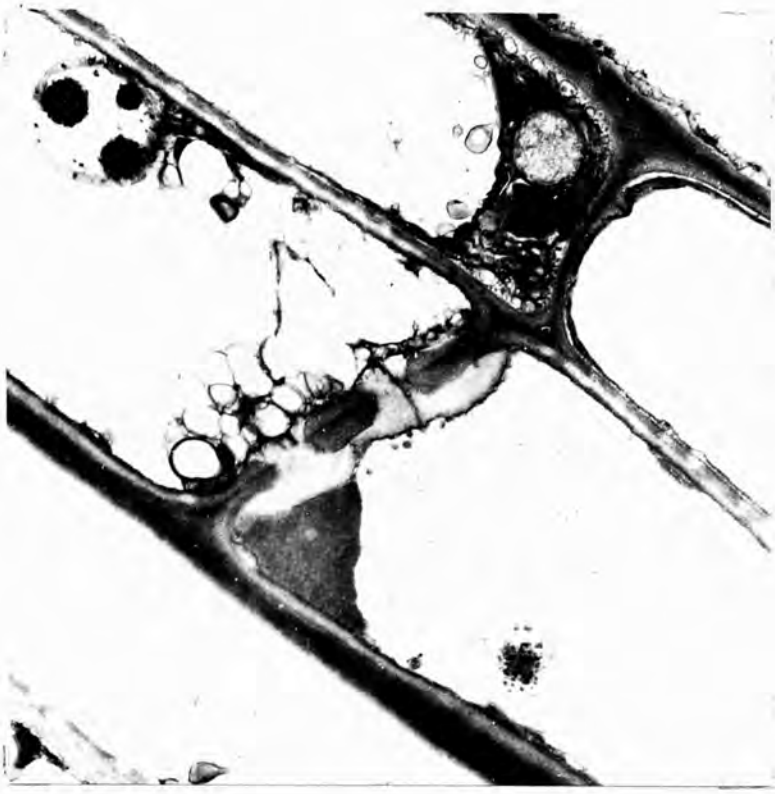


Plate 112 Helianthus hypocotyl: A magnified part of plate 111. The dark mass in the corner of the plate is not resolved. The sieve tube appears obsolescent and closed with definitive callose. X 15,000.

Plate 113 Helianthus hypocotyl: An obliquely cut sieve plate showing plastids and mitochondria. The callose seems to completely close the pores, but the organelles appear normal. Note radial structure in cell wall. Normal treatment, stained with uranyl acetate and lead citrate. X 11,250.



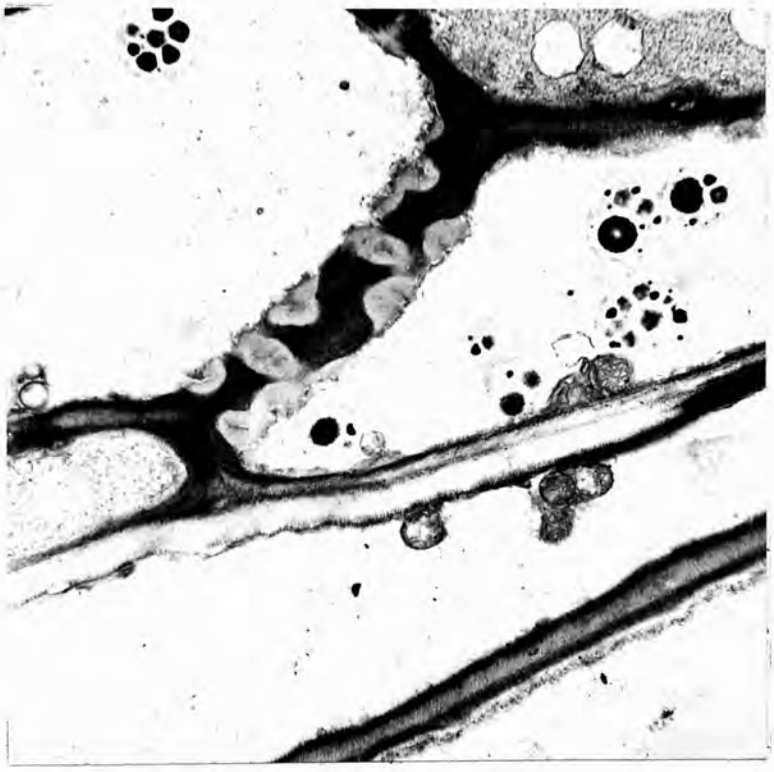
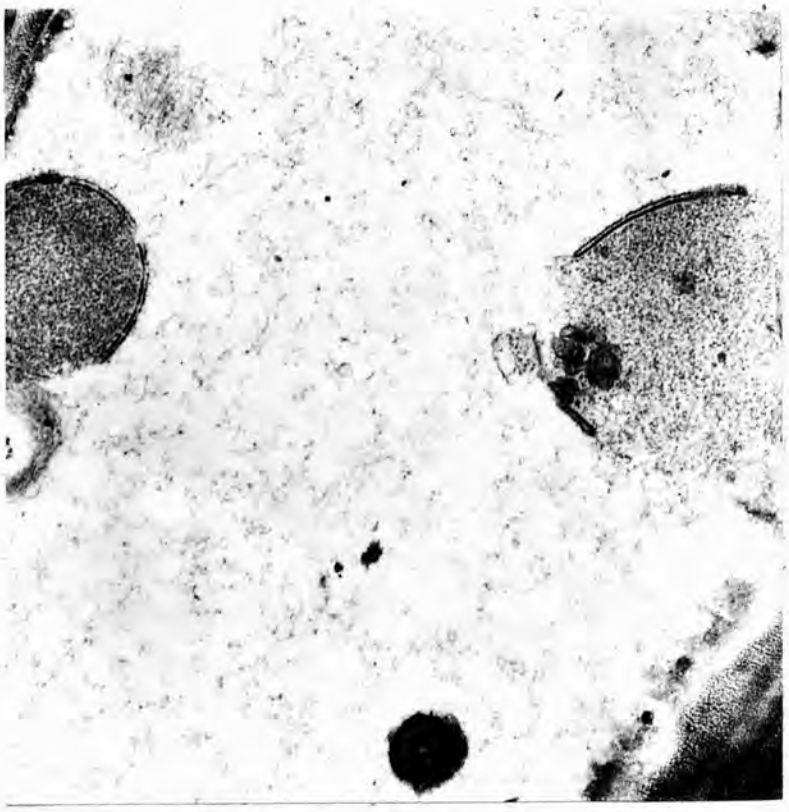


Plate 114 Helianthus hypocotyl: L S of sieve tube showing two inclusions bounded perhaps by endoplasmic reticulum (compare plate 1). Slime fibrils are dispersed in the lumen. Note the mitochondrion below, and the striations in the wall. Normal treatment, stained with uranyl acetate and lead citrate. X 22,500.

Plate 115 Ditto. L S of young sieve tube considerably off-axis. In the cell to the left numerous microtubules can be seen running just inside the wall. Ribosomes are abundant in the cytoplasm. X 45,000.



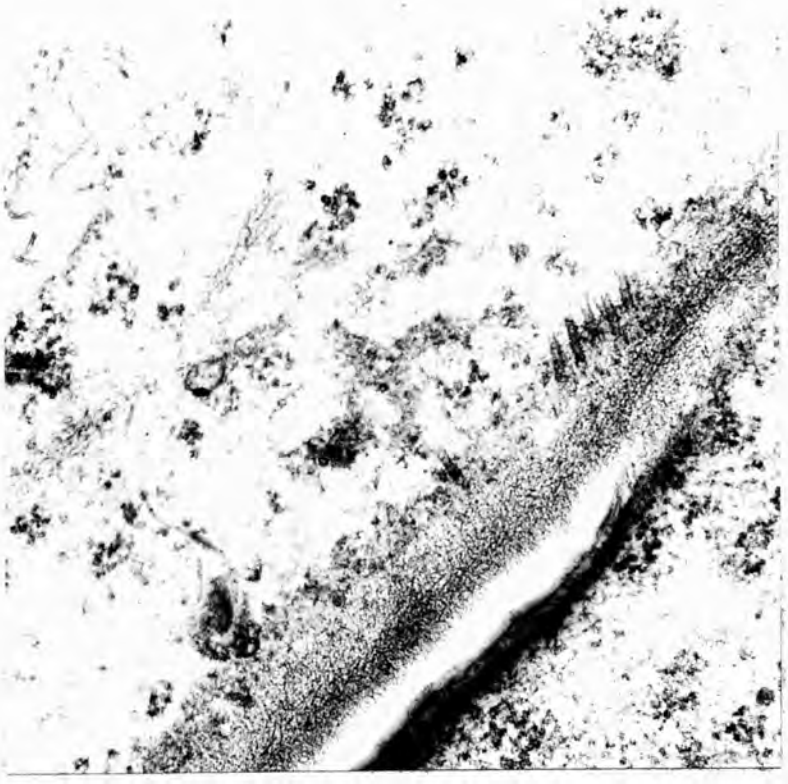


Plate 116 *Saxifraga* stolon: L S of sieve tube. Slime fibrils are dispersed in the lumen. At the lower end a plasmodesma is cut obliquely. Note the membranous structure in the tubes on left and right (compare *Helianthus*, plates 98, 100, 101 and 103). Normal treatment, stained with lead citrate. X 30,000.

Plate 117 *Heracleum* internode: L S of sieve plate with plugged pore showing what is probably a turgor-release artefact (movement to right). Normal treatment, stained with lead citrate. X 30,000.

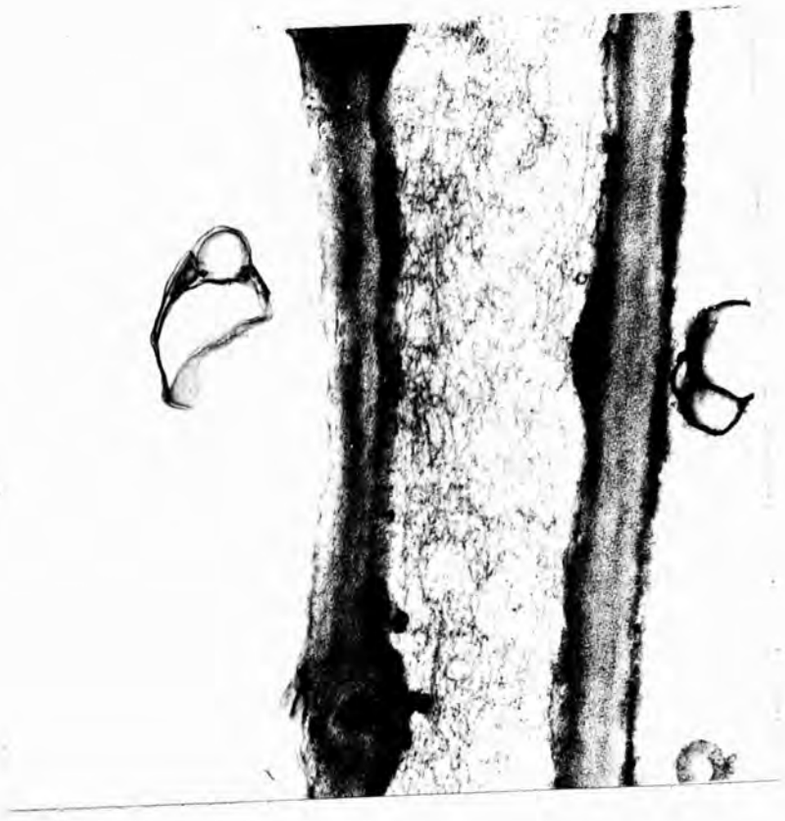


Plate 118 Saxifraga stolon: L S of sieve tube. Lamellar stacks are associated with mitochondrion-like body. Note banded fibrils and wall structure. Normal treatment, stained with lead citrate. X 45,000.

Plate 119 Ditto. Lamellar stacks are anchored on the sieve plate very near to a pore. Note the clearly-banded fibrils. X 45,000.

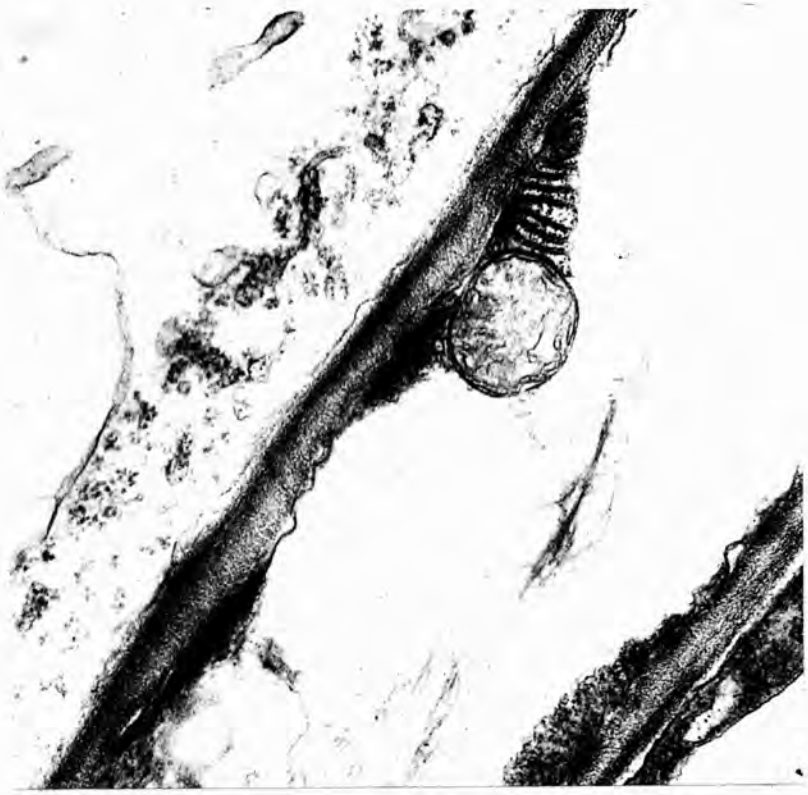
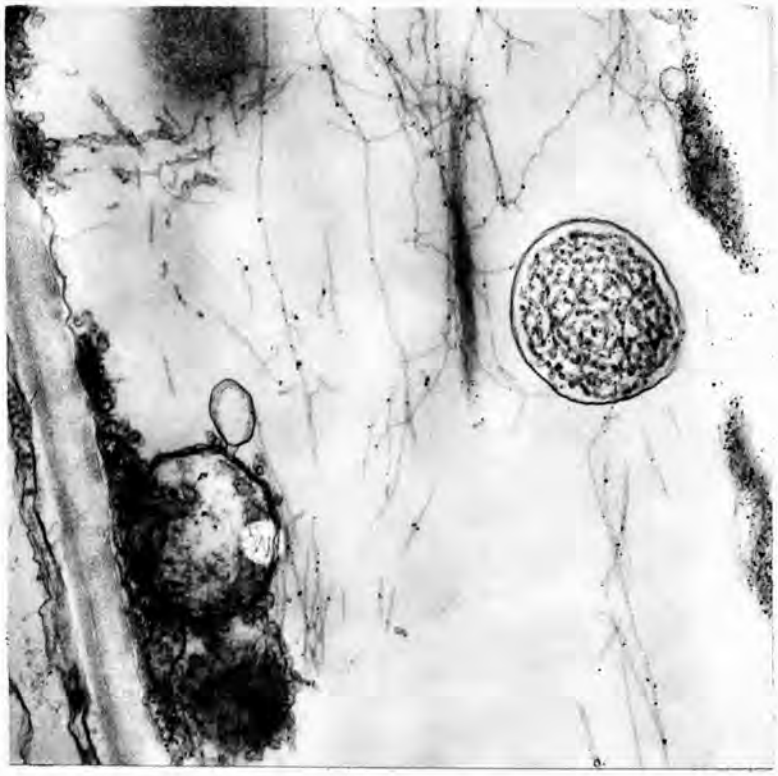


Plate 120 Saxifraga stolon: L S of sieve tube showing multi tubular bodies. Compare the structure on the right hand side with plates 83 and 114 and one on left with 101a and 101b. Normal treatment, stained with lead citrate. X 45,000.

Plate 121 Ditto. Showing a curvilinear membranous structure cut transversely and a multi-vesicular body. Compare plates 83, 84 and 101a, 101b. X 60,000.



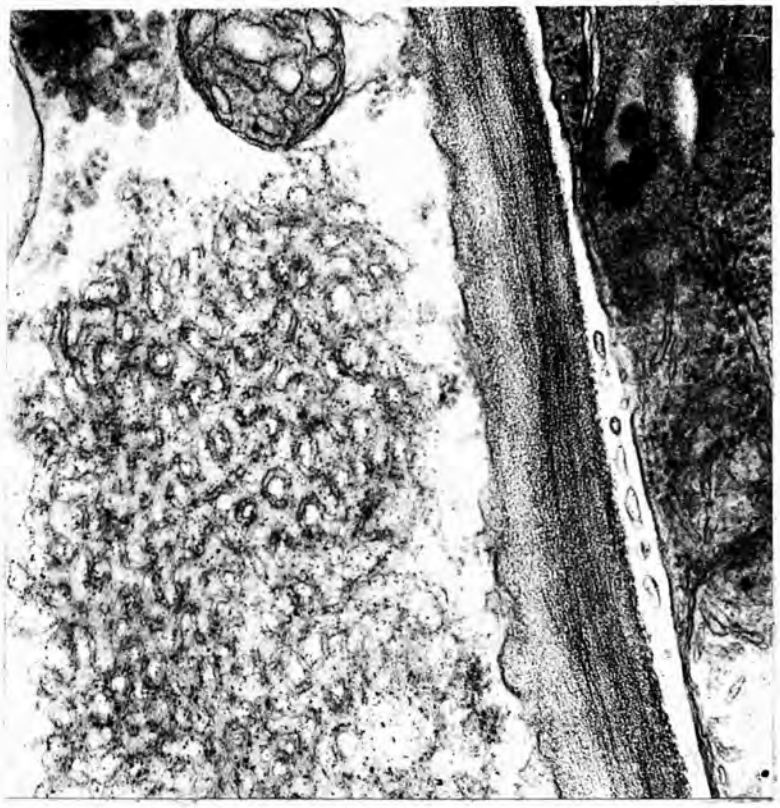


Plate 122 Saxifraga stolon: L S of sieve tube showing part of a disintegrating slime body. Note the lamellar structure in the adjacent tube which is cut at nearly glancing incidence. Normal treatment, stained with lead citrate. X 37,500.

Plate 123 Ditto. L S of sieve tube, a slime body in process of disintegration. Lumen is filled with fibrils. Note the lamellar structure in the adjacent tube which cut at somewhat glancing incidence. The section is probably serial to plate 122. X 11,250.

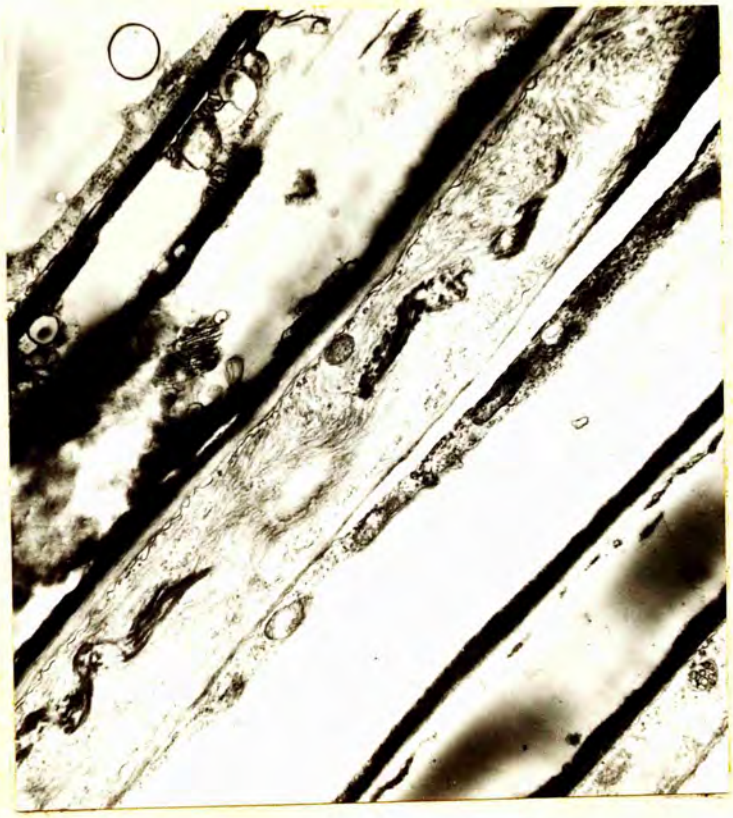


Plate 124 Saxifraga stolon: L S of a young sieve plate. A median nodule is formed and perforation of plate has started. Plastids with large starch grains are present on either side. The tonoplast can be seen around vacuoles; the endoplasmic reticulum is breaking into tubules and dictyosomes can be seen near the plate. Normal treatment, stained with lead citrate. X 15,000.

Plate 125 Saxifraga stolon: L S of companion cell. A large mitochondrion and a multivesicular body can be seen. Note that the vesicles are enclosed in two membranes externally. X 60,000.

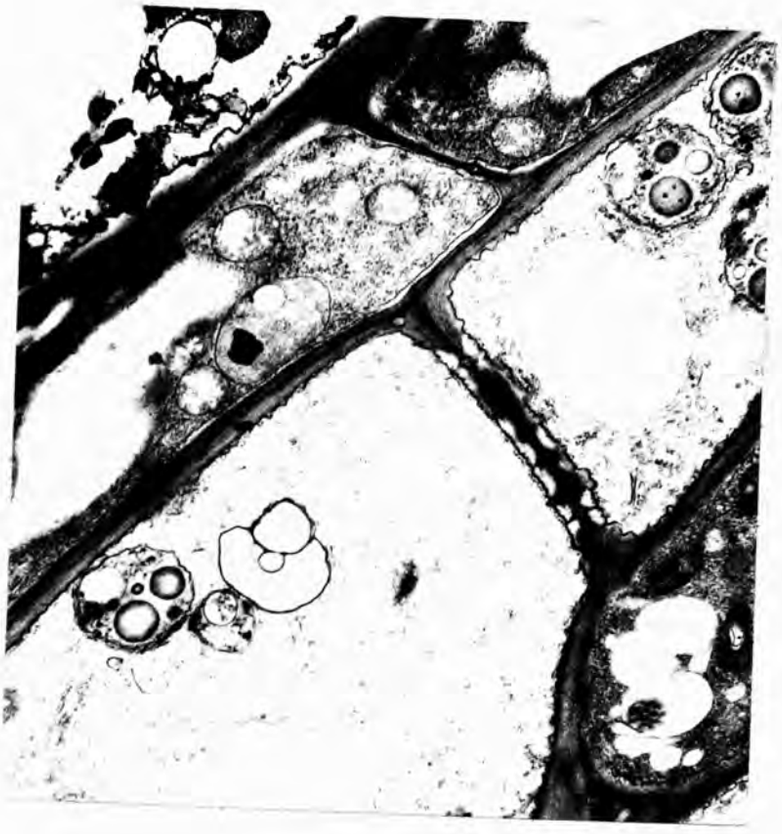
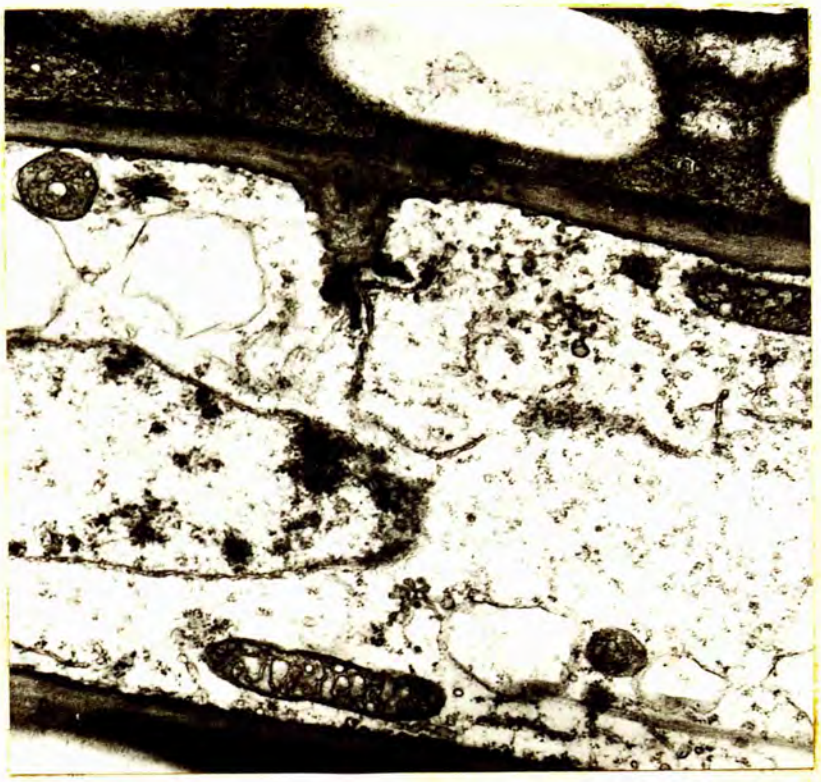


Plate 126 Saxifraga stolon: L S of a young sieve tube showing part of a nucleus, several mitochondria, endoplasmic reticulum, dictyosomes and other membrane systems. Ribosomes, fibrillar material and a large number of small vesicles can also be seen. Normal treatment, stained with lead citrate. X 22,500.

Plate 127 Ditto. L S of young sieve plate - an early stage of sieve plate formation. Callose platelets have developed on the site of a future pore. Sieve tube is filled with numerous organelles. The endoplasmic reticulum appears mostly tubular and vesicular. X 15,000.



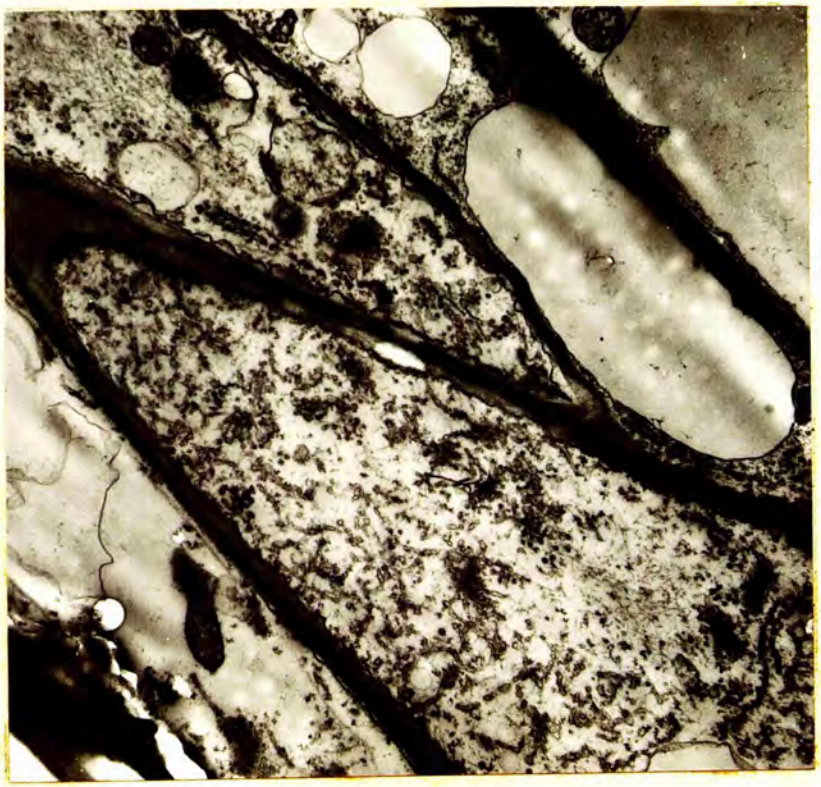
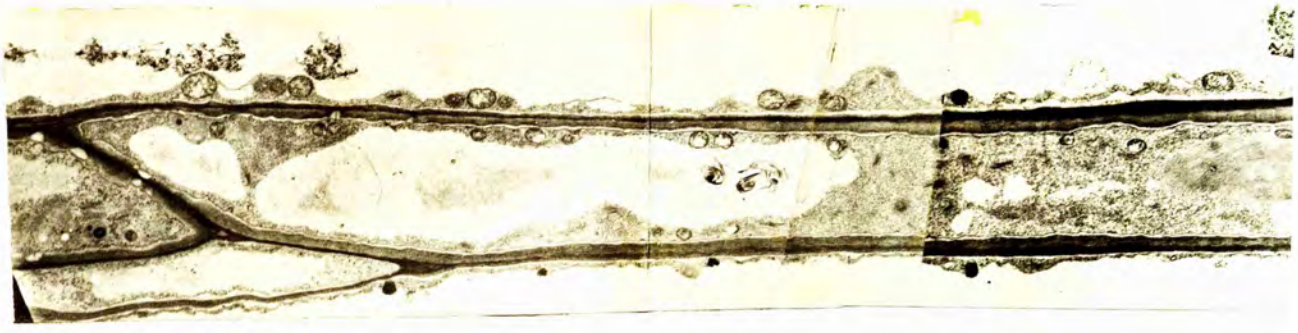
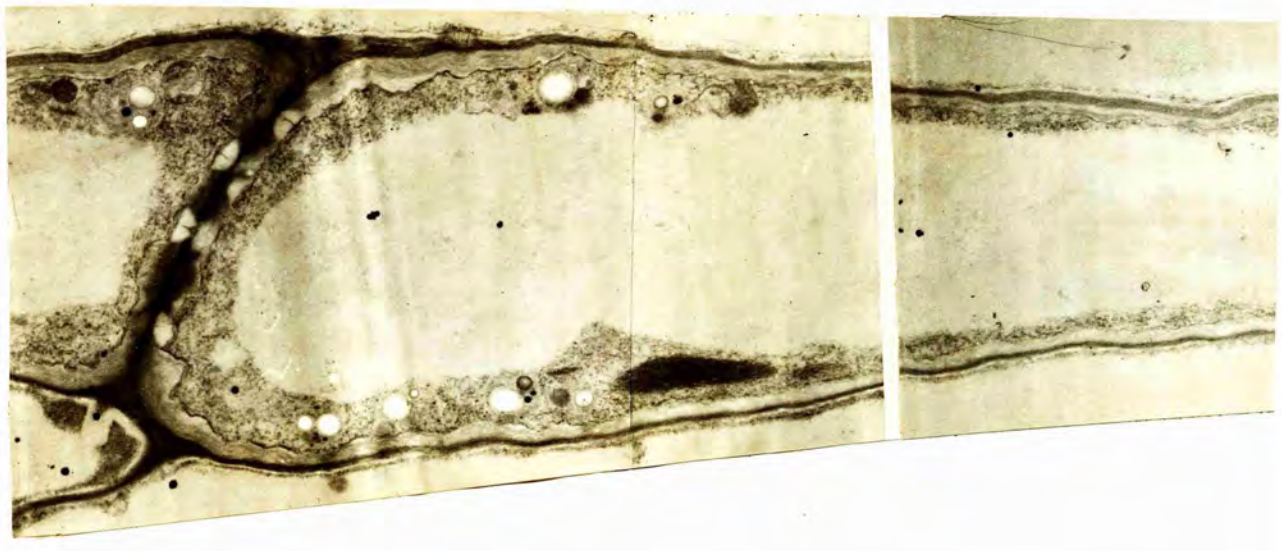


Plate 128 Helianthus hypocotyl: L S of whole young sieve tube showing an early stage of development. Callose platelets are deposited on the site of future sieve plate pores, the middle lamella being still intact between the platelets. Vacuoles occupy a major part of the tube, at some places almost filling the cavity and leaving only a thin layer of cytoplasm adjacent to the cell wall. Plastids and mitochondria are abundant mostly very near the wall. The cytoplasm is rich in ribosomes. An oblong slime body and a weakly staining nucleus can be seen. Note the denser layer of cytoplasm around the slime body. A part of the companion cell is apparent near the middle of the element. A large number of plasmodesmata connect it with the sieve tube below. Many mitochondria and a few plastids with osmiophilic granules are present in the companion cell. The length of the whole sieve tube is about 125 µm and the width 5-6µm. Normal treatment, stained with lead citrate and uranyl acetate. X 6,250.

Plate 129 Ditto: part of a young sieve tube showing a stage close to the previous one. A small slime body can be seen to the left and a larger one to the right. Ribosomes are abundant. The nucleus is very close to the large slime body and is beginning to lose its staining power. It appears to contain some fibrillar material. A nucleolus is present. Stained with lead citrate and uranyl acetate. X 12,500.





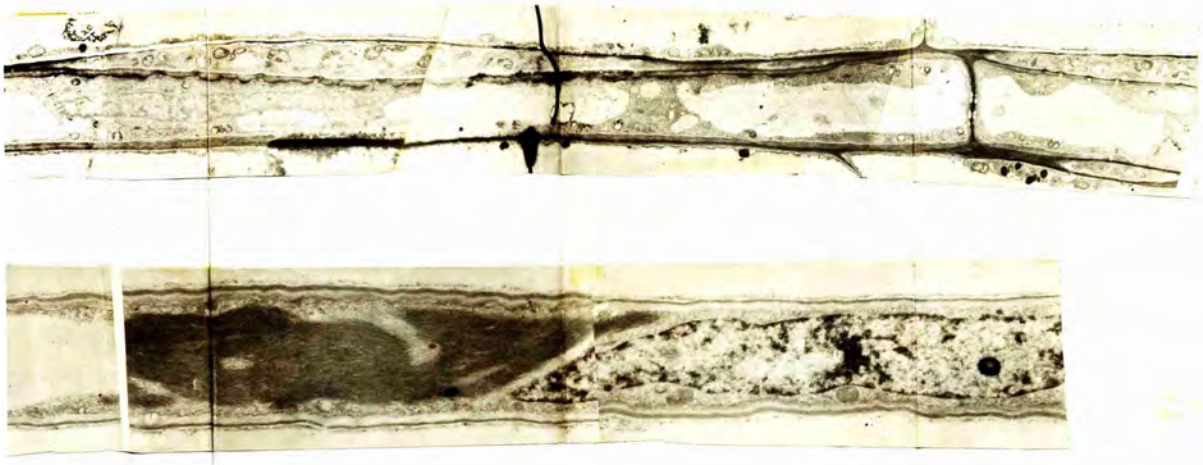
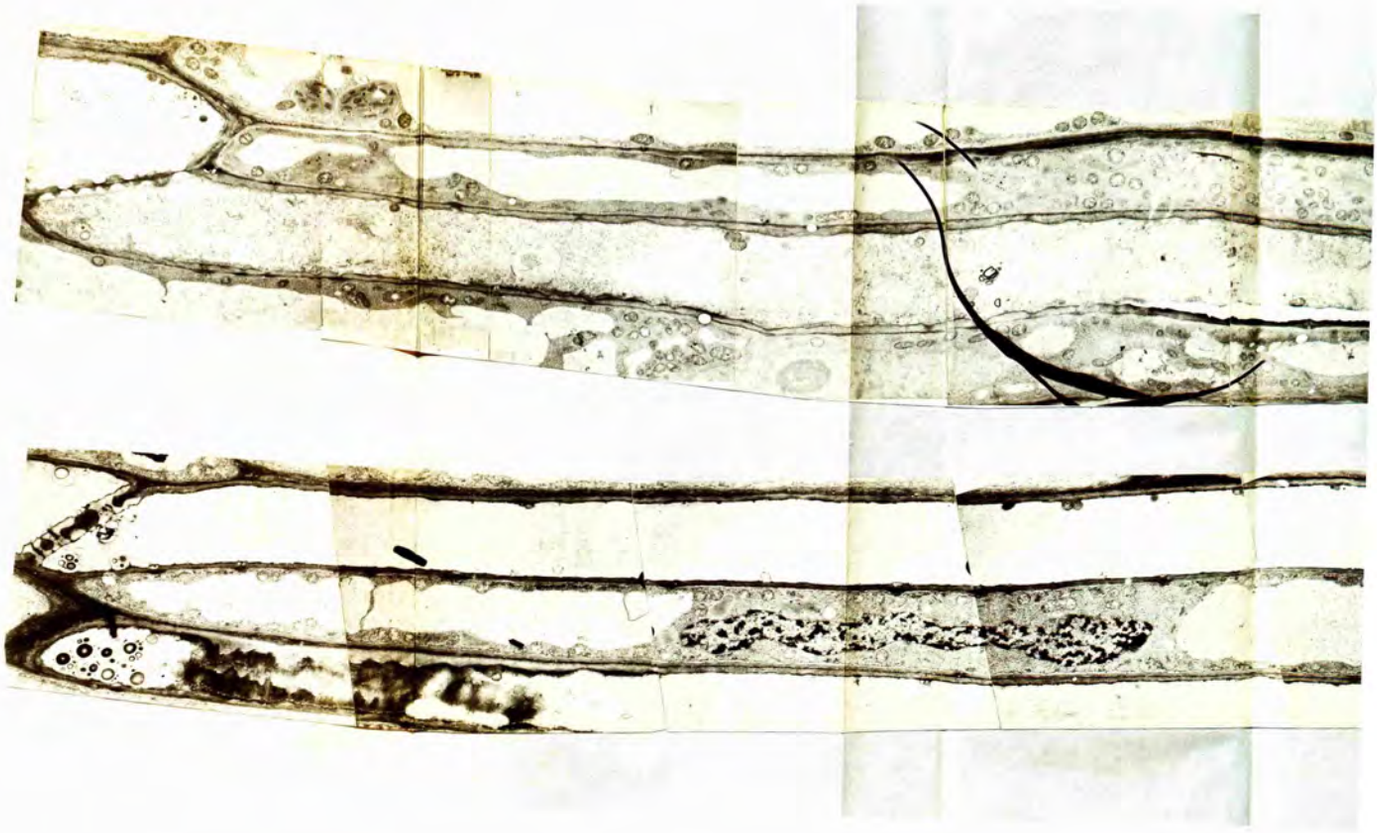


Plate 130 Helianthus hypocotyl: L S of a young sieve tube and companion cells of a stage later than plates 128 & 129. Vacuoles have dispersed from the sieve tube and the slime body is dispersing. The nucleus has lost its density but it can be identified by its membrane which is still present. Its shape is becoming irregular. Plastids, mitochondria and a few dictyocomes with large vesicles can be seen and ribosomes are still fairly abundant. Companion cells are present on both sides of the sieve tube. These cells are rich in mitochondria. Plastids are also present in the companion cells and at least in one, starch grains can be seen. Plastids are small in size and possess osmiophilic granules (compare with the chloroplasts in the parenchymatous cells above). The length of the sieve tube is about 130 µm and the width 5 µm in the middle of the tube. Stained with lead citrate and uranyl acetate. X 7,500.

Plate 131 Ditto: L S of sieve tubes and companion cells. These are again older than the preceding, and probably mature. The upper sieve tube seems almost empty except for dispersed fibrils, a few mitochondria (?) along one wall in the centre and some plastids near the sieve plates. The sieve plate on the left hand side is heavily callosed; two pores densely plugged with slime can be seen. The right hand side plate also has a thick callose deposit. The section has missed the pores. The sieve tube in the bottom is sectioned off-axis. Plastids are present both at the ends and centre. Note the layered appearance of the wall when cut at glancing incidence. A file of two companion cells belonging to the upper tube can be seen. The cells are vacuolated and show typical plasmodesmatal connections to the sieve tubes whose walls appear thinner than in previous stages. Sieve tube is approximately 140 µm long and 4 µm in width. X 7,500.



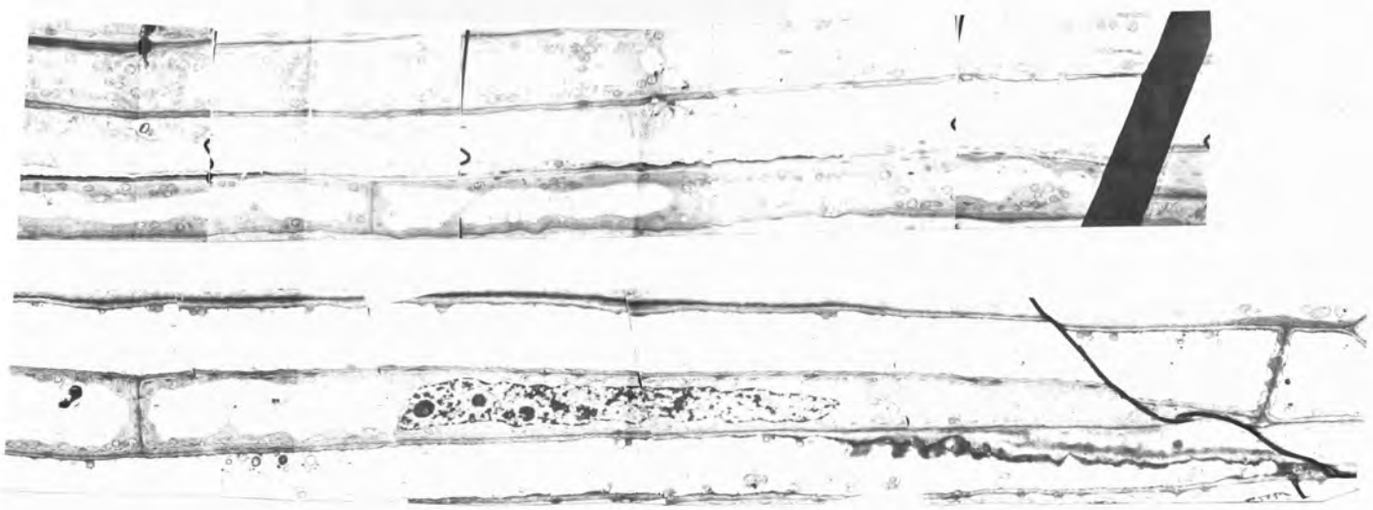
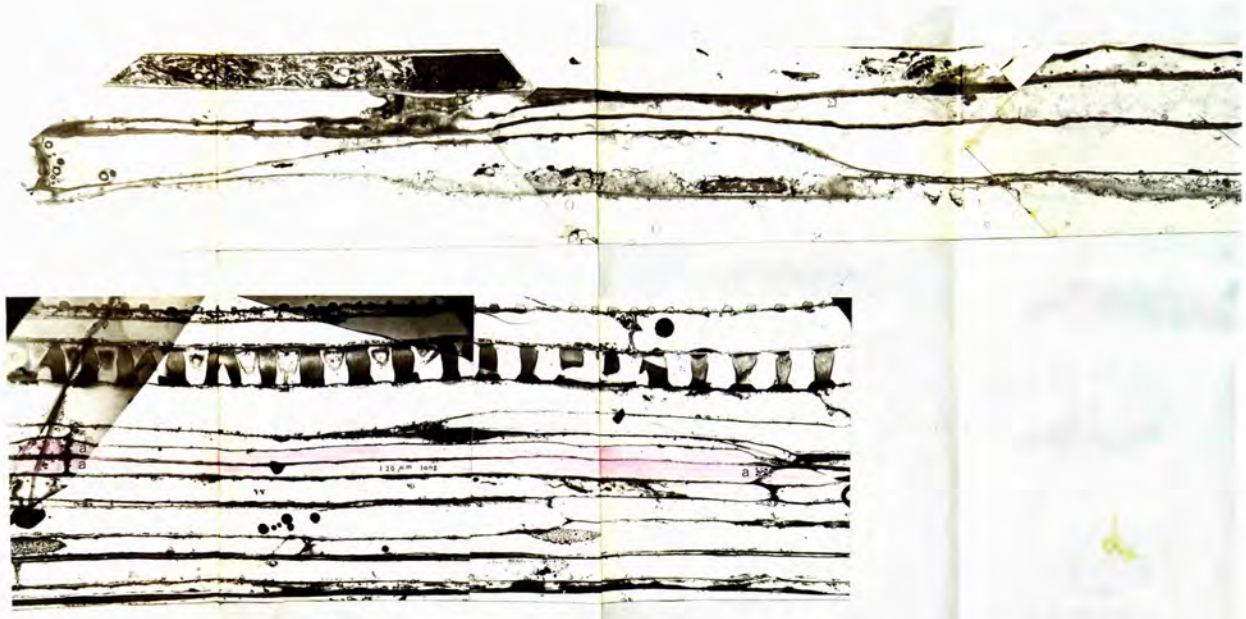


Plate 132 Saxifraga stolon: L S of mature sieve tube and parenchymatous cells. Plastids and starch grains together with slime fibrils are present near the sieve plates. Note vesicles in the tube near the walls. The length of the tube is about 100 µm and the width 3 µm. Normal treatment, stained with lead citrate. X 7,500.

Plate 133 Ditto: L S of vascular bundle - a low power electron micrograph showing two complete sieve tubes, xylem vessels and parenchymatous cells. Sieve tubes are coloured in red and measure approximately 120 µm in length and 3·5 µm in width. Photographed at 80 KV. X 2,500.



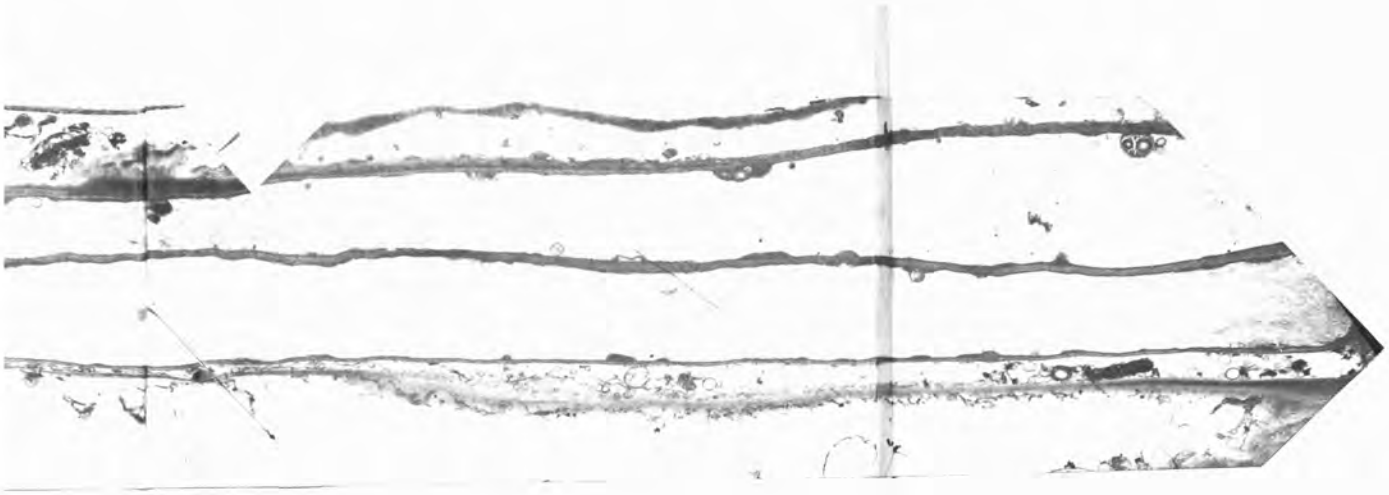


Plate 134 Saxifraga stolon: L S of phloem showing one complete tube element and at least five in parts (coloured red). All the sieve plates marked a appear polarised in the same direction. The complete tube element is about 180 µm in length and 5 µm in diameter. Note long coarser slime strands near the sieve plate and in the lumen. A large number of vesicles (plastid envelope ?) can also be seen. Note also the scarcity of intact plastids in the sieve tube. Parenchymatous cells contain tannin bodies. Normal treatment stained with lead citrate, electronmicrographed at 80 KV. X 2,500.

Plate 135 Ditto: L S of phloem showing one complete sieve tube element and three in parts. All sieve plates are plugged with slime. Plates a, b and d seem to indicate movement to the left. Plate c seems most easily interpreted as showing movement to the right. However, most plastids appear ruptured and these movements may reflect artefacts. The complete tube element is cut much off-axis in the middle. The tube is about 290 µm long and 3·5 µm in diameter. X 2,500.


a
a
c
b
a


a
a
a
d

Plate 136 Saxifraga stolon: L S of phloem showing a complete sieve tube element and two partial sieve elements. Complete element is about 250 µm in length, the other two seem to be of similar length. Starch grains are present near the sieve plates and scattered in the lumen, suggesting damage. There are no open pores. Normal treatment. Stained with lead citrate. Photographed at 80 KV. X 2,500.

Plate 137 Ditto: L S of phloem showing a complete sieve tube element and at least two in parts. All sieve plates are plugged with slime. Starch grains are mostly free in the lumen, suggesting the preparation damage. Complete element is 156 µm long and about 3 µm in diameter. X 2,500.


250 µm tone