STRUCTURE AND FUNCTION IN THE PHLOEM OF *SALIX CAPRAEA*

Thesis presented
by
Mrs. Urmila Mishra
for
the Ph.D. degree in the
University of London

Department of Botany,
Bedford College,
Regents Park,
London, N.W.1.

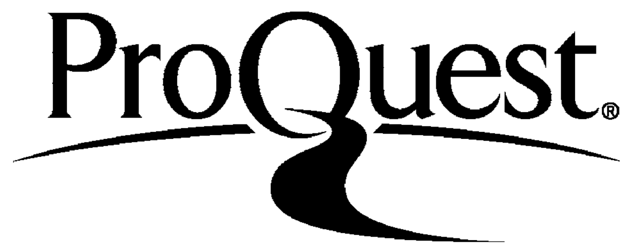
1967

ProQuest Number: 10098139

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.



ProQuest 10098139

Published by ProQuest LLC(2016). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code.
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ABSTRACT

A study has been made, by light and electron microscopy, of the structure of the phloem tissue and sieve tubes of *Salix capreea*, and the relevance of the findings to the electro kinetic theory of transport has been discussed. The anatomical features of the phloem at the cellular level do not seem particularly favourable to the theory; on the other hand, the ultra structure of the sieve tubes, and in particular the prevalence of "slime" in this species and many other species being composed of banded fibrils, seems to favour the electro kinetic theory at least as much as any other. A supporting calculation is given.

ACKNOWLEDGEMENTS

I take this opportunity of expressing my deep sense of gratitude towards Dr. D. C. Spanner, for his kind supervision, valuable advice and encouragement throughout the course of this work; also to Bedford College for the award of financial assistance (Irene Marshall Scholarship).

My thanks are also due to Mr. R. L. Jones for printing all the micrographs and to Miss E. M. Trotman for typing this thesis.

LIST OF ABBREVIATIONS

Cc	-	Companion cell
ca	-	Callose
CHL	-	Chloroplast
D	-	Dictyosomes
ER	-	Endoplasmic reticulum
f	-	Slime fibrils
ld	-	Lipid droplets
lm	-	Lamellar bodies
M	-	Mitochondria
N	-	Nucleus
Nu	-	Nucleolus
P	-	Pores
Par	-	Parenchyma cell
Pd	-	Plasmodesmata
Pl	-	Plasmalemma
Pt	-	Plastids
Sg	-	Starch grains
SP	-	Sieve plate
ST	-	Sieve tube

T	-	Tonoplast
Ta	-	Tannin
V	-	Vacuole
Ve	-	Vesicles
W	-	Cell wall

CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	ii
LIST OF ABBREVIATIONS	iii
CHAPTER I - INTRODUCTION	1
1. THE QUESTION OF MECHANISM	4
2. ORIENTATION OF PRESENT STUDY	7
3. PLAN OF THE WORK	9
CHAPTER II - LIGHT MICROSCOPY	10
1. MATERIAL AND METHODS	12
2.- (i) General Description of the bark of *Salix Capraea*	18
(ii) Fibres	20
(iii) Rays	20
(iv) Outer layers of bark	22
3. ANALYSIS OF PHLOEM AS AN ELECTROKINETIC CHANNEL	24

		Page
	A. DEFINITION OF TERMS	
	(i) Cambial Initial	24
	(ii) Cambial Zone	24
	(iii) Phloem Initial	24
	(iv) Tiers	26
	(v) Columns	26
	B. ANALYSIS	
	(i) Tiers	28
	(ii) Columns	49
4.	MISCELLANEOUS OBSERVATIONS	
	(i) Structure of sieve elements wall	56
	(ii) Contents of sieve elements	56
	(iii) Companion cell	64
	(iv) Phloem parenchyma	67
CHAPTER III -	REVIEW (OF PHLOEM ULTRA STRUCTURE)	78
CHAPTER IV -	ELECTRON MICROSCOPY	107
	1. PREPARATION OF TISSUES FOR THE ELECTRON MICROSCOPE	112

		Page
(i)	Fixation	115
(ii)	Post-fixation and Dehydration	120
(iii)	Embedding.	120
	Resin mixtures	125
	EPON	125
	Araldite	135
	Methacrylate	138
(iv)	Sectioning Araldite blocks	141
(v)	Staining	144
(vi)	Examination in Microscope	147

2. OBSERVATIONS

(i)	Introduction	153
(ii)	The nucleolus	153
(iii)	Sieve tube wall	170
(iv)	Plasmodesmata	170
(v)	Mitochondria	175
(vi)	Plastids	180
(vii)	Plasmalemma	185

		Page
(viii)	Tonoplast	192
(ix)	Endoplasmic reticulum	199
(x)	Sieve plate	228
(xi)	The Slime	242
(xii)	Companion cell	266
(xiii)	Phloem parenchyma	271

CHAPTER V - MICRO-AUTORADIOGRAPHY OF CAMBIAL ACTIVITY

(i)	Introduction	277
(ii)	Administration of CO_2^{14}	277
(iii)	Autoradiographic technique	278
(iv)	Results	279

CHAPTER VI - DISCUSSION 283

SUMMARY 290

REFERENCES 293

C H A P T E R I

INTRODUCTION

INTRODUCTION

Translocation, by common consent one of the classical interests of plant physiology, has been an object of study for a great many years; in fact ever since the time of Malpighi (1675 and 1679) and Stephen Hales (1769). Interest in the subject has waxed and waned since then, though it has never really ceased. It revived strongly when, with the quantitative work of Dixon, Mason and Maskell and others in the 1920's it became apparent that a great problem was involved in deciding how the conducting tissues maintained the very high rate of transport which careful measurements of various sorts confirmed that they do. Interests thus aroused were fostered by the arrival of powerful new physical methods of investigation of which radicactive isotopes, chromatography and electron microscopy merit special mention. It is not surprising that these were early applied to the problem; what is surprising to anyone ignorant of the experience of science is that they have not solved it, and indeed that the solution seems sometimes as far off as ever. However a very great deal of new information has been acquired and the flow of new facts continues, for at the moment many facets of the problem are the concern of active research. In particular there is considerable emphasis

on the relation of structure to function, the electron microscope being the pre-eminent tool in the investigation of the former and radioisotopes that in the study of the latter. We still await the time when the provision of the right interpretative principle will reduce all the knowledge we have acquired to order, and meanwhile there seems to be no alternative to continuing to amass facts while we seek for it. This is the justification for the present study.

THE QUESTION OF MECHANISM

One of the principal ends of the investigation of such a phenomenon as translocation is to gain an understanding of how the process is actually carried out. A knowledge of the structure of the transporting channels is in a sense merely preliminary to this. Thus it is relevant to this study to list the mechanisms for translocation which at the present time seem worthy of consideration. They are as follows:-

1) The pressure flow or Münch hypothesis (Münch, 1930; Crafts, 1961)
2) The electrokinetic theory (Fensom, 1957; Spanner, 1958)
3) The transcellular strand (Protoplasmic streaming) theory (Thaine 1964; Canny 1962).

Besides these, there are several other theories of a less clearly enunciated character, such as the theory of activated diffusion first suggested by Mason and Maskell (1928) and now sponsored by the Russian school (Kursanov, 1956); and the theory of movement along interfaces (van den Honert, 1932; van Overbeek, 1956). At least the activated diffusion theory however is too vague in outline at the moment for it to be effectively criticised;

and the theory of van den Honert, while it may be applicable to the movement of such substances as auxins is almost certainly highly inadequate to account for such massive transport as that of sugars. Thus effectively the contenders are limited to the three mentioned, and of these the favourite is certainly the first.

Having enumerated the possible mechanisms the question arises as to whether one only of these need be considered as operative in a given situation. Might not two or even three be jointly responsible, each contributing *significantly* to the total function? Or to vary the question a little, might it not be the case that with several mechanisms contributing, the balance between two or more mechanisms might be different as between one species and another? Might not a plant like *Cucurbita* with large and possibly open sieve plate pores conduct its translocation predominantly by a Münch mechanism while another, such as *Salix*, uses in the main an electrokinetic one; and still another one a protoplasmic streaming mechanism? These questions cannot be settled a priori; but there does seem experimental evidence for the fact that different mechanisms may be simultaneously operative. Thus Palmiquist (1938), investigating the possibility of

concurrent two-directional movement in the same phloem strands found that fluorescein would enter mature leaves of french-bean through the sieve tubes while the latter were exporting sugar. This would seem to be a clear case of sugar transport in one direction with simultaneous transport of fluorescein in the opposite direction by a different mechanism, for sugars apparently never enter mature leaves in noticeable quantities. Again Biddulph and Cory (1957) in an elegant investigation on french beans using radio-isotopes and autoradiographing the outer layers found what they considered was definite evidence of two mechanisms operating together.

Thus it cannot be assumed in respect of translocation either that all plants are alike or that in a single plant translocation is a unitary process. This means that there is still a need for extending both the width and depth of our factual knowledge. In spite of the considerable work done in both the optical and electron microscopic fields on phloem structure,investigations such as the present one still fulfil a real purpose.

ORIENTATION OF THE PRESENT STUDY

The present study embodies work done using several different techniques in particular optical and electron microscopy. What connects the different parts is a common orientation in the direction of the electrokinetic theory, though the findings have relevance to other theories also. It may be useful therefore to indicate what are the special requirements of the electrokinetic theory in the realm of structure. They are two:

1) The theory requires that the flow should traverse a membrane structure containing pores of a suitable degree of fineness. It may be surmised that their diameter should be of the order of 100 A or so; and in anatomical forms this means that the theory demands that the sieve plate pores in functioning elements be occupied by stationary cytoplasm, fibrillar slime or something comparable. It might be added that in pores large enough to satisfy the requirements of the pressure-flow hypothesis, the electrokinetic forces which could be developed would be too small. On the other hand in very small pores, such as might be consistent with an observation that adjacent sieve tube elements were

separated by a continuous plasma membrane, the resistance would be electrokinetically too high. Thus there is a crucial requirement here which it should be possible for the electron microscope to settle.

2) It also necessitated by the theory that there should be a continuous pathway through living parenchymatous cells from one sieve tube to the next in parallel with the sieve plate. In the original description of the theory given in 1958 this was shown as provided by the companion cells. This might be the most efficient arrangement; but the presence of ordinary phloem parenchyma cells linking companion cells not in direct continuity with one another would seem to be adequate for the theory. Further, this living pathway would have to provide a sufficient cross-section to sustain an appreciable ion flux (Fensom and Spanner, unpublished); and the investigation of this point was one of the main objectives of the present work.

PLAN OF THE WORK

The plan of this thesis falls into five parts.

Firstly there is an account of original studies with the light microscope on the phloem of *salix capraea*, the objectives of which were to investigate the special relationships of the sieve tubes and the living cells contiguous with them; and to provide a background for the interpretation of electronmicrographs.

Secondly there is a review of the electron microscopical studies of phloem structure to date, starting with the pioneer work at Leeds in 1955 (Hepton and Preston & Ripley).

Thirdly there is an account of the present electron microscopical investigations, which are concerned principally with sieve tube structure.

The thesis continues with a short account of some investigations with radioactive tracers designed to ascertain when sieve tubes were being laid down; and finally concludes with a discussion of the results obtained.

C H A P T E R I I

OPTICAL MICROSCOPE STUDIES

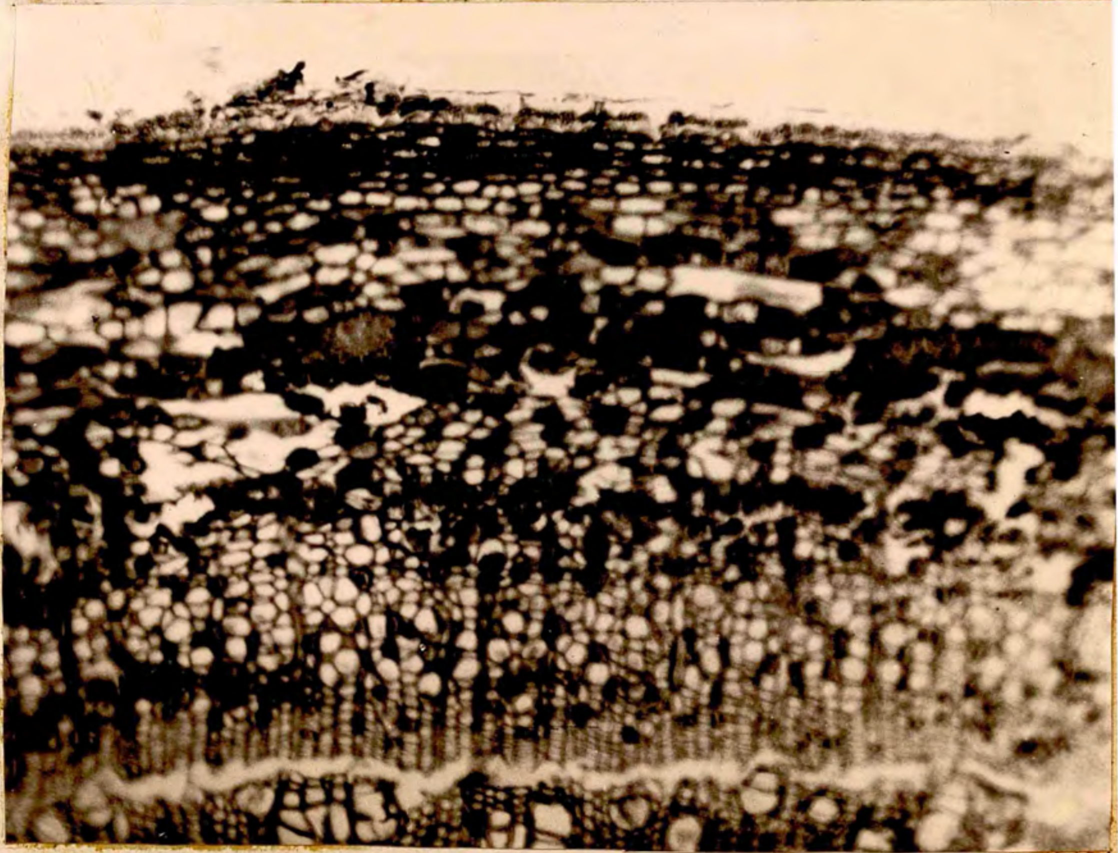


PLATE 1 Transverse section (T.S.) showing the bark, tangential bands of phloem fibres, secondary phloem, cambium and xylem. About X200.

OPTICAL MICROSCOPE STUDIES

MATERIAL AND METHODS

Two year old twigs were collected from a young tree in the Botanical garden at various times of the year. These were fixed in Craf III for a period of 24 to 30 hours. They were aspirated while in the fixative. The pieces of tissue which were excised with a sharp razor were very small, measuring about 5mm cube. They were embedded in Ester wax (Steedman, 1960). This wax shows good penetration of woody material and has proved far superior to paraffin wax for embedding and holding such tissues during sectioning.

Before embedding, the fixed material was washed thoroughly, then dehydrated in a graded series of Tertiary Butyl alcohol (Johansen 1940). The dehydrated material was passed from a mixture containing equal parts of tertiary butyl alcohol and liquid paraffin through three to four changes of fresh ester wax over a period of two to three days prior to embedding.

By using this technique 6μ thick sections could be cut fairly readily with a MINOT rotary microtome using a steel knife.

Sections were stained with tannic acid - iron alum

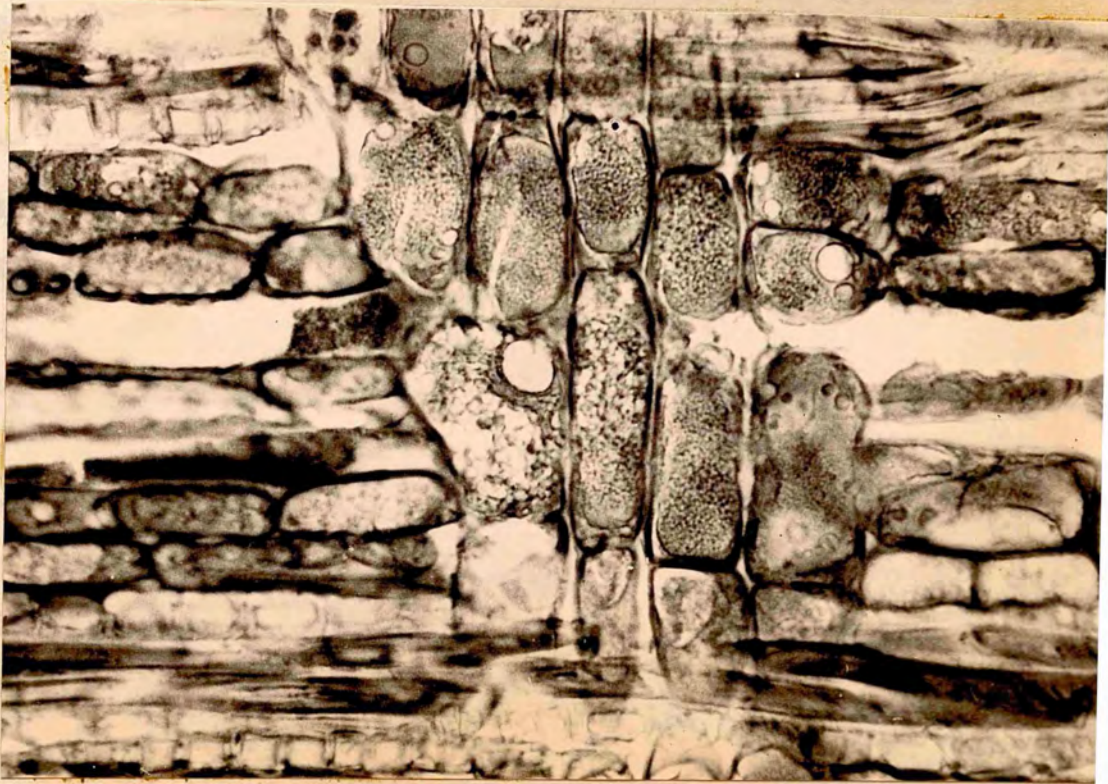


PLATE 2 Radial longitudinal section (R.L.S.) showing the ray cells (oriented vertically in the plate) and the crystal containing cells (oriented horizontally). About X700.

with safranin and orange G (Sharman 1943). Permanent slides were made by mounting in Canada balsam.

Auxiliary polarized light studies were carried out mainly to emphasise the phloem fibres and the crystal containing cells in optical photomicrographs. This was accomplished by a very simple arrangement using a piece of "polaroid" over the condenser as polarizer and another over the objective as analyser. Quite vivid effects were produced.

Eschrich's and Currier's (1964) method was employed to show callose deposition on the sieve plates. Fresh material was used to cut free-hand sections. The sections were then stained with resorcinol blue, which specifically stains the callose cobalt blue. Sections were mounted in the stain and photographed in colour on Ilford "Ilfocolor" using a daylight filter. The exposure given was one tenth of a second. Colour photography was also used in the case of permanent sections stained in Sharman's combination. The sieve tube walls take a blue-black colour. The nuclei are stained blue-black with bright red nucleoli. The freshly extruded nucleolus stains a red colour but at a later stage it stains a yellow.

In addition to this some macerated material was also

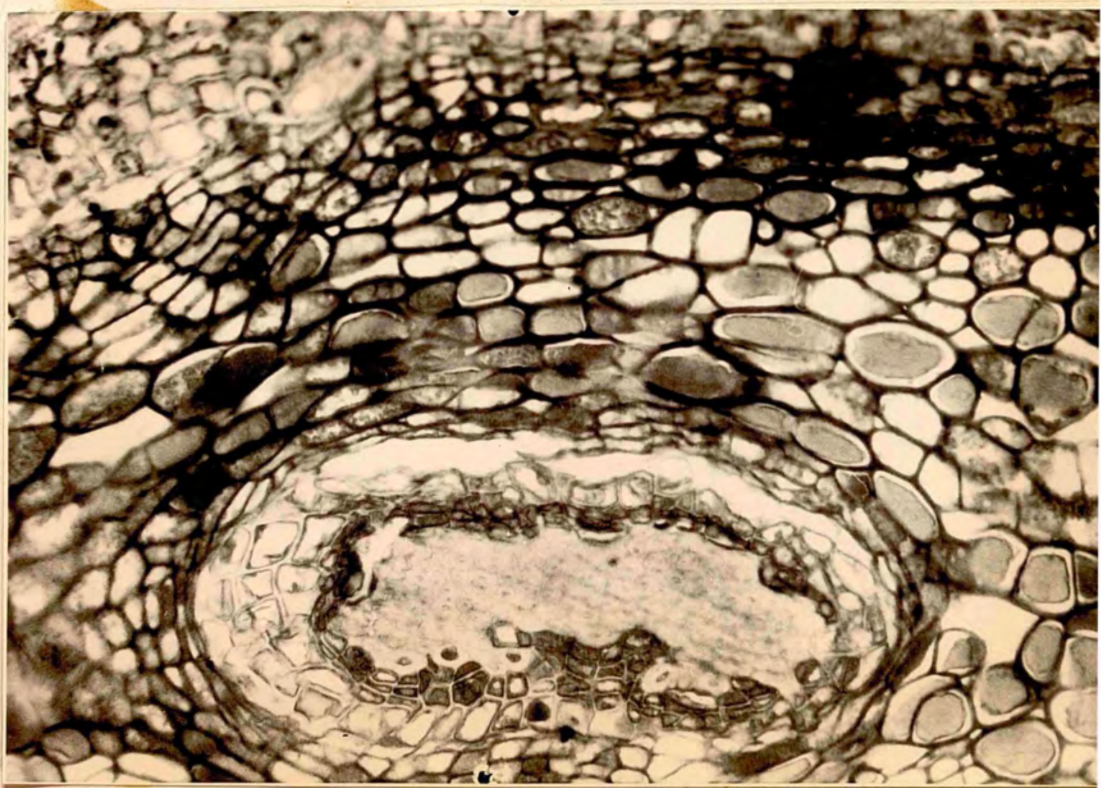


PLATE 3   Primary phloem fibres invested by local cork cambium. About X400.

prepared. For this the phloem part of the bark was removed with a sharp razor blade, left in 5% chromic acid for 48 hours then crushed with a glass rod. It was washed thoroughly and stained with methylene blue. Phase contrast pictures were taken using a green filter.

PLATE 4 T.S. under crossed polaroids showing the tangential bands of phloem fibres. About X200.

GENERAL DESCRIPTION OF THE BARK OF SALIX CAPRAEA

The bark of Salix capraea is fairly typical of a woody angiosperm and consists of periderm, cortex, patches of primary phloem fibres and phloem, yearly increment of secondary phloem, and cells belonging to the cambial zone (Plate 1). The secondary phloem possesses fairly conspicuous tangential bands of phloem fibres; crystal-containing cells are closely associated with them (Plates 2 and 6). Both the phloem fibres and the crystals almost filling the adjacent parenchyma cells, show up very clearly under crossed polaroids (Plates 4 and 5). Curiously no crystal-containing cell is associated with the primary phloem fibres (Plate 3). The sieve tube walls do not stand out in polarized light, as is sometimes the case (Bisalputra and Esau, 1964); presumably this is due to their lack of an obvious nacreous thickening. The xylem walls on the contrary stand out very conspicuously.

Tannin is very abundant in the phloem parenchyma even in fairly close proximity to the cambium (Plates 6 and 7). As mentioned below it is also abundant in the ray cells, but only within the phloem. There did not appear to be any evidence that the parenchyma of phloem formed early in the

PLATE 5 Tangential longitudinal section (T.L.S.) under crossed polaroids showing the phloem fibres and crystal containing cells. About X200.

year contained less tannin than that formed later (Evert 1963).

Intercellular spaces are hardly noticeable in the younger phloem, but as the tissue ages they become conspicuous, until eventually with the increase in circumference consequent on growth large scizo-lysigenous cavities appear in the obsolescent tissue (Plate 8).

Fibres

As just mentioned secondary phloem fibres occur in fairly well marked tangential bands serving to delimit the annual increments of phloem. Differentiation of the fibres by intrusive growth followed by wall thickening begins to be early June. Plate 4 shows a transverse section taken between crossed polaroids. The material was collected and fixed on the 15th June, 1965. It is obvious from the fact that the thickening fibres are some distance away from the cambial zone, that the fibre initials were formed a considerable time earlier.

The crystal-containing cells associated with the secondary fibres are juxtaposed in continuous longitudinal series which might be referred to as "septate fibres".

Rays

The rays are all uniseriate and hetero-cellular

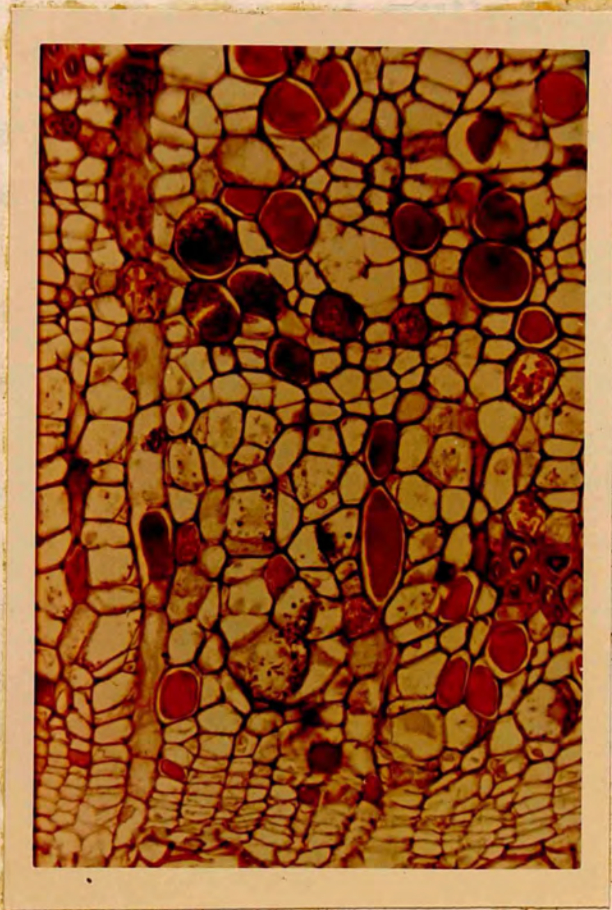


PLATE 6 T.S. of phloem. A secondary phloem fibre bundle is shown on the right and numerous tannin filled cells, some very close to the cambium. About X310.

i.e. their upper and lower edges are formed of vertically-elongated cells in contrast to the horizontally-elongated cells forming their middle portions. The ray cells have profusely pitted walls and all of them (except those newly formed) contain abundant tannin (Plate 2). Commonly there are two to four radial rows of conducting tissue cells between the rays (Plate 1).

Outer layers of Bark

In the outer layers the bundles of primary phloem fibres gradually become invested with a layer of periderm (Plate 3). These bundles with continued growth of the stem eventually break loose and give the bark its "stringy" appearance. In younger stems the parenchyma below the epidermis develops collenchymatously and contains numerous chloroplasts (Plate 8).

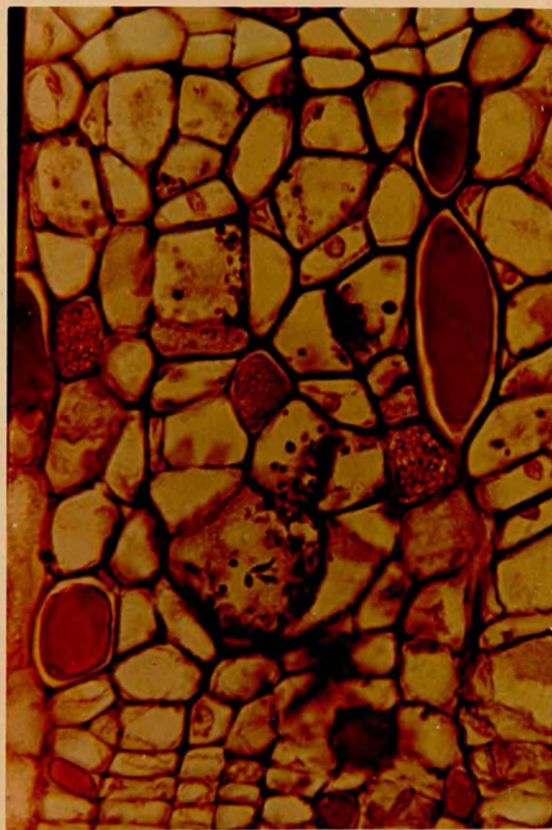


PLATE 7 High magnification of part of Plate 6. Several sieve plates can be seen. Plastids are seen accumulated near the plates. Note the denser cytoplasm of the companion cells. About X750 (oil immersion).

ANALYSIS OF PHLOEM AS AN ELECTROKINETIC CHANNEL

Before embarking on the analysis of the phloem cell by cell, it will be helpful to define the terms which will be employed. This is done as follows (Esau and Cheadle,1955).

Cambial Initials

These are the presumably single-layered cylindrical sheets of cells which, by dividing radially in both the inward and outward directions, give rise to the new xylem and phloem.

Cambial Zone

Normally it is very difficult to distinguish between the cambial initials and their immediate derivatives on either side. Consequently all these cells, of similar appearance are referred to collectively as the Cambial Zone or as cambial cells.

Phloem Initials

Cambial cells destined to become part of the phloem are referred to as phloem initials or phloem mother cells. In this sense a sieve tube or tubes with the associated companion cells will be spoken of as having originated from the same mother cell.

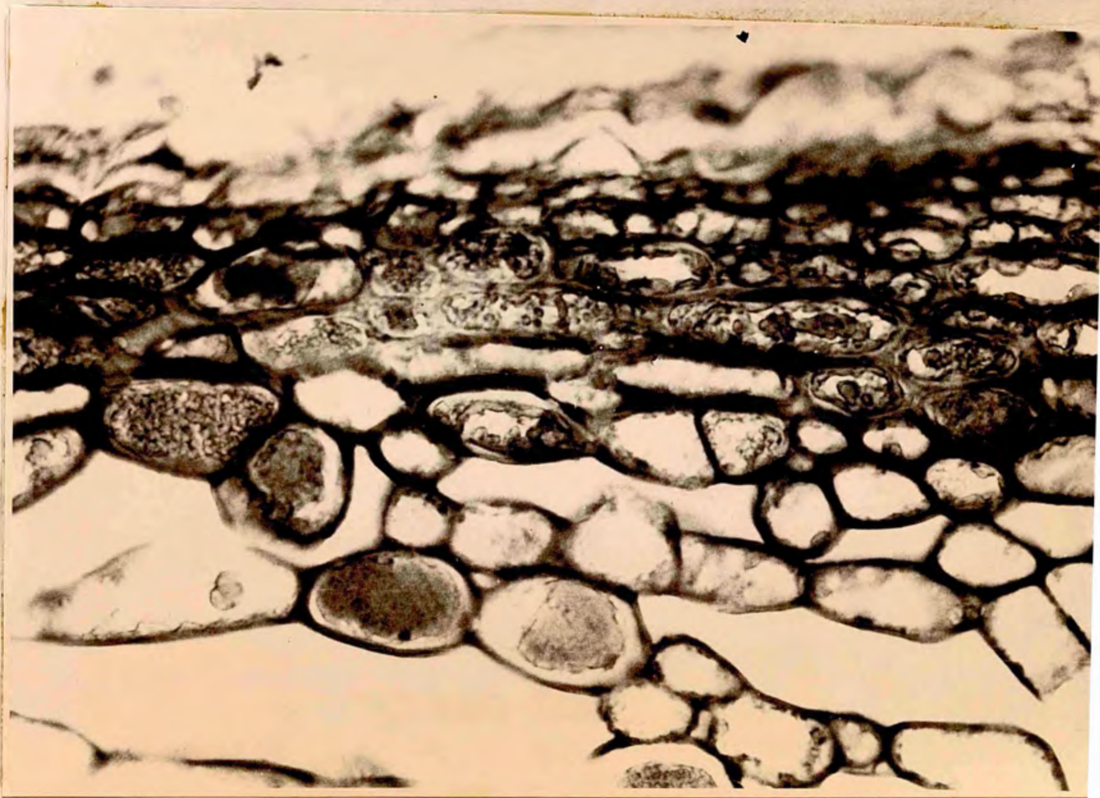


PLATE 8 T.S. showing collenchyma cells with chloroplasts near the epidermis. Some inter cellular spaces are also seen. About X700.

Tier

The products of the division of a single fusiform cambial initial will form a radial row of cells, not necessarily everywhere one cell wide (tengentially) or one cell deep (longitudinally). Such a row will be called a tier.

Columns

Longitudinally, while the cambial initial of one tier does not correspond exactly with initials above and below it, it will often be possible to trace more or less continuous columns of sieve tube elements along the stem. The connection is physiological rather than ontogenetic.

Rays

Ray cells will not be spoken of in the above terms but will be referred to as such.

PLATE 9 T.S. showing level I of tier I. This tier (shown coloured yellow) is analysed in Fig.1. About X700.

ANALYSIS OF TIERS

The present study is directed not so much to gaining knowledge of the phloem structure of Salix capraea for its own sake or for reasons of comparative anatomy or phylogeny but rather to provide an anatomical basis for assessing the adequacy or otherwise for this species of the electrokinetic theory of phloem transport. For this purpose it is necessary to trace the living cells in contact with the sieve tube elements along the files of the latter with a view especially to seeing whether they could possibly provide an adequate pathway for the flux of potassium ions suggested by the theory. In pursuance of this programme, serial transverse sections of the phloem of two year old twigs were cut at a thickness of 6μ (there was insufficient material to use older branches which from some points of view would have been preferable). Photomicrographs of the serial sections are shown in Plates 9 to 16. These represent every other section, but recourse was had to the intermediate sections at frequent intervals during the analysis.

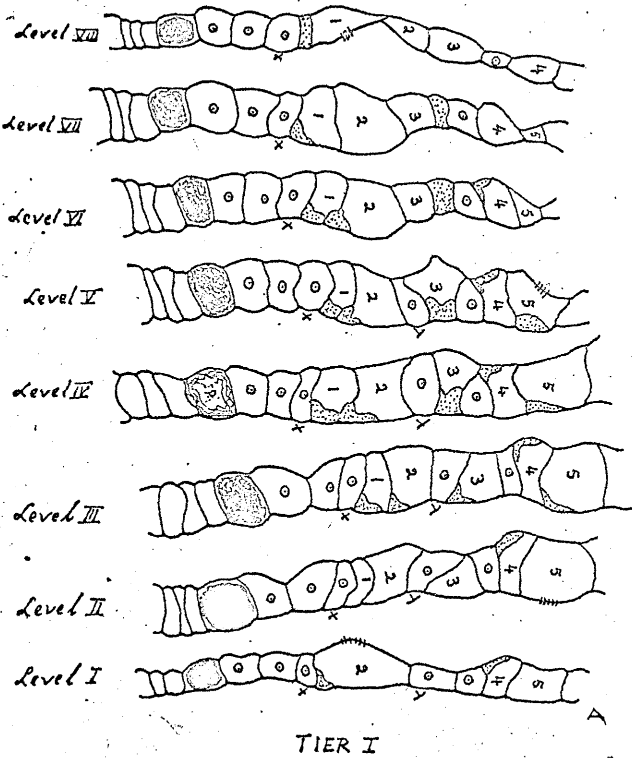
The tiers were analysed by first of all selecting on the uppermost micrograph several radial rows with well-marked outlines. These were designated A, B, C and so on.

PLATE 10 T.S. showing level II of tier I.
About X700.

Each tier to be analysed was then followed down through successive stem sections labelling it in each with the same letter. Eventually of course it narrowed and finally disappeared as the section reached the lower extremity of the fusiform initial giving rise to the tier. Having thus found how to bring the photomicrographs into vertical correspondence adjacent less well-marked tiers could be labelled (as a, b, c and so on) and similarly traced from section to section. Since the cambium of _Salix capraea_ is not storied the tiers do not all end at the same level, and this means that as files are followed downwards tiers will make their appearance between others already given designations. These new ones were given designations such as a_2, a_3 (between a and b) or c_1, c_2 (between c and d). Having thus traced a contiguous number of tiers down through an adequate length of stem individual sieve tubes could be treated in a rather similar way within tiers, being allocated numbers instead of letters. Thus d_2 would represent a sieve tube element belonging to the tier of d. On this process it was often helpful or even necessary to consult intermediate photographs or even have recourse to the original sections.

PLATE 11 T.S. showing level III of tier I.
About X700.

The final process involved grouping the cells within a tier in such a way as to give recognition to the fact that a phloem mother cell derived from a cambial initial usually divides into several cells before it matures, the object being to trace the history of each cell in the tier back to the original derivation from the cambium.



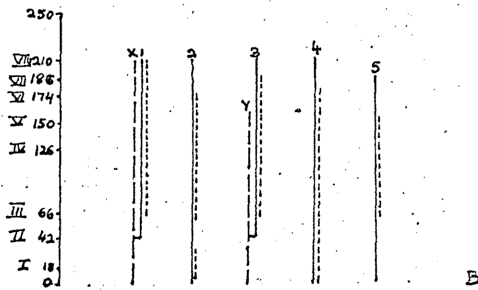


FIG 1

FIG.1 - Analysis of Tier I. Drawings I, II, III, IV, V, VI, VII, VIII of (A) illustrate the tier in transverse sections taken at levels indicated as I, II, III, IV, V, VI, VII and VIII at (B). In (A) parenchyma cells are with nuclei; companion cells stipled; sieve elements numbered; sieve plates hatched. In (B) the numbered solid lines are sieve tubes, sparsely broken lines are parenchyma and frequently broken lines are companion cells. Numbers from 0 to 250 in (B) represent length in microns.

PLATE 12 T.S. showing level IV of tier I. About X700.

TIER - I

This tier has been coloured yellow in Plates (9-16) and tracings at eight levels (spaced as indicated) are shown in the attached drawing (Fig.1, A) together with a diagram of the longitudinal distribution of the cells (Fig.1, B).

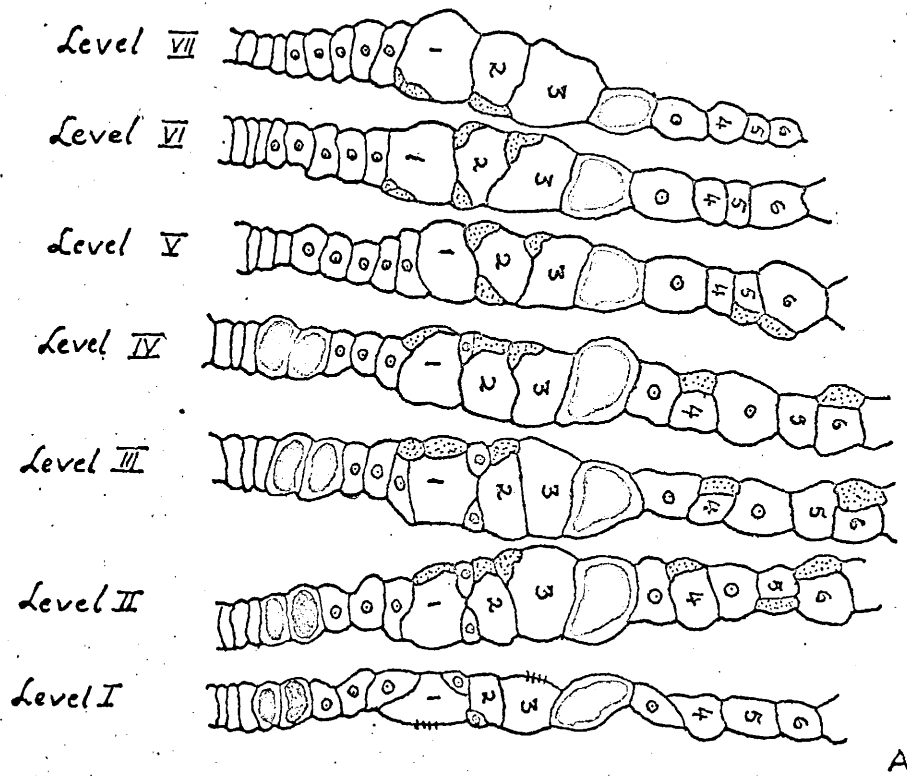
Referring to Fig.1 the cambial zone is at the left, represented by four undifferentiated cells. The portion of this tier shown contains five sieve tubes (the numbered cells). Next to the cambial zone, there is a strand of parenchyma cells containing tannin.

The sieve tubes have been numbered from 1 to 5 in each of the eight transverse sections, which are designated "level VI etc" to agree with the transverse photomicrographs.

Each of the ontogenetically related assemblages 2, 4 and 5 were clearly derived from one phloem initial, that acted as sieve tube mother cell. It is apparent from the sections in Fig. 1-A that the companion cells usually occupy a position in between the radial and tangential walls of the sieve tube, though they may occasionally occupy either the radial or the tangential wall of a sieve tube (see Fig.1 in A - cells III - 5; VI - 3 and VIII - 1).

However, ontogenetic relationship is not always quite

PLATE 13 T.S. showing level V of tier I.
About X700.



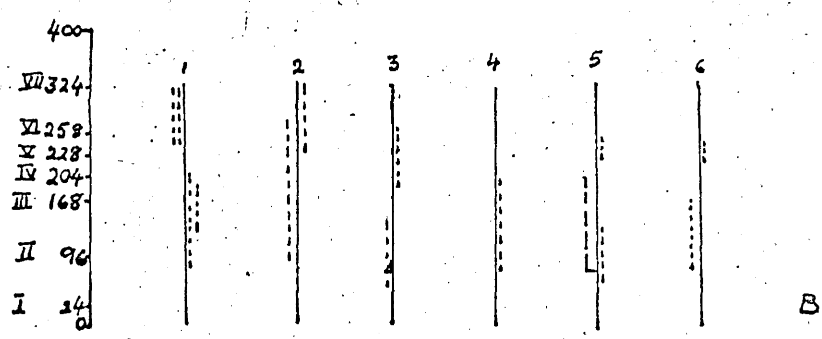


FIG.2 - Analysis of Tier II, details as given for FIG.1.

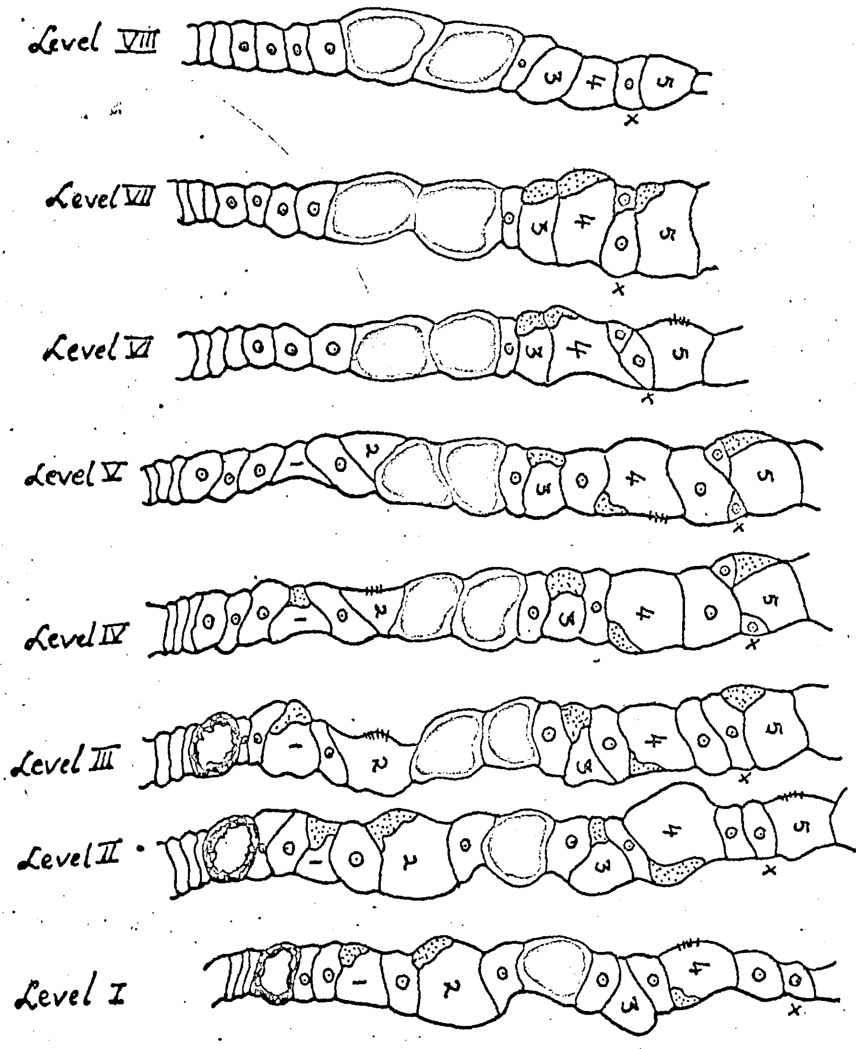
PLATE 14 T.S. showing level VI of tier I.
About X700.

so obvious; if assemblage 3 had not been studied throughout, its companion cell (VI and VII in Fig.1 A) would have been mistaken for a parenchyma cell (as it looks quite large in VI).

The complicated assemblages 1 and 3 (Fig.1 in A) are interpreted as follows. The first periclinal division of the phloem initial giving rise to the assemblage 1 resulted in the formation of a longer and a shorter cell. The longer cell served as the precursor of the parenchyma strand X that extends below the sieve tube as shown in Fig.1 (I of A). The shorter cell became the sieve tube mother cell which in turn divided to give rise to the sieve tube 1 and its associated companion cell. The same sort of division is also found in the assemblage 3, where the phloem initial divided by an oblique wall to form a longer and a shorter derivative. Unlike the previous one, in this case the shorter derivative acted as the precursor of the parenchyma strand Y in Fig.1 and the longer cell served as the mother cell of the sieve tube 3 and its associated companion cell.

TIER - II

The portion of this tier shown (Fig.2) contains six



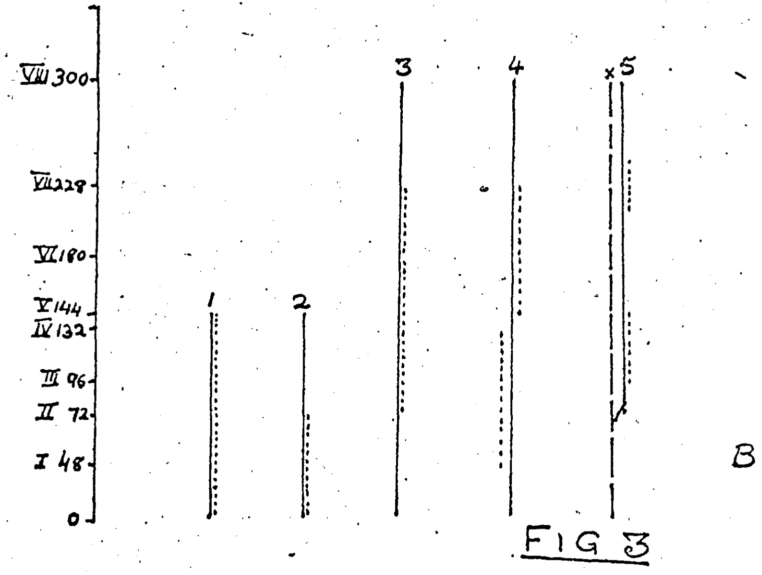


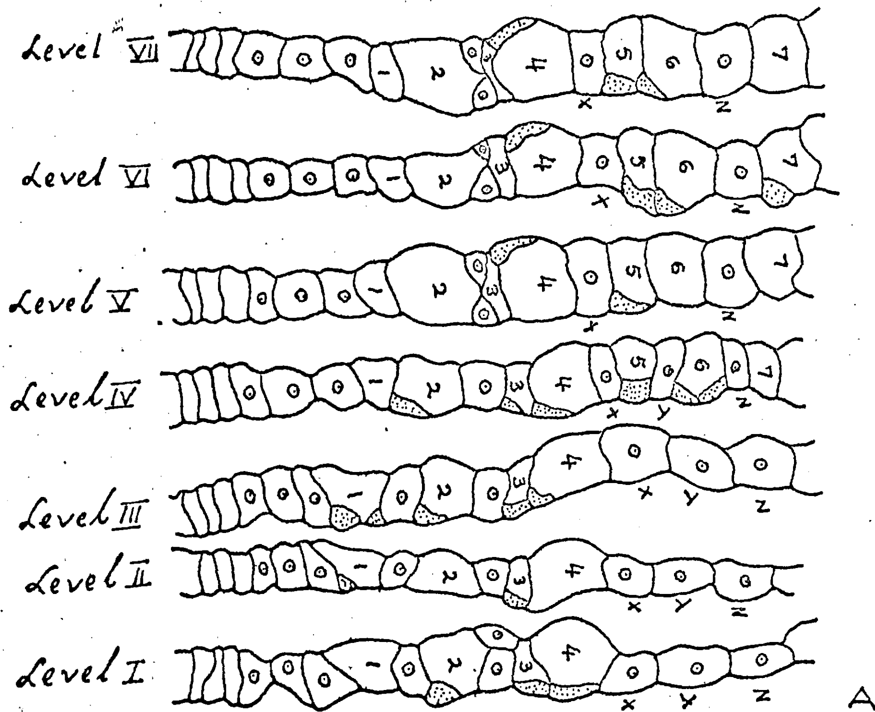
FIG.3 - Analysis of Tier III, details as given for FIG.1.

PLATE 15 T.S. showing level VII of tier I. About X700.

sieve tubes. Each of them is associated with more than one companion cell, except sieve tube 4 (Fig.2 at B). Each of the assemblages 1, 3 4 and 6 has been derived from one phloem initial. The phloem initial acted solely as the mother cell of the sieve tubes and their companion cells. Sieve tube 5 (Fig.2), its associated companion cells and the parenchyma cell in contact with it have however been derived from the same phloem initial. The phloem initial in the assemblage 5, first divided to give rise to two unequal cells. The longer derivative acted as the immediate precursor of the sieve tube 5 and its associated companion cells. The shorter one acted as the precursor of the parenchyma strand.

TIER - III

The portion of the tier shown here in (Fig. 3) contains five sieve tubes. Each of the assemblages 1, 2, 3 and 4 and their associated companion cells have been derived from a single phloem initial. Sieve tubes 4 and 5 have two companion cells each. In the case of assemblage 5, however, the phloem initial gave rise not only to a sieve tube and companion cells but also to the parenchyma strand X , as in the case of assemblage 5 of the tier II. This



TIER IV

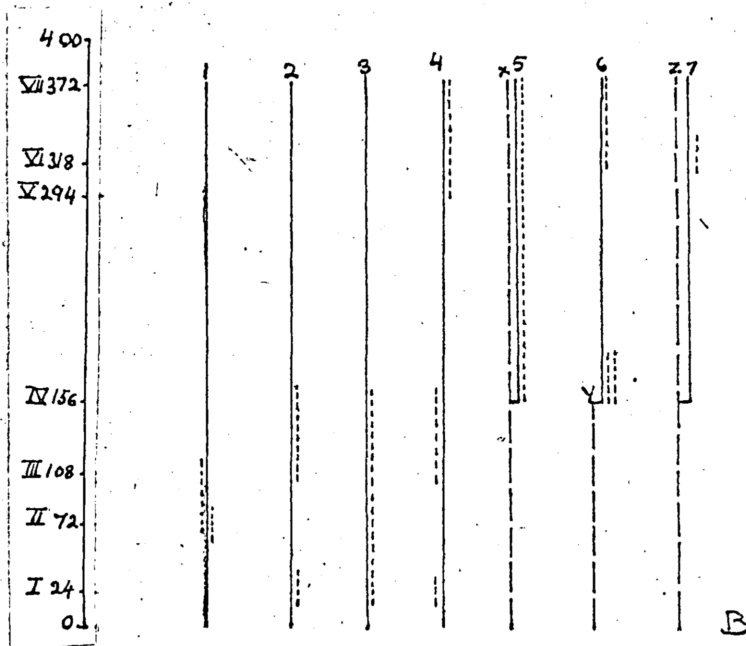


FIG.4 - Analysis of Tier IV, details as given for FIG.1.

PLATE 16 T.S. showing level VIII of tier I.
About X700.

parenchyma strand is longer than the sieve tube 5. Evidently the phloem initial underwent an oblique division to give rise to a longer and a shorter derivative. The longer one formed the parenchyma strand which can be traced down below the sieve tube 5. Interestingly, parenchyma cells have become tannin filled very close to the cambial zone.

TIER - IV

This tier contains (Fig. 4) seven sieve tubes. Towards the left (Fig.4 at A) there are three undifferentiated cells, which form the cambial zone. Next to these there are three parenchyma strands. Sieve tubes 1, 2, 3, 4 and their associated cells are each the exclusive products of one phloem initial, no parenchyma cells sharing their ontogeny. By contrast sieve tube 5, its associated companion cell together with the parenchyma strand X (Fig. 4 at A at the level IV) have been derived from the same initial. The phloem initial first dividing periclinally gave rise to a longer and a shorter derivative. The longer derivative served as the precursor of the parenchyma strand (X) and the longer derivative acted as the precursor of the sieve tube 5 and its associated companion cell. An exactly similar sequence of divisions took place in the assemblage 7.

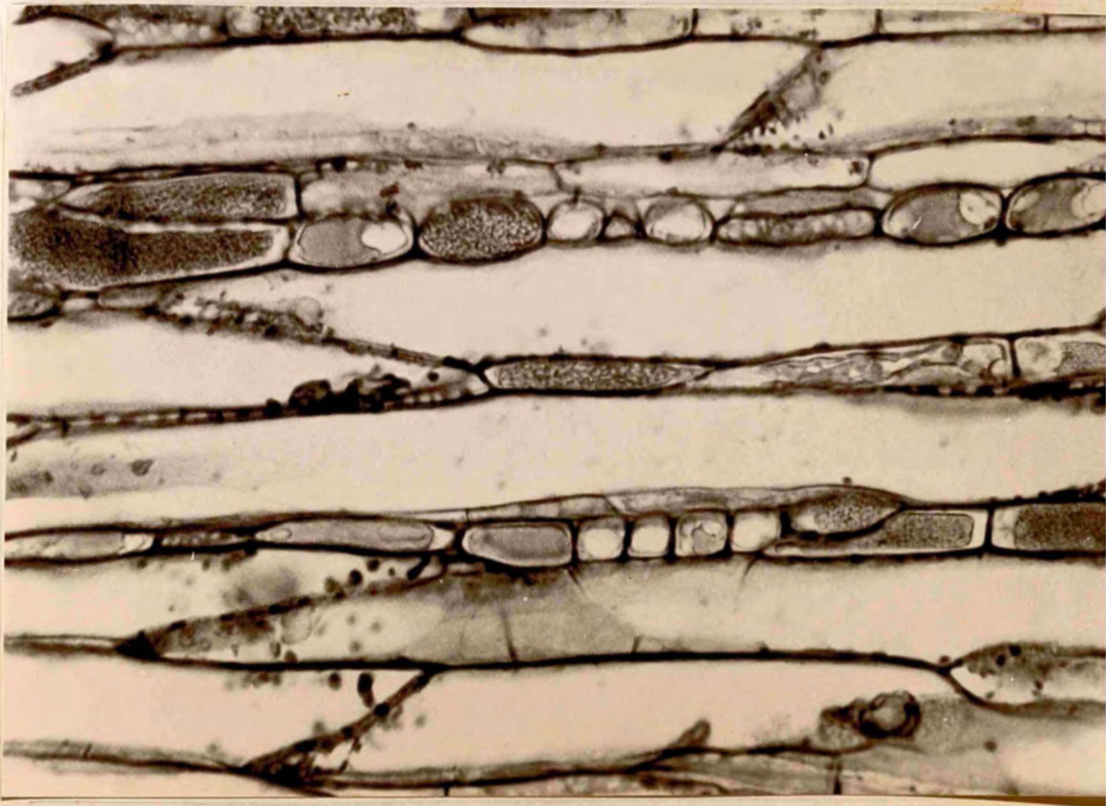


PLATE 17 T.L.S. showing sieve tubes with very oblique sieve plates, nucleoli and a slime plug. Companion cells can also be seen. About X1500 (oil ~~emulsion~~ immersion).

But in the assemblage 6, the two derivatives resulting from the periclinal division of the phloem initial were almost equal in size (Fig. 4 at B). One of them served as the precursor of the parenchyma strand Y, while the other acted as the immediate precursor of the sieve tube 6 and its associated companion cells.

From these analyses it is clear that there is a considerable diversity in the pattern of divisions undergone by a single phloem initial. This is similar to the state of affairs found in Calycanthaceae by Cheadle and Esau (1958) and Evert and Derr (1965).

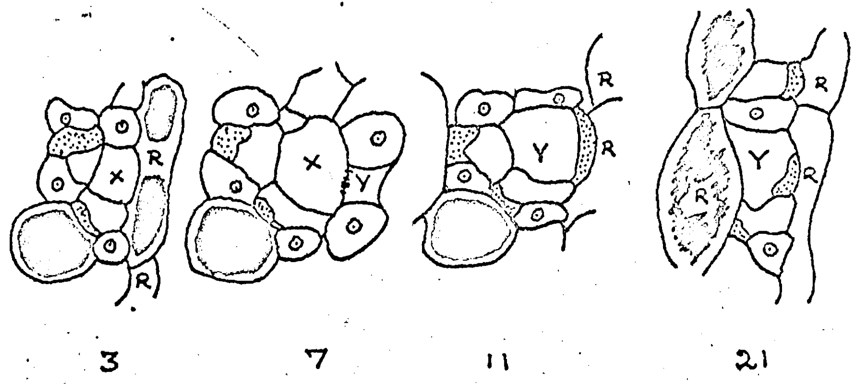
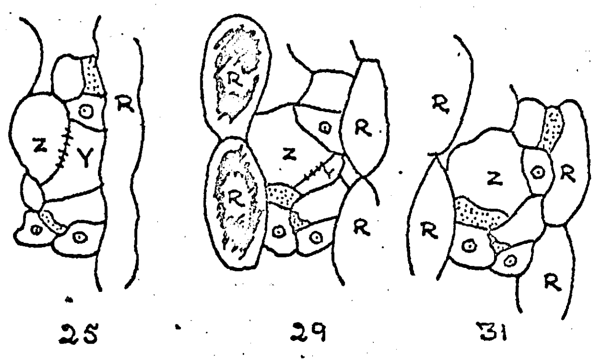


FIG.5



COLUMN I

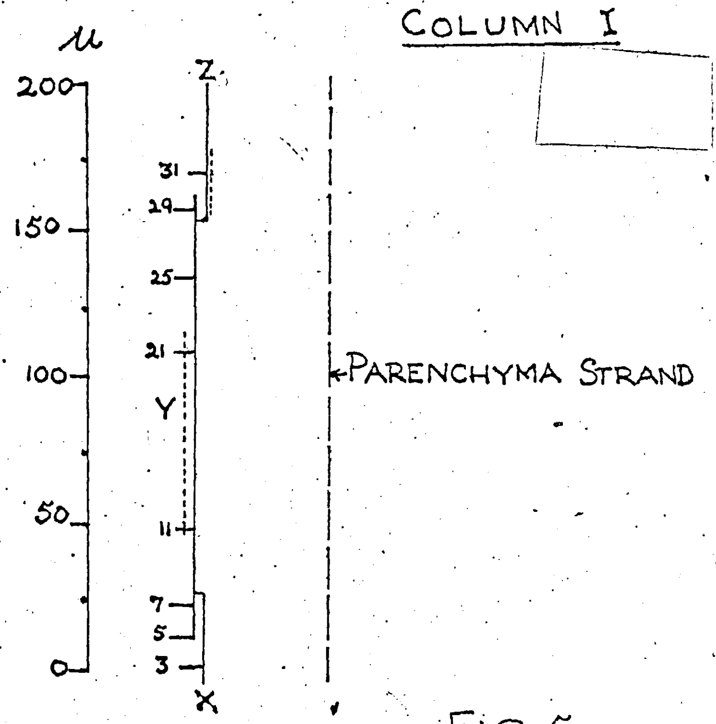


FIG 5

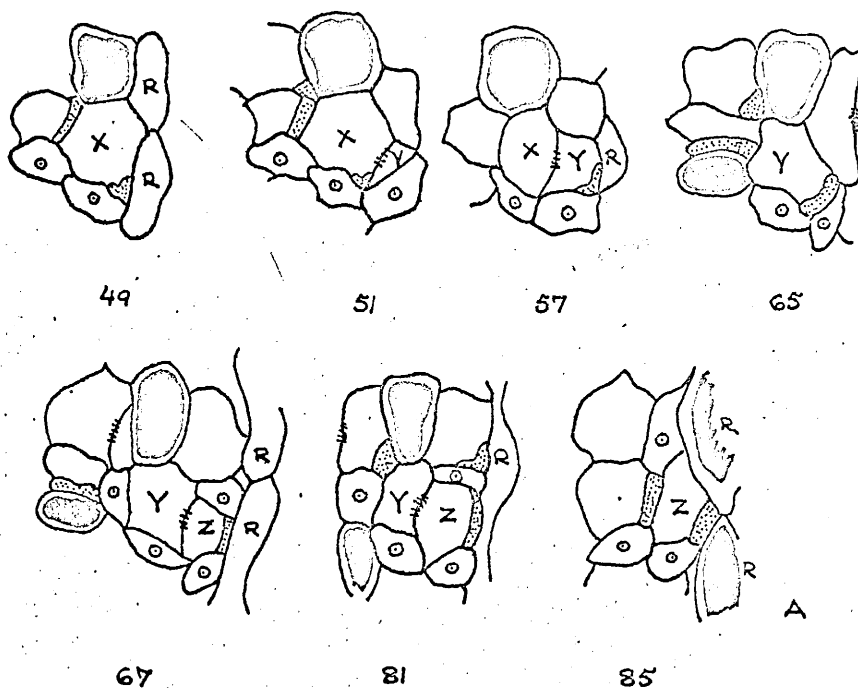
Analysis of sieve tube column I. A micron scale has been given at the left. Details as given for FIG.1.

ANALYSIS OF SIEVE-TUBE COLUMNS

The object of this analysis is to elucidate the relation of the living cells - companion and parenchyma - to the sieve tubes in order to throw light on the question of whether they constituted an adequate "return path" for the potassium ions as envisaged by the electrokinetic theory of translocation. Longitudinally while the cambial initials do not correspond exactly to simple unambiguous columns, it is often possible to trace fairly definite 'functional' columns of sieve tube elements down the axis and this has been done in the analysis which follows.

Column 1

This is analysed in figure 5. The number below each drawing (e.g. 7, 25) is the ordinal number of the serial section from which it was copied. Since the serial sections were 6 μ thick the distance between the sections can be at once found (in the example given it is $(25 - 7) \times 6 = 102\ \mu$). Sieve tube element X overlaps element Y to which it is joined by a sieve plate between 5 and 7; similarly Y overlaps Z between 25 and 30. It can be seen that the companion cells do not 'run across' the sieve plates as was originally envisaged in the



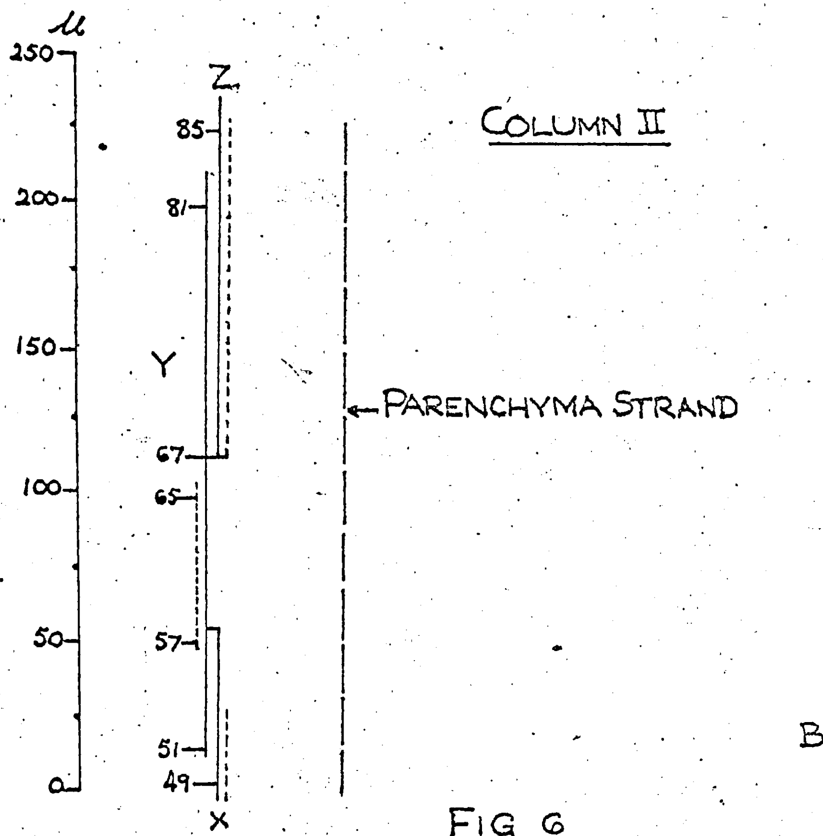


FIG 6

FIG.6 - Analysis of sieve tube column II, details as given for FIG.1.

electrokinetic theory (Spanner, 1958). On the other hand there is a continuous strand of parenchyma cells running the full length of X, Y and Z shown and in immediate contact with them.

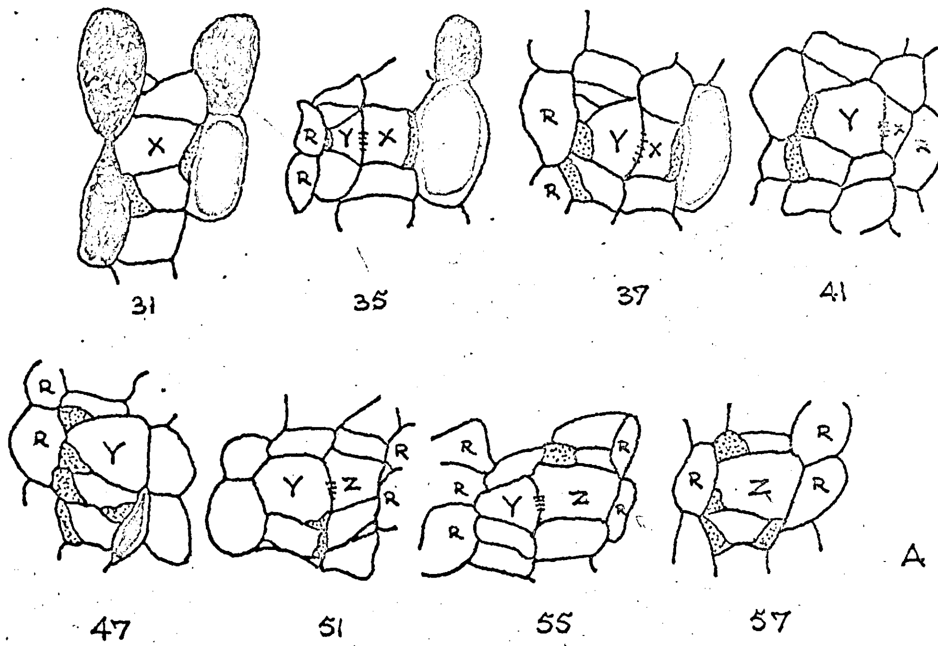
Column 2

Here again it is obvious that the sieve plates are not accompanied laterally by a continuous row of companion cells linking the sieve tube elements; but again there is a continuous strand of parenchyma cells, the latter in contact with both the sieve elements and the companion cells. The two sieve tubes Y and Z intercommunicate with a very oblique sieve plate and companion cell coverage is in fact co-extensive with this.

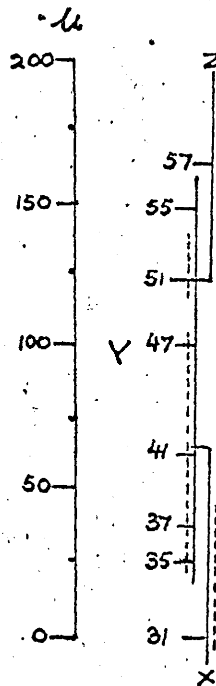
Column 3

Here the picture is a little different. There are no parenchyma cell strands though the companion cell longitudinal coverage is a little greater. However, where the companion cells overlap at the sieve plates they are not in lateral contact with one another.

The conclusions to be drawn from this limited analysis of longitudinal connections are not very far-reaching. Superficially the companion cell distribution would not



COLUMN III



B

FIG 7

FIG.7 - Analysis of sieve tube column III.

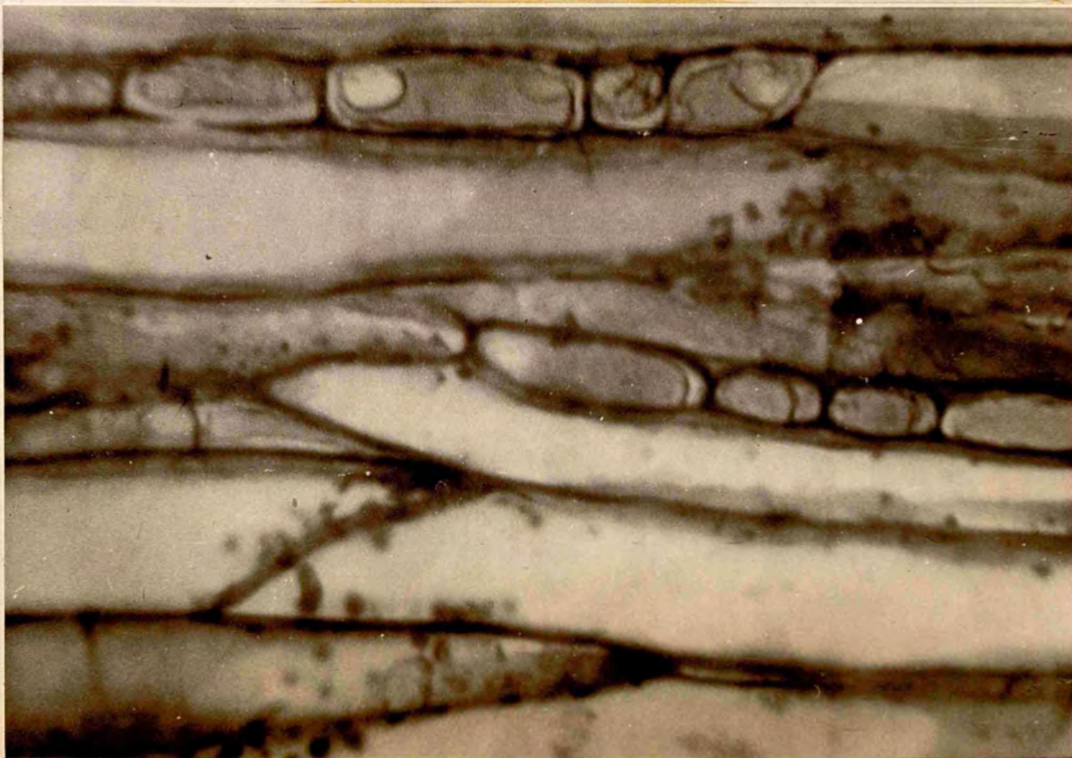


PLATE 18 T.L.S. showing sieve tubes. Note the thin wall between adjacent sieve tubes and wall between sieve tube and companion cell. About X1500 (oil ~~emulsion~~ immersion).

seem to lend strong support to the electrokinetic theory. The parenchyma strands would seem to be a possible return path for ions; but even then the route would appear to be much less direct than the theory originally envisaged. The tannin-filled cells are probably excluded, and the ray-cells unlikely too to be of much significance in this connection.

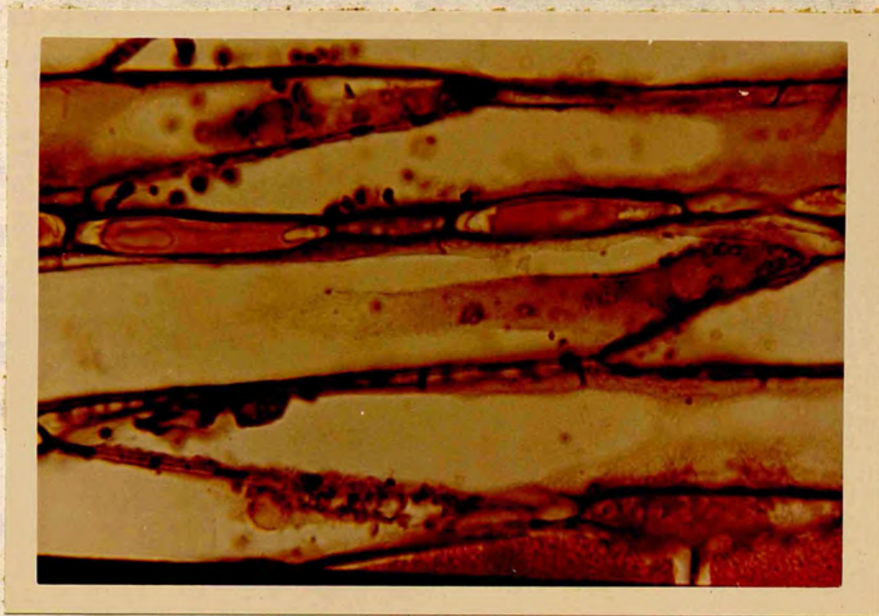


PLATE 19 T.L.S. showing the extruded nucleoli (stained yellow) near the sieve plates and a slime plug. Note the dense cytoplasm of the companion cells. About X750 (oil immersion).

OBSERVATIONS

It is rather essential to get oneself equipped with all the details available of the structure of any organ through conventional optical microscopy before embarking on electronmicroscopy.

The staining technique of Sharman (1943) was found to be very good for the phloem of Salix capraea.

Structure of sieve elements wall -

There are numerous pit fields on the sieve element side of the common wall between the sieve element and its companion cells (Plates 17, 18 and 19). This common wall is very thin. Sieve areas between adjacent sieve elements are also abundant (Plate 18).

Sieve plates are very oblique and occur in the end walls (Plates 17, 18 and 19) of the sieve elements. Sieve plates are compound (Plates 20 and 21).

Callose deposits were well shown by Eschrich's and Currier's (1964) method, which stains them a cobalt blue colour with resorcinol blue (Plates 22, 23 and 24).

Contents of the sieve element -

Here cytologic findings will be dealt with very briefly.

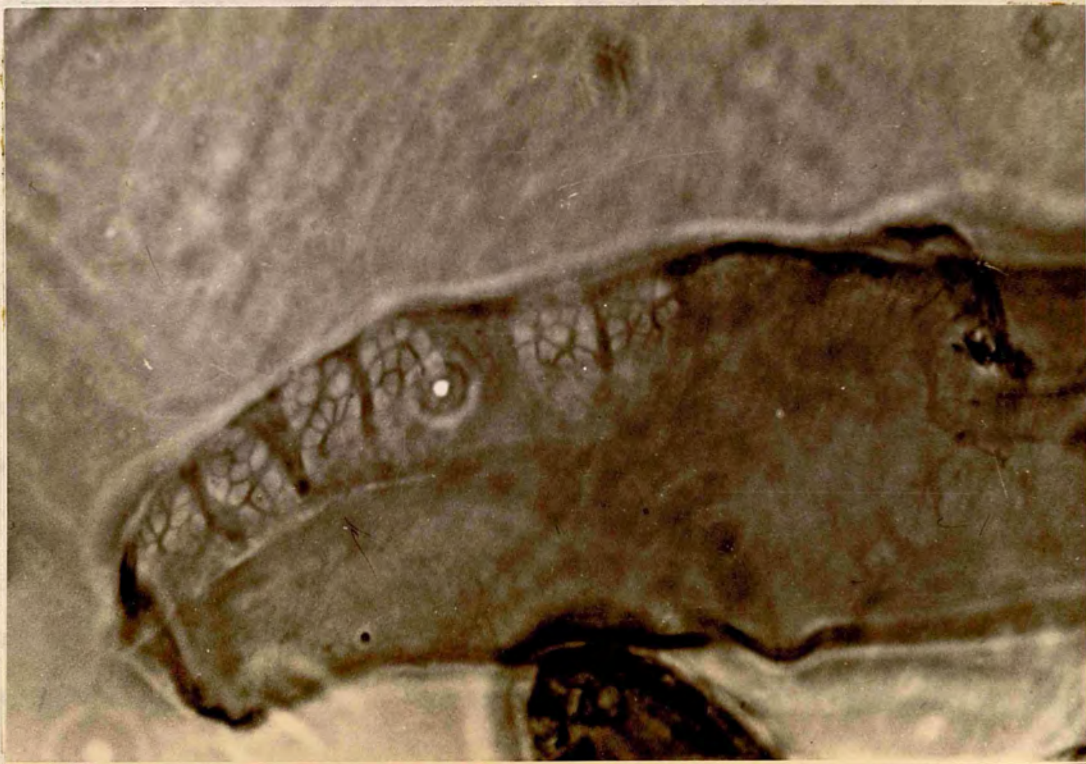


PLATE 20 Single sieve tube member showing the compound nature of the plate; prepared by a maceration technique. About X2,500 (oil ~~emulsion~~ immersion).

Protoplast -

Sieve element differentiation begins after the immediate precursor has divided to give rise to the mother cells of the sieve element and associated companion cells. Colour plate 25 shows an early stage in this process, the dividing walls being still very thin.

In the fusiform initials more than one nucleus has been observed (Plates 25, 26, 27 and 28).

In the colour plates 29 and 30, one nucleus is seen towards the end wall of a phloem initial, and another nucleus nearer the middle of the same cell, the latter being in the process of extruding its nucleolus. A thin wall can be seen near the emerging nucleolus presumably dividing the cell into the mother cell of the sieve element and its companion cell. Possibly in salix capraea the nucleolus may be extruded rather early in the development of the sieve tube element. The chromatic material of the nucleus disintegrates after the release of the nucleolus. Immediately after the companion cell is cut off, the sieve element undergoes rapid radial expansion (Plates 19 and 29). In salix the extruded nucleolus persists for quite a long time,

PLATE 21 R.L.S. showing compound sieve plates, starch and tannin containing parenchyma cells. About X750 (oil immersion).

apparently throughout the functional life of the sieve tube (Plates 31 and 32). The nucleolus looks quite small when it is inside the nucleus. But after its extrusion it gets larger and assumes a serrated outline, and at the same time its colour (in Sharman's stain) changes from red to yellow (Plate 19). Before the nucleolus finally disappears it looks disorganized and a bit elongated (Plates 31, 32 and 33).

Slime is apparent in the form of slime plugs near the sieve plate in a number of micrographs (Plates 17 and 19). Formation of slime plugs is interpreted as an artefact by most workers. It is difficult to say with confidence, in the present work, what was the location of the slime in the functioning cells, but there are many indications of it in the immature elements (Plates 25, 28 and 34). Strands are frequently seen (Plates 32 and 34) and these may be similar to the strands seen by Thaine (1964), and Evert and Derr (1964). The mature sieve elements are enucleate, though as just mentioned the nucleolus persists.

Numerous granules can be seen clustering mostly near

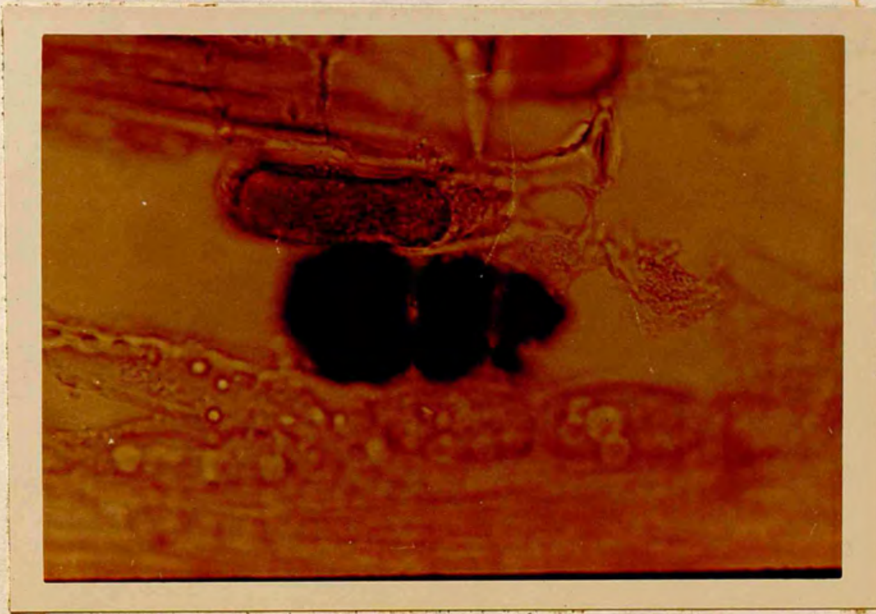


PLATE 22 R.L.S. showing callose deposition on the sieve plate. The callose is stained a cobalt blue colour with resorcinal blue. About X750 (oil immersion).

PLATE 23 R.L.S. showing callose on sieve plates and sieve areas stained with resorcinol blue. Abaut X750 (oil immersion).

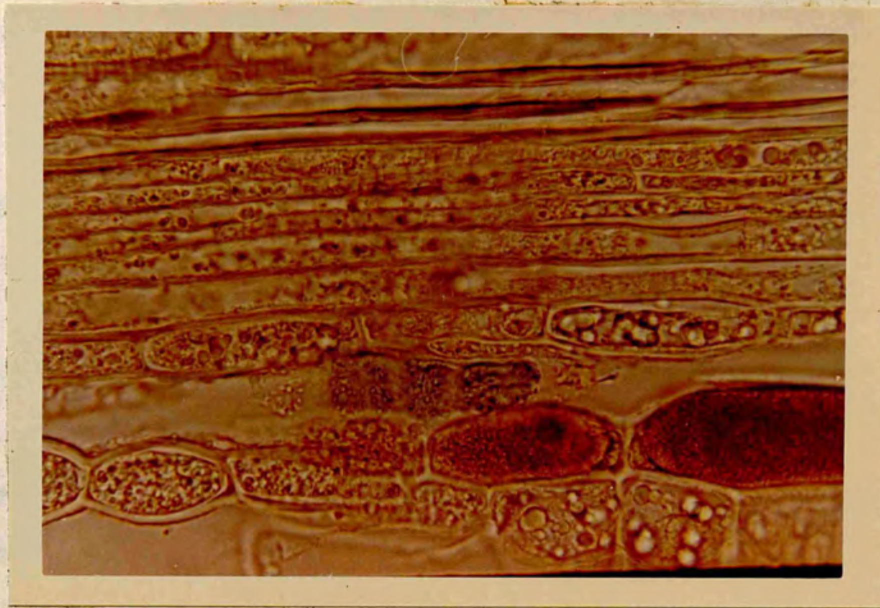


PLATE 24 R.L.S. showing callose on sieve plate stained with resorcinol blue. About X750 (oil immersion).

the sieve plates. Presumably the larger ones are plastids. Some of the smaller ones are undoubtedly starch grains released from broken plastids during preparation, though some of the smallest may be mitochondria (Plates 7, 19, 34).

Companion Cell

Considerable attention has been paid to the relation between companion cells and sieve elements. Many workers now think that the companion cell plays a most important role in the activities of the enucleate sieve element.

The protoplast of the companion cell is dense unlike the associated sieve elements. (Plates 6, 7 and 19). It is a nucleate cell even at maturity. The cytoplasm looks quite homogenous. The common wall between the sieve tube and companion cell is very thin.

Length -

The companion cells vary greatly in their shape, size and orientation. The lengths of some of the companion cells (c.f. Cheadle Esau, ~~Cheadle~~ on Calycantyaceae 1958) and their associated sieve elements are given as follows.

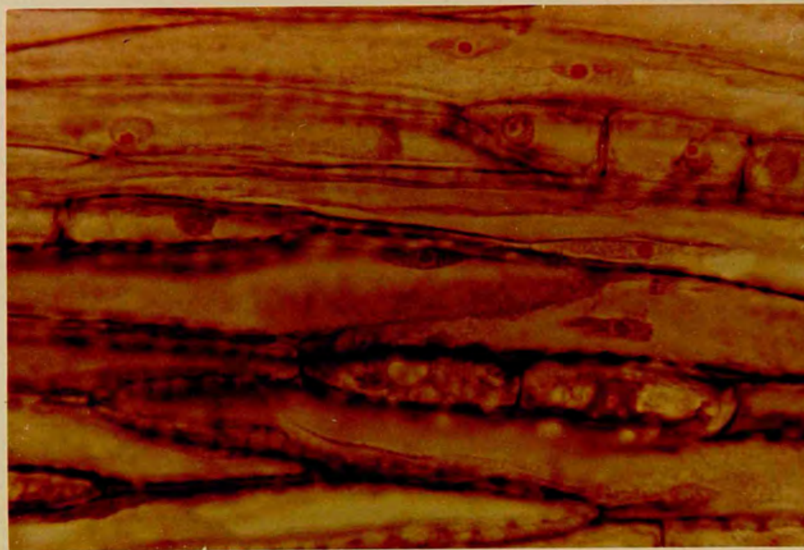


PLATE 25 T.L.S. showing sieve tubes of different ages. In young cells more than one nucleus can be seen. Slime appears as the cell matures. About X750 (oil immersion).

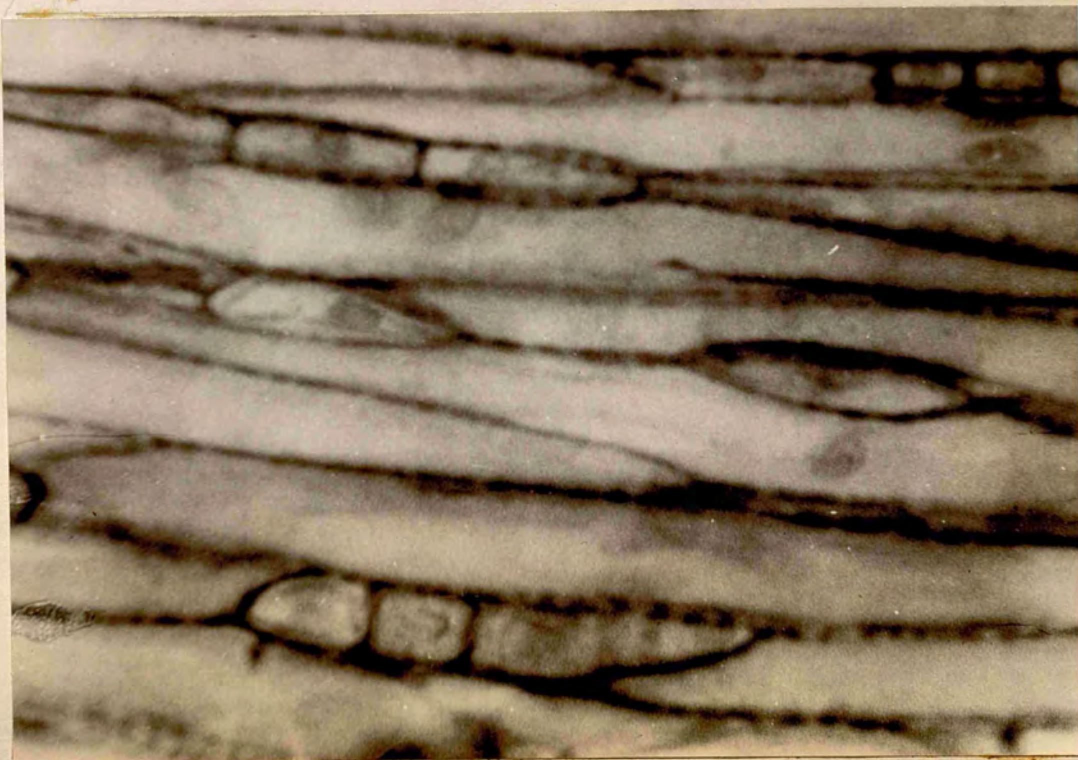


PLATE 26 T.L.S. of young sieve tubes. The pit fields are clearly shown. About X1,500 (oil immersion).

Lengths of 21 sieve elements in microns are arranged in order of decreasing length with their associated companion cells.

Sieve element	Companion Cell	Sieve element	Companion Cell
372 μ	120 μ	324 μ	186 μ
372 μ	96 μ	324 μ	120 μ
372 μ	48 μ	324 μ	110 μ
324 μ	108 μ	252 μ	78 μ
324 μ	102 μ	216 μ	54 μ
324 μ	96 μ	210 μ	180 μ
324 μ	90 μ	210 μ	108 μ
324 μ	84 μ	210 μ	84 μ
324 μ	72 μ	210 μ	18 μ
		192 μ	134 μ
		144 μ	72 μ

These measurements were made from transverse sections 6 μ thick.

Parenchyma Cells

Although much consideration has been given to the relation between companion cells and sieve elements, until recently little attention has been paid to the physiological

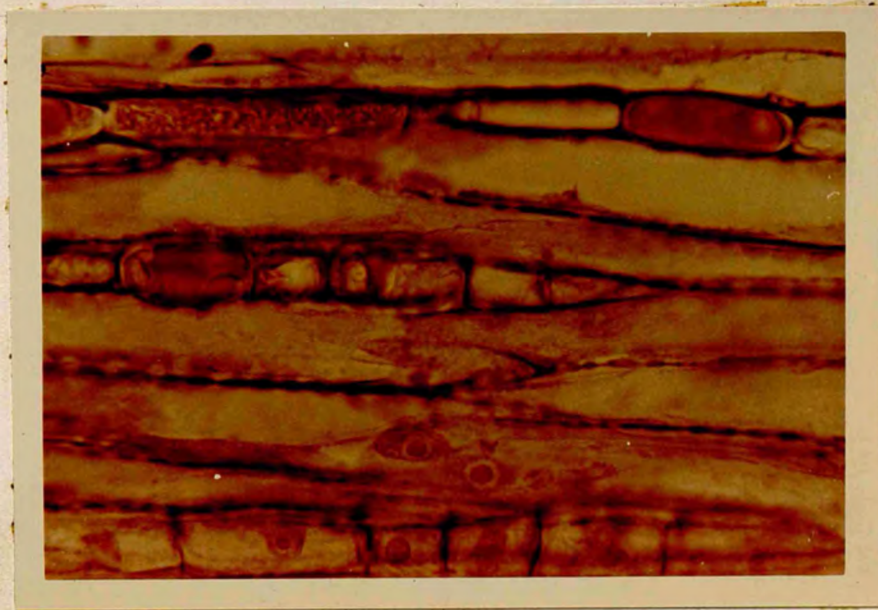


PLATE 27 T.L.S. showing young and newly matured cells. Note the pitted walls between adjacent sieve tubes which represent the primary pit fields and later sieve areas. The nucleoli are not yet extruded. They stain reddish as in the parenchyma cells of the strand at the bottom. About X750 (oil immersion).

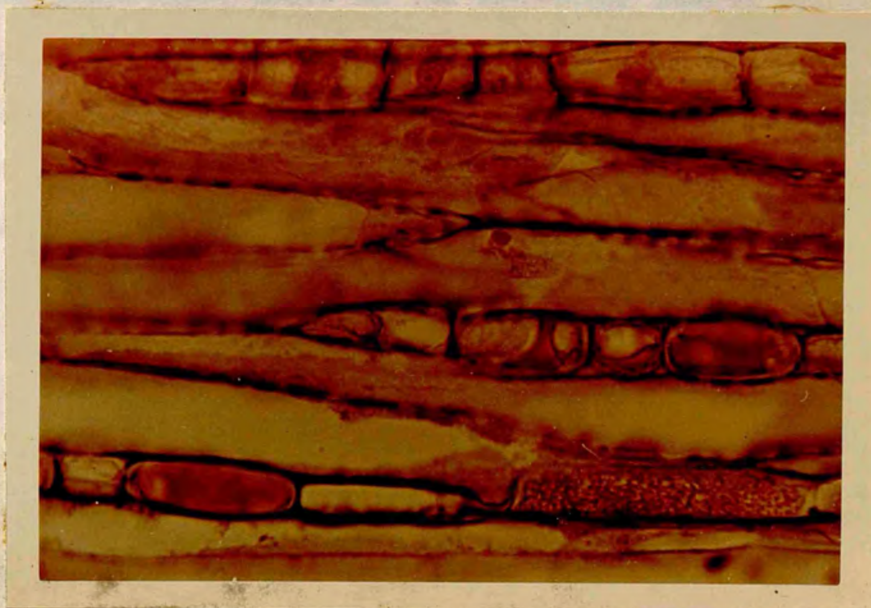


PLATE 28 T.L.S. Recently released nucleolus (in the centre) where the nucleolar material still stains reddish. The chromatin of the disintegrating nucleus is still visible. About X750 (oil immersion).

relation between parenchyma cells and sieve elements.

Cytological details of the parenchyma cells will be discussed under electron microscopic observations.

The parenchyma cells in the secondary phloem of *salix capraea* can be divided into two types. Some which are derived from the phloem initials which give rise to only parenchyma cells and some which are derived from phloem initials which serve also as the precursors of the sieve elements. Both the types are seen in tier I (Fig.1). The parenchyma strand in between sieve tubes 3 and 4 (Fig. 1) belong to the first group but those immediately to the left of sieve tube 1 and in between 2 and 3 belong to the 2nd group.

In addition to the above grouping parenchyma cells can be classified according to their contents; crystal containing cells (Plate 2), tannin containing or starch containing cells (Plates 6, 21 and 24).

The crystal containing cells are confined to tangential bands in the secondary phloem of *salix*. They are always associated with the phloem fibres (Plates 2,4 and 5). There is no such obvious pattern, however, in the distribution of the tannin-filled cells.

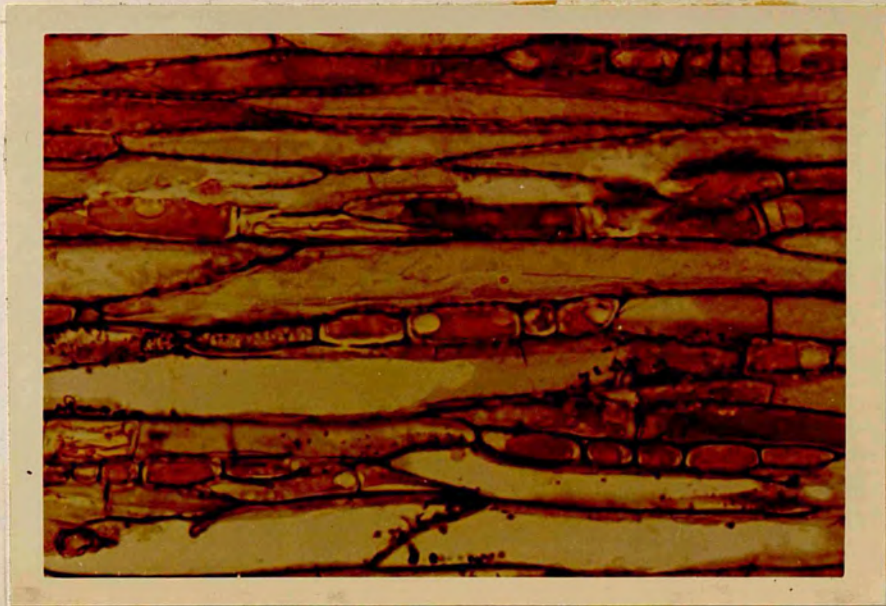


PLATE 29 T.L.S. showing young and matured cells. In the centre a nucleolus seems to be in the process of being released from the nucleus (cf.Plate 28). About X310.

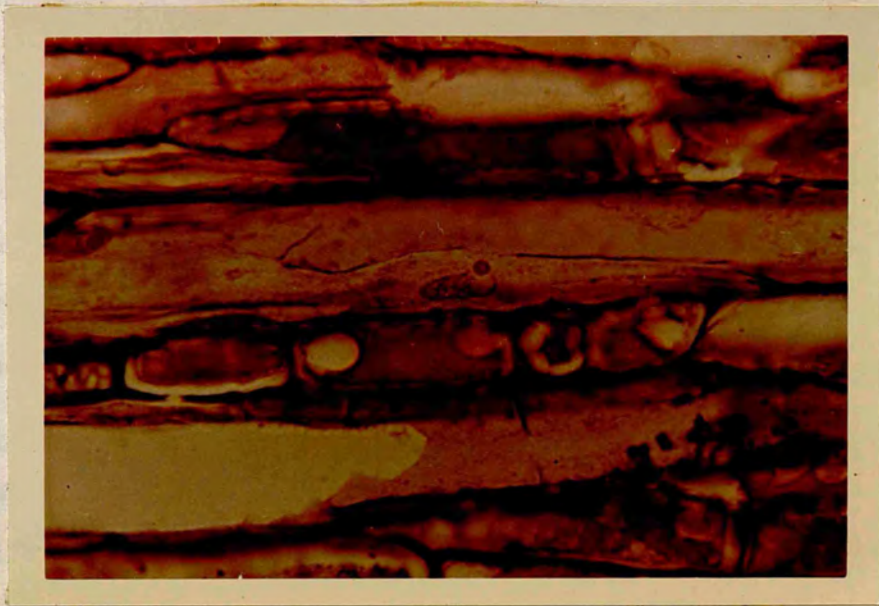


PLATE 30 Higher magnification of Plate 29. This shows clearly the nucleolus emerging from the nucleus. A newly formed oblique-longitudinal wall is also visible. On the left there is another nucleus near the end-wall of the same cell. About X750 (oil immersion)

In some the parenchyma strands are longer than their ontogenetically related sieve elements (Figs.1, 3 and 4).

Below are given the lengths of some sieve elements with their associated strands of parenchyma cells.

<u>Lengths of 7 sieve elements in microns arranged in order of decreasing length with their associated parenchyma strands</u>

Sieve elements	Parenchyma strands
324 μ	204 μ
324 μ	132 μ
264 μ	372 μ
264 μ	176 μ
252 μ	300 μ
192 μ	210 μ
192 μ	150 μ

These measurements were made from serial transverse sections 6 U thick which naturally limits their accuracy.

PLATE 31 T.L.S. showing persistent nucleolus even in an old sieve tube. Note the disorganized appearance of the nucleolus which stains yellow. About X750 (oil immersion).

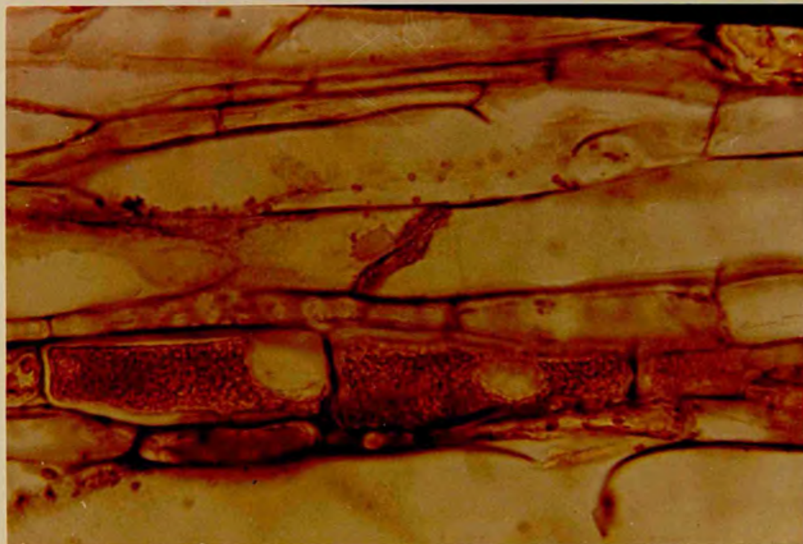


PLATE 32 T.L.S. showing nucleolus in an old cell probably in the process of disintegration. Note the longitudinal plasmatic strands with plastids in one sieve tube (upper centre). About X750 (oil immersion).

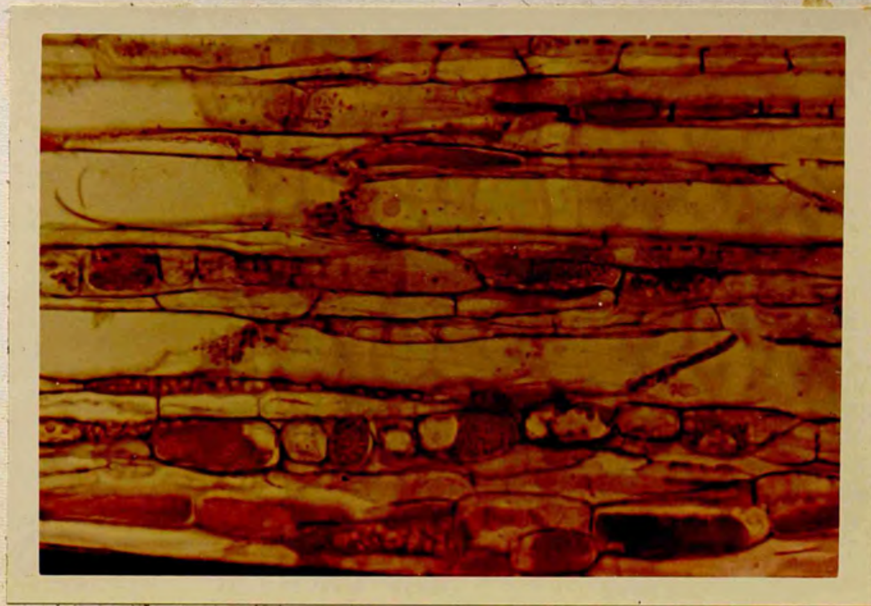


PLATE 33 T.L.S. showing old cells and comparatively young cells both with extruded nucleoli. Compare the appearance of nucleoli on both types of cell.
About X310.

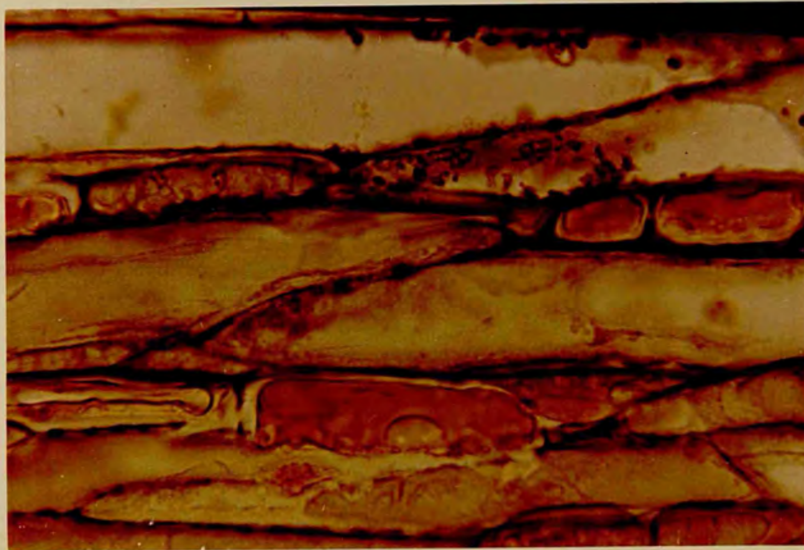


PLATE 34 T.L.S. showing some longitudinally oriented strands in the sieve tubes. Starch grains, some within plastids are congregated near the sieve plates. About X750 (oil immersion).

CHAPTER III

REVIEW

In this Chapter are summarised the main conclusions from electron microscopical work on the phloem particularly as it bears on the question of the mechanism of translocation.

NO	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
1	1948	Hüber, B & R.W.Kolbe		Sieve tubes	First attempt to examine sieve elements with electron microscope. This was before glass knife was invented or before plastic embedding media were first used.		Early study adding little to optical work. (Quoted by Kollmann, 1964)
2	1951	Küster, E.	_Cucurbita species_				
3	1952	Volz, G	_Betula species_	Pores	Narrow sieve area pores (0.5-2u). In the region of middle lamella pores of sieve areas are closed by a thin membrane. (Quoted by Kollmann, 1964)		She used macerated material. Her photographs do not support her conclusion. (Johnson's thesis 1967,Aberdeen University)
4	1955	Hepton, C.E.L., R.D. Preston & G.W. Ripley	_Cucurbita species_	Sieve plate structure	Cytoplasm continuous through sieve pores in tubes presumed to be functional. No vacuolar continuity across sieve pores.	Rejects Münch hypothesis (Pressure gradient required is too large), protoplasmic streaming & interface theories.Vital process must be important.	Stem turgor reduced before fixation. Sections metal-shadowed. A preliminary study.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
5	1956	Eschrich, W.					
6	1959	Beer, M.	"Cucurbit"	Fine structure of phloem	Mature sieve tubes appear to be open with no indication of membrane across them. Cytoplasm penetrates through the sieve pores. Slime bodies granular bounded with double membrane.	No conclusion	Plasmodesmata between sieve tubes & companion cells are highly developed. Mature sieve tubes contain no mitochondria, ER or nucleus, but some vesicles. The companion cells have a nucleus & densely packed with the cytoplasmic particles, mitochondria, microsomes, but no starch or chloroplast.
7	1959	Schumacher, W. & R. Kollmann	*Passiflora coerulea*	Sieve tube protoplast.	Sieve plate pores plugged "homogeneously" with microtubular cytoplasm.	Cytoplasmic "bridges" can hardly be passive. Electro-kinetic theory offers a possible explanation.	Tonoplast, Mitochondria, golgi bodies, plastids & starch grains are present in the sieve tubes.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
8	1960	Duvat, R.	*Cucurbita pepo*	Ontogeny of sieve tube	No vacuolar continuity. Pore fibrils 80-150 A° down to 40 A° (Englemann, 1963)	Electrokinetic theory a possibility, pressure flow is discarded.	
9	1960	Hepton, C E.L. & R.D. Preston	*Pinus sylvestris*, *Sorbus aucuparia*, *Vitis vinifera* & *Cucurbita pepo*	Comparison of sieve tubes of both Angiosperms & Gymnosperms	Sieve plate pores filled with cytoplas. No vacuolar continuity.	Probably an active transport. Electro-kinetic theory a possibility.	Turgor reduced prior to fixation.
10	1960	Kollmann, R.	*Passiflora coerulea*	Phloem	Sieve plate pores plugged densely with cytoplasmic threads, 100-150A diameter, perhaps tubular with walls 36-55 A.	Active role of sieve element is probable, because of highly differentiated protoplast.	Nucleolus composed of fine fibres (or tubular ?) of diameters 115 x 175 A packed hexagonally and over 5000 A long. Lumen contains cytoplasmic threads of diameter 70-130 A. Mitochondria & plastids present, but few.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
11	1960	Ziegler, H.	*Heracleum mantegazzianum*	Phloem strand	Pores filled with solid cytoplasm (no central lumen) E.R. (presumably) prominent in latter.	No conclusion	No intact mitochondria and no tonoplast over sieve plates. Phloem strands prepared by freezing in liquid air and drying.
12	1961	Duloy, M., F.V. Mercer & N. Rathgeber	*Cucurbita pepo*	Translocation and submicroscopic anatomy of phloem.	Sieve plate pores are open with thin cytoplasmic lining. Slime (fibrillar) forms connections through pores.	Sieve tubes apparently metabolically inert. Vacuolar continuity constitutes them an open conduit. (Münch hypothesis?)	Sieve tube has thin parietal layer of membranous cytoplasm. Slime dispersed throughout lumen. Scarcity of mitochondria and plastids.

NO	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
13	1961	Esau, K. V. I. Cheadle	*Cucurbita maxima*	Sieve plate	Appearance of sieve plate pores under electron microscope variable. Vacuole sometimes appears continuous, at other times pore seems filled with solid or fibrillar connecting strands.	No conclusion	Slime plug is an artefact and it is vacuolar not cytoplasmic in nature.
14	1961	Kollmann, R. & W. Schumacher	*Metasequoia glyptostroboides*	Dormant phloem	Immature sieve tubes possess dense cytoplasm in the winter. Many thin tubules of E.R. pass longitudinally through cytoplasm of sieve cells.	No conclusion	

NO	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
15	1961	Parker, J. & D. E. Philpott	Macrocystis pyrifera	"Sieve plate" ultra structure	"Sieve plate" pores obstructed with electron dense material resembling E.R.	Mass-flow may be theoritically possible. Though there appear to be no unobstructed pores. Perhaps cytoplasm necessary to retain carbohydrates.	Occasional chromoplasts (?) which reduced tetrazolium. "Radioactive ions do not seem to move faster than simple diffusion."
16	1962	Esau, K. V.I. Cheadle & E.B. Risley.	Cucurbita maxima, & Vitis viniferea	Ontogeny of sieve plate pores	Pore is not simply an enlarged plasmodesma. It is formed by removal of wall material probably in way of a plasmodesma.	No conclusion	Perforation of plates occurs after the nucleus disintegrates, but in Cucurbita, after slime bodies disaggregate. Paired platelets of callose first appear across compound middle lamella. E.R. becomes applied to these & perforation begins at centre of platelets. These fuse & thus pore is lined with callose from start.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
17	1962 (a)	Esau, K. & V. I. Cheadle	_Cucurbita maxima_	Tonoplast in sieve tube	Tonoplast is disorganized during nuclear disintegration.	No conclusion	Maturation also involves dispersal of slime and perceptible modification of E.R., dictyosomes and mitochondria.
18	1962 (b)	Esau, K. & V. I. Cheadle	_Cucurbita maxima_	Mitochondria in phloem	Reduced number of mitochondria with smaller internal differentiation in mature sieve tubes. Appear degenerate, but not inactive.	No conclusion	Mitochondria normal in immature sieve tubes and in companion cells. Latter functionally integrated with sieve elements.

NO	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
19	1962 (a)	Kollmann, R. & W. Schumacher.	*Metasequoia glyptostroboides*	Fine structure of connecting strands in sieve areas, fixed from January to April.	Cytoplasmic threads in sieve areas are 5 - 6 times broader than plasmodesmata in pit fields, but have some structure - i.e. they are lined by plasmalemma and traversed by numerous structure of E.R. equal in size to those of plasmodesmata. This agrees with earlier studies on *Passiflora*.	No conclusion	The connecting strands are more highly differentiated plasmodesmata only.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
20	1962 (b)	Kollmann, R. & W. Schumacher	*Metasequoia glyptostroboides*	Reactivation of phloem in spring. (Fixed from February to March)	Perhaps due to increase in water uptake protoplast (of phloem cells) appears to be "loosened". Its E.R. changes remarkably; the thin tubules become dilated, branched, expanded into cisternae and disintegrated into vesicles.	No conclusion	No corresponding changes in albuminous cells of parenchyma and rays.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
21	1962	Mehta, A.S. & D.C. Spanner	*Nymphoides peltatum*	Sieve tubes	Functioning sieve plate pores filled with solid dark staining material, which appears to be full of E.R. tubules.	Electro-kinetic theory favoured.	Nacreous wall prominent. Specialized E.R. in sieve tubes oriented spirally and becoming finer near sieve plate.
22	1963 (a)	Buvat, R.	*Cucurbita pepo*	Tonoplast & vacuole	Tonoplast disappears on maturation; no true vacuole remains.	Movement must occur within a cytoplasmic (non-vacuolar) phase	Ribosomes present in "upper" cell.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
23	1963 (b)	Buvat R.	*Cucurbita pepo*	Wall and plasma membrane of sieve tube and plate.		No conclusion	Formation of pores similar to that described by Esau et al 1962
24	1963 (c)	Buvat, R.	*Cucurbita pepo*	RNA in slime bodies	Slime bodies contain RNA, suggesting they should be regarded as "cytoplasmic".	No conclusion	Slime bodies appear like nucleolus, but are not identical.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
25	1963 (d)	Buvat, R.	*Cucurbita pepo*	Ontogeny and ultra structure of sieve tubes	Pores occupied with "hyaloplasmic network" of cytoplasmic nature	Doubtful if it could be entirely passive. Might be a mass flow with external motive power	Sieve tubes show polarity even prior to perforation of plates. Slime bodies show marked resemblances to nucleoli.
26	1963	Engleman, E.M.	*Impatiens sultanii*	Slime substance in sieve tubes.	Dense pore-filling substance in dicot sieve plates seems to be fibrillar rather than membranous, though most often grannular-amorphous (fibril diameter 100-120A in pores, 170-250A in lumen)	No conclusion	Some pores contain 350-1250 A diameter light areas (too great for E.R.?). Slime might be regarded as either "vacuolar" or "cytoplasmic".

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
27	1963	Eschrich, W.	*Cucurbita ficifolia*	Callose in primary phloems	Material in pores, a lipo protein reticulum continuous with similar material in lumens and no denser.	No conclusion	Hexose phosphate very abundant in sieve tube sap; sucrose low. Slime bodies appear fibrous or microtubular (tubules up to 170 A in diameter). Fixative injected into central petiolar cavity. Internal metaphloem appears callose free.
28	1963	Kollmann, R. & W. Schumacher	*Metasequoia glyptostroboides*	Fine structure of connecting strands in sieve areas, fixed from March to October.	Confirms results of Kollmann & Schumacher (1962a). Connecting strands differ in size & probably in fine structure from some highly specialized angiosperm sieve tubes.	No conclusion	Large median nodule about 3μ diameter, sends many connecting strands 500-850 A in diameter to sieve cell protoplasts.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
29	1964	Falk, H.	Tetragonia expansa	Origin of slime	Slime bodies of Tetragonia are modified plastids. They are fibrillar.	No conclusion	Sieve tube plastids contain a dense ring-shaped inclusion (cf. Beta vulgaris, Esau 1964; McGivern 1957). Sometimes plastids break down & release these which disintegrate into fibrillar slime.
30	1964	Kollmann, R. & W. Schumacher	Metasequoia glyptostroboides	Changes in sieve cells during growing season. (March to November).	During differentiation a remarkable increase in E.R. occurs. At stage of maximum extension of E.R. the tonoplast disintegrates. Dictyosomes disintegrate concurrently.	No conclusion	Sieve cells in Metasequoia function at their earliest stage of development.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
51	1964	Kollmann, R.	Various	Review work on sieve element protoplast	Fine structure of cytoplasm in sieve plate pores may be either tubular or fibrillar.	It has not yet been proved that the "mature" sieve tubes are really conducting.	There are "essential differences" between coniferous sieve areas and sieve plates of the higher angiosperms. Final changes in sieve tube fine structure may be a result of, rather than a pre-requisite for translocation.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
32	1965 (a,b)	Behnke, H.D.	*Dioscorea* spp	Phloem, especially nodal ganglia	Cytoplasm of all conducting cells (sieve tubes and "nodal sieve elements") are completely uniform, i.e. all contain plastids, numerous mitochondria, a lattice-like body, E.R. and network of fibrils (100 A diameter) but no nucleus or tonoplast.	Mass-flow is discarded. Must be a complex mechanism, requiring an active share in control by the protoplast.	Function of sieve tubes at ganglia taken over by much more numerous (X 100) sieve plate pores and smaller "nodal sieve elements". Plasmatic fibrils sometimes with E.R. pass through sieve pores.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
33	1965	Bouck, G.B. & J.Cronshaw	*Pisum sativum*	Sieve tube differentiation.	Sieve plate pores plugged with finely fibrous material (slime ?) possibly synthesised by E.R. but not derived from plastids (cf. Falk, 1964).	Highly likely that plugged sieve plates are site of active pumping, with perhaps cytoplasm mediated transport through sieve cell. Streaming and surface flow also possibilities.	Ribosomes not associated with surface of cisternae. Microtubules evident in early ontogeny but disappear after formation of nacreous wall. Cisternae, longitudinally oriented and edges attached perpendicularly to wall surface. Tonoplast disintegrates after formation of plate.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
34	1965 (a)	Engleman, E.M.	*Impatiens sultanii*	Wound reaction in sieve elements.	Slime plugs and dense connecting strands in pores seem to be artefacts.	No conclusion	Callose present in sieve elements killed within 4 seconds of cutting. Further callose develops only after 5 minutes and only within 15 elements from cut surface. Callose definitive after 30 minutes.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
35	1965 (b)	Engleman, E.M.	*Impatiens sultanii*	Development of sieve elements	Cytoplasm and fibrillar slime disperse in lumen as "mictoplasm" which with plasmalemma is continuous through pores, where slime is denser.	Possibly protoplasmic "streaming of the tidal type" under pressure gradient.	Nucleoli (1 or 2) extruded but disappear usually with tonoplast, nucleus and dictyosomes.
36	1965 (a)	Esau, K.	*Beta vulgaris*	Sieve element plastids	Plastids contain a ring of proteinaceous fibrils recalling *Tetragonia* (Falk, 1964) (after OSO_4 or Glutaraldehyde but not $KMnO_4$)	No opinion expressed.	Plastids do not seem to break down until sieve tubes collapse. Relation of contents to slime uncertain.
37	1965 (b)	Esau, K.	*Vitis spp*	Anatomy and cytology of phloem.	Sieve plate pores filled with slime	No opinion expressed.	Slime possibly is involved in removal or reactivation of callose.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
33	1965 (*)	Esau, K and V.I. Cheadle	1) *Cucurbita maxima* 2) *Beta vulgaris* 3) *Elodea s.p.* 4) *Robinia pseudoacacia* 5) *Phaseolus vulgaris*	Cytological studies on phloem.	The connecting strands appear to be solid. Possibly sieve plate pores are filled with slime. E.R. very much vesicular.	No opinion expressed	Mostly $KMnO_4$ fixation. Origin of pores from paired callose platelets. Plasmodesmata branched on C.C. side. Slime bodies of *Robinia* persistent C.f. *Phaseolus*
39	1965	Evert, R.F. and L. Murmanis	*Tilia americana*	Structure of secondary phloem	Pores filled with fibrillar strands of slime. Fine fibrils of latter organised into coarser ones which form "transcellular strands". Pores traversed by such strands, contrast plasmodesmata by E.R. tubules.	Energy required for transport is possibly released in sieve element protoplast with movement of assimilates "in association with or along surfaces of strands". "Many streams of assimilates flowing through a more or less stationery fluid medium."	

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
40	1965	Parker, B.C. and J. Huber	*Macrocystis pyrifera*	Fine structure of the "sieve tube"	Resembles higher plants in presence of fibrillar slime & callose. When "Nitrogen-frozen, osmium fixed" pores are not more densely filled than the lumen.	Tentatively as Englemann (1965 b). "Sieve tubes" metabolically independent as they lack companion cells or any other such connected cells.	Many more organelles present than in higher plants, but no nucleus or tonoplast. Protoplasmic connections to other cells absent. Fixation in $KMno_4$ or osmium.
41	1965	Wark, M.C. and T.C. Chambers	*Pisum sativum*	Sieve element ontogeny (secondary phloem)	"Connecting strands are continuities of endoplasmic reticulum."	Regards a younger stage than usual as the functional one. No opinion on mechanism.	Fibro tubular body, structurally distinct from slime body, mediates extrusion of nuclear material.
42	1965	Wark, M.C.	*Pisum sativum*	Fine structure of companion cell & phloem parenchyma.	Sieve element is connected to companion cell by numerous complex plasmodesmata; no connections to parenchyma (as in *Acer* and *Tilia*).	Probably the young sieve elements are functional and the companion cells regulate the lateral movement of assimilates in and out of sieve elements.	Cytoplasm of companion cells and phloem parenchyma cells little changes during ontogeny.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
43	1965 (a)	Wooding, F.B.P. and D.H. Northcote	*Acer pseudoplatanus* & *Pinus pinea*	Association of E.R. and plastids.	Close and permanent sheathing of plastids by ER observed in companion cells of *Acer* and resin canal cells of *Pinus*; transitory in sieve tubes and leaf callus cells.	No opinion	No indication has been observed of an origin of a slime body from plastid.
44	1965 (b)	Wooding, F.B.P. and D.H. Northcote	*Acer pseudoplatanus*	Fine structure of companion cell	Connections with sieve tubes complex. Cytoplasm rich in ribosomes and rough ER "Spherosome" aggregates found (lipid synthesis?)	No firm opinion. Either the companion cell is directly concerned with translocation (electro-kinetic theory) or acts to preserve the functional integrity of the sieve tube.	Parenchyma not connected to sieve tubes.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
45	1966	Evert, R F., L. Murmanis & I.B. Sachs	*Cucurbita maxima*	Ultra structure of primary phloem.	Central cavity of sieve elements traversed by numerous strands, which run from cell to cell through the sieve pores derived from slime bodies. Slime sometimes appears tubular rather than fibrillar. Slime strands seem to be universal in higher plants. Presence in exudate not to be considered, evidence that it moves with stream.	No opinion	There seems to be an internal delimiting membrane in sieve tube. Slime bodies sometimes membrane bound - homologous with Falk plastids ? Many pores traversed by slime early in development. Did not test slime for RNA but seems sceptical. Slime bodies appear in *immature* companion cells.
46	1966	Johnson, R.P.C.	*Nymphoides peltatum*	Sieve tube contents	Pores filled with fibrillar slime	No opinion	$KMnO_4$ is an unsuitable fixative for sieve tubes. It causes granular precipitate and obscures fibrils.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
47	1966	La Fleche, D.	*Phaseolus vulgaris*	Ultra structure and cytochemistry of flagellar-like inclusions in the sieve tube.	Remarkable "crystalline" slime body of 25A fibrils transversely striated (100A) of protein nature after sometimes in mature element it becomes "hairy", looses striation.	No definite opinion. Uncertain role of flagellar inclusion in conduction.	The crystalline protein typical of Papillionaceae may be a reserved (food) substance. "Curviform tubules" are also present.
48	1966	Murmanis, L. & Evert, R.F.	*Pinus strobus*	Sieve cell ultra structure.	Strands derived from slime bodies traverse central cavity of mature sieve element and are continuous through sieve pores. Pores always traversed by endoplasmic membrane.	No opinion.	Young slime bodies possess double-layered membrane. They often appear very much like nucleus. *Pinus* is very similar to *Metasequoia*.

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
49	1966	Northcote, D.H. and Wooding, F.B.P.	*Acer pseudoplatanus*	Development of sieve tubes	Pores are filled with fibrillar material derived from slime bodies. Fibrils may arise from ribosomes. Fibrillar material seen in the pores are thicker than those in the lumen.	Mass flow would be possible if the *in vivo* sieve pores contained a meshwork of longitudinal fibrils. Perhaps sol-gel transformation occurs, and lipoprotein material circulate continuously in sieve tube system.	Some of callose shown to be deposited after wall formation, wound reaction likely.
50	1966 (a & b)	Srivastav, L.M. and T.P.O'Brien	*Pinus strobus*	Ultra structure of cambium & its vascular derivatives (Secondary phloem)	Similar to the findings of Kollmann on *Metasequoia* cytoplasm of albuminous cells "extraordinarily rich" in mitochondria & rough ER.	No opinion	
51	1966	Tamulevich, S.R. and Evert, R.F.	*Primula obconica*	Ultra structure of sieve tube.	Fibrillar slime oriented longitudinally appear to be continuous from cell to cell through the pores. Internal delimiting membrane in sieve element present.	No opinion	Slime is tubular. It is distributed in the lumen as a "lipoprotein network . (c.f.Buvat 1960 & Eschrich, 1963")

NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
52	1967	Behnek, H.D. and Dörr, I.	*Dioscorea* spp, *Cuscuta odorata*, *Primula obconica* & *Cucumis sativus*	Development & fine structure of the sieve tube filaments.	Filaments appear to originate in ground plasm (ribosomes ?) and they are tubular about 120-150A in diameter.	No opinion	Plastids of young sieve elements show osmophilic inclusions in contrast to those of parenchyma. Fibrillar net work never surrounded by membrane.
53	1967	Esau, K., Cronshaw, J. & Hoefert, L.L.	*Beta vulgaris*	Relation of virus to the movement in sieve tube.	Virus particles occur throughout the lumen of sieve tube (usually absent from vacuoles of parenchyma) in pores of sieve plate & in parenchyma.	Virus particles appear to move completely.	Most of the infected sieve elements have mitochondria, plastids, ER & plasma membrane normal for mature sieve elements. Some appear degenerating.
54	1967	Johnson, R.P.C.	*Nymphoides peltatum*	Fine structure of sieve tube.	Pores in functioning sieve tubes almost certainly traversed by slime fibrils, abundant also in lumen (confirmed by freeze-etching).	Favours electro-kinetic theory.	Fibrils of two series, larger (180-220 A diam.) appear to fray out into smaller (80-100A). Latter banded & seem to aggregate, with bands aligned, in pores callose forms also with glycerol treatment for freeze-etching.

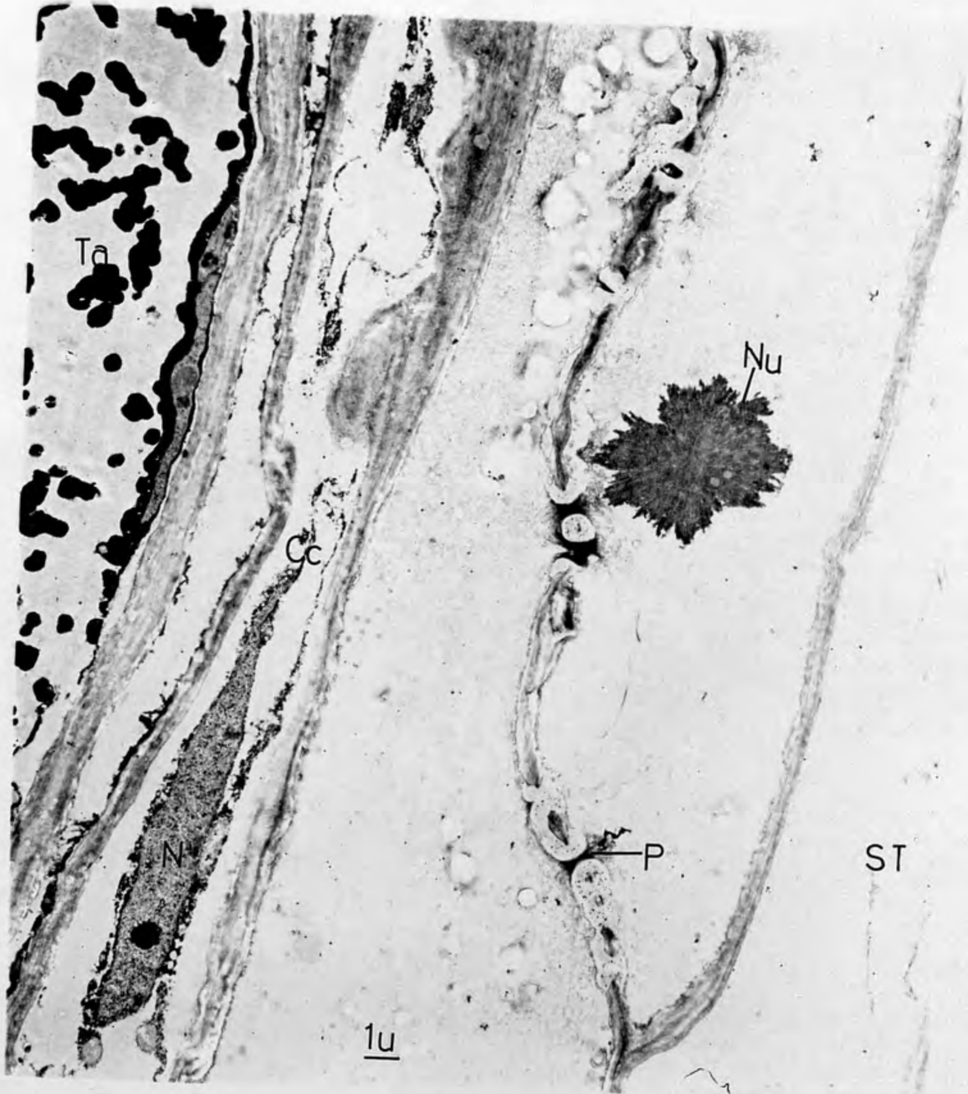
NO.	DATE	AUTHOR	SPECIES	COVERAGE	MAIN CONCLUSION	REMARKS ON MECHANISM	MISCELLANEOUS REMARKS
55	1967	Murmanis, L. & Evert, R.F.	Pinus strobus	Parenchyma cell of secondary phloem.	End (transverse) walls of axial parenchyma becomes perforated (cytomixis).	No opinion	Plastids show some differences from usual.
56	1967	Ziegler, H. & Ruck. I	Laminaria spp	Fine structure of phloem. "Trumpet cell".	The trumpet cells have a sieve plate, with 20-30,000 normal plasmodesmata with 50-60 per μ^2	No opinion	Callose is probably present in pores & on plate.

C H A P T E R   I V

ELECTRON MICROSCOPY

PLATE 35

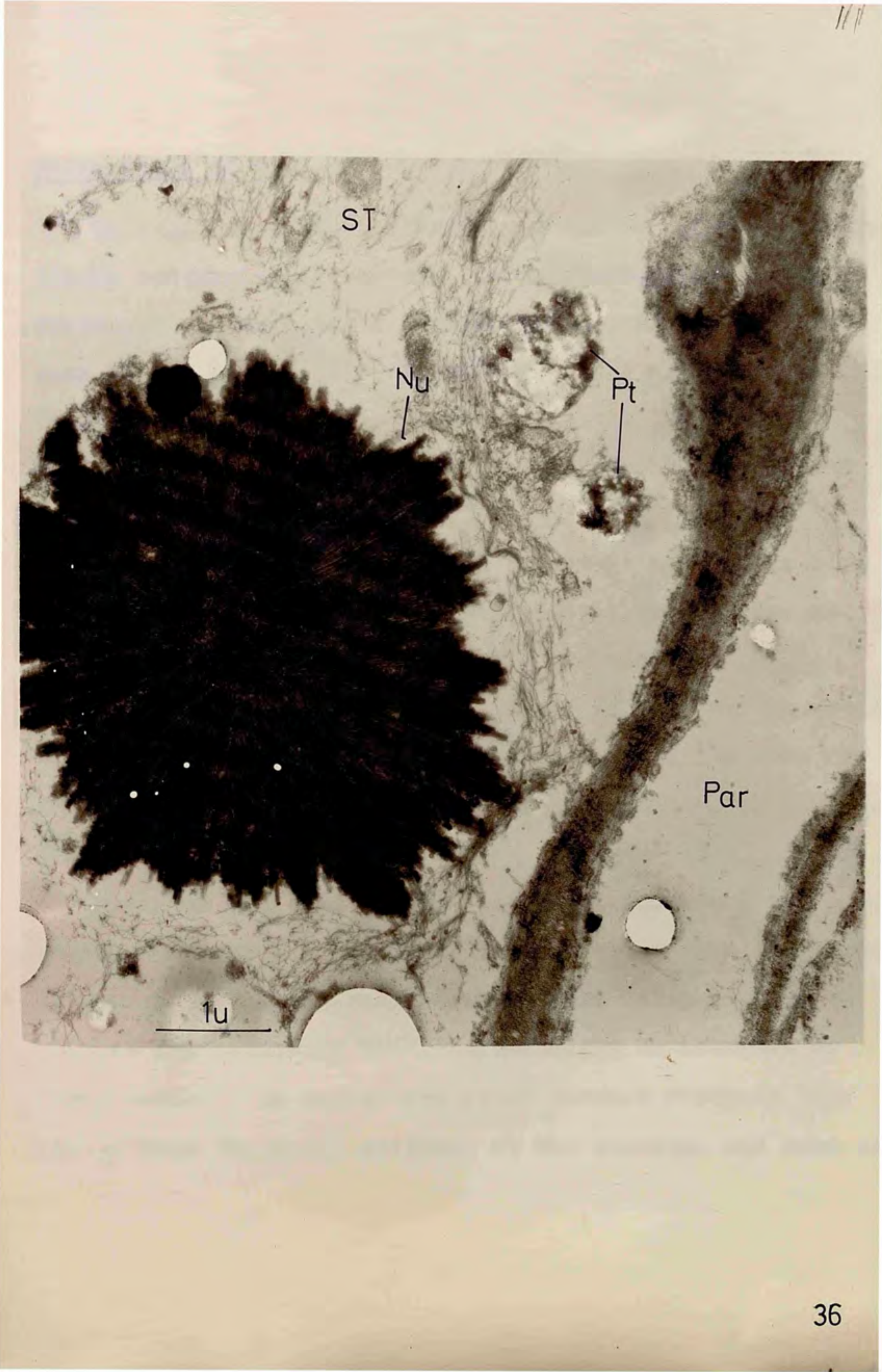
Tangential longitudinal section, showing two sieve tubes and a nucleate companion cell at the left. In the centre near the sieve plate a nucleolus can be seen. Stained with lead citrate, Magnesium uranyl acetate and lead citrate. X5,000.



35

PLATE 36

T.L.S. showing a nucleolus,
plastids, and slime fibrils.
Stained with lead citrate,
uranyl acetate and lead citrate.
X20,000.


ST
Nu
Pt
Par
1u

PREPARATION OF TISSUES FOR THE ELECTRON MICROSCOPE

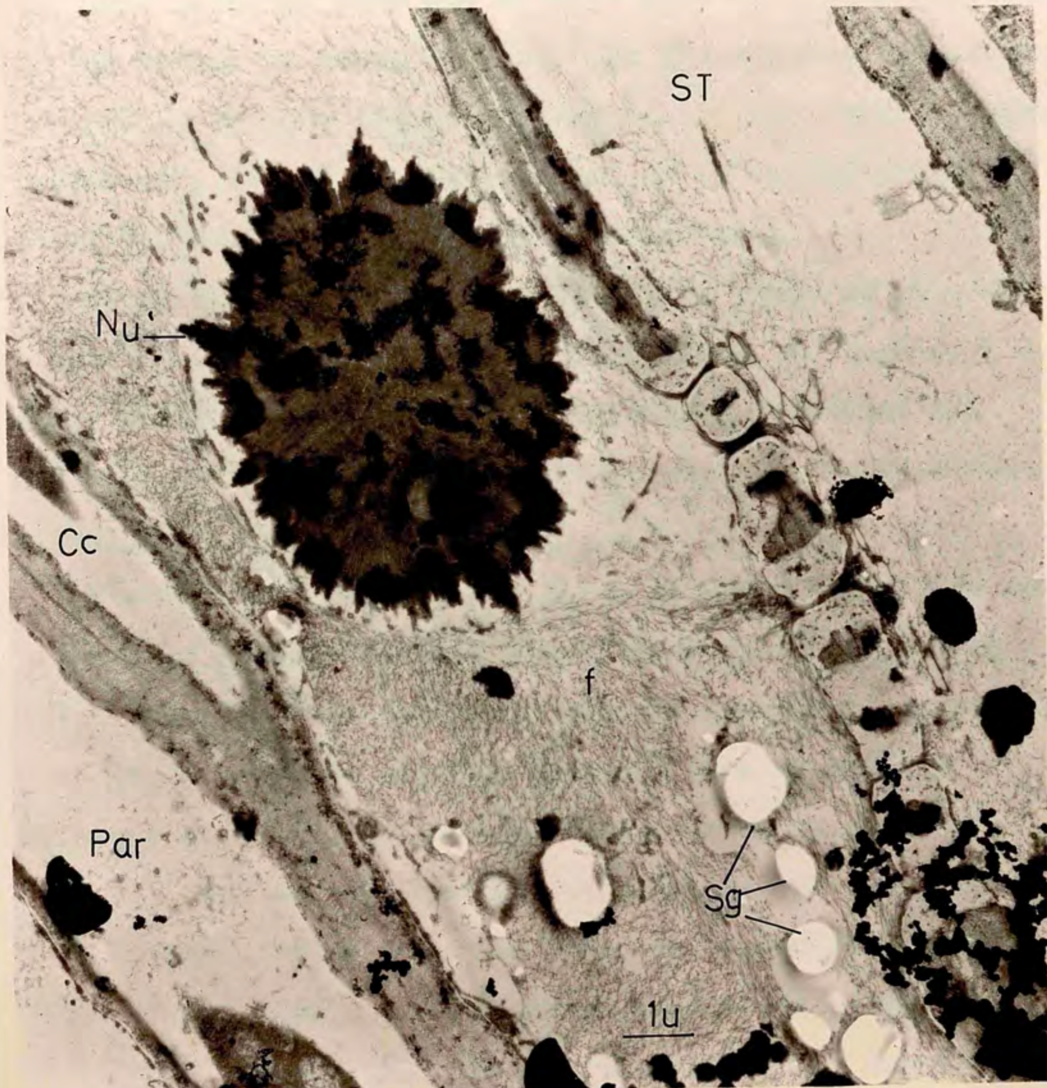
On account of its higher turgor and peculiar structure tissue containing sieve tubes is well recognised as being difficult to fix without introducing artefacts, especially such as may be caused by a sudden release of pressure. This problem had therefore to be faced, and in the main the methods used to surmount it were fairly well-established ones.

Longitudinal strips of bark (about 2" x ½") were cut with a sharp razor blade direct from the intact stem and placed at once in 0.25 M Mannitol, containing 1/15 M phosphate buffer of pH7. The object of this was to reduce the turgor of the tissue before excising small pieces for fixation. The procedure followed earlier workers (~~Currier~~, Esau and Cheadle 1955).

Mannitol was used instead of sucrose to avoid any possible uptake of the latter which might reduce the osmotic potential of the external milieu, there being a priori reasons for believing that sucrose might be absorbed by sieve tubes. In summer the outer tissues separate very easily from the woody cylinder at the cambium, and most of

PLATE 37

T.L.S. showing a nucleolus near a sieve plate. Stained with lead citrate, uranyl acetate and lead citrate. X10,000.


ST
Nu
Cc
f
Par
Sg
1u

the work reported here was in fact done on tissue collected in the spring or early summer. In the dormant season the separation is more difficult, and in any case winter phloem proved very hard to infiltrate and embed satisfactorily which further handicapped its investigation.

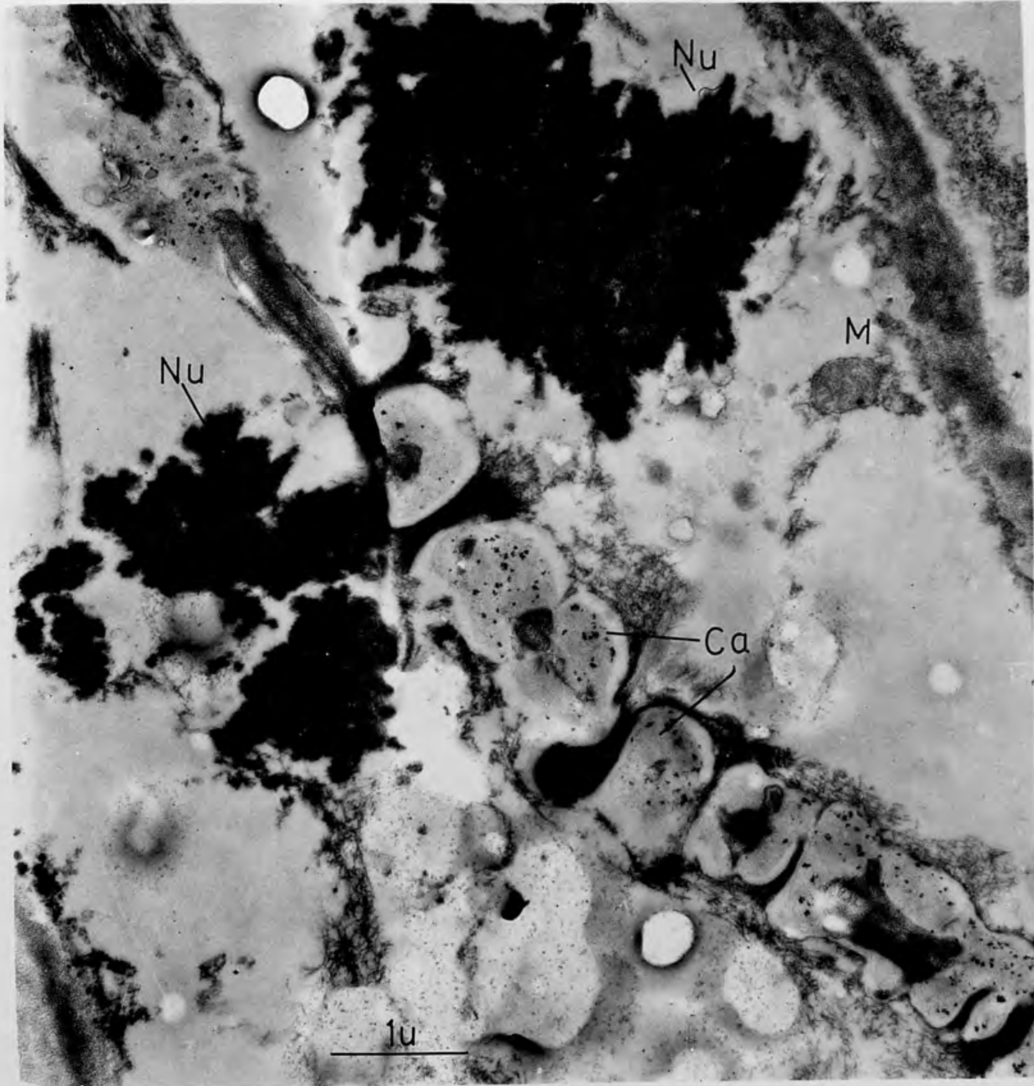
After an hour in the Mannitol small cubes of phloem tissue of about 0.5 mm edge were cut from the bark with a sharp razor and transferred to fixative, sometimes the cutting was performed after transferring to fixative.

Fixation

Fixation was carried out in 4% glutaraldehyde made up to contain 0.25 M Mannitol and m/15 phosphate buffer of pH7. The vial was aspirated several times under a vacuum pump (to a pressure slightly greater than the vapour pressure of water). Normally this served to get rid of all air; but material collected in winter proved exceptionally difficult to handle and even days under vacuum failed to clear the lumens of the phloem fibres, which showed up as silvery streaks when the tissue was finally embedded in Araldite. Sectioning of such material was accordingly very difficult and after many attempts, was abandoned.

PLATE 38

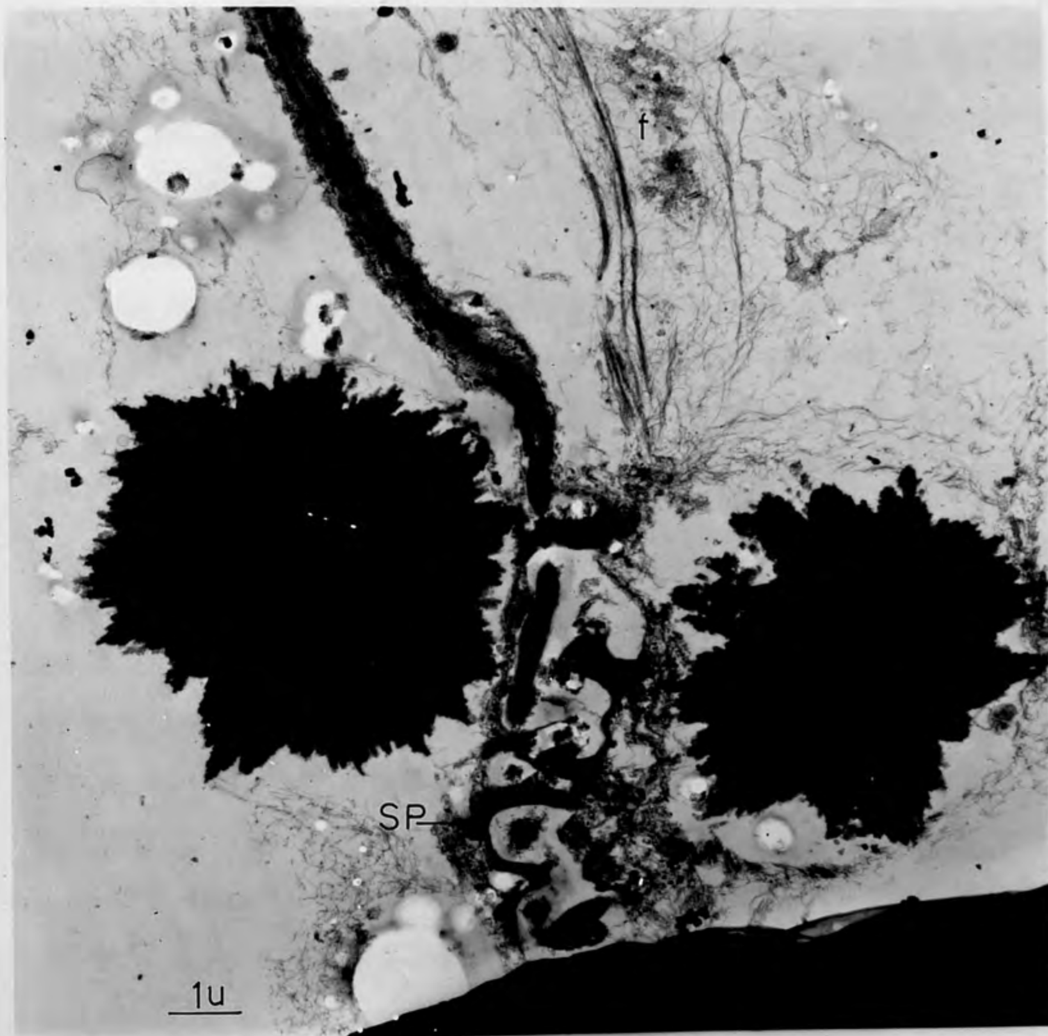
T.L.S. of sieve plate showing two nucleoli, one on either side of the plate, cut, off-centre. Note the upper one has more than one centre. Stained with lead citrate, Magnesium uranyl acetate and lead citrate. X20,000.



38

PLATE 39

T.L.S. of sieve plate showing two nucleoli one on either side of the plate, and slime strand. Stained with lead citrate, uranyl acetate and lead citrate. X10,000.



39

Fixation in glutaraldehyde was carried out for four hours. The specimens were than washed very thoroughly overnight in phosphate buffer, or for shorter periods on a slowly-rotating tumbler.

Post-fixation and Dehydration

The tissue blocks were next post-fixed in 1% Osmium tetroxide containing the same buffer for one hour. From this they were washed thoroughly in plain buffer and dehydrated in the usual way in a graded series of alcohols.

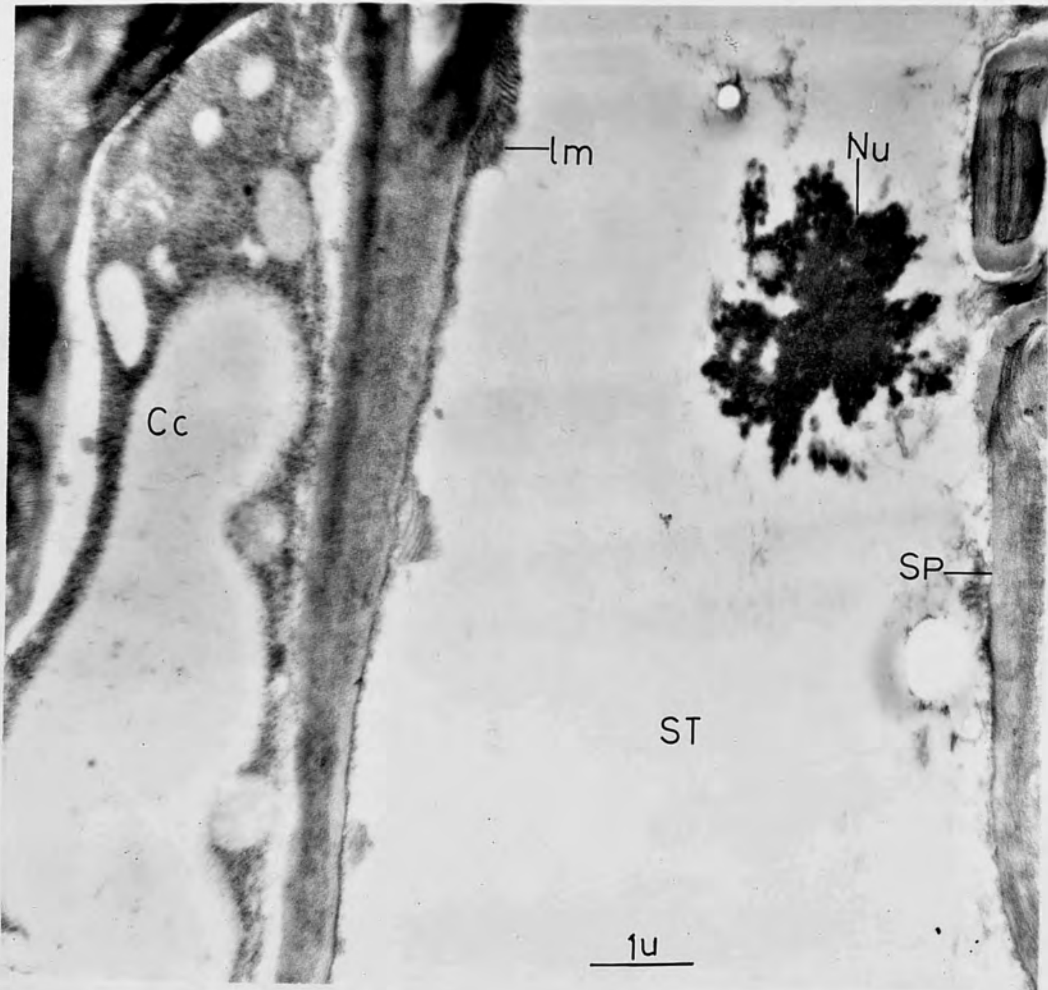
All the solutions were used at room temperature. Glutaraldehyde was obtained either from L. Light and Company or Taab Laboratories as a 25% solution and de-acidified before use by shaking with a small quantity of barium carbonate and filtering (Kay, 1965).

Embedding

The dehydrated blocks were transferred through a 50:50 mixture with alcohol to water-free propylene oxide, a reactive liquid residual traces of which can enter into chemical combination with the polymerising resin (Luft 1961). After one or two changes of propylene oxide resin mixture (see as follows) was added according to the following schedule:

PLATE 40

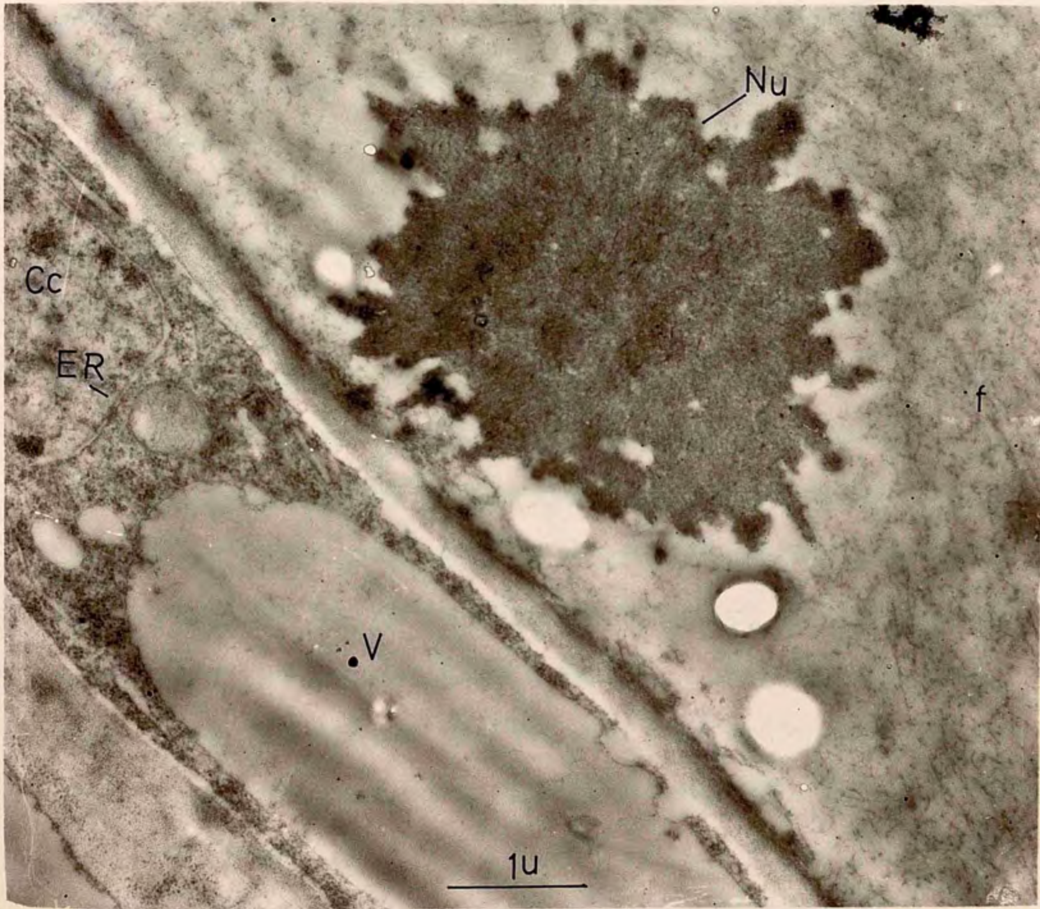
T.L.S. sieve tube and companion cell showing nucleolus cut off-centre and lamellar bodies near the wall. Stained with lead citrate. X15,000.



40

PLATE 41

T.L.S. of sieve tube and companion cell showing a nucleolus and slime fibrils in the cell lumen. Vacuole, mitochondria endoplasmic reticulum can be seen in the companion cell. Stained with lead citrate. X20,000.


Nu
Cc
ER
f
V
1u

i) Propylene oxide + resin 2:1 for 2 hours
ii) - ditto - 1:1 overnight
iii) - ditto - 1:2 for 18 hours
iv) Pure resin, change twice daily for 2 to 3 days, with evacuation to remove air and propylene oxide during the first change.

Where appropriate the materials which were in 1½" x 1" polythene-capped glass specimen tubes, were "tumbled" on a disc revolving at a slow-speed; where this could be done times could be shortened to an hour or two.

For polymerisation the resin mixture was at first transferred to small polythene BEEM capsules; later it was found preferable to polymerise in drops of resin spotted on a sheet of polythene 1/16" thick. Orientation was simpler, and the tissue blocks could later be cut out and stuck to resin blanks with Araldite adhesive for mounting in the microtome.

Heat treatment was carried out at 40° overnight followed by about 48 hours at 60°C.

Resin mixtures - EPON

The resin mixture tried first was Epon (Epikote 812 in Britain) as recommended by Luft (1961). It was made

<u>PLATE 42</u>

T.L.S. sieve tube showing
a nucleolus cut off-centre,
starch grains and slime
fibrils near the plate.
Stained with lead citrate,
Magnesium uranyl acetate and
lead citrate. X15,000.

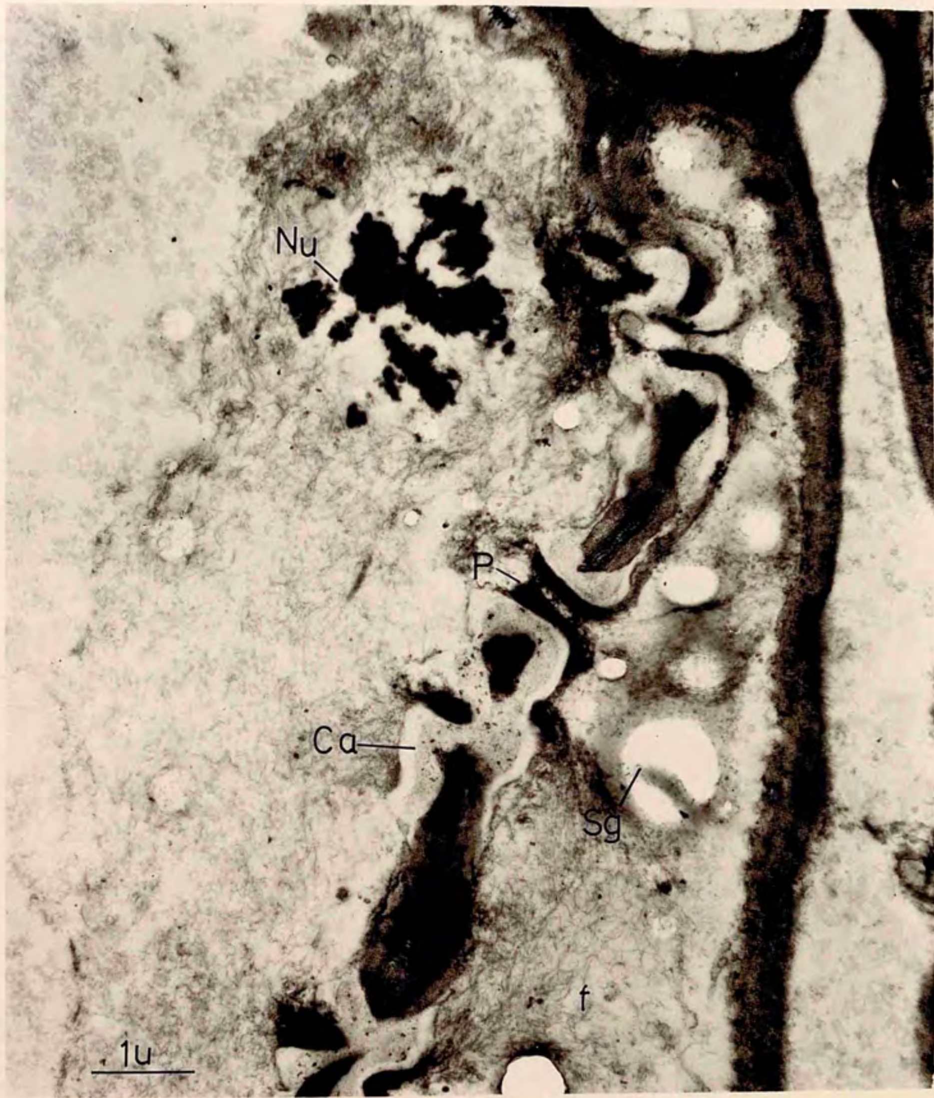
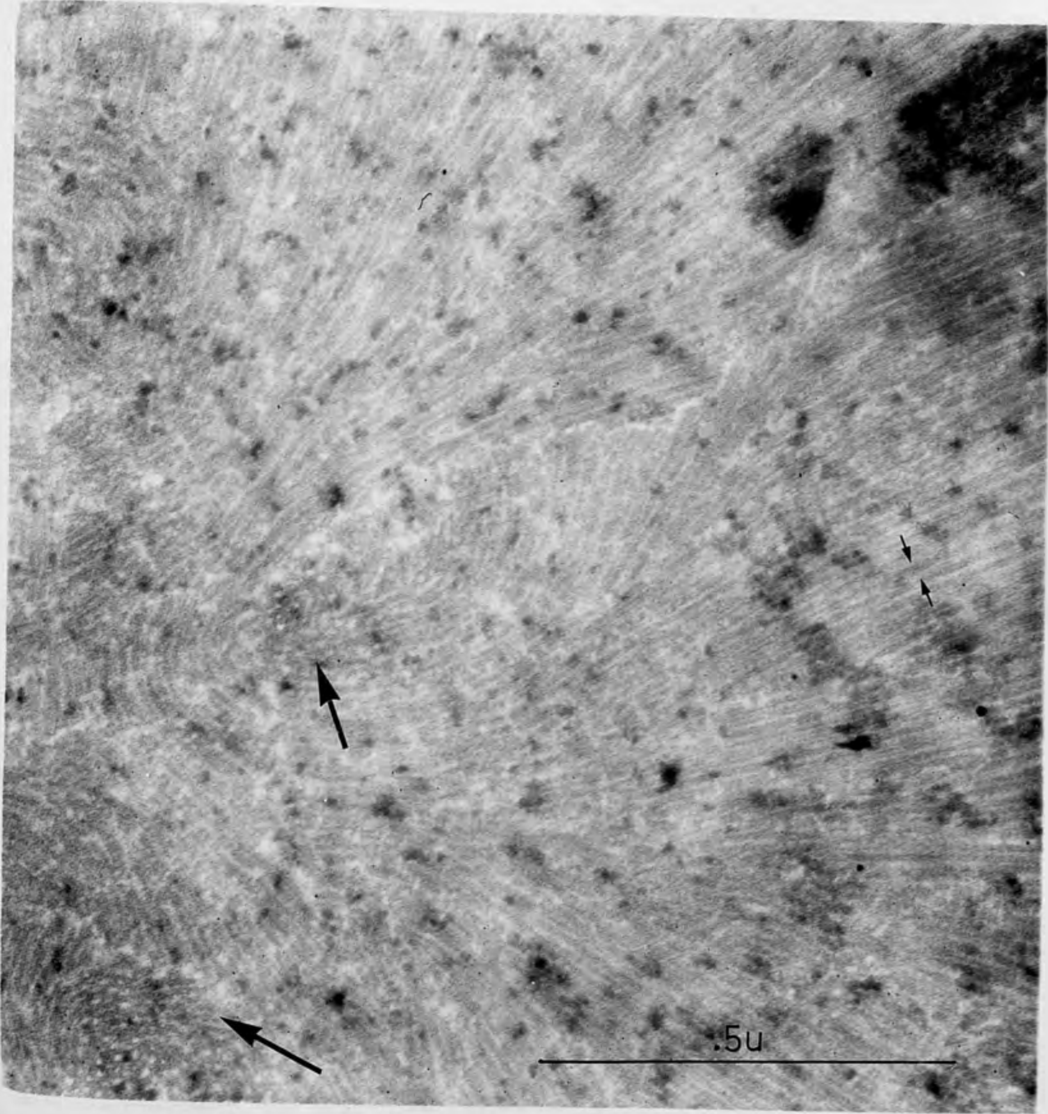

Nu
P
Ca
Sg
f
1u

PLATE 43

Fine structure of sieve tube nucleolus shown in plate -36 X120,000 taken at X60,000.



43

up as follows:

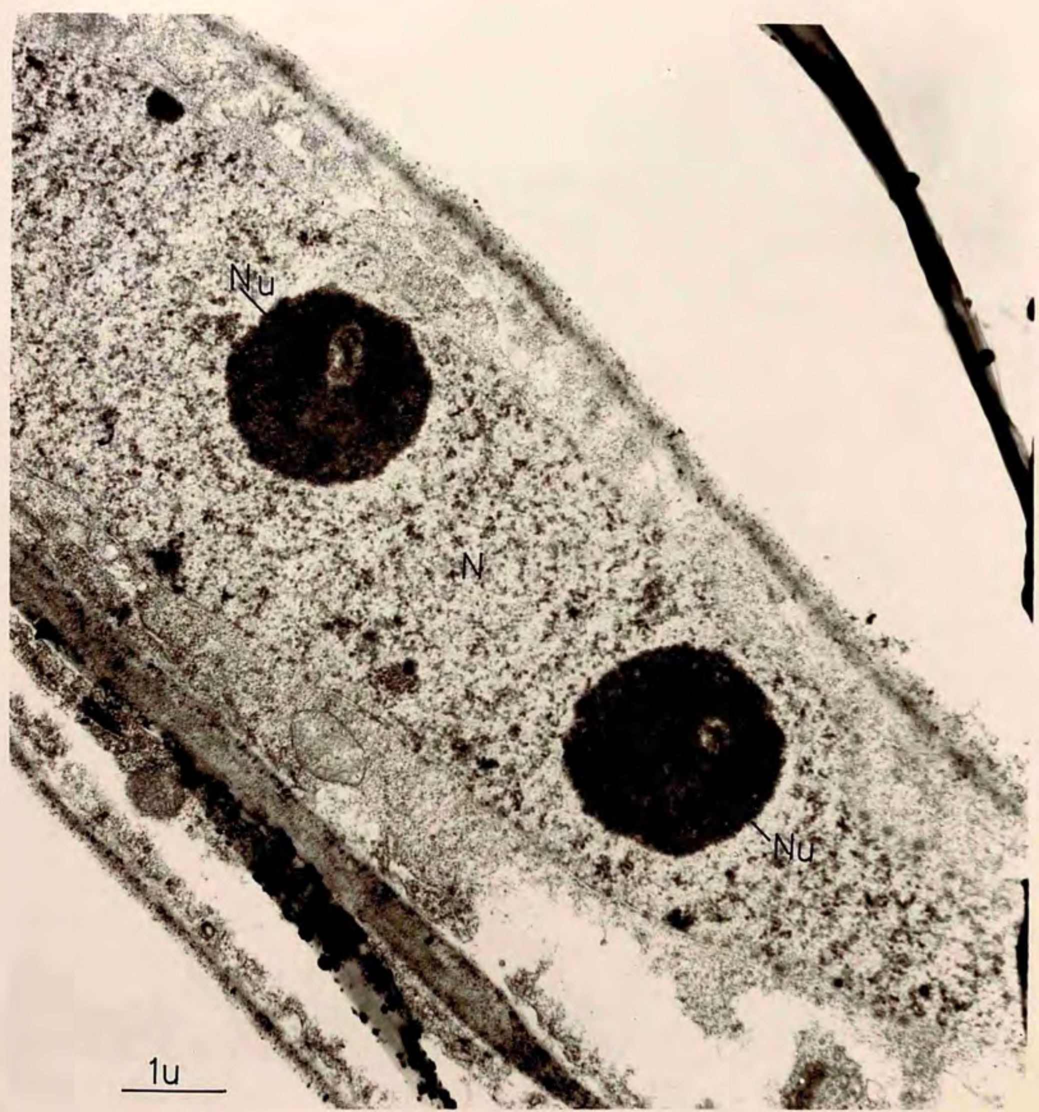
<u>Mixture A</u>	
Epikote 812	62 ml
Dodecenyl succinic anhydride (DDSA)	100 ml
<u>Mixture B</u>	
Epikote 812	100 ml
Methyl nadic anhydride (MNA)	89 ml

Each mixture was stored at 4°C in a polystyrine specimen tube with polythene cap. When required for use the tubes were allowed to warm to room temperature before opening (as Epon is hygroscopic to a small extent) and then mixed in the ratio of 3:7 with about 1.5 to 2% of DMP 30 (2, 4, 6 tri (dimethyl amino-methyl) phenol) added as accelerator.

Experience with Epon was for one reason or another not very successful. This may have been due partly to the effort to section the difficult dormant tissue, and partly to the resin being moist. Pease (1966) mentions the liability of Epon to this latter defect and hints that possibly for this reason it is not so regularly successful. It was also found that Epon sections could not be "expanded" with xylene vapour when floating on water in the knife bath;

PLATE 44

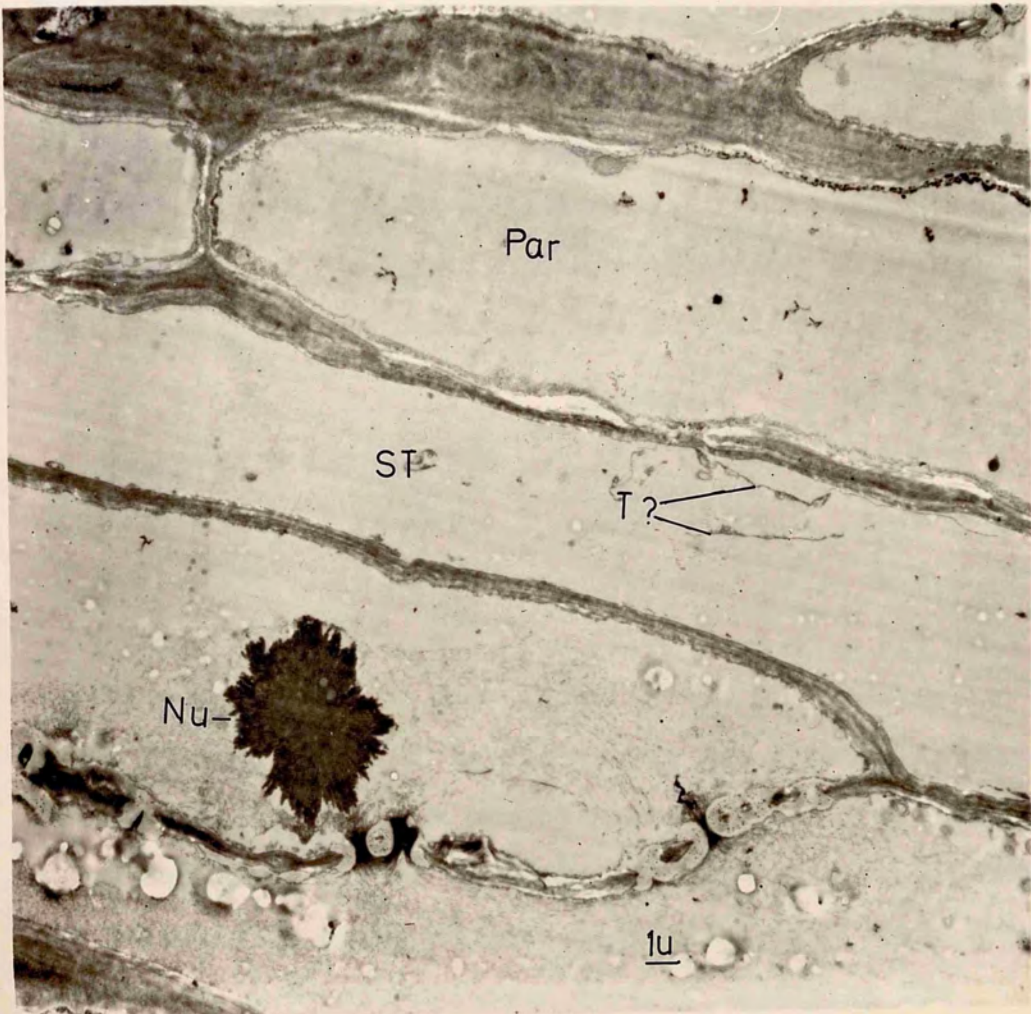
T.L.S. of a young cell from
cambial zone showing nucleus
with two nucleoli. Note the
latter show no radiating
structure. Stained with lead
citrate, uranyl acetate and
lead citrate. X15,000.



44

PLATE 45

T.L.S. showing two adjacent
sieve tubes, a phloem paren-
chyma cell, a nucleolus near
the sieve plate. Stained
with lead citrate, Magnesium
uranyl acetate and lead citrate.
X5,000.


Par
ST
T ?
Nu-
1u

further the resin seemed to be a little unstable under the electron beam.

ARALDITE

For these reasons, and after considerable effort, attention was turned to Araldite. Pease recommends this as being one of the most reliable embedding materials available. The mixture used was as follows (Kay 1965):

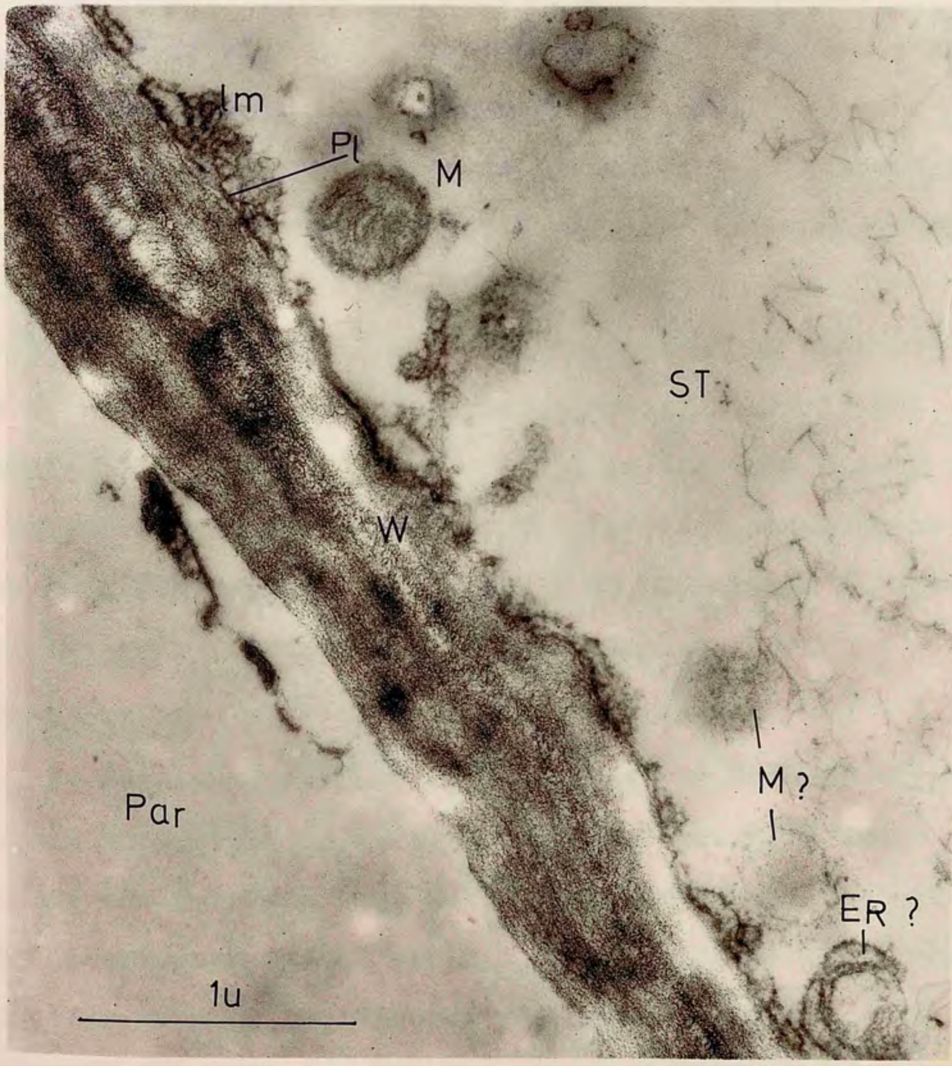
	By volume	By weight
Araldite M (or Cy 212)	10.0 ml	11.3 g
DDSA	10.0 ml	10.0 g
DMP 30	0.5 ml	0.5 g
Dibutylphthalate	1.0 ml	1.0 g

It was found more convenient to mix the relatively small amounts required by dispensing the constituents gravimetrically using a Torbal balance. This method was quick and precise, and avoided soiling measuring glassware, the ingredients being dispensed directly from their original containers. It necessitated however measuring the densities and these are recorded below:

Araldite M	1.135	g per	c.c.
DDSA	0.999	" "	"
DMP30	0.970	" "	"
Dibutylphthalate	1.038	" "	"
MNA	1.237	" "	"

PLATE 46

Longitudinal section through thick wall of a sieve tube. Note the mitochondria,lamellar bodies, plasmalemma and note also the parenchyma cell lacks fibrils. Stained with lead citrate, Magnesium uranyl acetate and lead citrate. X40,000.


Im
Pl
M
ST
W
M ?
Par
ER ?
1u

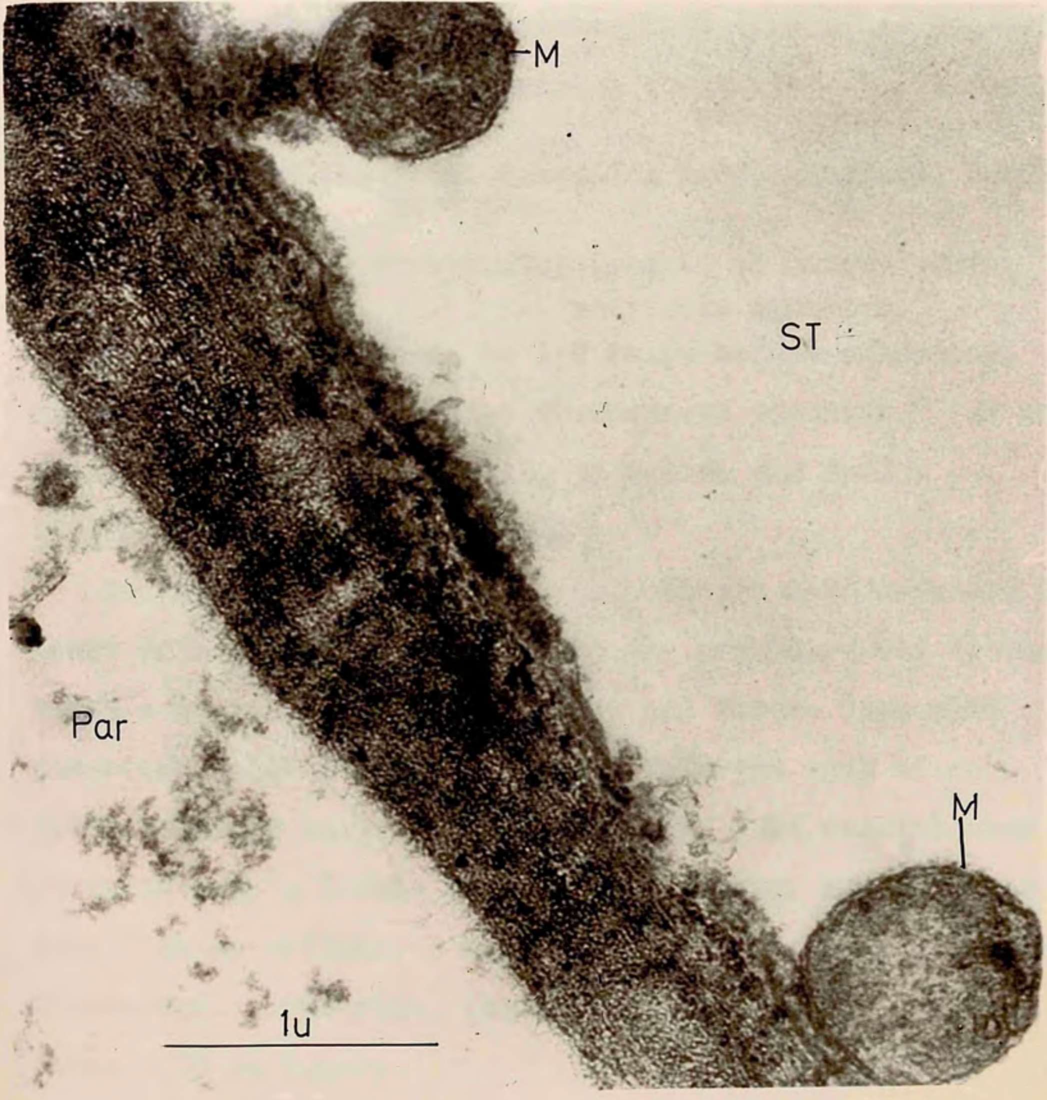
The constituents, now in a 1½ x 3" polystyrine specimen tube were mixed very thoroughly either by stirring with a ½" glass rod for 10 minutes and then "tumbling" at a slow speed for an hour or so, or by subjecting to agitation with a piston which fitted the tube with only a small clearance, followed by tumbling. Both methods cause the trapping of many small air bubbles, and these were got rid of by holding the mixture under a vacuum about 1" off absolute for 5-10 minutes. The tube was then closed with a polythene cap and stored in the freezing compartment of the refrigerator till required. It was always allowed to warm to room temperature before opening.

METHACRYLATE

As mentioned earlier phloem fibres in dormant tissue proved impossible to infiltrate, even when held under vacuum for nearly a week both at the aqueous stage and also when dehydrated and in resin. To some extent this may have been due to the viscous nature of the resin, and attempts were made to try the recent technique of using Methacrylate cross-linked with styrene. Details were supplied by Dr. E. C. Cocking of Nottingham. The mixture was as follows:

PLATE 47

Longitudinal section showing
the thick wall of sieve tube,
and two mitochondria. Note
the double membrane of the
mitochondria. Stained with
lead citrate, uranyl acetate
and lead citrate. X40,000.



47

70% (by volume) butyl methacrylate (inhibitor removed)

30% (by volume) vinyl benzene ("styrene" -inhibitor not removed)

1-2% (W/v of the total embedding medium mixture) benzoyl peroxide.

Approximately 1 teaspoonful (per 20 ml medium) $CaSO_4$ anhydrous granular.

The mixture was made up 1-2 hours before embedding, swirling gently to dissolve the benzoyl peroxide. It was centrifuged just before using to remove the $CaSO_4$.

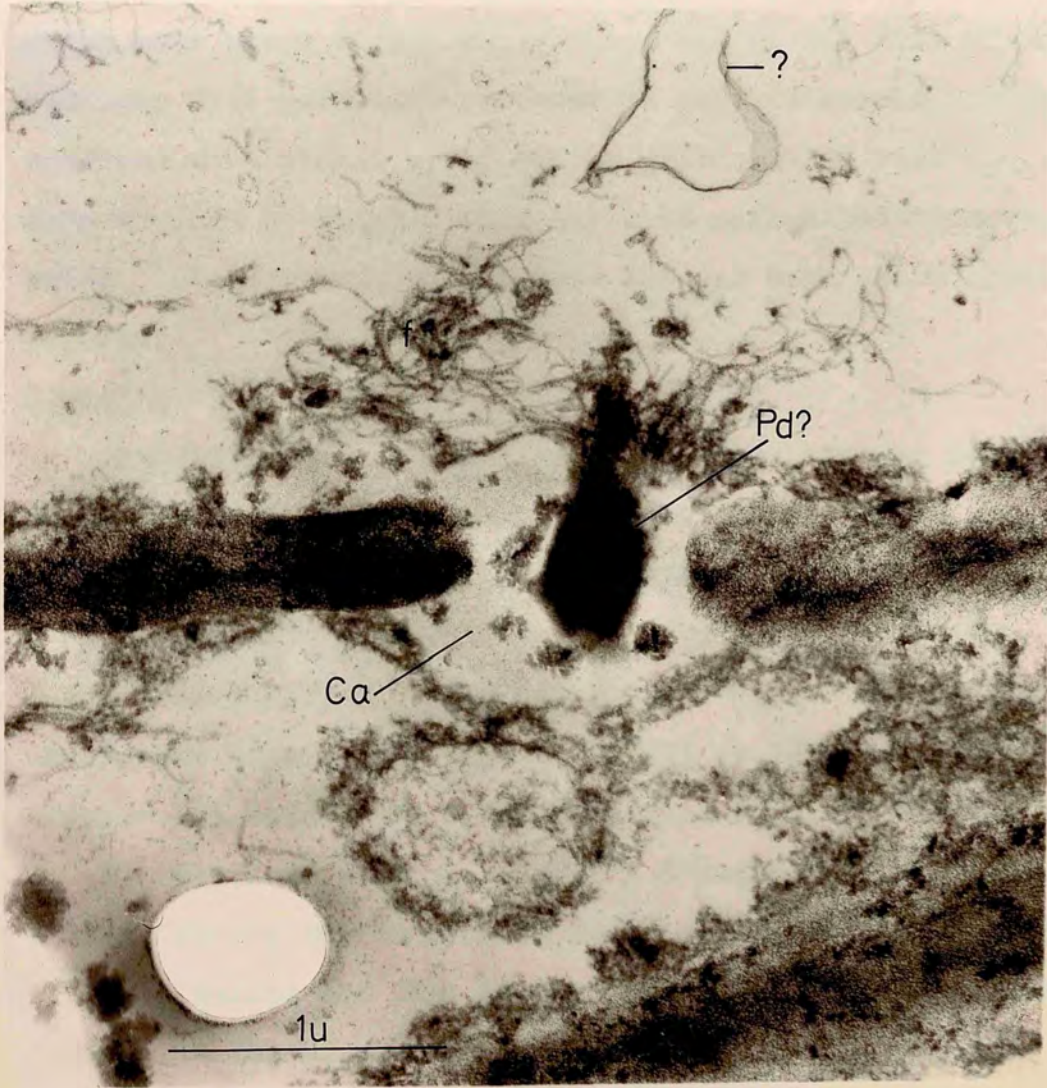
Sectioning Araldite blocks

In later work where the tissue blocks were embedded in drops of resin on a flat surface the procedure was to cut these out with an x-acto microsaw and fasten them with domestic araldite adhesive to the enlarged apex of an araldite blank cast in a BEEM capsule. So mounted they required only a little additional trimming, and this was done with an ordinary 3-hole razor blade under a binocular microscope. The final facet was usually quite small, about 0.33 mm square.

Sections were cut on a Huxley microtome with motor drive. Glass knives made by an LKB knife-maker were used, and the flotation liquid was plain distilled water. The

PLATE 48

Possibly the face view of a sieve area. Note the callose deposition and the slime fibrils. Stained with lead citrate, uranyl acetate and lead citrate. X40,000.


?
f
Pd?
Ca
1u

araldite sections were expanded by holding over them a camel hair brush dipped in xylene; but in the case of Epon sections this procedure produced no obvious result. The sections were picked up on copper grids coated with a formvar film on which a thin layer of carbon had been evaporated. Most often, the formvar had not been subsequently removed.

Staining

Araldite-embedded sections require staining to improve their contrast. Three schedules were used:

1) Sections were stained in freshly prepared Reynold's lead citrate for 25-30 minutes, washed thoroughly with 0.02N Sodium Hydroxide and CO_2 free distilled water, stained in freshly prepared 1% uranyl acetate in 70% alcohol for 10 minutes and then given a final washing in distilled water.

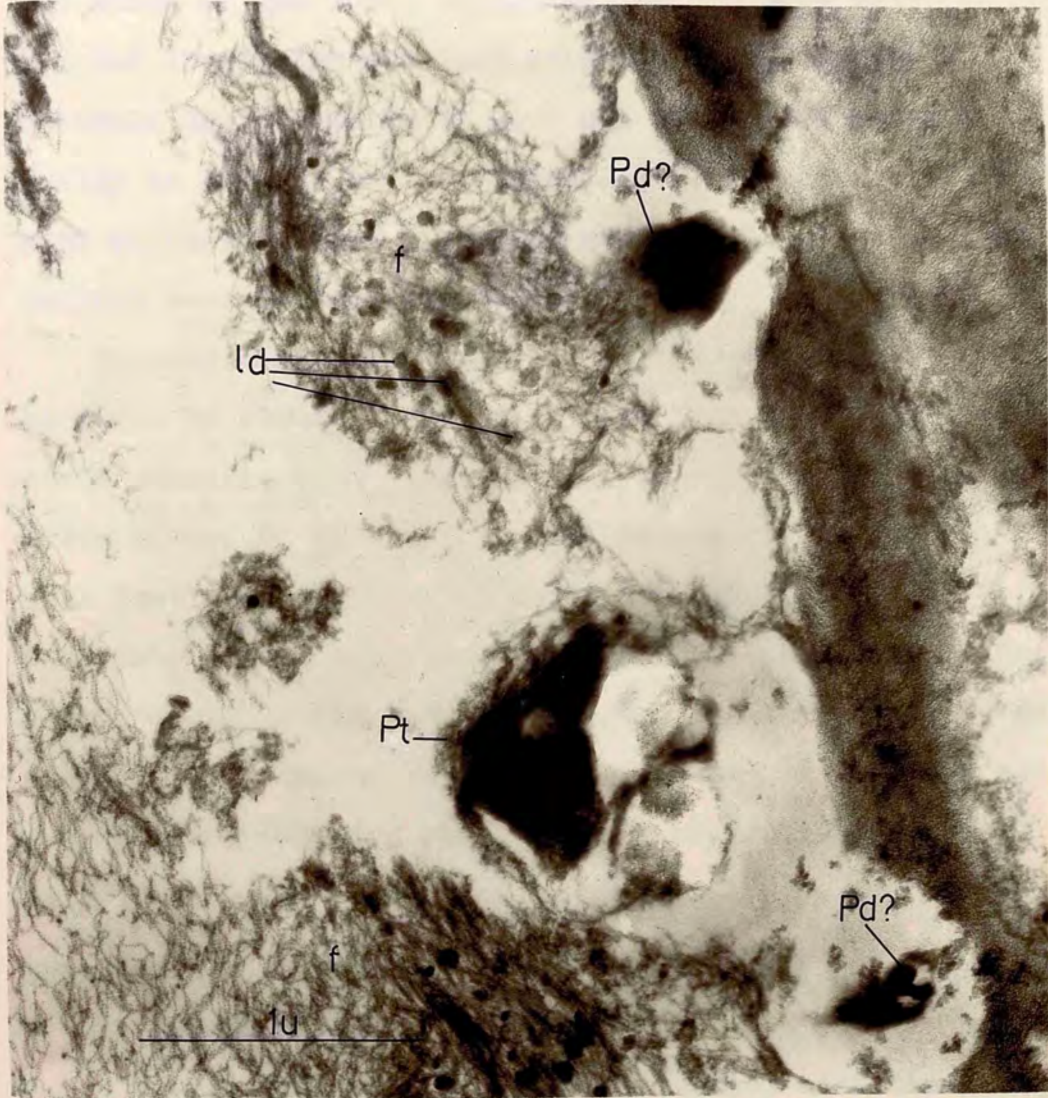
2) Sections were placed for 2-3 minutes in lead citrate, washed, placed for 30 minutes in 1% uranyl acetate in 70% alcohol, washed and then stained for a further 1-2 minutes inlead citrate before final washing.

3) Similar to (2) but 2% uranyl magnesium acetate in 70% alcohol (Frasca and Parks, 1965) was substituted.

It is difficult with the amount of results to hand to

PLATE 49

Possibly the face view of a sieve area. Note the slime fibrils, lipid droplets, plastid and callose deposition. Stained with lead citrate, uranyl acetate and lead citrate. X40,000.


Pd?
f
ld
Pt
Pd?
f
1u

say which of the three schedules was the most satisfactory, but the impression was that schedule No.(2) proved best. Schedule No.(3) was quite good but it did not stain so deeply as No.(2), and the advantage of lower contamination rate claimed for the magnesium acetate did not seem in the present case to outweigh this.

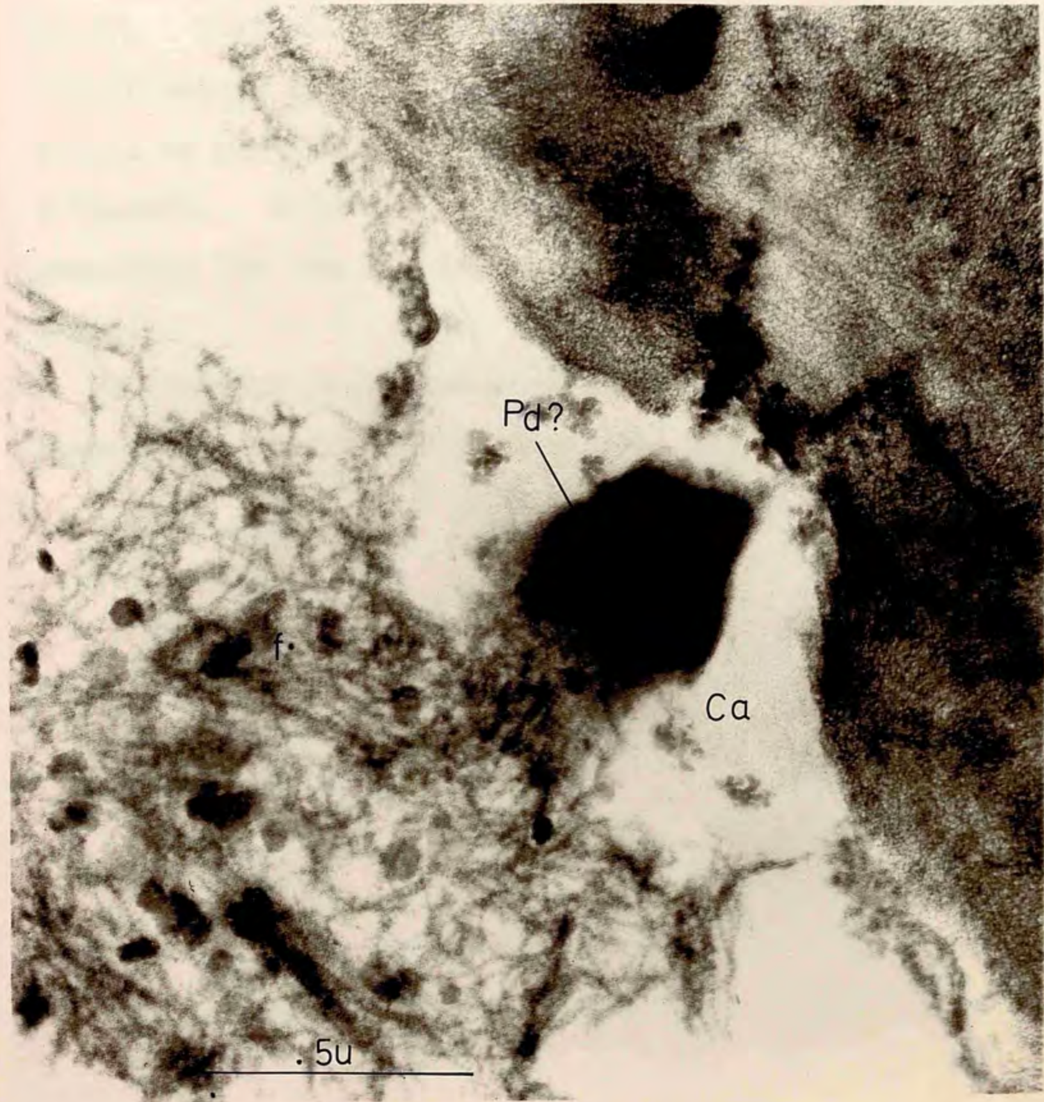
Treatment in the staining solutions was carried out at first by floating the grids face downwards on drops of stain standing on dental wax in a CO_2-free atmosphere. This often seemed to result in contamination of the specimens with insoluble deposits. Later the method of W. J. Dougherty (1967) was successfully used. Here the grids remain wholly submerged except when distilled water is concerned, and so never have to pass through the surface of the staining solution where lead or uranium precipitates collect.

Examination in Microscope

The sections were examined in a new AEI EM6 B microscope. A lot of preliminary breakdowns especially with the specimen airlock occurred with this and as a result the work was often held up. Normally, sections were examined at

PLATE 50

A portion of Plate 49 magnified to show the slime fibrils, lipid(?)droplets. Taken at X40,000. X80,000.

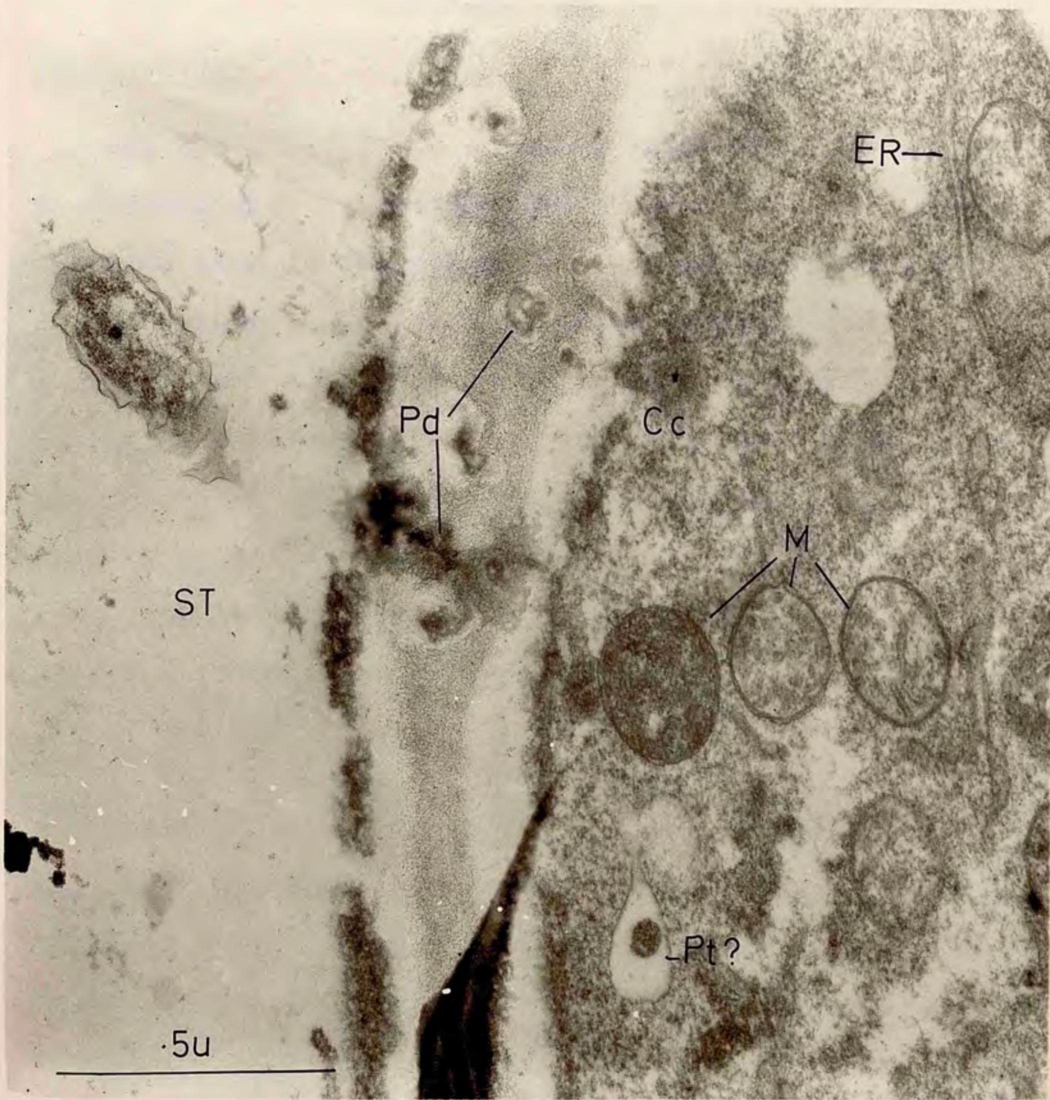

Pd?
f.
Ca
.5u

60 KV. Initially the instrument was adjusted for high magnification work and suffered badly from chromatic change of magnification at low magnifications (up to about x 10,000). This was rectified in July 1967 and subsequently the low magnification performance was much improved.

Photographs were taken on Ilford N50 plates and processed in Ilford ID 36 phenidone and Kodafix.

PLATE 51

Longitudinal section showing a sieve tube and a companion cell. Note the branching of plasmodesma towards the companion cell side and the dense cytoplasm of the companion cell with ER, Monitochondria. Stained with lead citrate, uranyl acetate and lead citrate. X80,000.


ER
Pd
Cc
M
ST
Pt?
·5u

OBSERVATIONS

Introduction

All the sieve tubes examined under the electron-microscope were apparently mature ones. Phloem fibres are laid down in positions fairly close to the cambium which renders the section of sieve tubes of differing ages for sectioning not an entirely straightforward matter, and this coupled with the time factor of the work prevented the original objective of an ontogenetic study from being realised. In the following description therefore it must be borne in mind that mature cells are being discussed. Their various features are considered in turn.

The Nucleolus

The sieve tubes of *Salix capraea* as usual contain no nucleus; but this species is one of the apparently numerous class in which the nucleus on disintegration extrudes (usually one) prominent and persistent nucleolus. This has already been illustrated in the chapter dealing with the light microscope investigations. Nucleoli have been described in electron microscope work in *Passiflora* (Kollmann 1960); *Cucurbita* (Buvat 1963 c); *Tilia* (Evert and Murmanis 1965); *Impatiens* (Engleman 1965 b). On

PLATE 52

Cross-section showing the fine branching of a plasmodesma between a sieve tube and a parenchyma cell. Stained with lead citrate, uranyl acetate and lead citrate. X120,000.

155

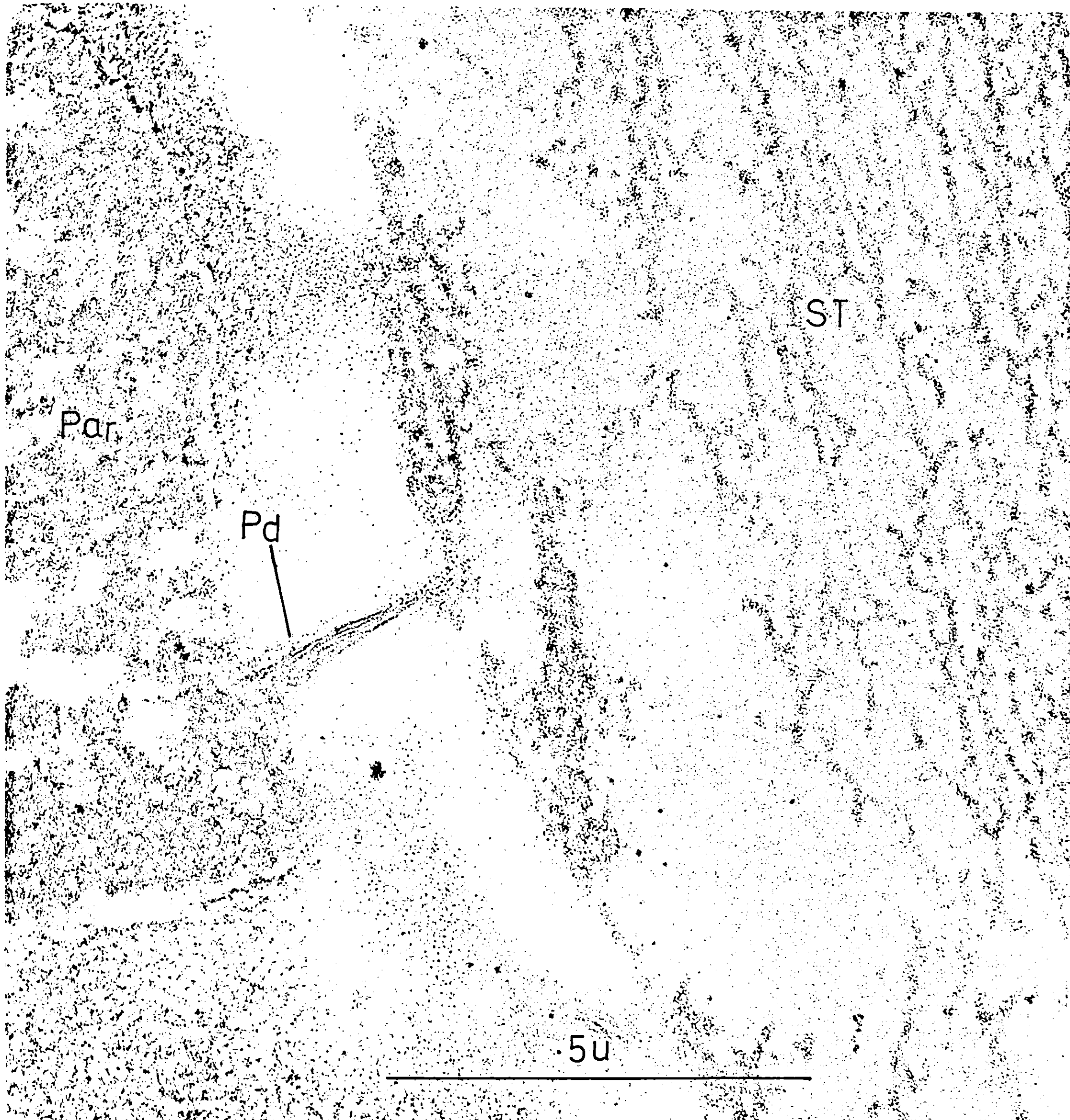
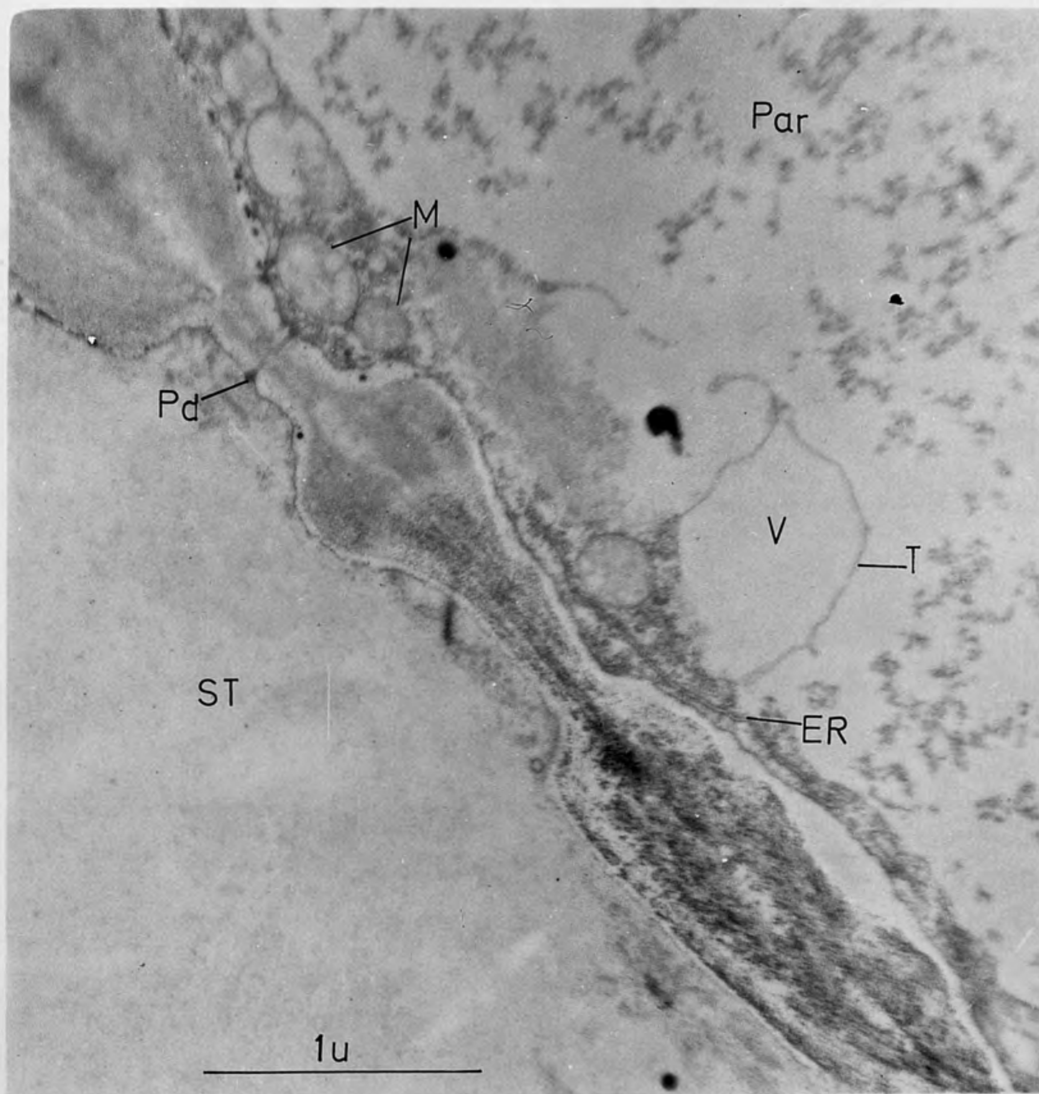


PLATE 53

Longitudinal section showing a plasmodesma between a sieve tube and a parenchyma cell. Note the mitochondria, ER and vacuole in the parenchyma cell. Stained with lead citrate. X40,000.



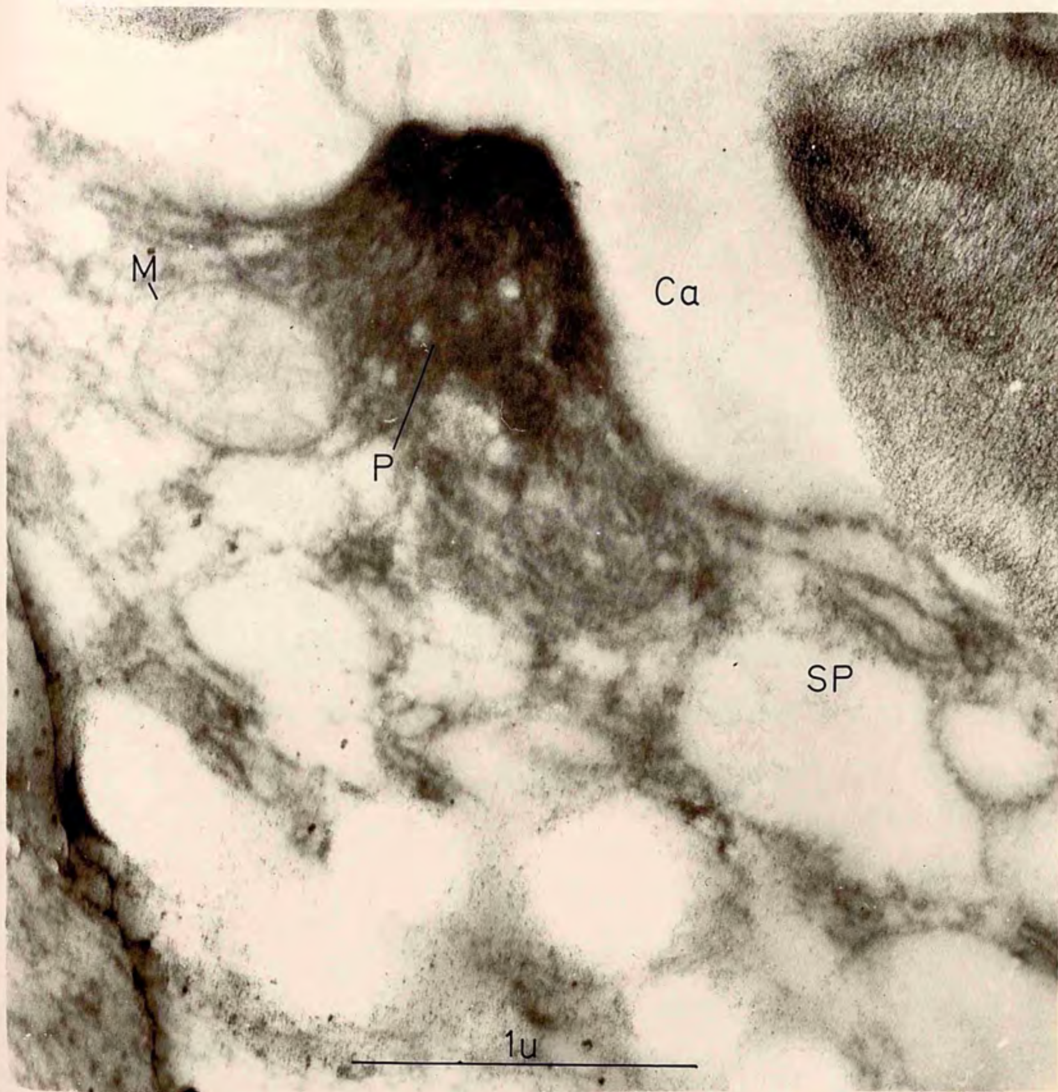
53

the other hand there are cases where these naturally conspicuous organelles are apparently absent, as in Nymphoides (Mehta and Spanner 1962; Johnson 1967), Pisum (Bouck and Cronshaw 1965; Wark and Chambers 1965) Acer (Northcote and Wooding 1966); Metasequoia (Kollmann and Schumacher 1960); Dioscorea (Behnke 1965 a); Vitis (Esau 1965 c); Beta (Esau et el 1967). Whether there is any constant difference in the contents of the sieve tubes of the latter group of species which correlates with the disappearance of the nucleolus is a question which it is perhaps premature to consider. It may be questioned, in passing, whether the structure described in such studies as the present is truly a nucleolus as understood by chromosome cytologists.

In appearance the nucleolus of Salix capraea is similar to previously - described sieve tube nucleoli. It appears irregularly spherical with a curiously radiating structure, the rays being of variable length and giving the surface its urchin-like contour (Plates 35-42 & 45). The radiating elements are parallel sided, not conical, and as a consequence they cannot all be traced back to a single centre. While in suitable sections the broad impression is of a

PLATE 54

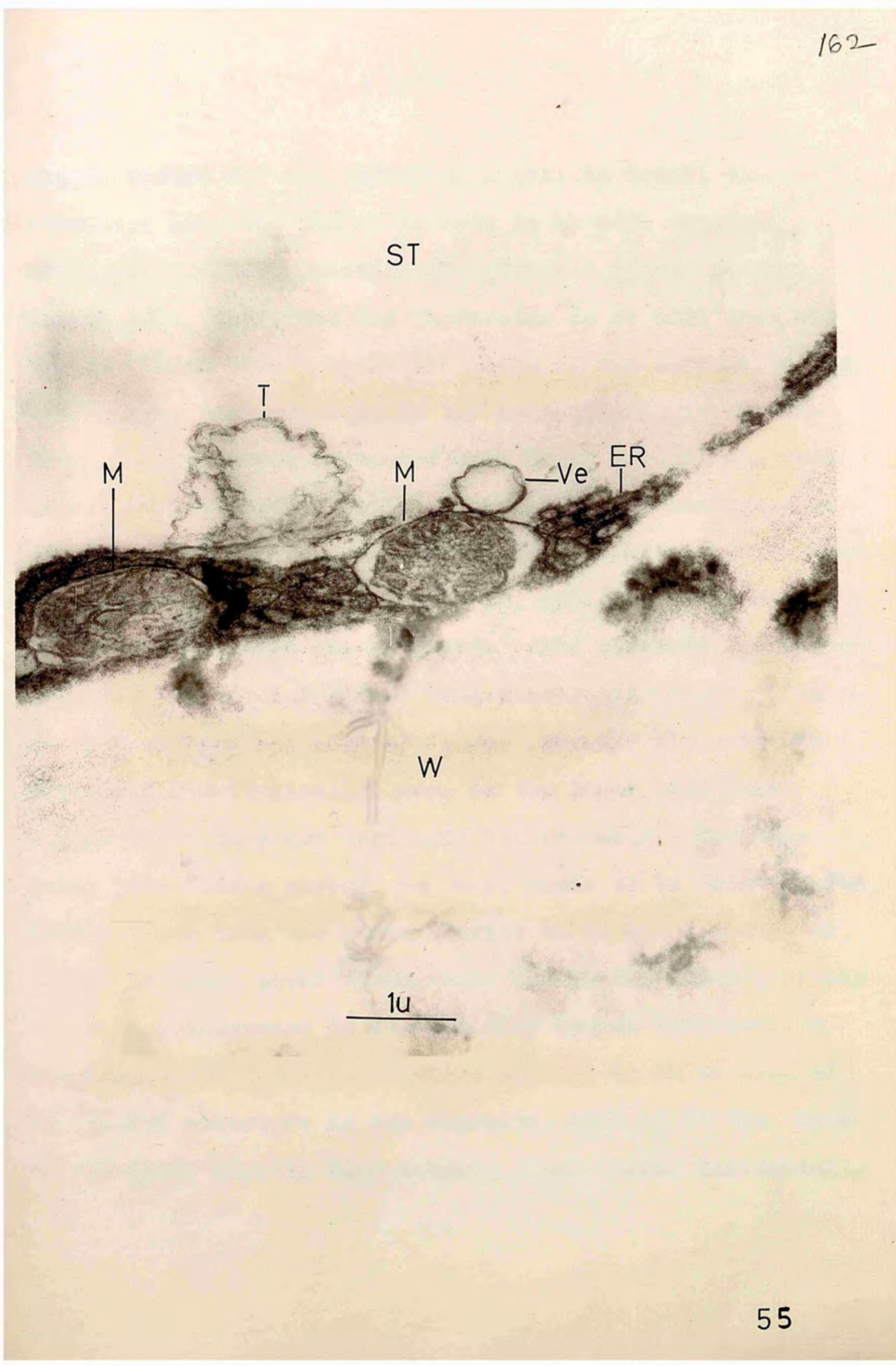
An oblique section showing
the slime fibrils and a
mitochondrion with swollen
cristae near the sieve plate.
Stained with lead citrate.
X50,000.



54

PLATE 55

Longitudinal section through a sieve tube wall showing two mitochondria, ER, a vesicle and membranous structure near the wall. Stained with lead citrate, uranyl acetate and lead citrate. X20,000.


ST
T
M
M
Ve
ER
W
1u
55

single centre for the radiating lines, in detail the structure near the centre is seen to be more complex, often giving the impression of a "finger print" pattern (Plate 43). Sometimes the impression is of more than one centre (Plate 38). Where the plane of the section passes fairly near the periphery of the organelle it is obvious that the appearance presented will be of several discrete islets of nucleolar material with the linear character of the elements much foreshortened. This is probably the case in the upper nucleolus of Plate 38, and in other cases illustrated (Plates 40, 41 & 42). The elements themselves appear to be tubular rather than simply fibrillar. This is most obvious in Plate 43, both where the elements are sectioned longitudinally, and in the lower left-hand corner where they are sectioned transversely. The diameter (see facing arrows ⟶ ⟵) seems to be about 60-70A, rather finer than the slime fibrils to be discussed later, though in lower power micrographs (Plate 36) owing probably to their coalescence in bundles they appear considerably coarser than the latter. There appears to be no sign of any banded structure in the elements, such as is the case in the slime fibrils (see later). There seems incidentally

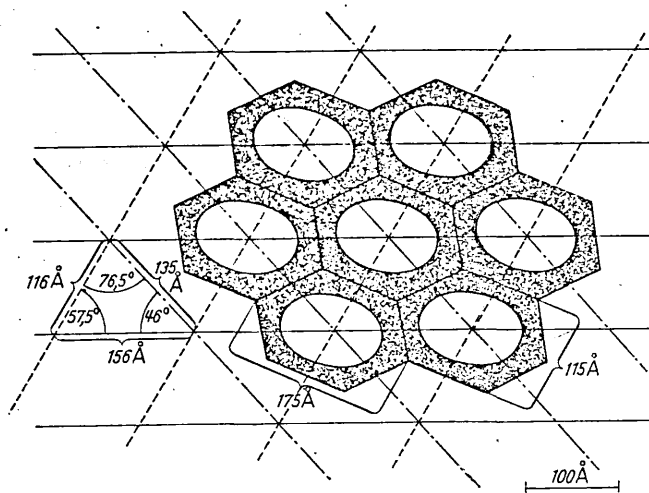


Abb. 6a. Schema des Nucleolus-Feinbaues (s. Text) im Bereich quergeschnittener Fibrillen

FIG.8 - Diagrammatic representation of Nucleolus in <u>Passiflora</u> presented by Kollmann (1960). Similar pattern observed in Salix.

to be no obvious connection between the nucleolus and the latter. The impression is that the nucleolus seems often to be surrounded by a narrow region free of fibrils (Plates 36,37,39 & 61) the latter having contact with it only at the ends of its rays. The 60-70 A dimension just noted may be compared with the figures given by Kollmann (1960) illustrated in Figures 5 and 6. It seems probable that the linear elements in the _Salix_ nucleolus are similar to those in _Passiflora_ nucleolus.

Within the body of the organelle are sometimes to be observed lighter (Plate 35) or darker (Plates 36 & 37) areas. These may be compared with the "core" observed in the nucleoli of _Tilia_ by Evert and Murmanis (1965) but how close is the homology it is hard to say.

One feature of the distribution of nucleoli in the sieve tube deserves notice. This is that while they are usually to be found in the region of the sieve plate (Plates 35,37,38,39,40,42 & 45 contrast 41) not infrequently there is a nucleolus on both sides of the plate (Plates 38 & 39). This makes it difficult to interpret their presence there as an artefact of pressure release.

Owing to the difficulties encountered earlier in embedding dormant phloem, and to the limited time the

PLATE 56

Longitudinal section showing
a sieve tube and a parenchyma cell.
Note the mitochondrion-like
structure. Stained with lead
citrate, Magnesium uranyl
acetate and lead citrate.X30,000.

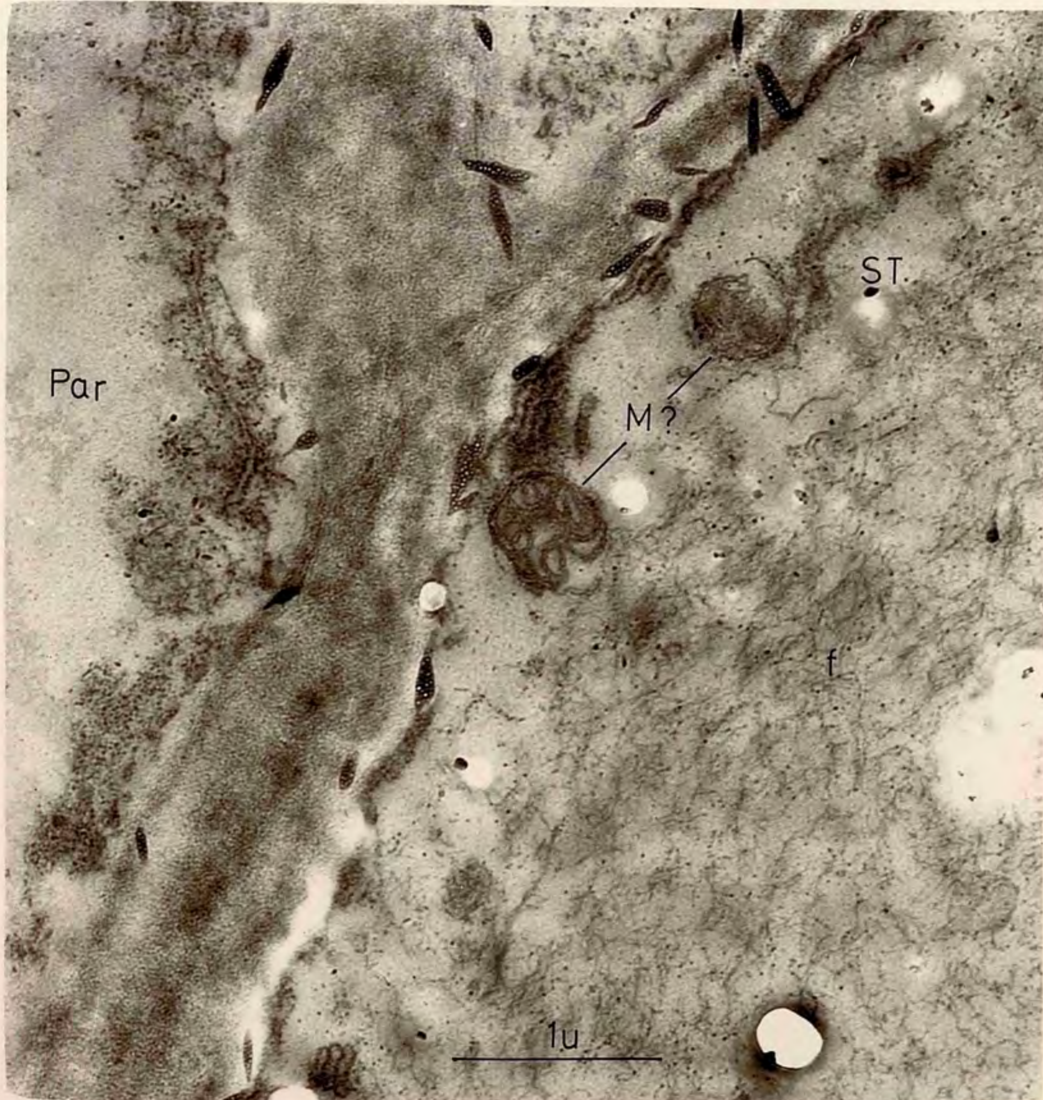
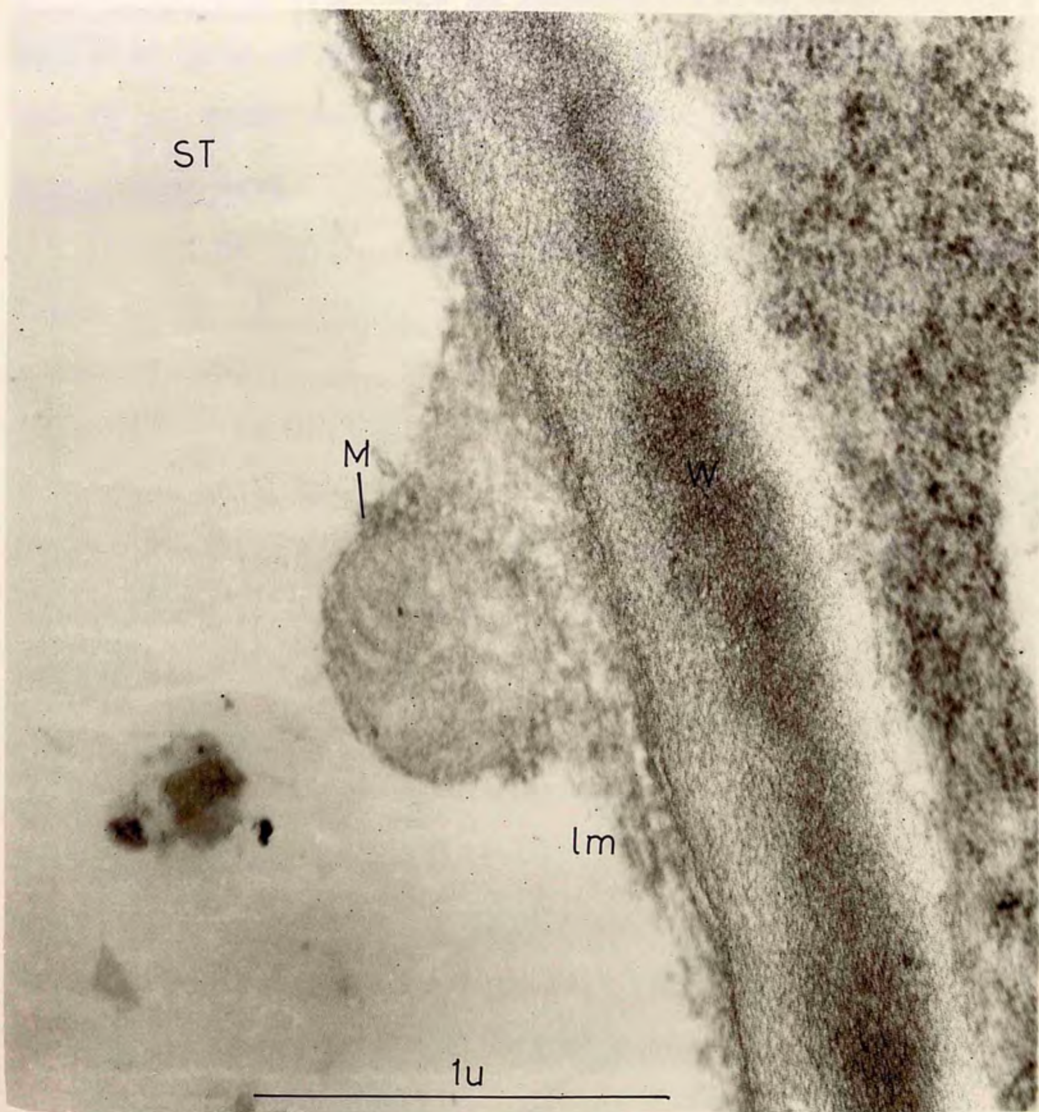

ST.
Par
M?
f
1u

PLATE 57

Longitudinal section through a sieve tube wall. Note the swollen cristae of the mitochondrion. Stained with lead citrate, uranyl acetate. X60,000.



57

microscope was available no comments can be made on the ultimate fate of the nucleolus. It may well persist till the sieve tubes become obsolete (Esau 1947).

Sieve tube wall

There is little unusual about the walls of the sieve tubes in Salix capraea. In the mature cells they are not outstandingly thick, certainly not nacreous (Plates 35,45, 46 & 47). Where the sieve tube is in contact with a companion cell the wall is much thinner (Plates 35,41,79, 90). Sometimes the wall between adjacent sieve tubes is thin (Plate 35 & 45) and sometimes too between a sieve tube and an adjacent parenchyma cell (Plate 45). No doubt these details represent ontogenetic relationship and are not necessarily functional or diagnostic.

Plasmodesmata

Plasmodesmata occur fairly frequently between the sieve tubes and companion cells. They are lined with callose at least in the prepared condition, though in view of the considerable evidence (e.g. Eschrich, 1963 a; Evert and Derr, 1964; Engleman, 1965) that callose formation in response to wounding is a very rapid process, some

PLATE 58

An oblique longitudinal section of the sieve tube showing two mitochondria. Stained with lead citrate, uranyl acetate and lead citrate. X40,000.

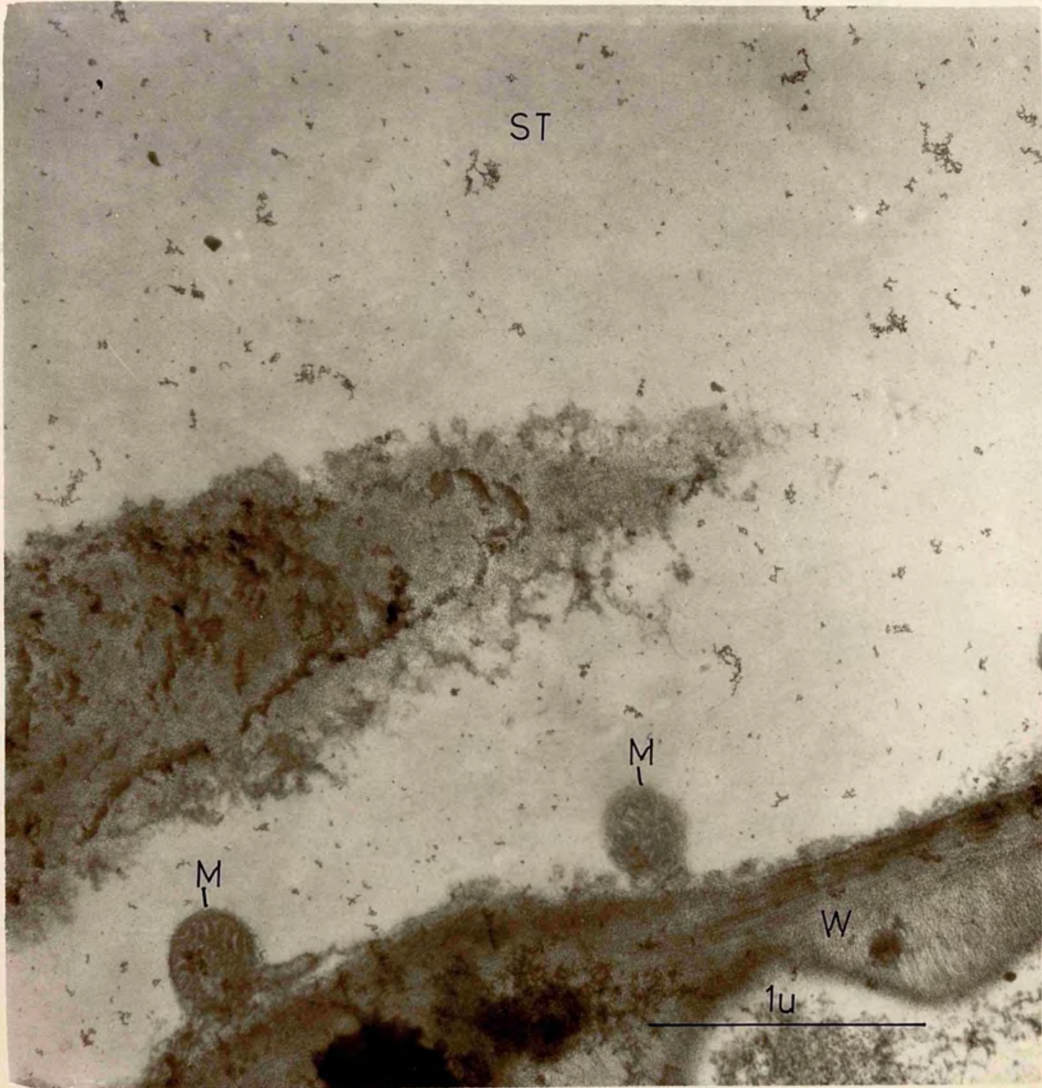
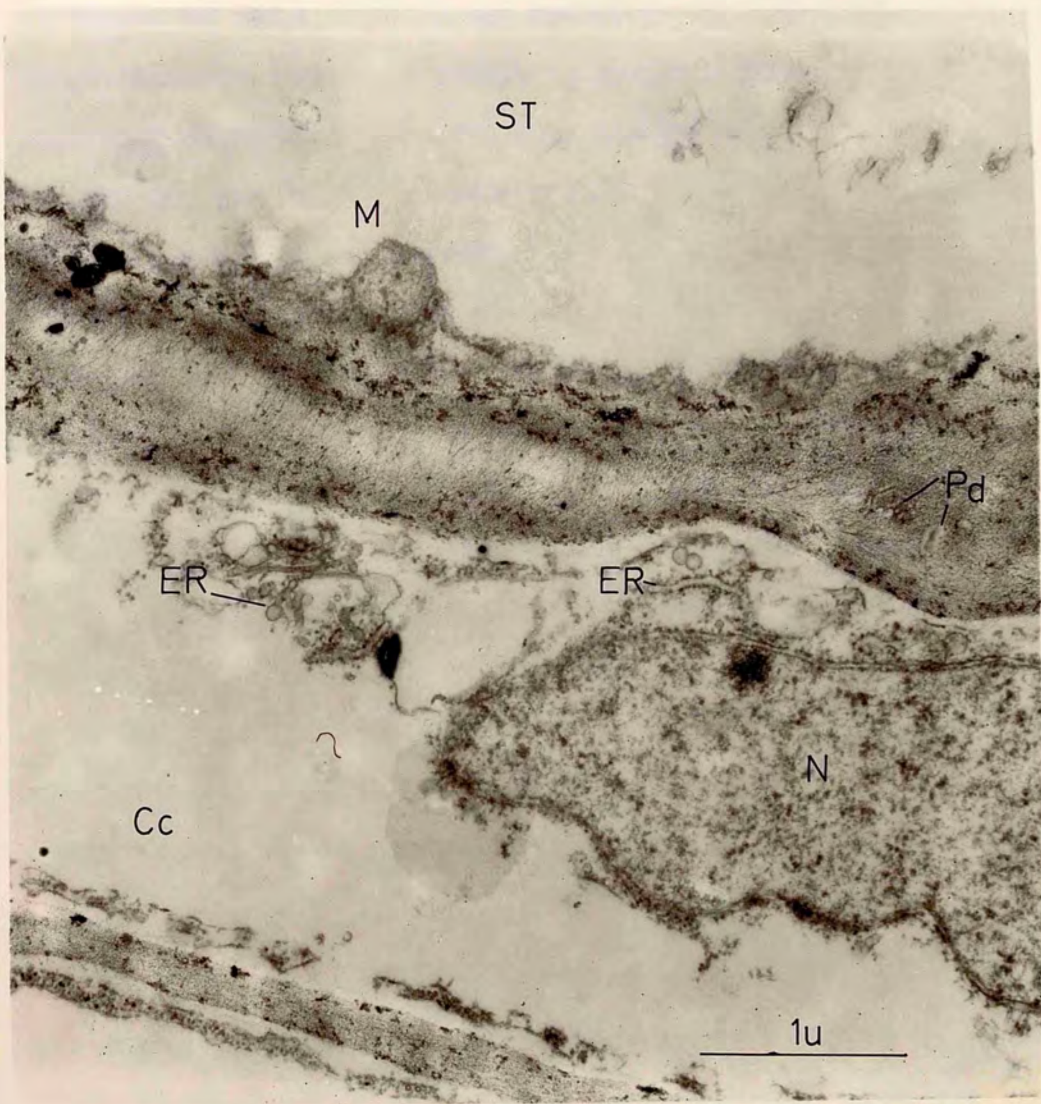

ST
M
M
W
1u

PLATE 59

T.L.S. showing a sieve tube and a companion cell. Note the nucleus, ER, in the companion cell. Stained with lead citrate, uranyl acetate and lead citrate X30,000.


ST
M
Pd
ER
ER
N
Cc
1u

at least of this callose is probably an artefact. Plasmodesmata were not found in numbers justifying a stronger adjective than "fairly frequently".

Plate 51 shows a rather oblique section through a plasmodesma joining a sieve tube and companion cell as has been often noticed by other workers (e.g. Evert, Murmanis and Sachs, 1966; Johnson, 1967); the structure is simple and wide at the sieve tube end, branches within the wall thickness and enters the companion cell as a number of distinct and much finer channels. Some of these latter ones shown in the photograph, cut transversely. Plates 52 & 53 each show a plasmodesma between a sieve tube and a simple parenchyma cell. It is obvious that the characteristic delta-like structure just described is absent here, or at most very rudimentary.

Mitochondria

Like other species, Salix capraea has mitochondria in its sieve elements though not abundantly (Plates 38,46,47,54, 55,56,57,58,& 59). It is to be noticed that the cristae of sieve tube mitochondria often show an unusual bloated appearance (Plates 46,47,54,55,57) and to some

PLATE 60

An oblique longitudinal section showing possibly a sieve area, plastid with starch grains, slime fibrils and lipid droplets. Stained with lead citrate, uranyl acetate and lead citrate. X40,000.

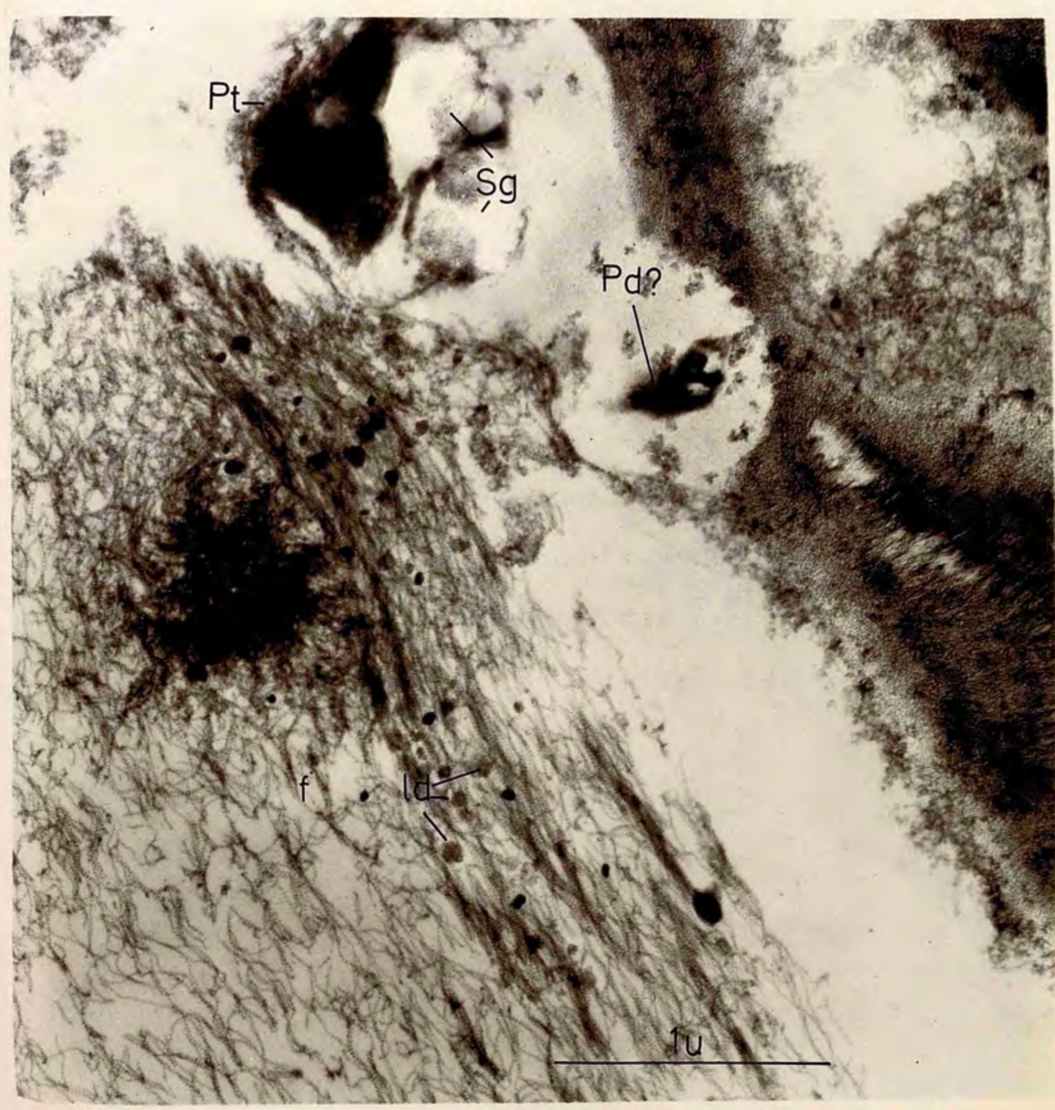
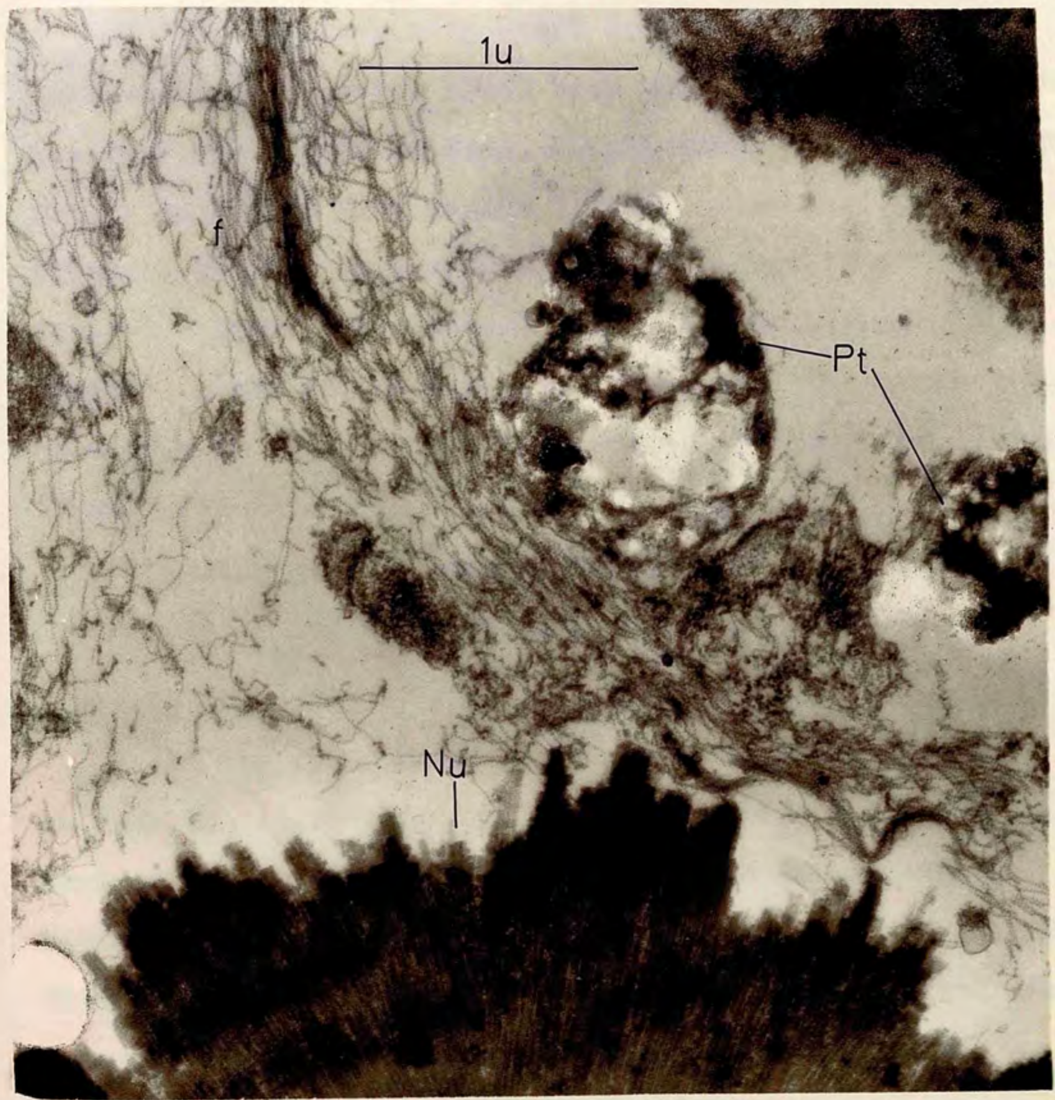

Pt
Sg
Pd?
f
ld
1u

PLATE 61

A portion of Plate 36 magnified to show 2 plastids and their possible connection with slime fibrils. X40,000.


1u
f
Pt
Nu

extent those of companion cells do too (Plate 90). Other workers (e.g. Johnson, 1967) have also found this and they are very noticeable in Esau, Cronshaw and Holfert's study (1967) on phloem from plants infected with beet yellow virus. To what extent this is an artefact is difficult to say. Sieve tube mitochondria, as usual, occupy the parietal position and are often found near the sieve plate. When seen in surface view the outer membrane often appears "fuzzy" (Plates 46,47).

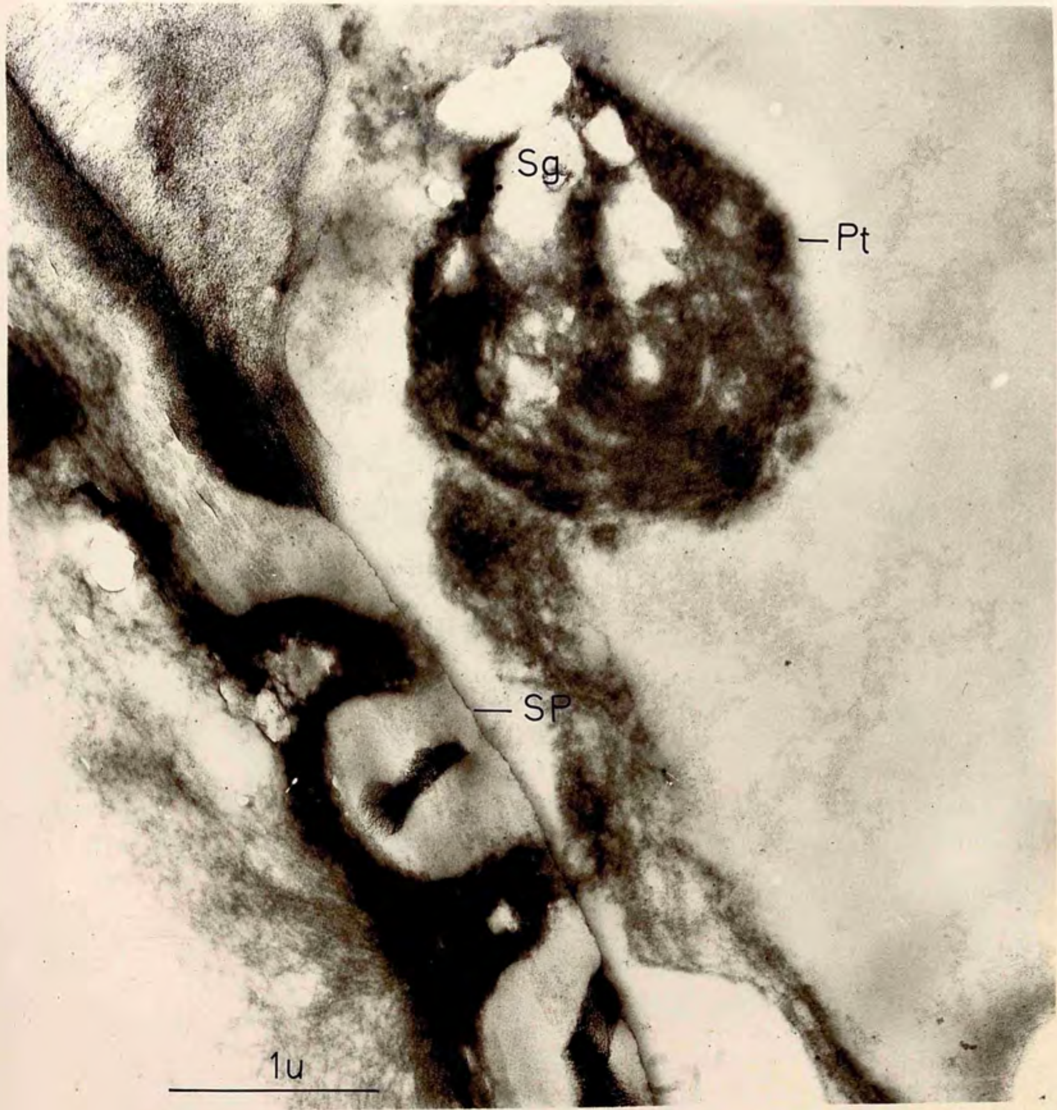
Companion cell mitochondria may possess the bloated appearance or may be more normal. (Plates 41 & 51)

Plastids

These are found near the parietal layer of the wall or near the sieve plate (Plates 49,60,61,62,64,66).They usually look very much disorganised. They have darkly stained regions and electron transparent regions. The latter are thought to be starch grains. Very often lots of released starch grains are found clustering near the sieve plate (Plate 65). Probably these are released during preparation, this appearance being thought to be an artefact by many workers. The outer membrane of plastids is sometimes

PLATE 62

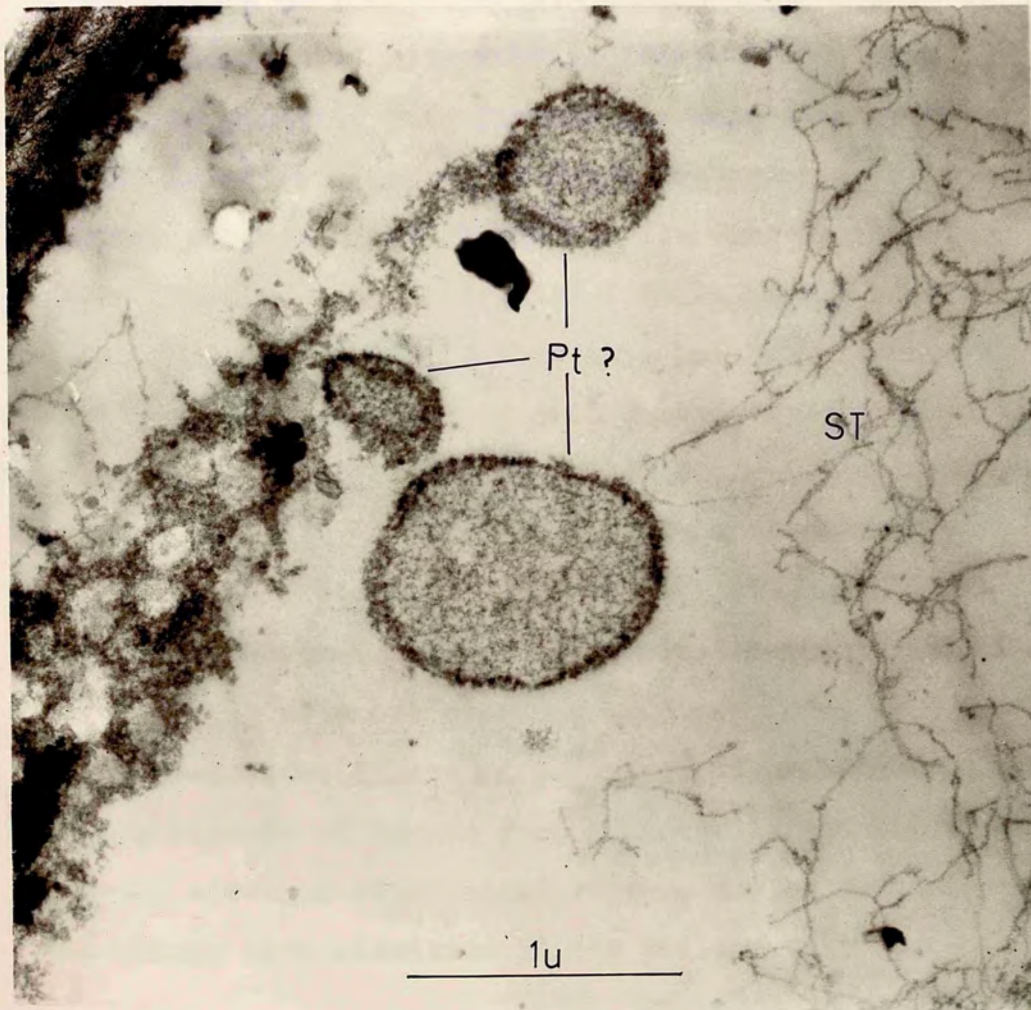
An oblique section through a sieve plate showing a plastid. Note the fibrillar appearance of the plastid. Stained with lead citrate, uranyl acetate and lead citrate. X30,000.



62

PLATE 63

Longitudinal section of a sieve tube showing three unidentified structures, apparently breaking. They might be degenerating plastids. Stained with lead citrate, uranyl acetate and lead citrate. X40,000.


Pt ?
ST
1u

found to be covered with protrusions like those of mitochondria (Plates 61,62,&64).Often they seem to be closely associated with fibrils (Plate 61). A breaking up of plastids into fibrils was suggested by Falk (1964) in sieve elements of Tetragona expansa. He further suggested that these fibrils were similar to slime fibrils. In Salix, the fibrils hardly seem to arise in the plastids in this way, but the micrographs of the plastids are hardly good enough to be critical. Some unidentified bodies of rather different appearance (Plates 63 & 71) are very similar to certain structures called plastids by Buvat (1963 a). It is difficult, however, to be certain of the identification.

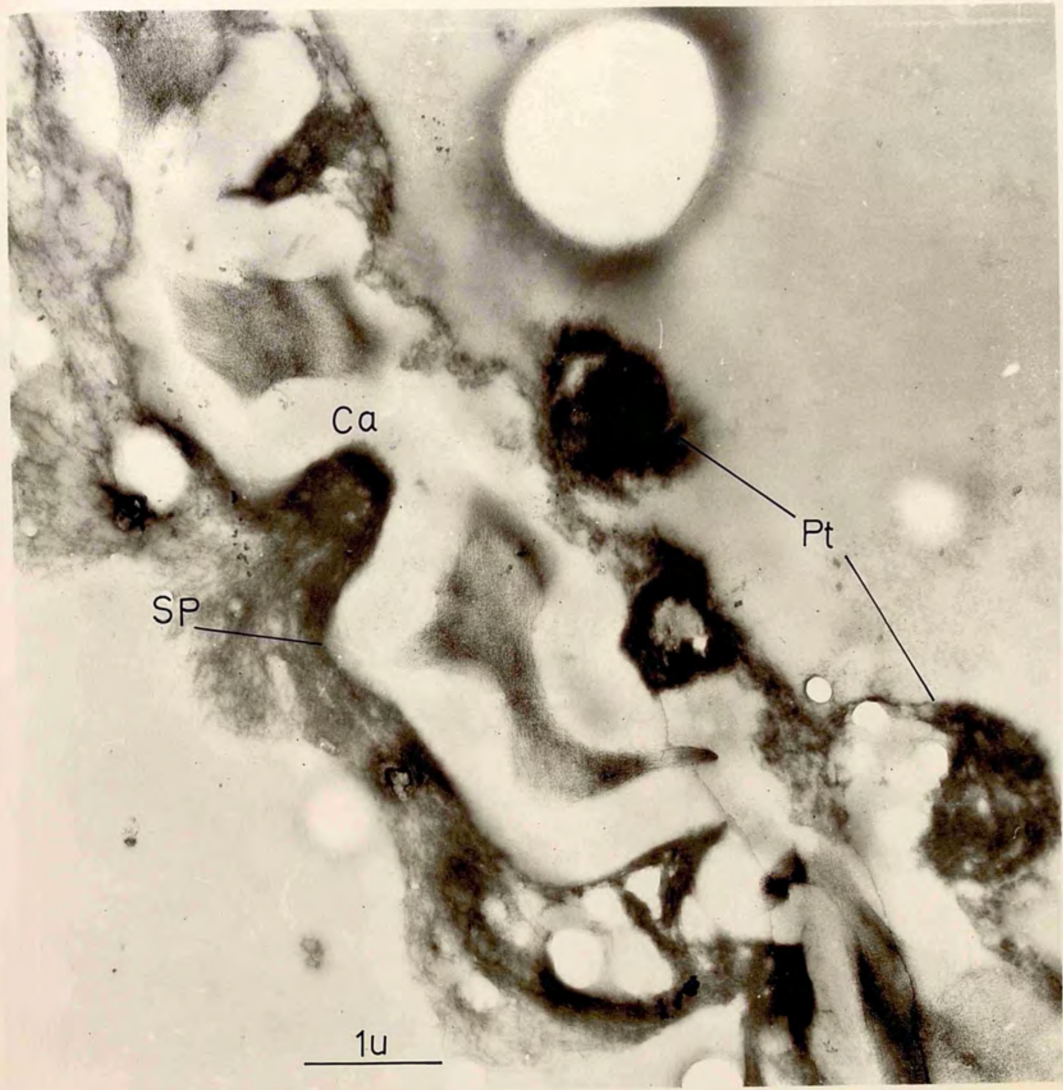
In Salix released starch grains are found inside the sieve plate pore (Plate 65). This might be due to the sudden release of turgor during manipulation. Alternatively, the electron-transparent regions may be sections of protruding irregularities in the callose cylinder.

Plasmalemma

The plasmalemma in Salix is quite similar to the ones found by other workers. It is composed of two dark layers separated by a lighter one in the middle (Plates 46, 67, 68, 69, 70 and 71). The plasmalemma appeared

PLATE 64

An oblique longitudinal section through a sieve tube showing two plastids near the sieve plate. Stained with lead citrate and uranyl acetate. X20,000.



64

PLATE 65

T.L.S. through sieve tube showing a sieve plate, slime fibrils and released starch grains. Note one starch grain in the pore. Stained with lead citrate and uranyl acetate. X20,000.

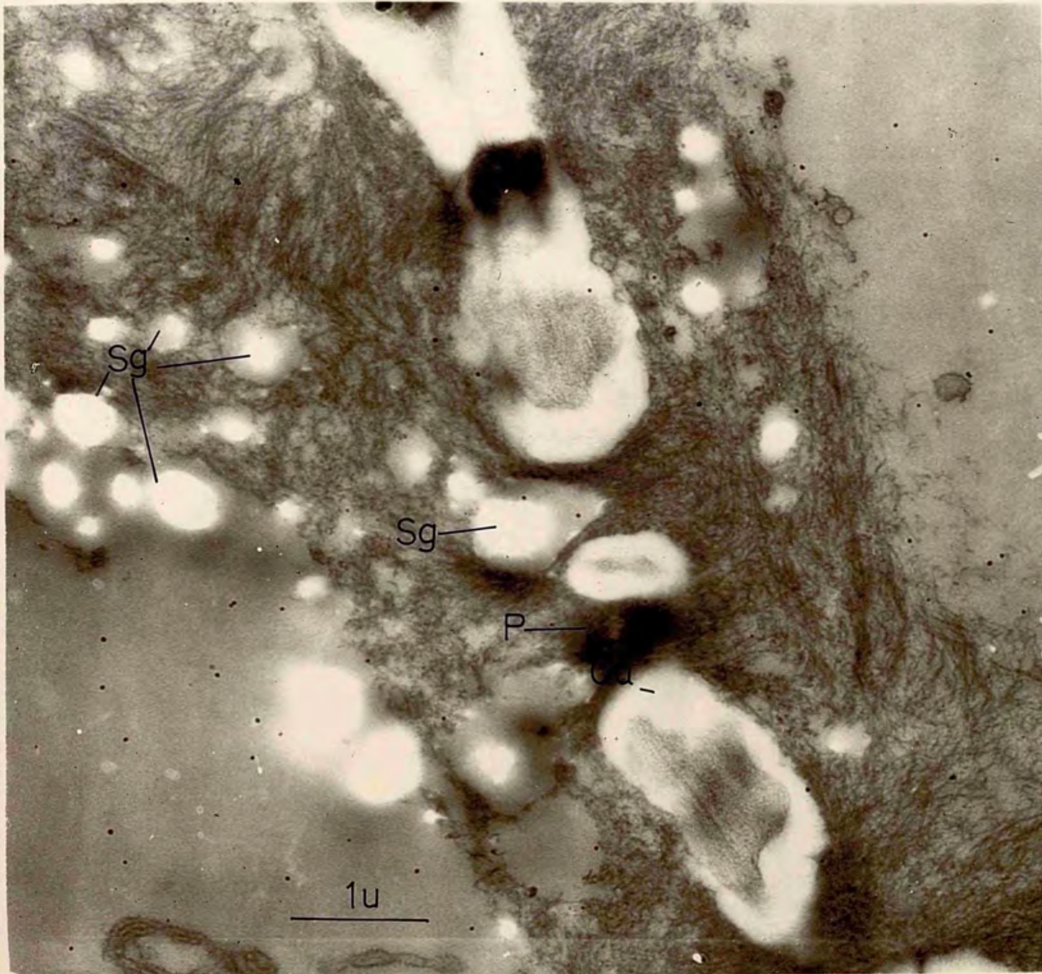
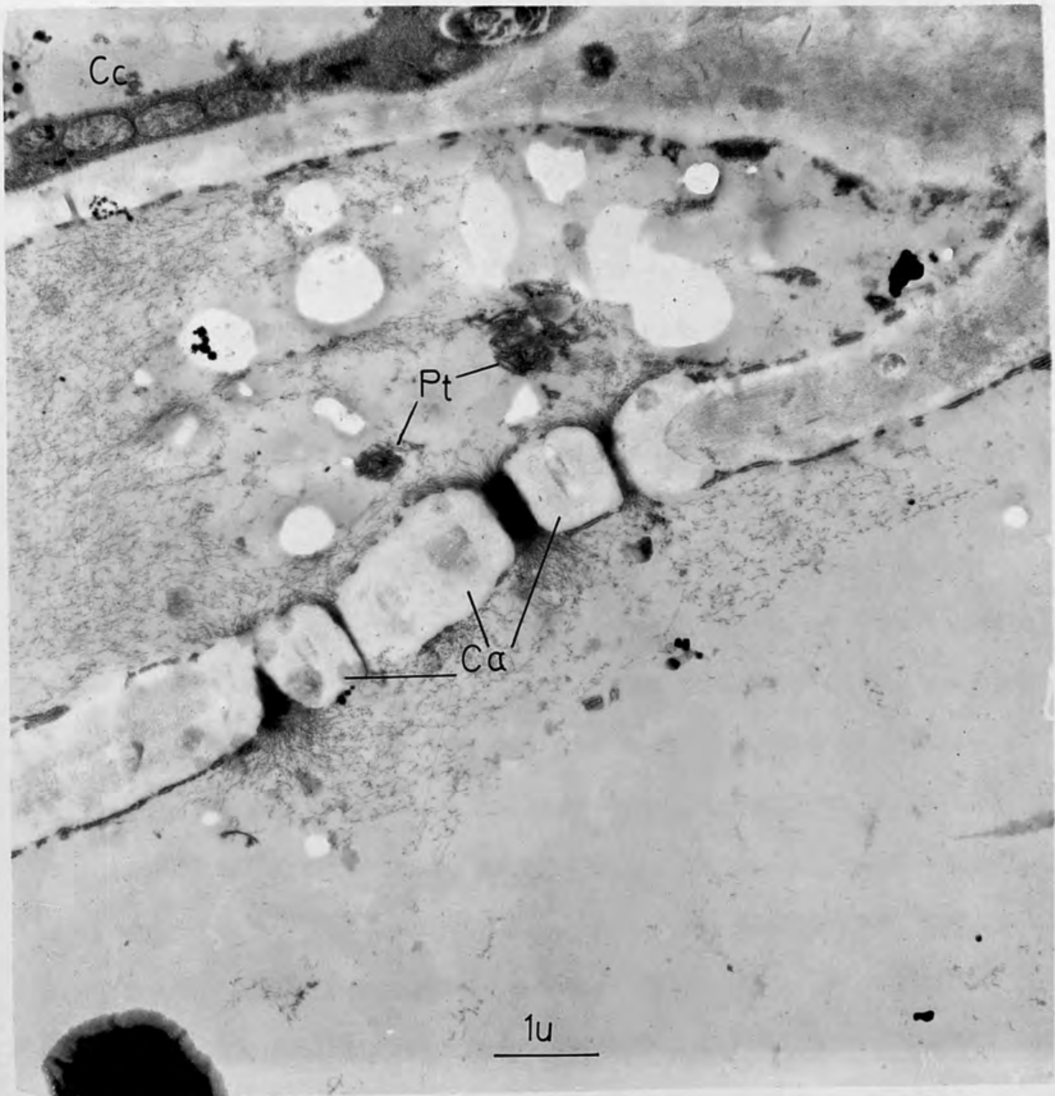

Sg
Sg
P
1u

PLATE 66

T.L.S. through a sieve tube, showing the sieve plate. Note the callose on the sieve plate and two apparently degenerating plastids. Stained with lead citrate, uranyl acetate and lead citra te. X15,000.


Cc
Pt
Ca
1u

to line the pores through the sieve plate and is continuous from one sieve tube to the next. (Plates 67, 69 70 and 71).

Tonoplast

On the moot point as to whether or not a tonoplast still exists in the functioning cell the present results throw little light. Most authorities agree that it does not; the use of the term "mictoplasm" presupposes this view. However Tamulevich and Evert (1966) have lately suggested that in Primula there is a membrane "which apparently separates parietal cytoplasm from the central cavity". Most micrographs in the present study support the view that the tonoplast has gone. The parietal organelles seem to have no membrane covering, and the slime fibrils penetrate well into the cell lumen (Plates 46, 52, 56 and 79). However there are cases where one cannot be so dogmatic (Plates 72 and 73). A priori it would not be surprising if in the general disorganisation and dismembranement of a fairly stable structure like the tonoplast fragments were left like flotsam in the cell lumen. Plates 45 & 48 may easily illustrate this possibility.

<u>PLATE 67</u>

T.L.S. showing a portion of a sieve plate. Note the plasma membrane lining the pore and a membranous aggregate. Stained with lead citrate, uranyl acetate and lead citrate. X60,000.

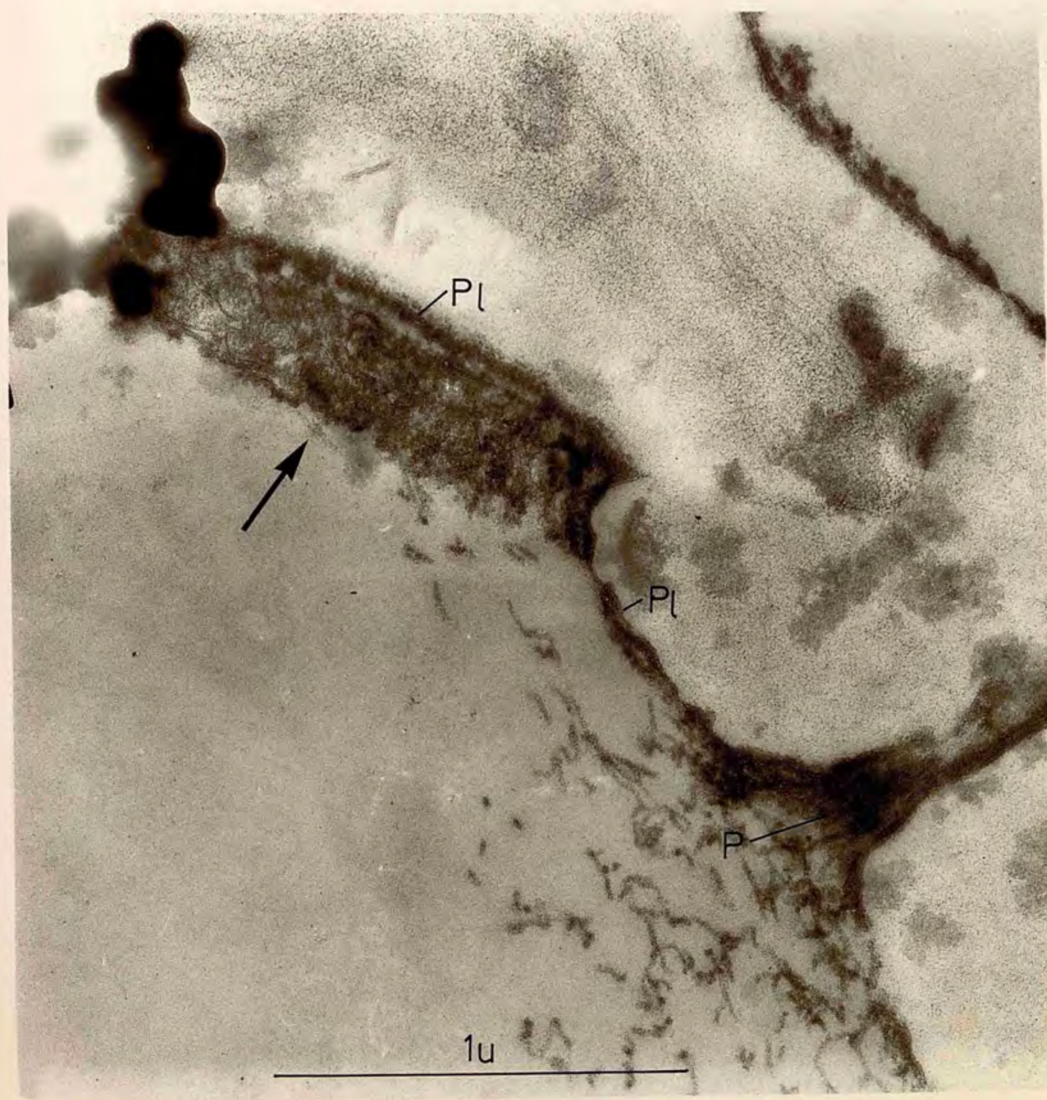
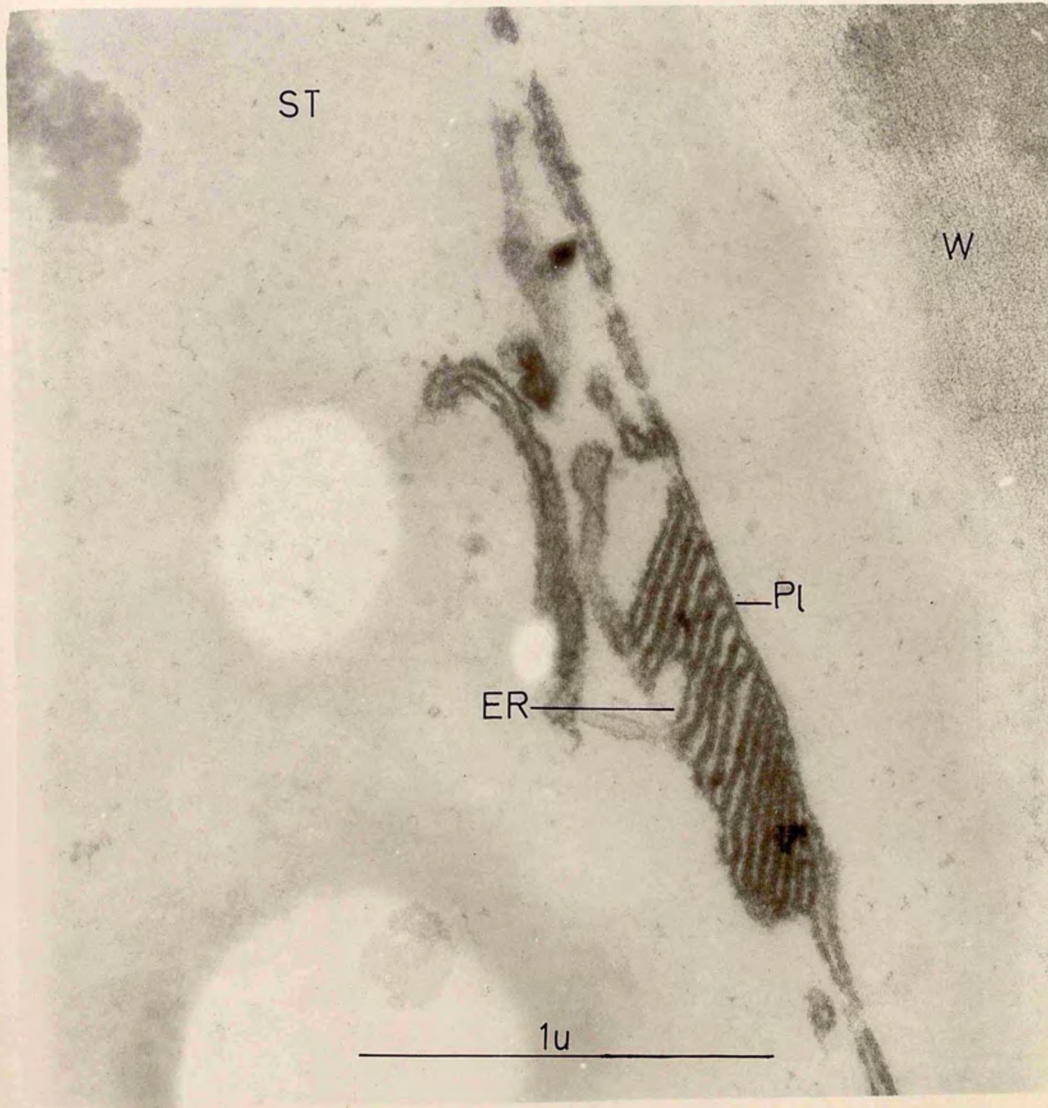

Pl
Pl
P
1u

PLATE 68

T.L.S. through a sieve tube wall. Plasmalemma is clearly seen with the trilaminar tubular structure possibly ER. Stained with lead citrate, uranyl acetate and lead citrate. X60,000.

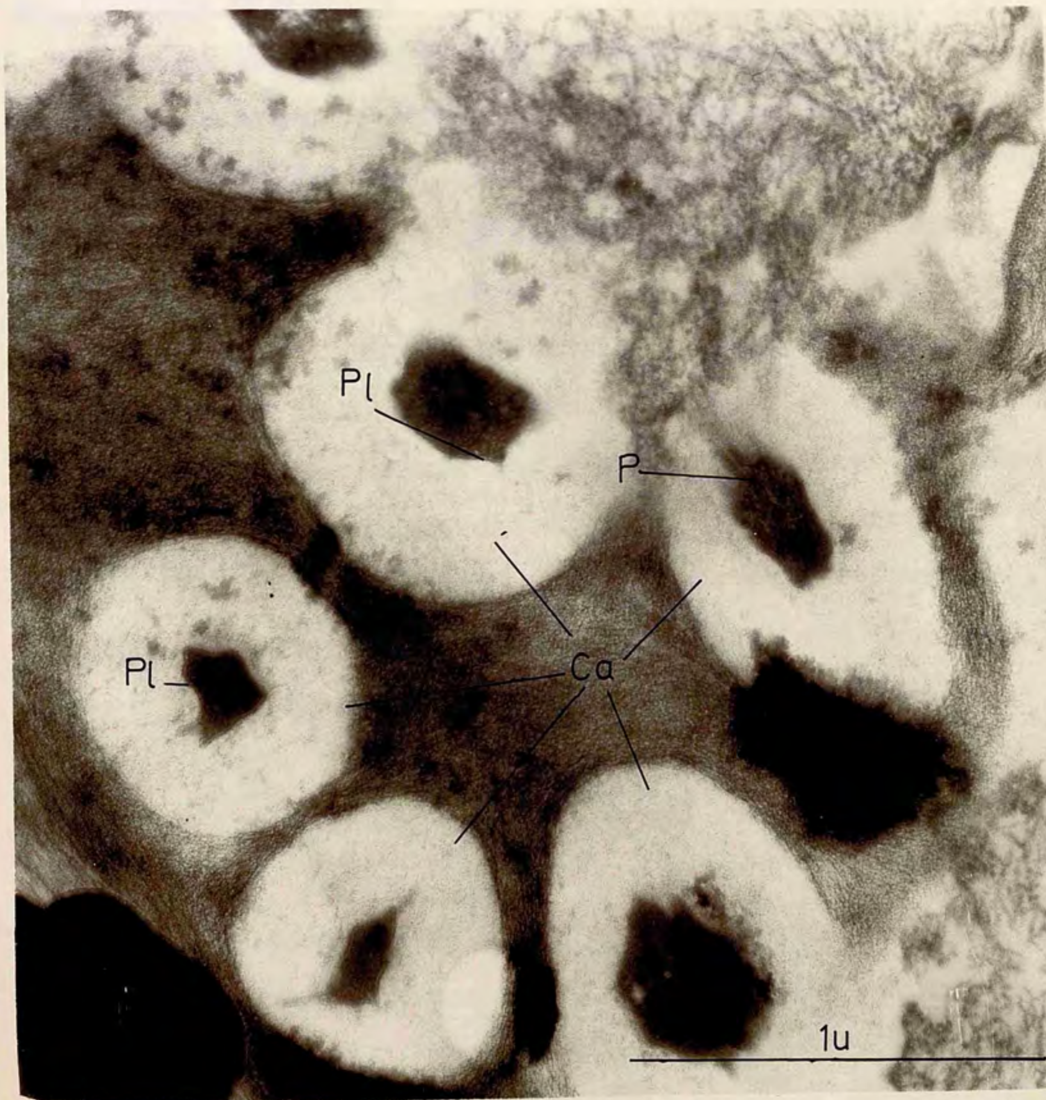


68

PLATE 69

Transverse section through a sieve plate. Note the plasma membrane lining the pores and the callose cylinders. Stained with lead citrate, uranyl acetate and lead citrate. X60,000.

198



Endoplasmic reticulum

Under this heading will be discussed a number of features revealed by the micrographs the nature of which is a little uncertain. It can be stated at once that in the mature sieve tube of Salix capraea the endoplasmic reticulum is not at all conspicuous. In a typical form the method of fixation and staining employed shows it fairly readily in the companion cells (Plates 41,51& 59) so that it should be revealed in the sieve tubes if present in them. Occasional traces only are found, however (Plates 55,68,74,77,78 &79). No such extensive developments as were shown in P i s u m (Bouck and Cronshaw,1965) or in Acer (Northcote and Wooding, 1966) were ever found, probably due to the fact that these authors were able to investigate ontogenetic sequences and so picked up earlier stages.

A considerable number of cases of what has been interpreted as rather non-typical endoplasmic reticulum were however found. Sometimes this was in the form of what appeared to be vesicles within vesicles (Plates 46,51 87 and 89), though this might possibly be due to remnants of other organelles, such as dictyosomes, though these commonly have disappeared by this stage. Other

PLATE 70

A portion of the Plate 66 has been magnified to show the sieve pore, plastids and the plasma membrane. X50,000.

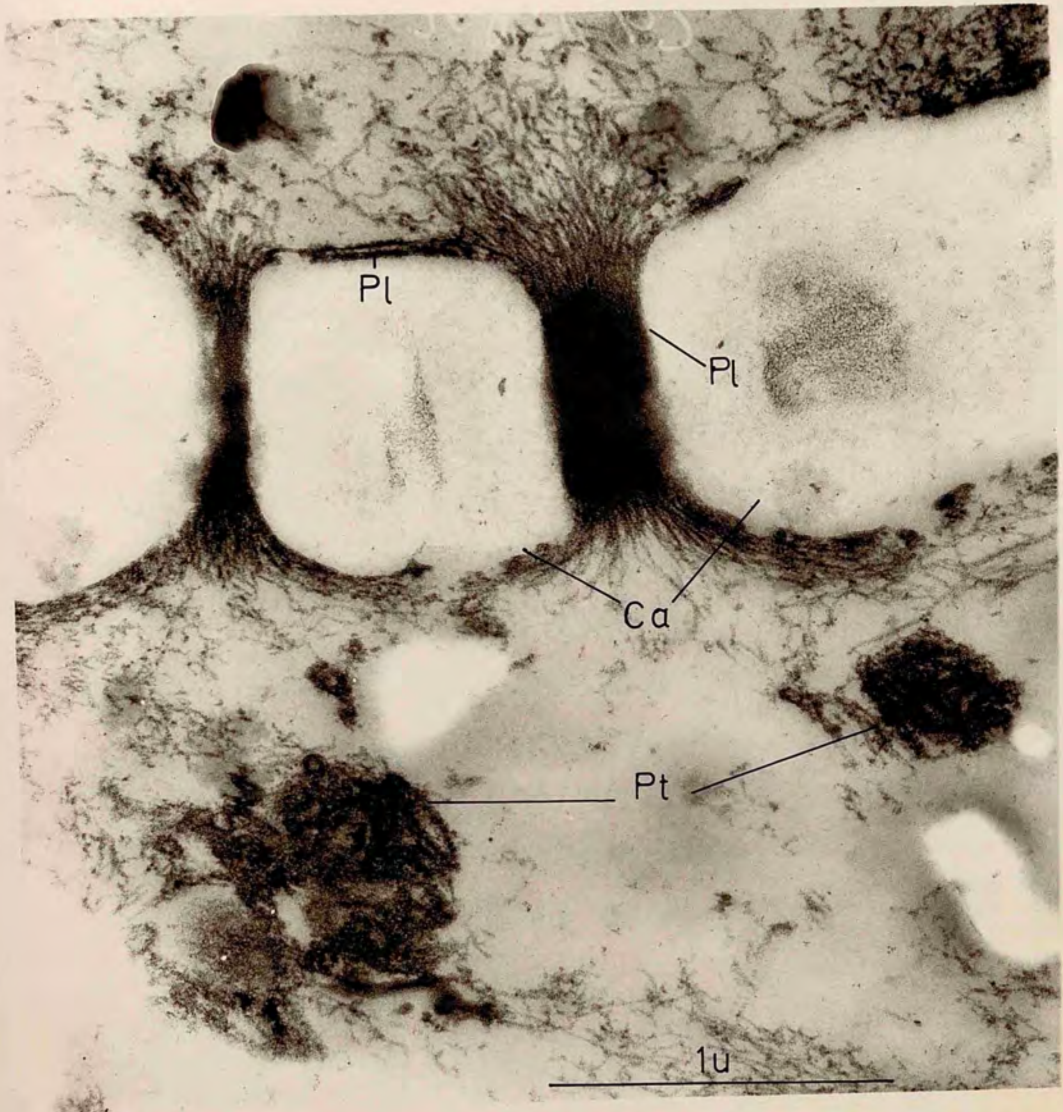

Pl
Pl
Ca
Pt
1u

PLATE 71

T.L.S. of a sieve tube
showing a portion of the
sieve plate lined with
plasmalemma. Note the
callose, banded fibrils
and possibly a plastid.
Stained with lead citrate,
uranyl acetate and lead
citrate. X40,000.

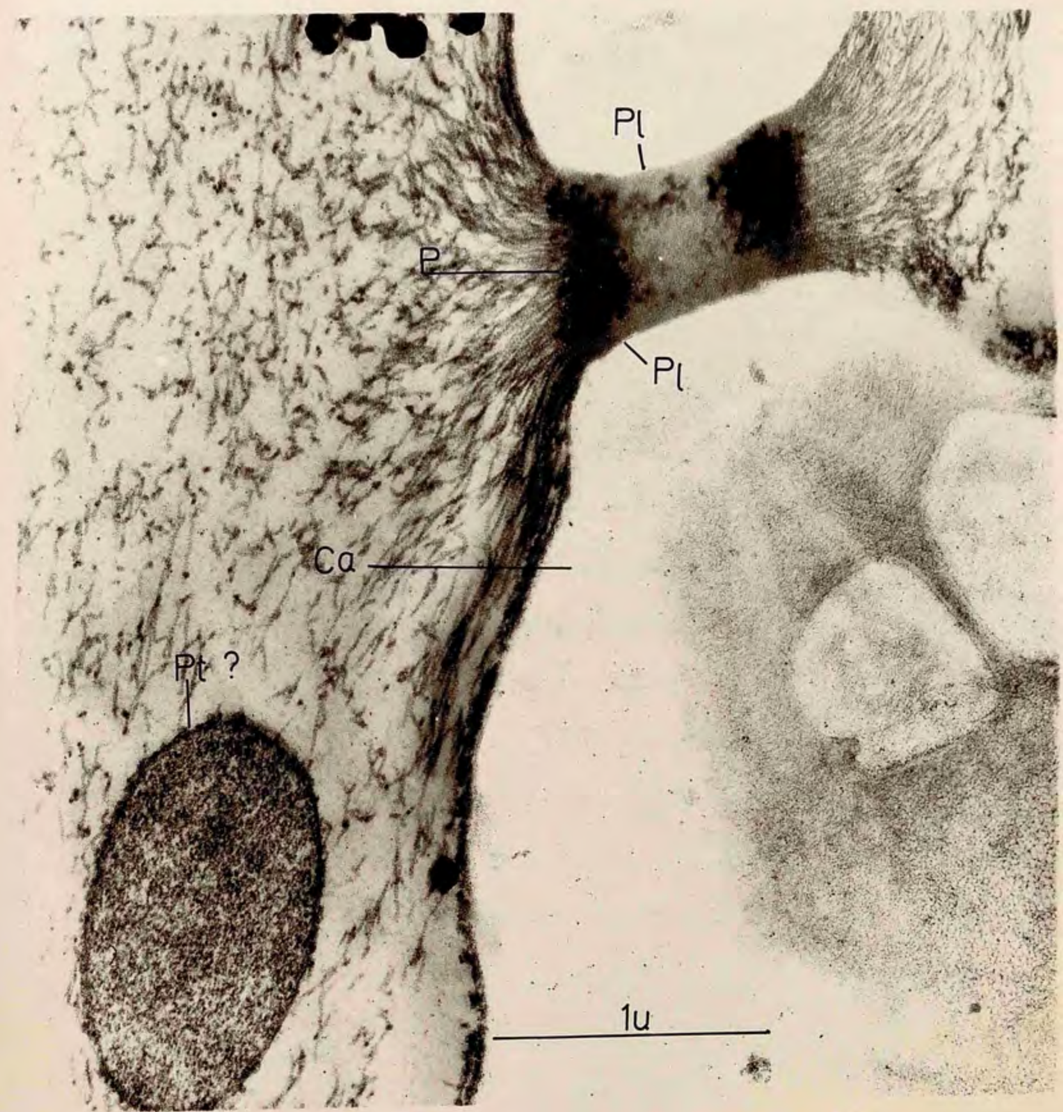
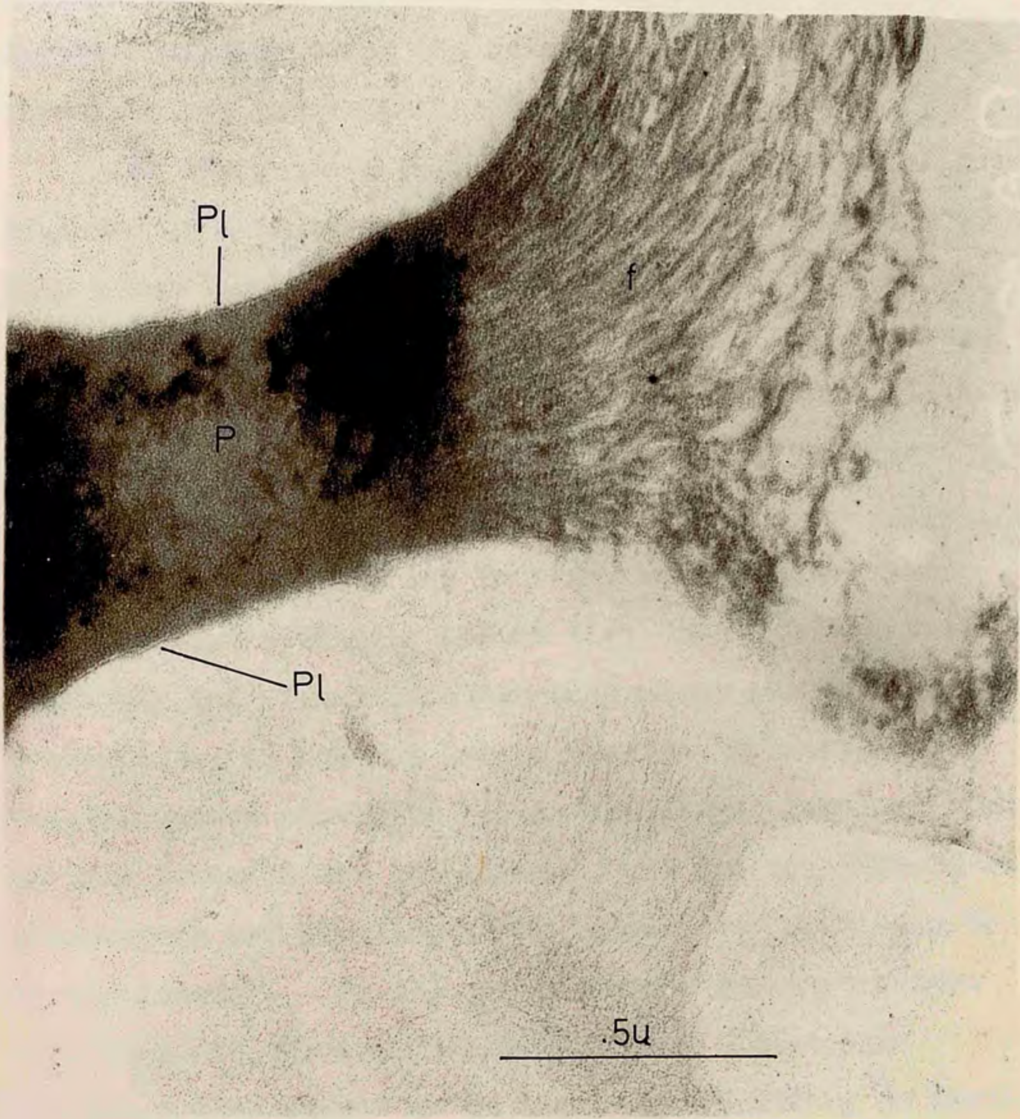

Pl
P
Pl
Ca
Pt ?
1u

PLATE 71a

A portion of Plate 71
has been enlarged to
show the banded fibrils
in the pore. X80,000.



71a

workers have found similar images in mature sieve tubes (Buvat, 1963 d ; Bouck and Cronshaw 1965) and identified them with the endoplasmic reticulum.

At other times what appeared to be reticulum occurred as short lamella stacked in formations more or less perpendicular to the sieve tube wall (Plates 40,46,56, 57,68 & 74) or parallel to it (Plates 46,55,57,74,77). These are rather similar to images found by Tamulevich and Evert (1966) in *Primula obconica* by Northcote and Wooding (1966) in *Acer pseudoplatanus* and by Johnson (1967) in *Nymphoides peltatum*. Northcote and Wooding, referring to these lamellar stacks in *Acer* remark that "Although the lamellar stack has individual lamellae equivalent in width to the plasmalemma, it has not proved possible to resolve any triple-layered structure in the individual lamellae such as that found in the plasmalemma, and the two structures, plasmalemma and lamellar stack, have never been seen to be continuous". In the present study the stacks have been found to possess roughly the same dimensions (80-90A x 150A apart) as in *Acer*; but contrary to Northcote and Wooding's results the laminae have been found to

PLATE 72

T.L.S. through s sieve tube and a parenchyma cell, showing a tonoplast-like structure extending into the cell lumen. A portion of Plate 45 magnified. X30,000.

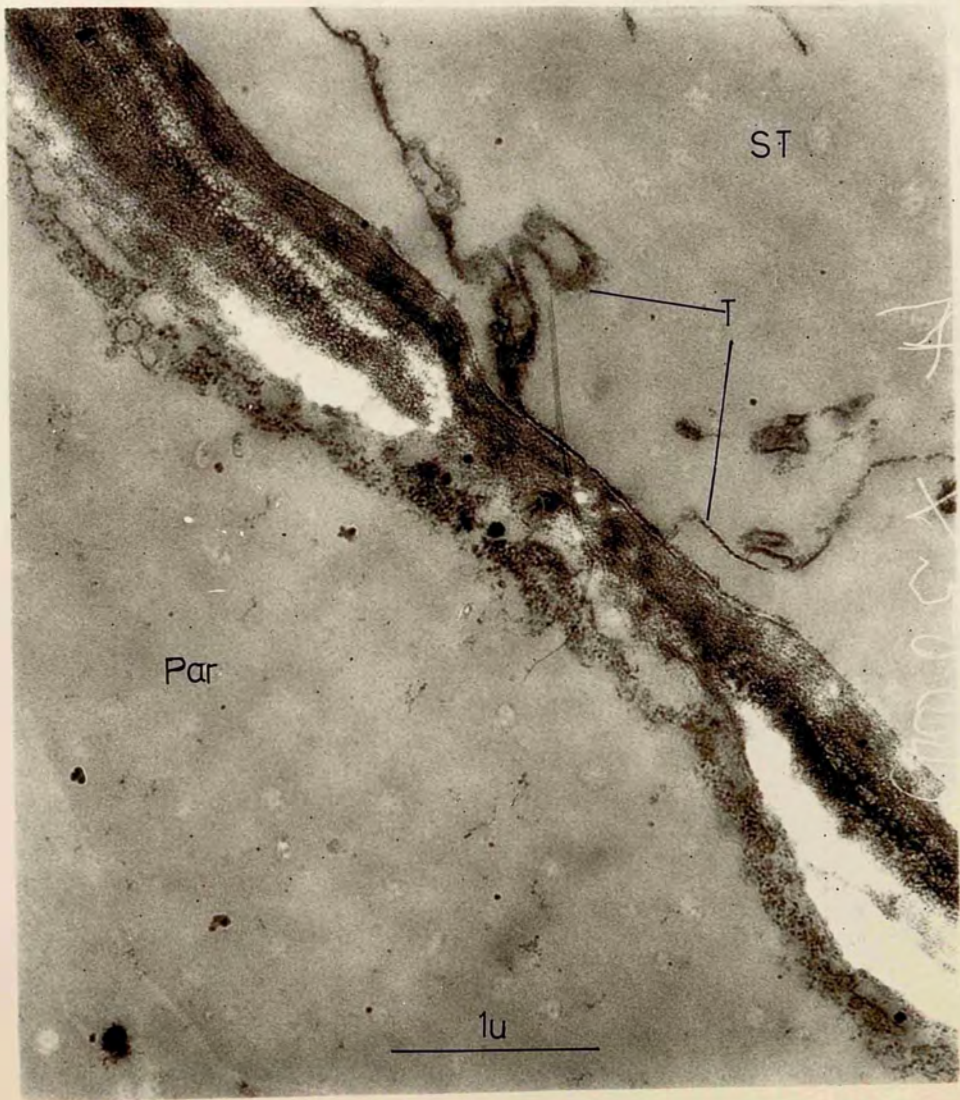
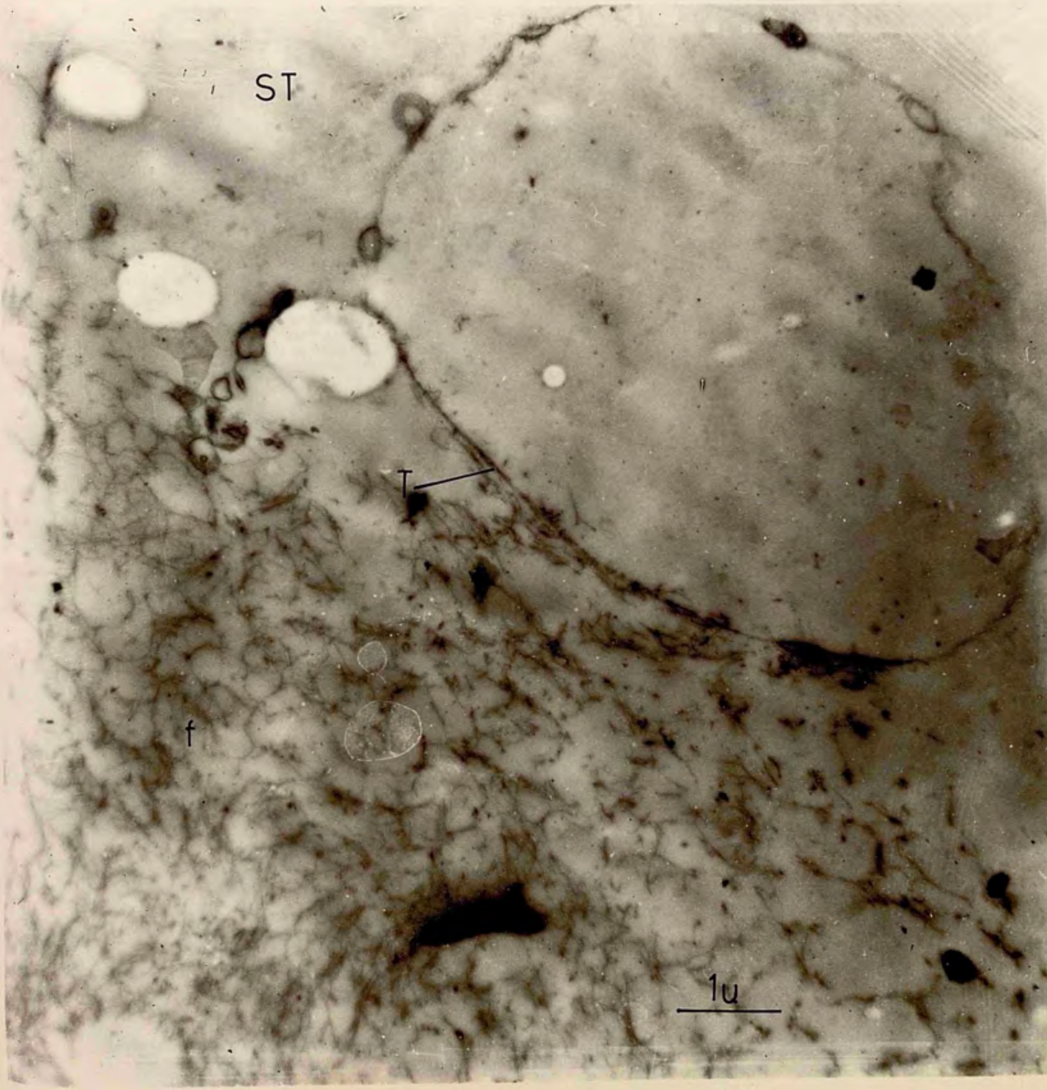

ST
T
Par
1u

PLATE 73

T.L.S. through a sieve
tube showing slime fibrils
and a tonoplast-like structure.
Stained with lead citrate.
X15,000.


ST
T
f
1u

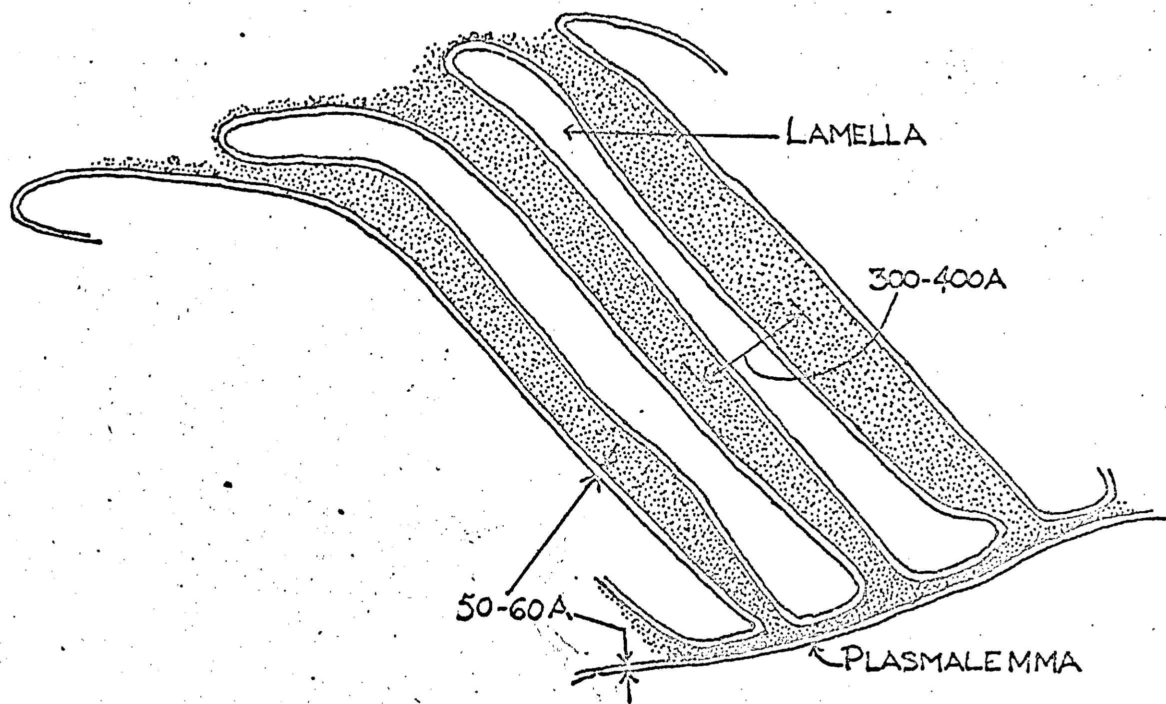


FIG 9

FIG.9 - Tri laminar lamellar body has been shown diagrammatically.

possess a trilaminar structure very similar to that of the plasmalemma (Plates 68,74,77). Whether this is ever continuous with the plasma membrane is difficult to say, but the detailed structure appears to be as shown diagrammatically in Figure 9. At one point only is there a suggestion of continuity with the plasmalemma, and this is not unambiguous. Plate 74 clearly shows a similar structure. It raises difficulties however with the previous interpretation since some of the paler areas seem to lie outside the membrane of the lamellae, in particular the two moon-shaped ones. This may perhaps be due to rupture during preparation. Some of the numerous small circular areas in the micrograph appear to be bounded by a plasmalemma-like membrane. They may be sections of plasmodesmata, though their direction does not seem to fit in with this suggestion - in view of the position of the wall surface one would have expected them to have shown a very elliptical section. It may be that they are isolated endoplasmic tubules though this again seems unlikely.

Whether these stacks of lamellae fulfil any vital function in the functioning sieve tube, or whether they

PLATE 74

T.L.S. through a sieve tube wall showing the tubular tri-laminar structure. Stained with lead citrate, uranyl acetate and lead citrate. X60,000.

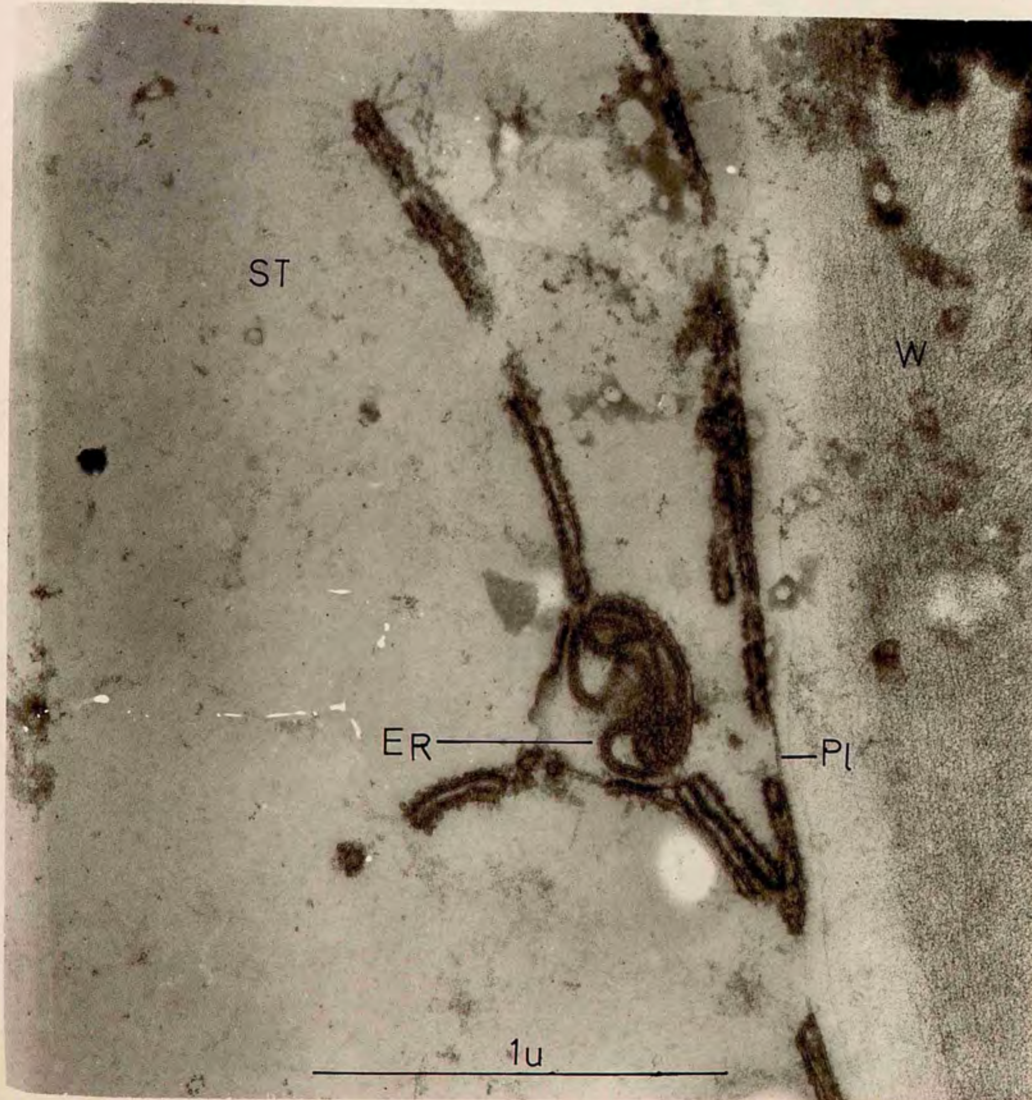
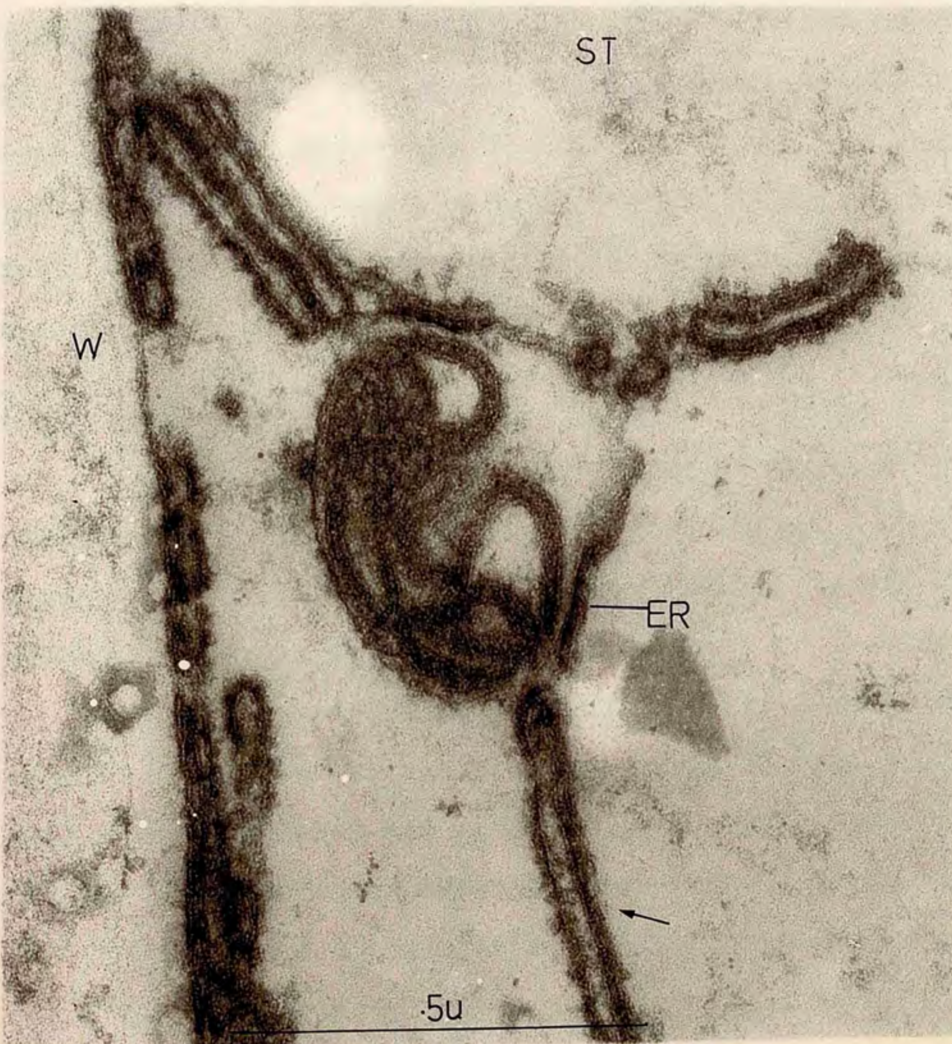

ST
W
ER
Pl
1u

PLATE 74a

A portion of plate 74 has been enlarged to show the trilaminar structure. Taken at X30,000, enlarged 4 times X120,000.



74a

are to be regarded as just a natural physicochemical rearrangement of fairly stable membrane detritus released by the obsolescence of the nuclear membrane and endoplasmic reticulum it is impossible to say. Plate 68 and Figure 9 indicate that dark material occurs external to the membrane-bound lamellae. Conceivably it might be in process of manufacture and extrusion into the cell lumen.

Tubular elements

Many workers have found evidence of tubular structures of various kinds in the sieve tube. Thus in *Nymphoides* (Johnson, 1967); in *Acer* (Northcote and Wooding, 1966); in *Pinus pinea* (Wooding, 1966).

In many cases these tubular elements formed very regular, almost crystalline arrays. In the present study none of these arrays has been found, possibly because young stages in the ontogeny have not been investigated. Possibly also for the same reasons no cases have been observed of Ledbetter and Porter's (1963) microtubules (cf. Plate 76)

PLATE 75

T.L.S. through a sieve plate
showing tubular structures
and electron transparent regions
probably callose inside the pore.
Stained with lead citrate,uranyl
acetate and lead citrate.X60,000.

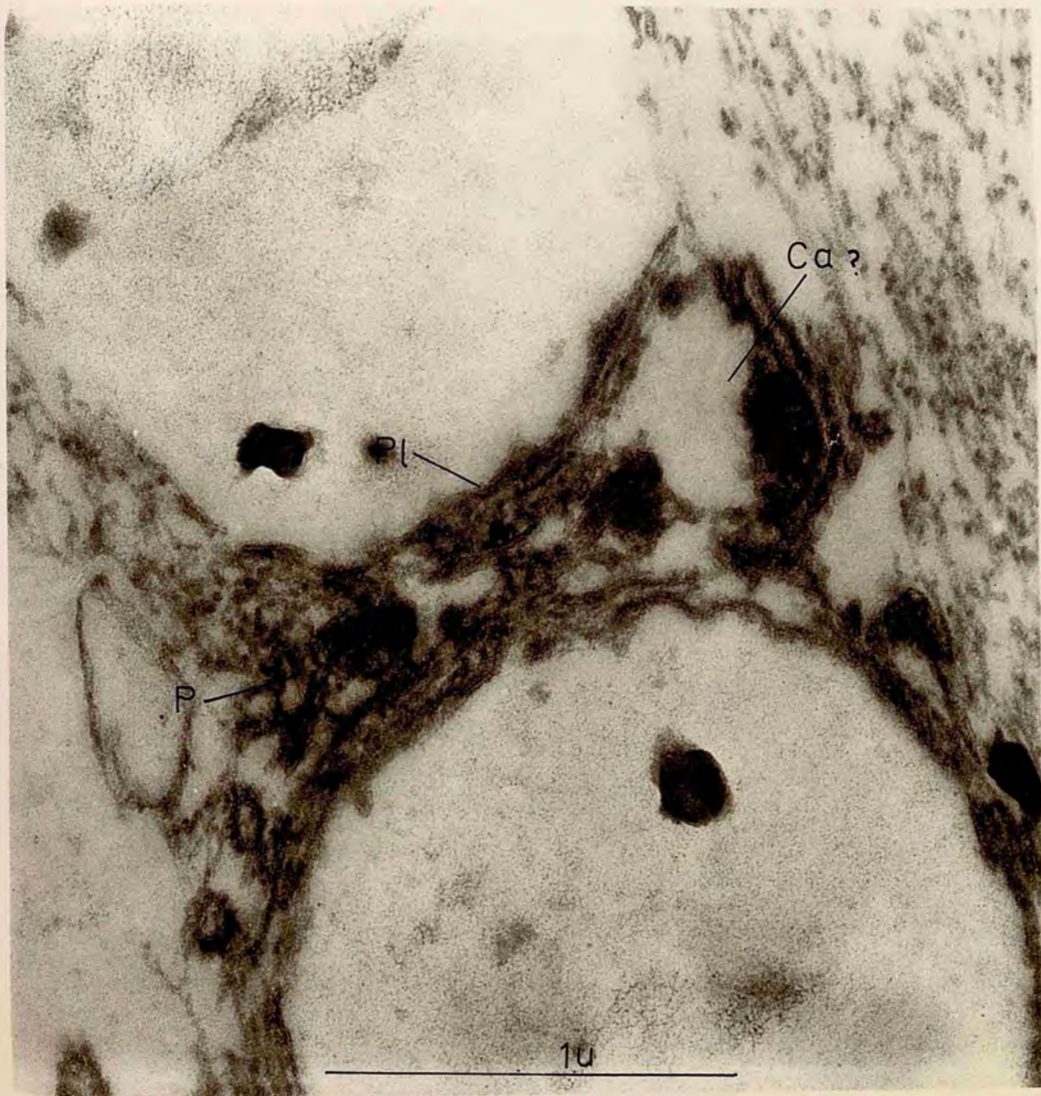

Ca ?
Pl
P
1u

PLATE 76

An oblique longitudinal section through a sieve tube showing tubular structure possibly micro-tubles (Mt). Stained with lead citrate, uranyl acetate and lead citrate. X60,000.

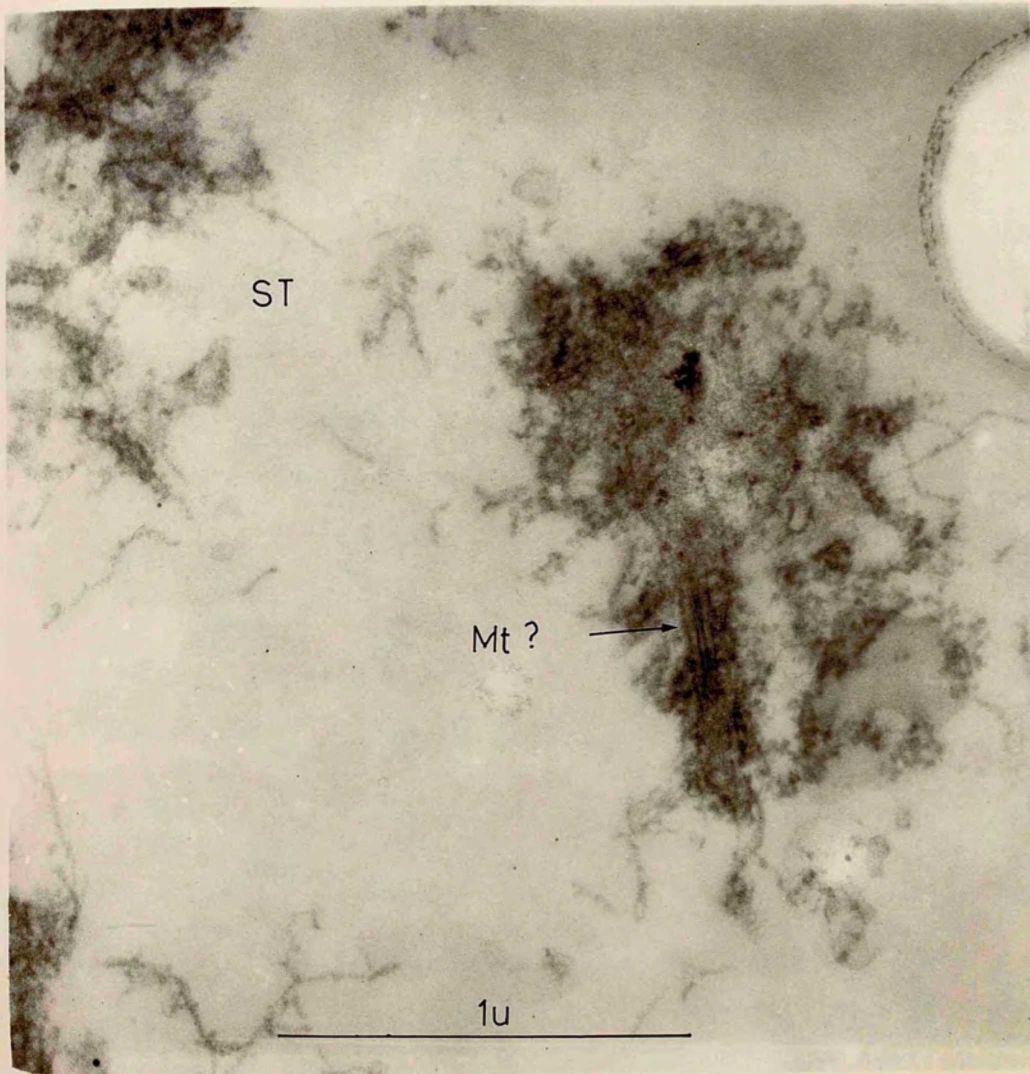
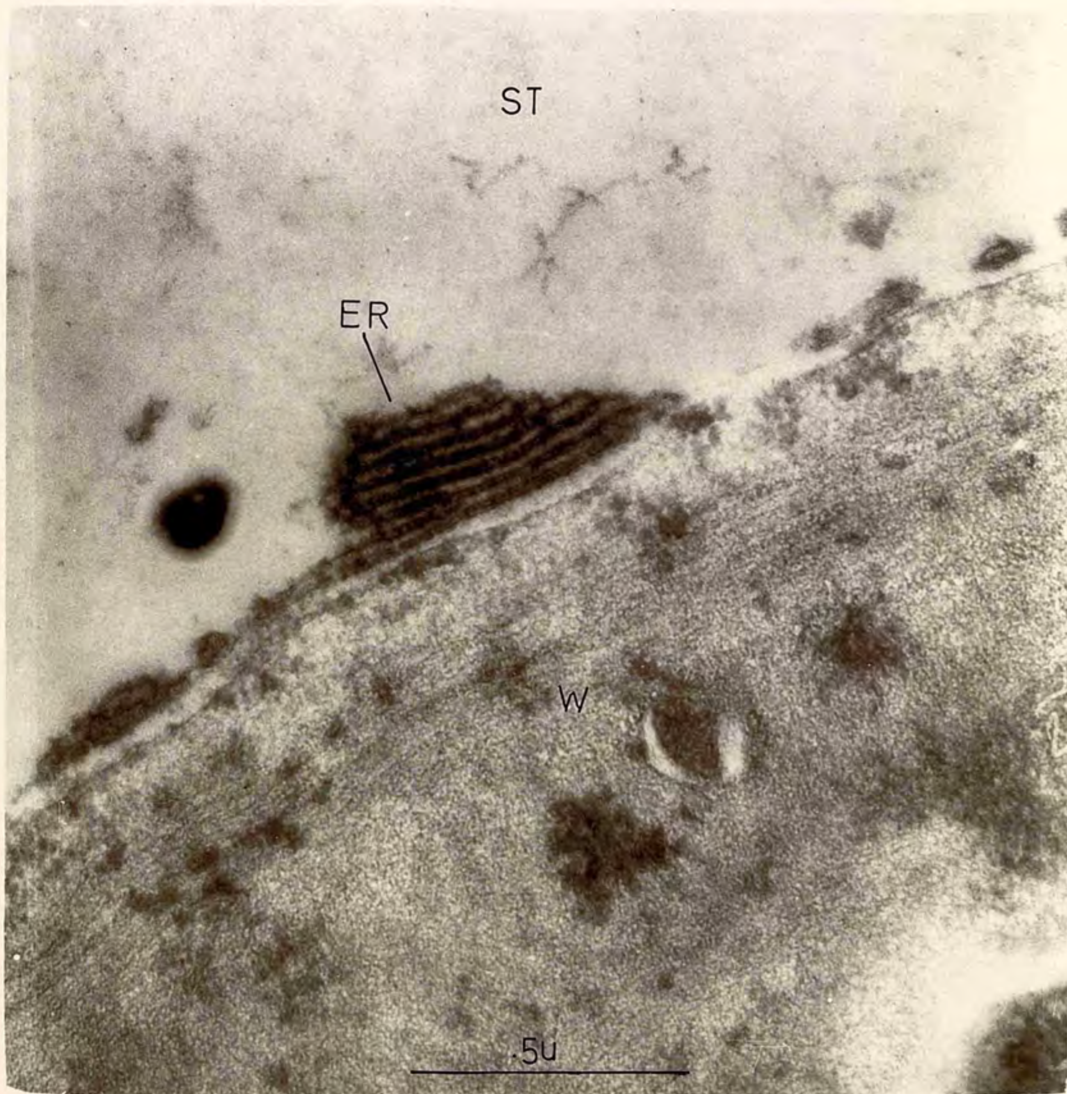

ST
Mt ?
1u

PLATE 77

T.L.S. through a sieve tube wall showing trilaminar tubular structure possibly ER. Stained with lead citrate, uranyl acetate and lead citrate. X80,000.



77

PLATE 78

An oblique longitudinal
section through a sieve
tube showing the tubular
structure (ER). Stained
with lead citrate, uranyl
acetate and lead citrate.
X40,000.

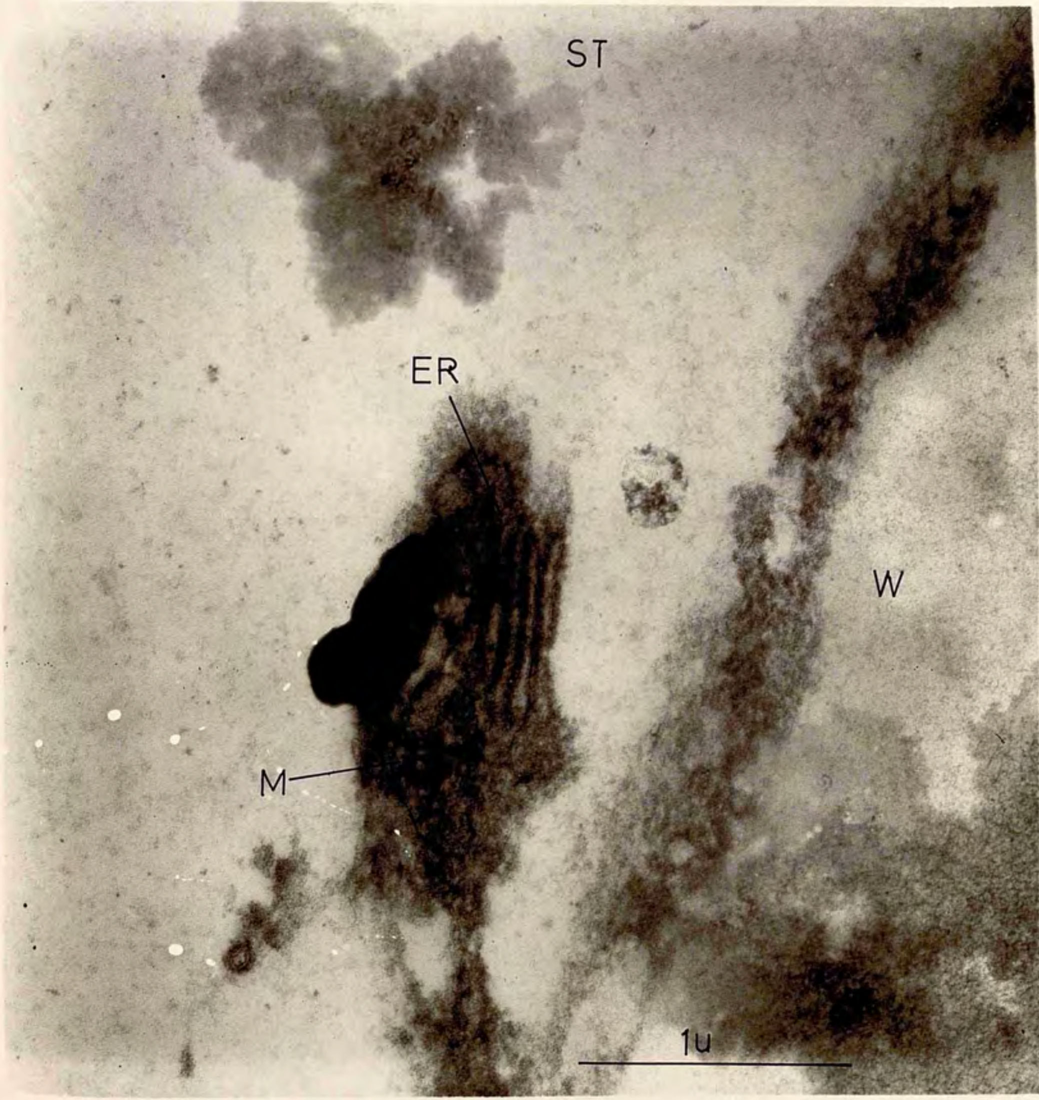
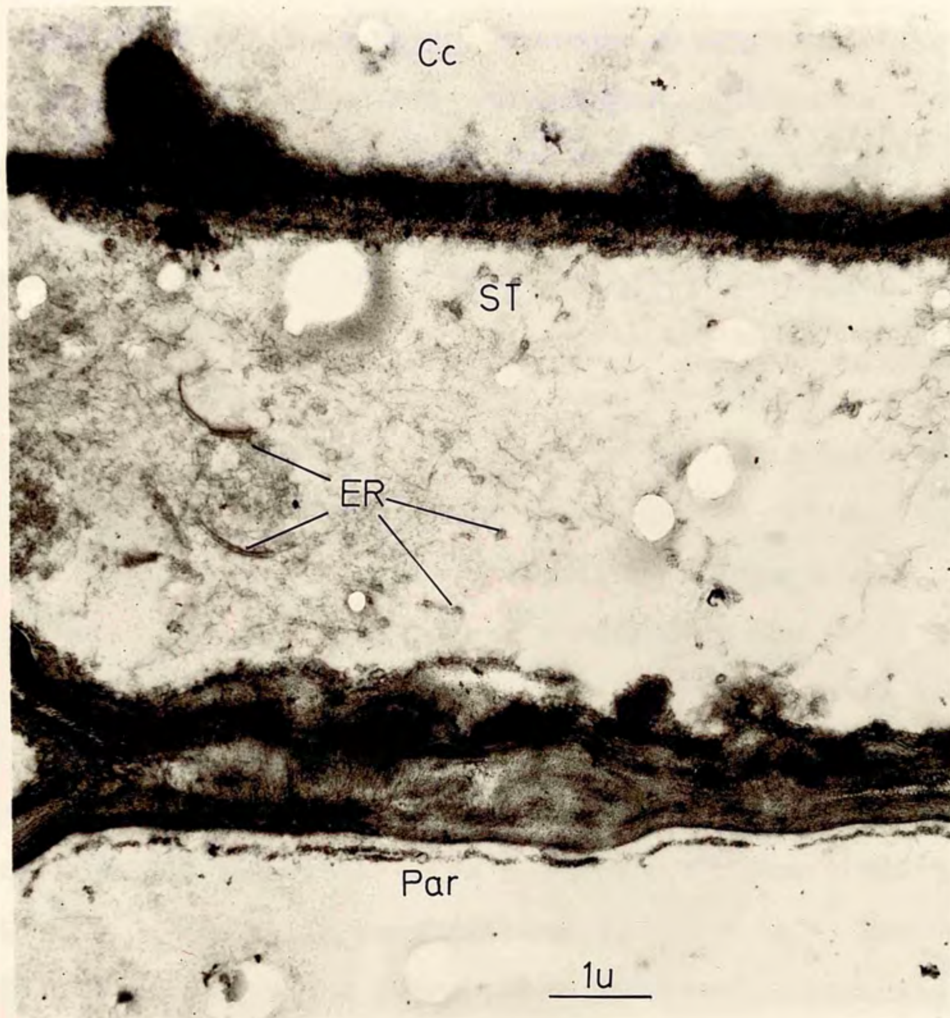

ST
ER
W
M
1u

PLATE 79

Longitudinal section showing a sieve tube, companion cell and a parenchyma cell. Note the slime fibrils and ER in the sieve tube. Stained with lead citrate, Magnesium uranyl acetate and lead citrate. X15,000.

227



THE SIEVE PLATE

The sieve plate in Salix is compound and very oblique. This is shown in the optical micrographs and also in Plates 35, 80, 81, 84 & 85. The last two micrographs indicate one of the broader cellulose regions which gives the plate its compound nature. In the present series of micrographs the sieve pores always show a very considerable amount of callose, probably due to the fact that the tissue was first fixed in glutaraldehyde alone for a period of an hour to four hours, and this fixative with its gentle action is known to permit enzyme action. Plates 35, 66, 67 & 81 show the callose in longitudinal sections of the pores and Plates 50 & 69 in transverse sections. There seems every reason to believe that much, if not most of the callose shown lining the pores in these micrographs was deposited as a result of the preparative techniques. Sometimes the callose is markedly layered (Plates 42, 80 & 81) though it is possible that the outer paler "callose" represents a shrinkage of the protoplast from the wall. This interpretation does not seem quite certain however (especially in Plate 42) and if it is not accepted it leaves open the question as to the difference between the two callose layers.

PLATE 80

T.L.S. through a sieve tube showing a compound sieve plate. Note the callose deposition on the sieve plate. Stained with lead citrate, Magnesium uranyl acetate and lead citrate. X10,000.

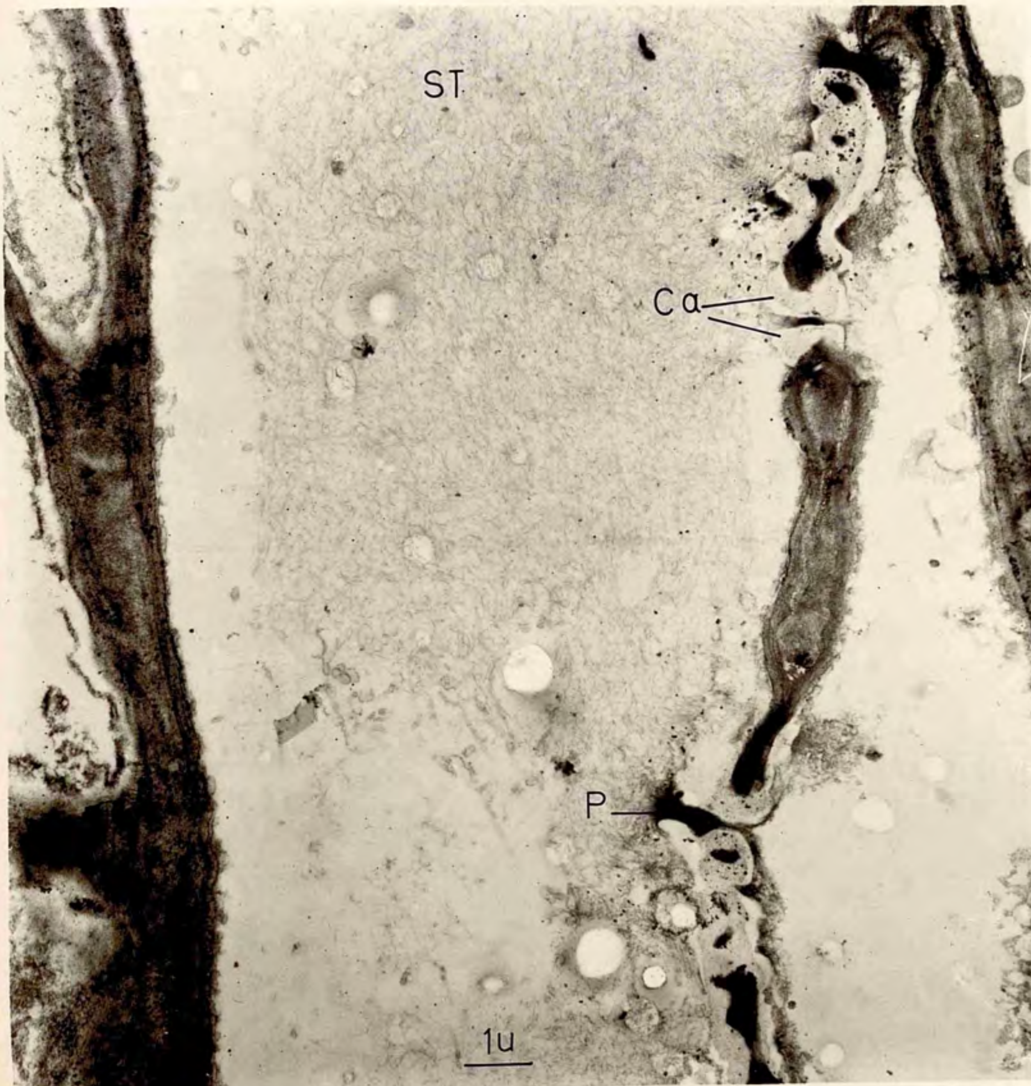
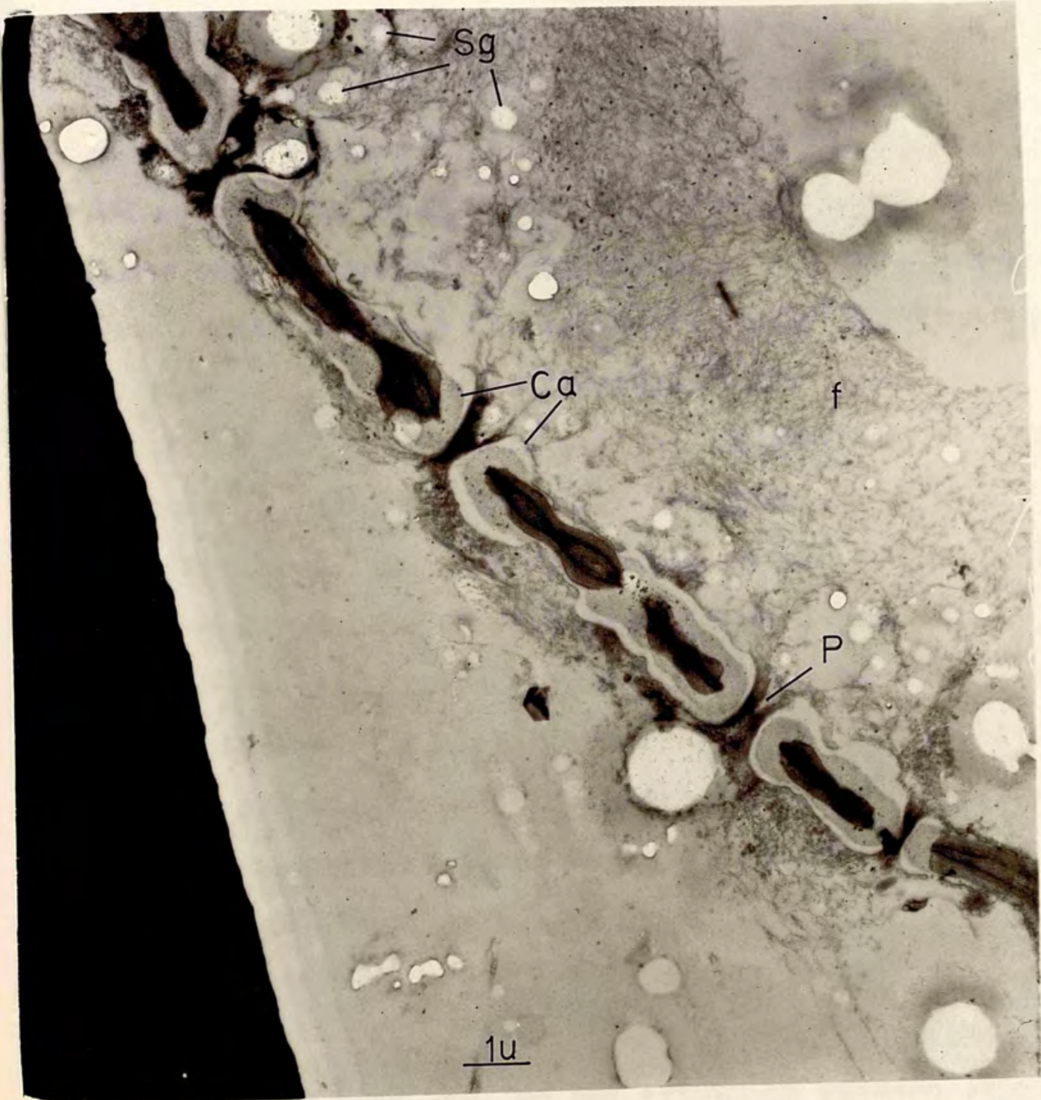

ST
Ca
P
1u

PLATE 81

T.L.S. through a sieve tube showing the compound sieve plate. Note the two (?) distinct layers of callose on the sieve plate. Stained with lead citrate, Magnesium uranyl acetate and lead citrate. X10,000.


Sg
Ca
f
P
1u

One possibility is that one represents the natural callose, and the other that provoked by manipulation; but if so the injury callose has done little to close the pores. Another possibility is that the darker layer nearer the cellulose bars represents injury callose laid down between excision of the specimen and immersion in the fixative (a period of sometimes up to 10 minutes). The paler layer would then be callose laid down in the presence of fixative; the absence of sugar transport and the presence of compressed fibrils in the pore might then restrict the thickness of the further layer laid down within the pore, and account for its narrowness. The callose frequently shows cytoplasmic threads remaining embedded in it (Plates 69 and 83) and occasionally as other workers (e.g. Esau, 1965 (b)) have remarked, the inner contour of the callose cylinder becomes stellate (Plate 69).

Unfortunately time did not permit an investigation of the interesting question of what effect different fixative treatments would have had, on the extent of callose deposition.

The pores of the sieve plate presented a fairly

PLATE 82

T.L.S. through a sieve plate showing only one pore. Note the pore looks almost empty. Stained with lead citrate and uranyl acetate. X60,000.

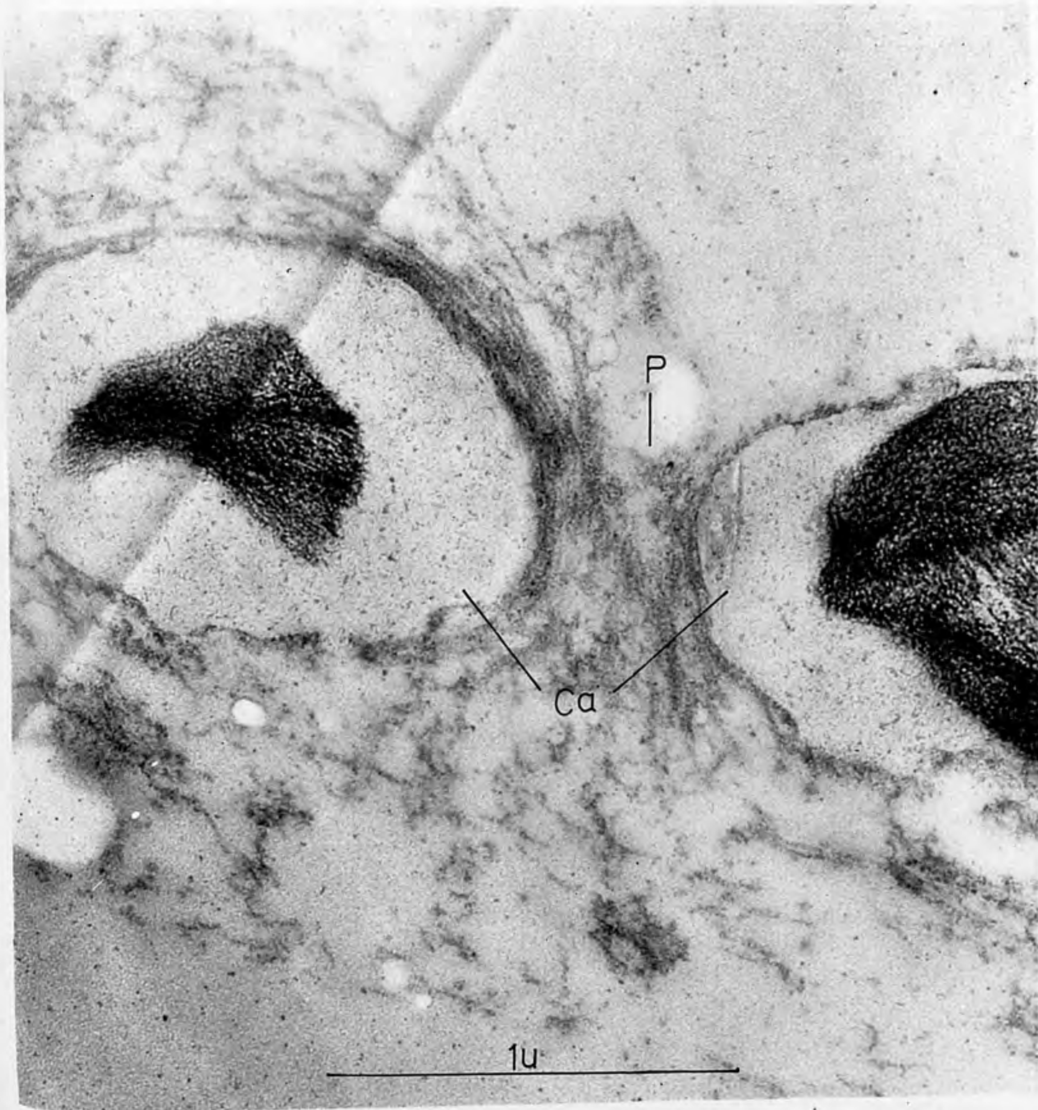

P
Ca
1u

PLATE 83

T.L.S. showing a portion of a sieve plate. Note the callose deposition on the plate. Stained with lead citrate, uranyl acetate. X30,000.

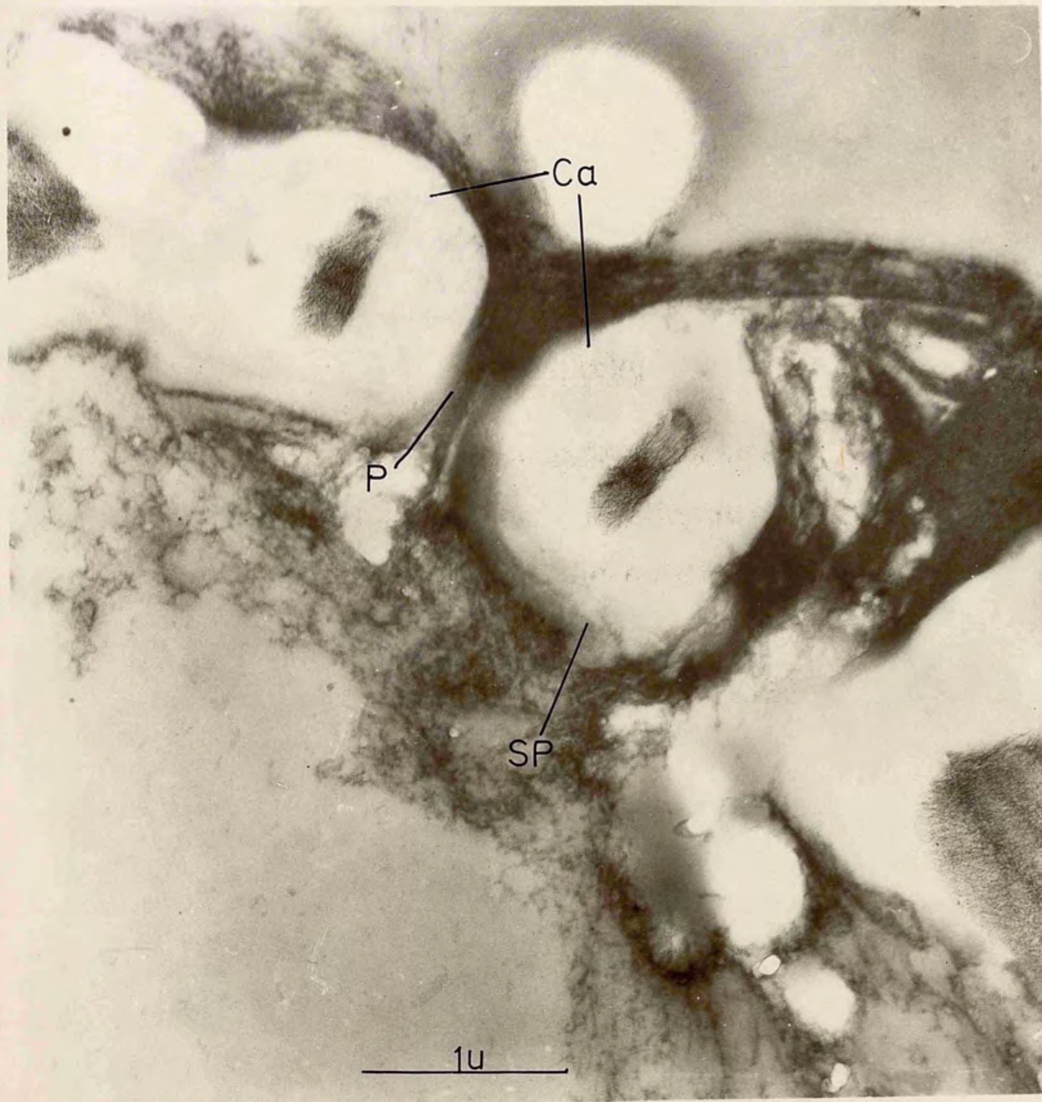

Ca
P
SP
1u

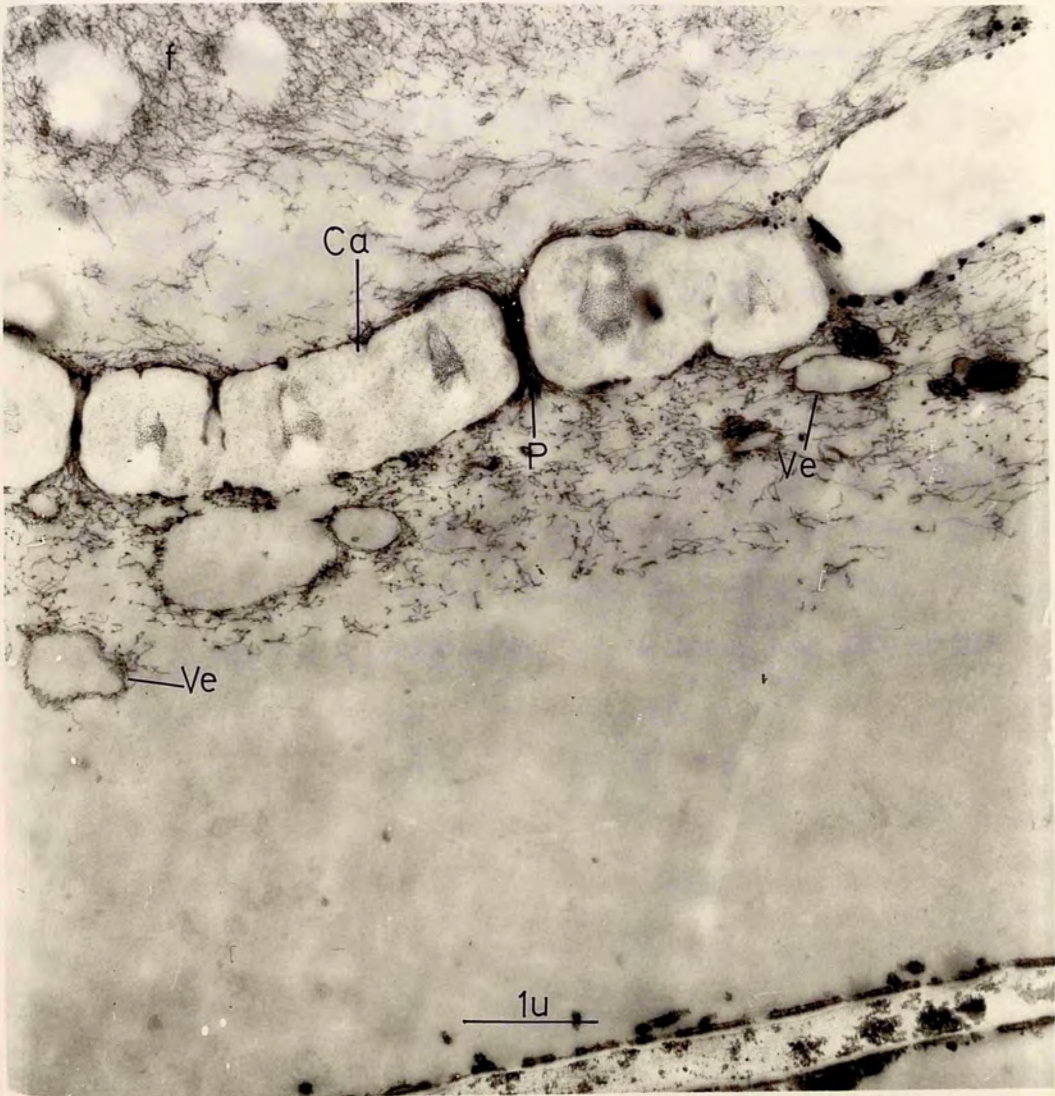
PLATE 84

T.L.S. of a sieve tube showing a
compound sieve plate. Note the
callose deposition on the plate
and the accumulation of slime
fibrils. Stained with lead
citrate and uranyl acetate. X20,000.

f
Ca
P
W
1u

PLATE 85

T.L.S. through a compound sieve plate showing callose and fibrillar slime. Fibrillar nature of slime is very well marked here. Note the small vesicles near the plate. Stained with lead citrate, uranyl acetate and lead citrate. X20,000.


f
Ca
P
Ve
Ve
1u

uniform appearance in this investigation. Almost universally they were shown as filled with electron dense material in the form of fibrils (Plates 35, 42, 54, 66, 67 71). Occasionally the contents appeared less dense (Plate 82), or of rather a different character (Plate 75). In the latter case the plate appears to be immature, and the contents of the pore to include elements of the endoplasmic reticulum. In addition to the fibrillar material (which is further discussed below) the pores are lined with a distinct plasmalemma continuous with that in the lumen of the sieve tube elements (Plates 69, 70 and 71).

The distribution of material between the two sides of the sieve plate is asymmetrical (Plates 35, 66, 80, 81) but not always extremely so (Plates 42 and 85). Sometimes as in Plate 66, the indications as to the previous polarity of movement across the plate seem rather contradictory. Certainly the general impression is that the pores are not occupied by the fibrils merely as a result of their preparative treatment, though the density of their contents may well reflect the artificial induction of callose.

THE SLIME

Probably the most significant constituent of the

PLATE 86

Longitudinal section of a sieve tube showing some banded fibrils. Arrows pointing to the bands which give a striated appearance. Stained with lead citrate, uranyl acetate and lead citrate. X120,000.

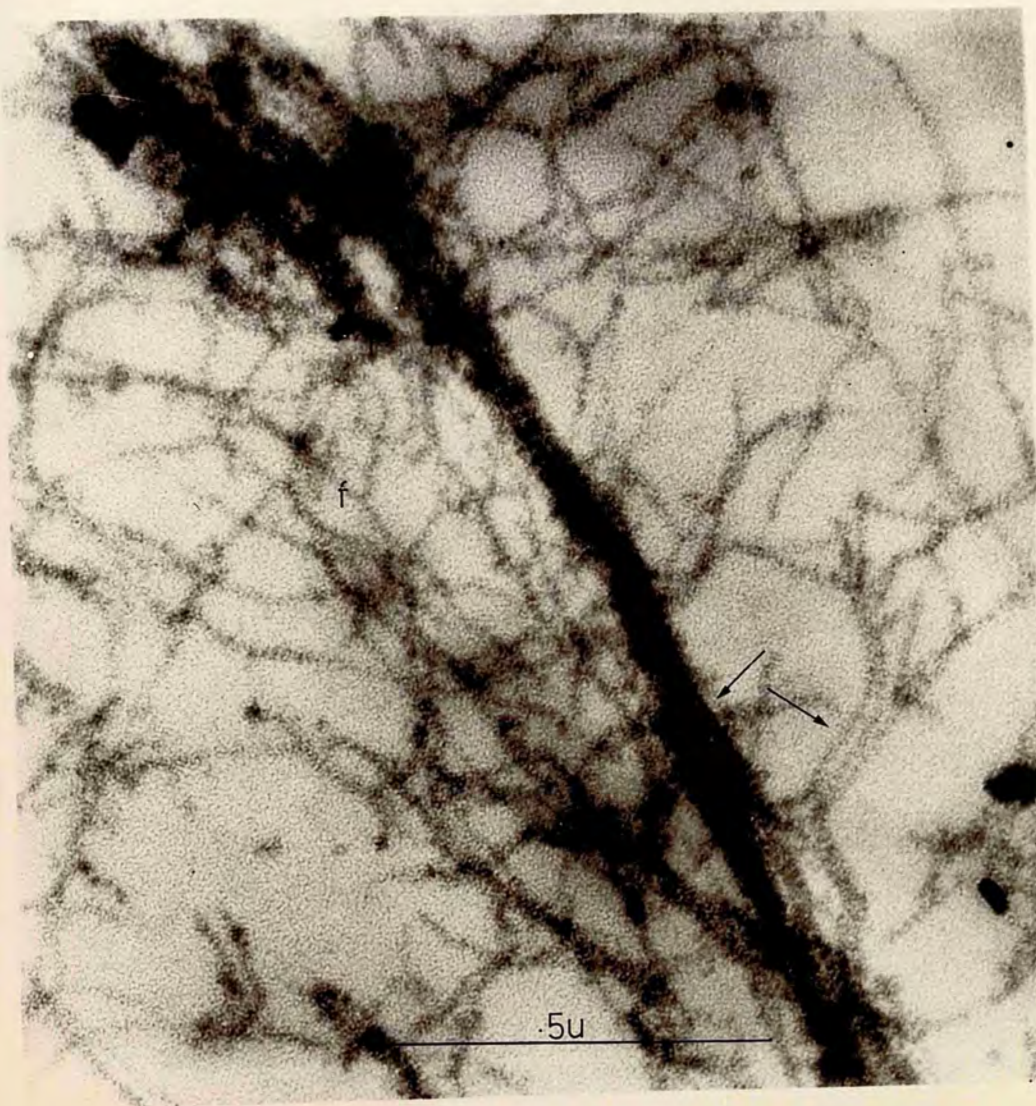

f
5u

PLATE 87

Longitudinal section of a sieve tube showing some banded fibrils and a coil like body, possibly ER. Note the larger slime which possibly frays out into smaller fibrils. Stained with lead citrate, uranyl acetate and lead citrate. X120,000.

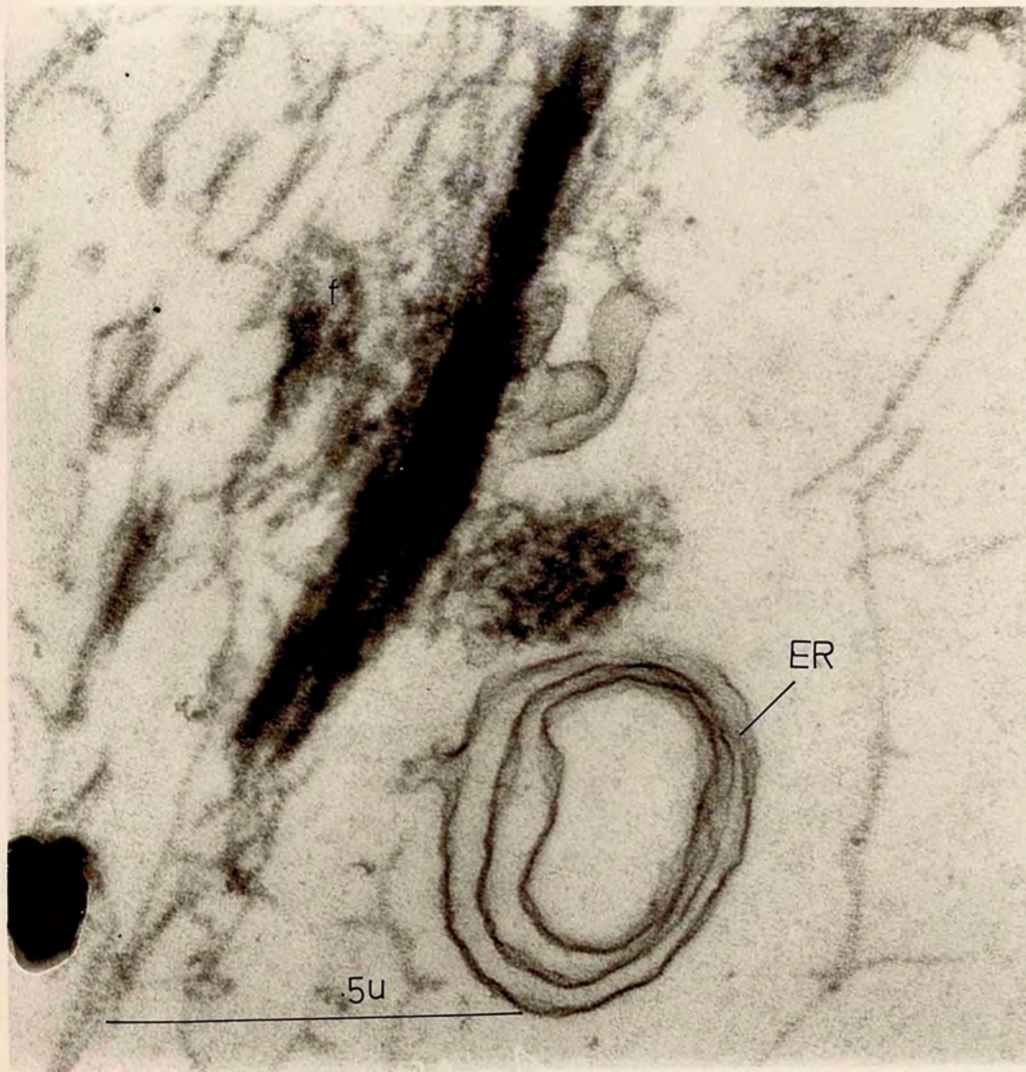
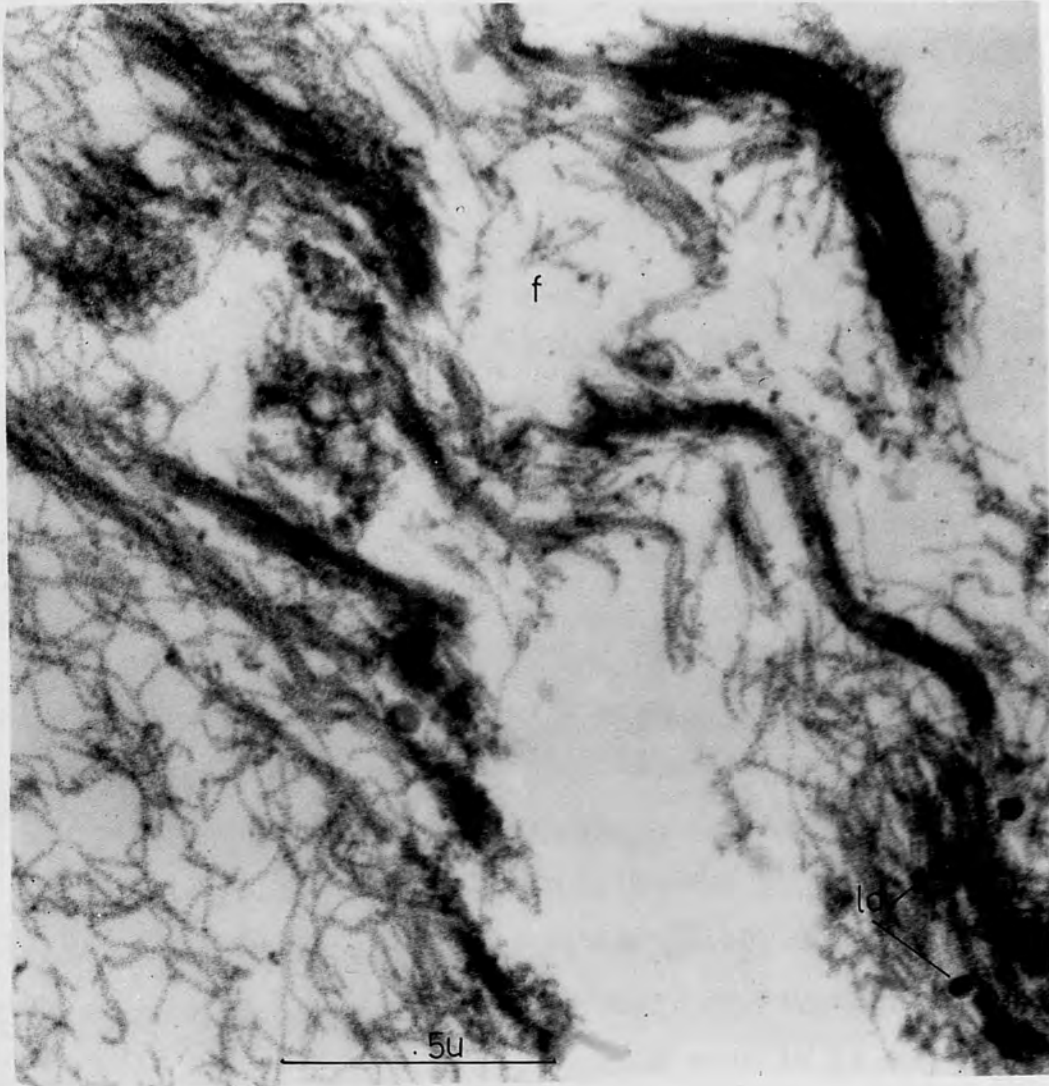

f
ER
5u

PLATE 88

Longitudinal section through a sieve tube showing banded slime fibrils. The larger ones seem to fray out into smaller fibrils. Note the lipid droplets.
Stained with lead citrate, uranyl acetate and lead citrate. X80,000

218

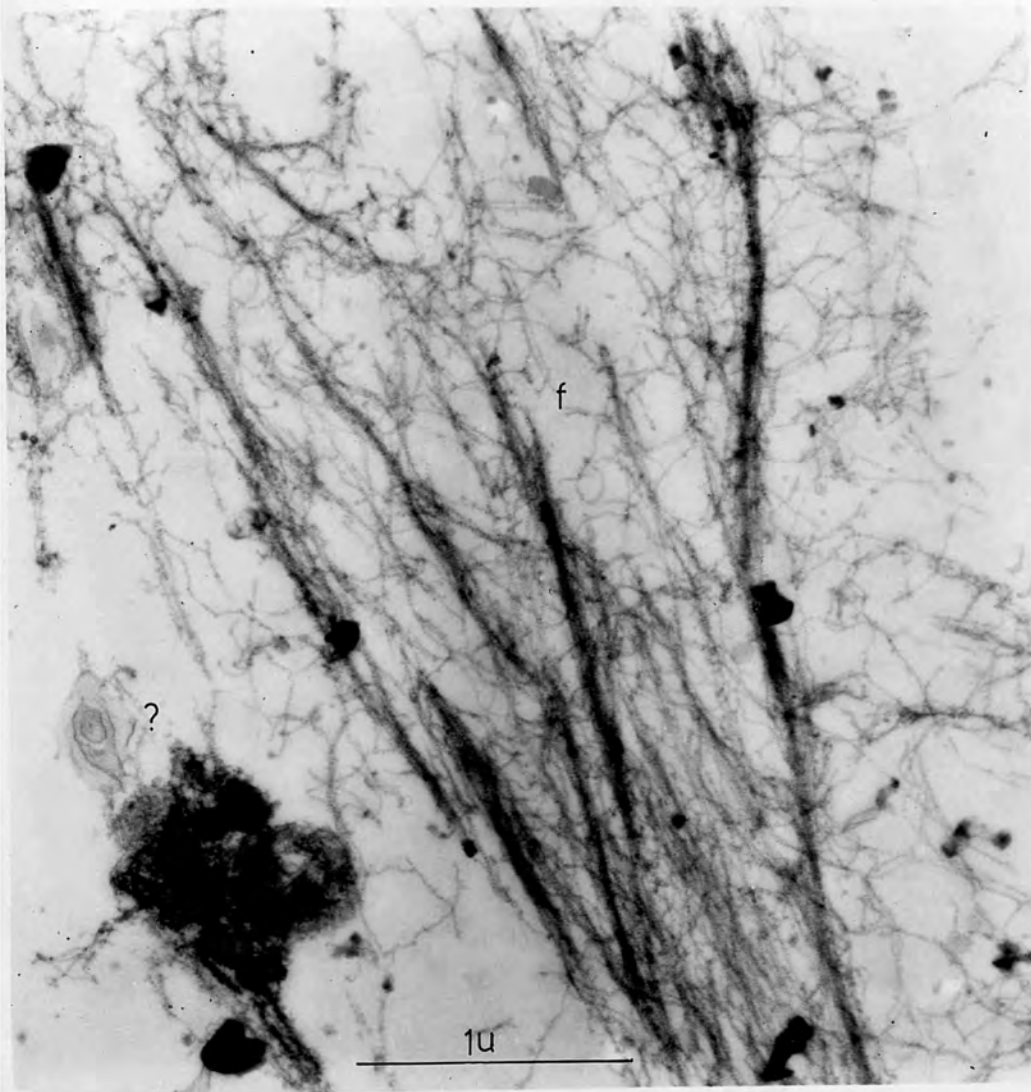


sieve tubes of _Salix capraea_ is the so-called slime. Owing to the fact that the sieve tubes observed were all fairly mature the slime was never observed in the form of typical 'slime bodies', and the present study therefore throws little light on its origin. It is, in fact, largely on the basis of the work of others (e.g. Esau and Cheadle, 1965; Murmanis and Evert, 1966) that the fibrillar substance observed in _Salix_ is identified with slime.

Fibrillar material was regularly observed in all sieve tubes (Plates 37,42,85) principally near the plates (Plates 65,66,71) but also less densely in the lumens (Plates 41,79, 91). The diameter of the fibrils was about 70-80 A. Very frequently the fibrils appeared aggregated into strands,which recall the slime strands of Evert and Murmanis (1965) in _Tilia_ and perhaps represent the controversial transcellular strands of Thaine (1964). Plates 39,60,86,88 and 89 among others show this feature very clearly, though whether it represents an earlier or a later stage in fibril development is uncertain. An opinion may be hazarded that it is an earlier stage, since it is not apparent near the apparently functioning sieve plate pores, and this supposition would fit in with the development of the fibrils from the larger fibres

PLATE 89

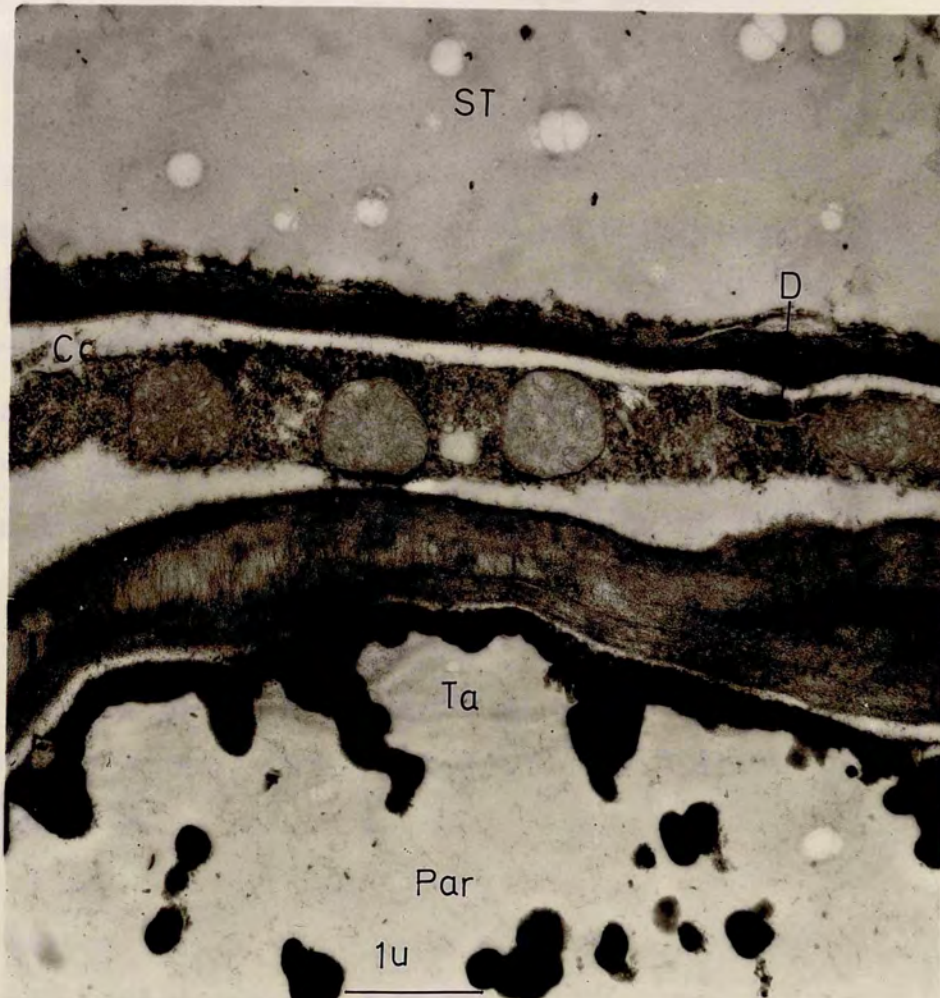
Longitudinal section through a sieve tube showing strands of slime fibrils. The larger ones breaking into smaller fibrils. Stained with lead citrate, uranyl acetate and lead citrate. X 40,000.



89

PLATE 90

T.L.S. showing a sieve tube, a companion cell and a tannin-filled parenchyma cell. Note the bloated appearance of companion cell mitochondria. Stained with lead citrate, uranyl acetate and lead citrate. X20,000.


ST
D
Cc
Ta
Par
1u

(suggested by Johnson, 1967) or from flagellar bodies (Lafleche, 1966).

A very interesting feature of the fibrils is their markedly banded appearance. This is noticeable almost everywhere in the present series of micrographs; Plates 71,86,87,88, 89 represent a sample. The period of the bands is about 140-150 A, this being made up of dark and light regions of about equal lengths. Northcote and Wooding (1966) and Johnson (1967) record a similar appearance in Acer and Nymphoides respectively (the period being about 100 A) and Lafleche records something similar in the "flagellar body" of Phaseolus. In agreement with Northcote and Wooding the fibrils appear to aggregate band to band when they traverse the pores (Plate 71a) or become grouped in larger strands (Plate 86). This renders their periodicity more evident, and strongly suggests the structure of Lafleche's flagellar body, although his fibrils were only 25 A in diameter. It may be mentioned that Johnson (1967) has observed the banding in freeze-etched material of Nymphoides. It is evidently therefore not a fixation artefact. Possibly however the dimensions of the fibrils are to some extent dependent on treatment; imbibition or

PLATE 91

T.L.S. showing a sieve tube and a companion cell. Slime fibrils are seen in the cell lumen away from the sieve plate. Note the dictyosomes, mitochondria and plastids in the companion cell. The companion cell cytoplasm looks shrunken. Stained with lead citrate, uranyl acetate and lead citrate. X30,000.

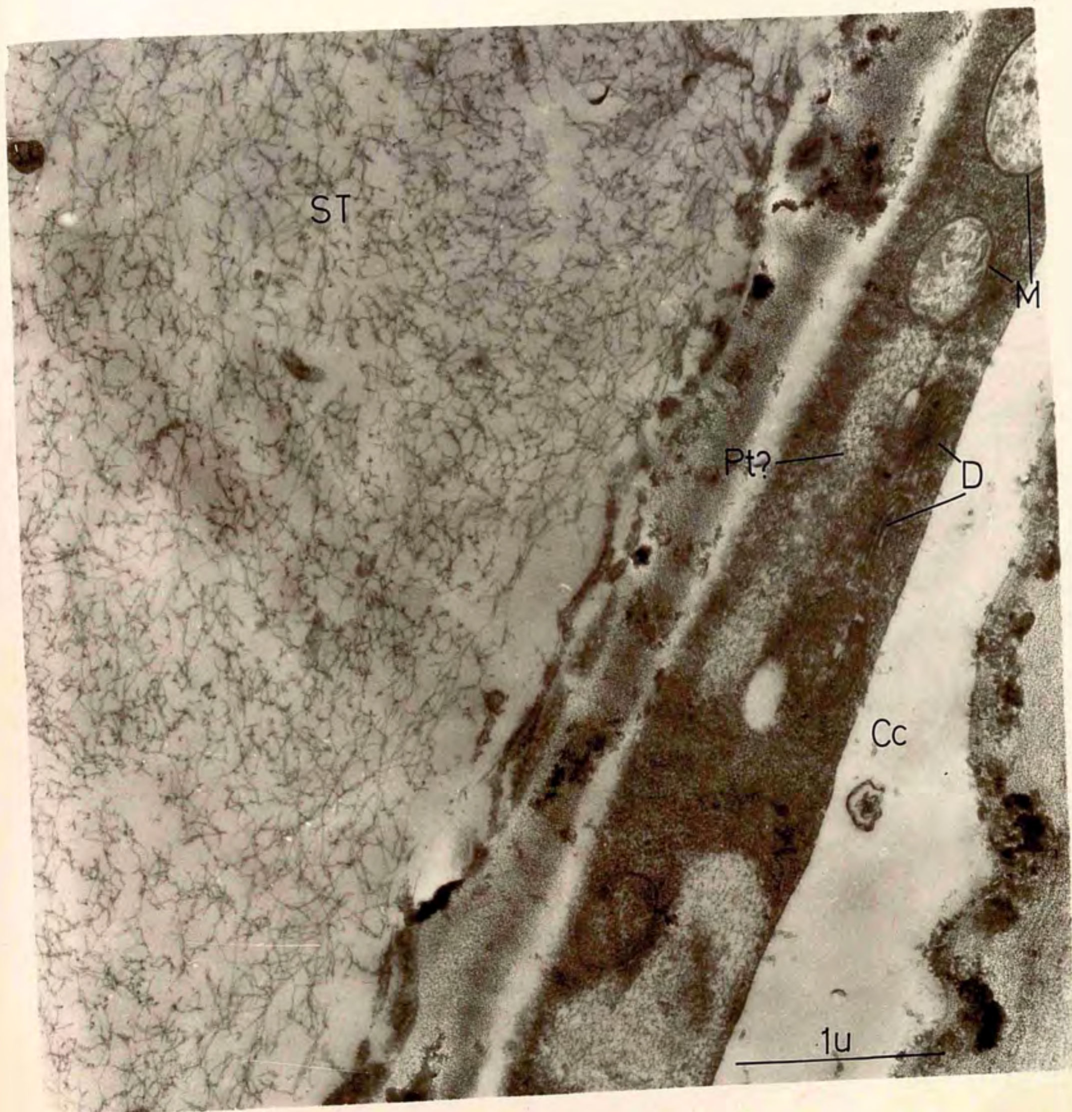

ST
M
Pt?
D
Cc
1u

PLATE 92

T.L.S. showing a sieve tube, companion cell and parenchyma cell. Note the denser cytoplasm of the companion cell. Both companion cell and parenchyma cell are nucleate cells. Stained with lead citrate, uranyl acetate and lead citrate. X15,000.

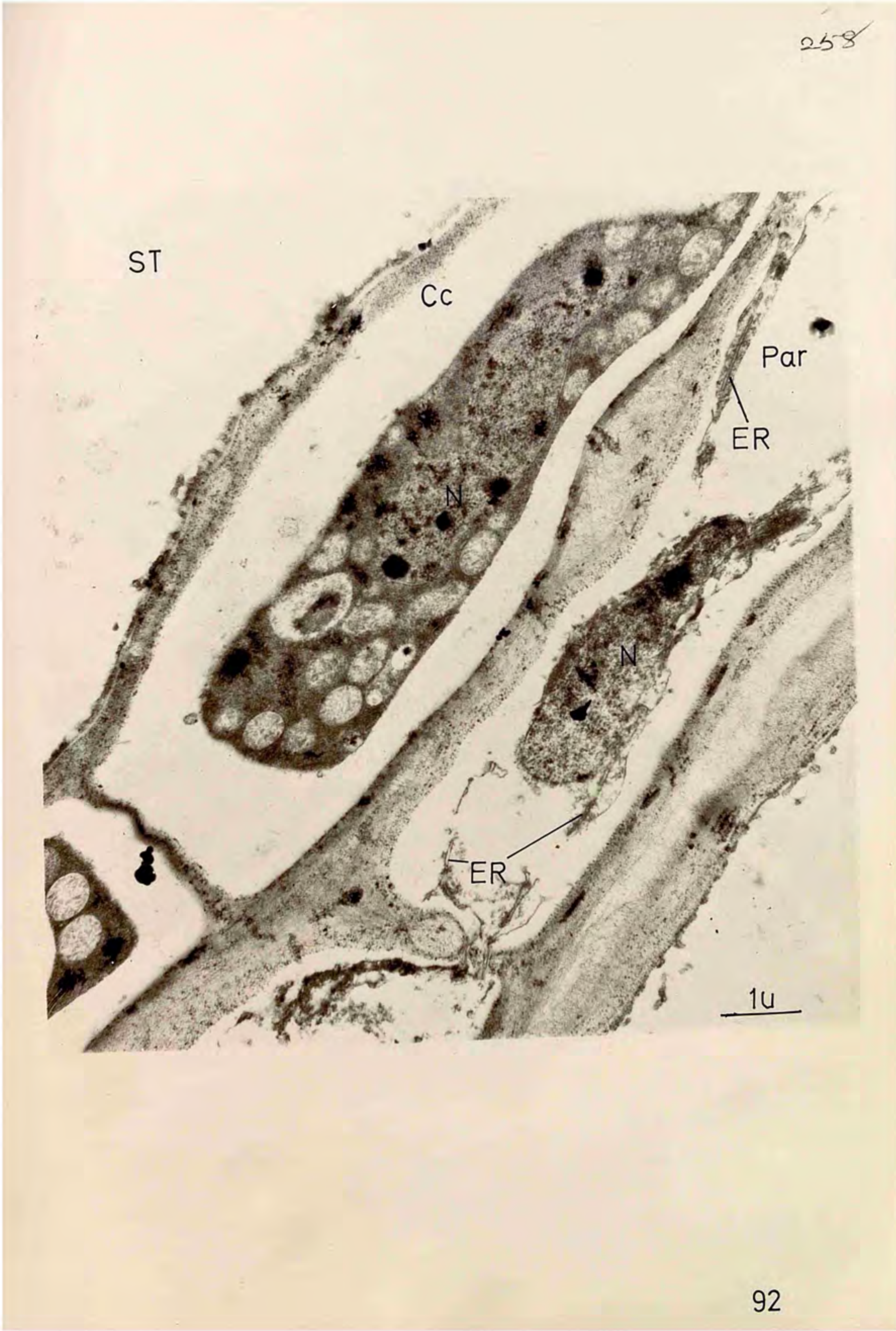
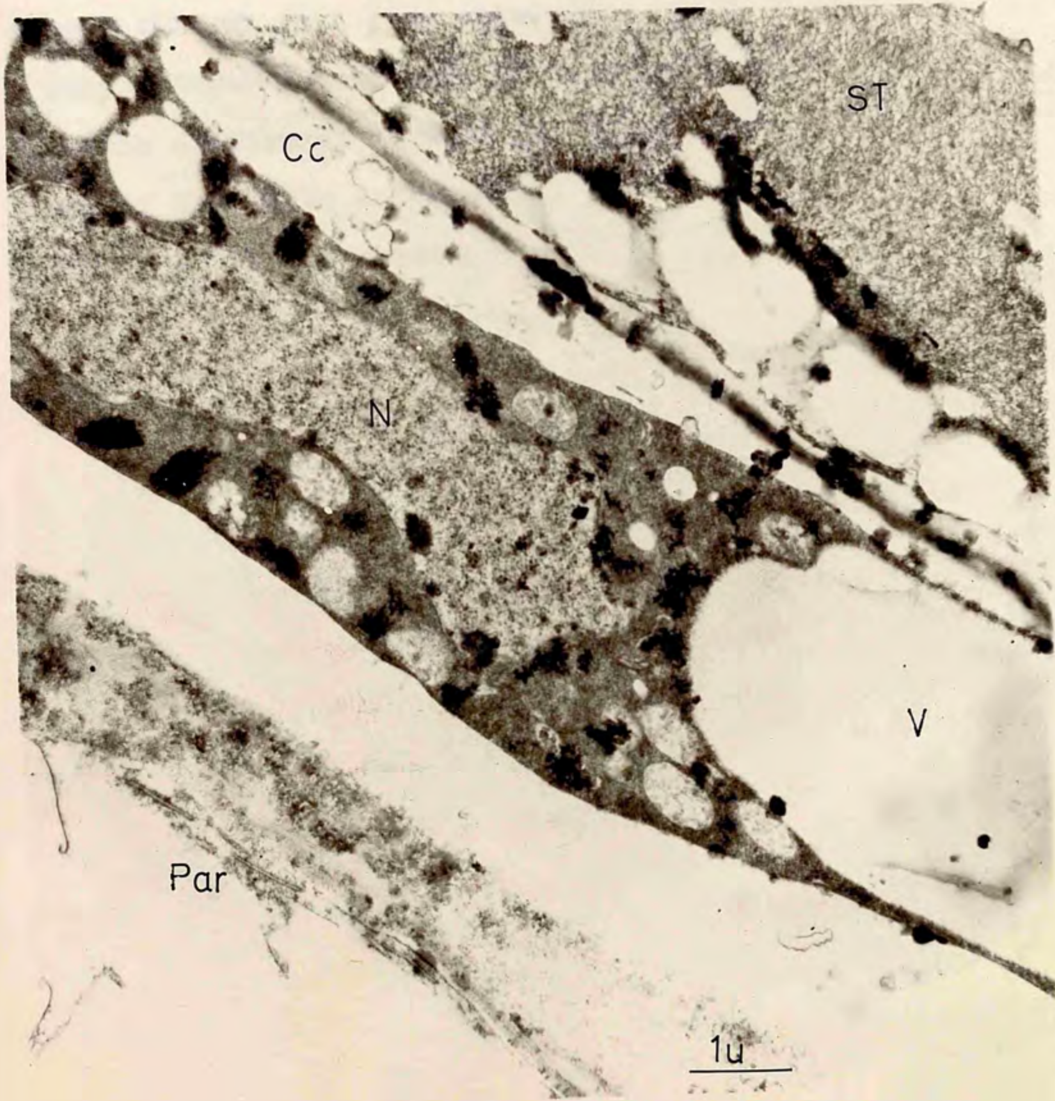

ST
Cc
Par
ER
N
N
ER
1u

PLATE 93

T.L.S. showing a sieve tube and a companion cell. Note the big vacuole and the long nucleus in the companion cell. Companion cell cytoplasm looks shrunken which is a fixation artefact. Stained with lead citrate, uranyl acetate and lead citrate. X15,000.

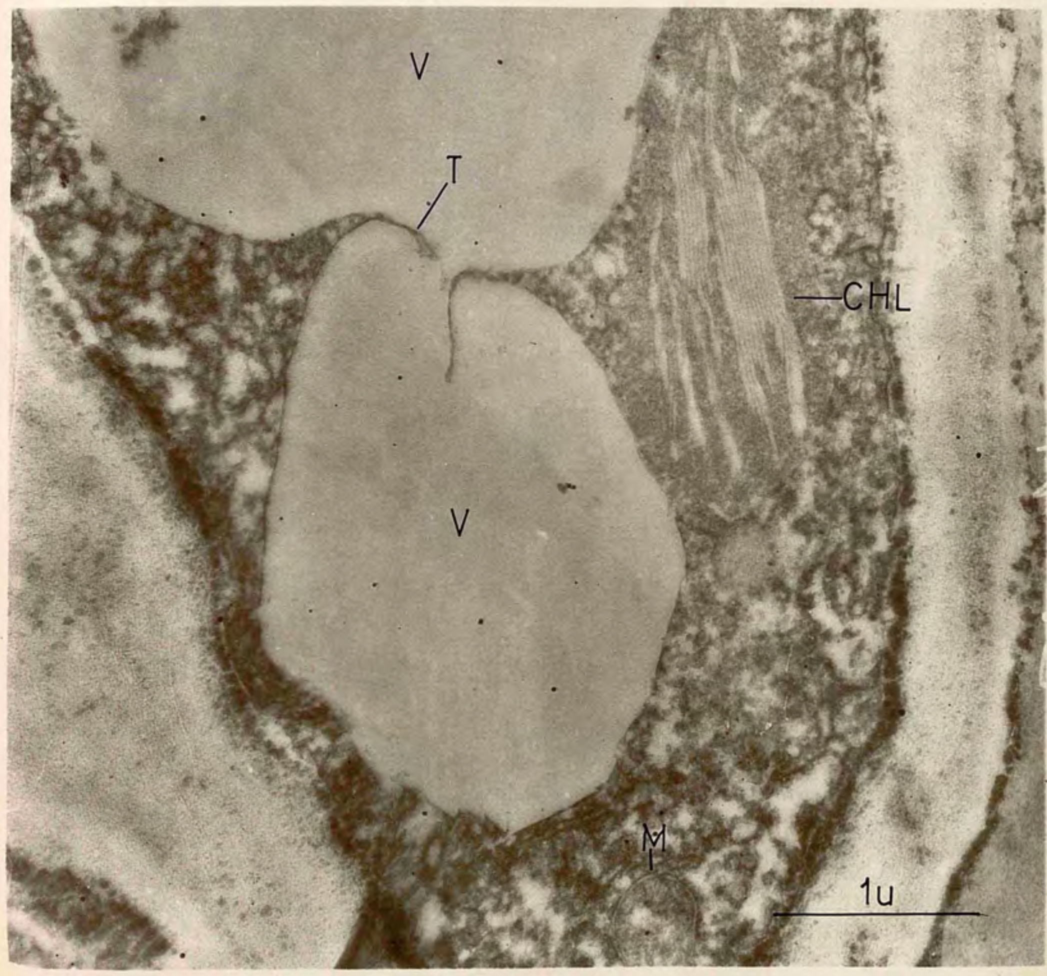


93

dehydration or such fine structures would probably be a very natural consequence of fixation procedures, so that the difference between the dimensions noted and those of Northcote and Wooding, and Johnson need occasion little surprise.

<u>PLATE 94</u>

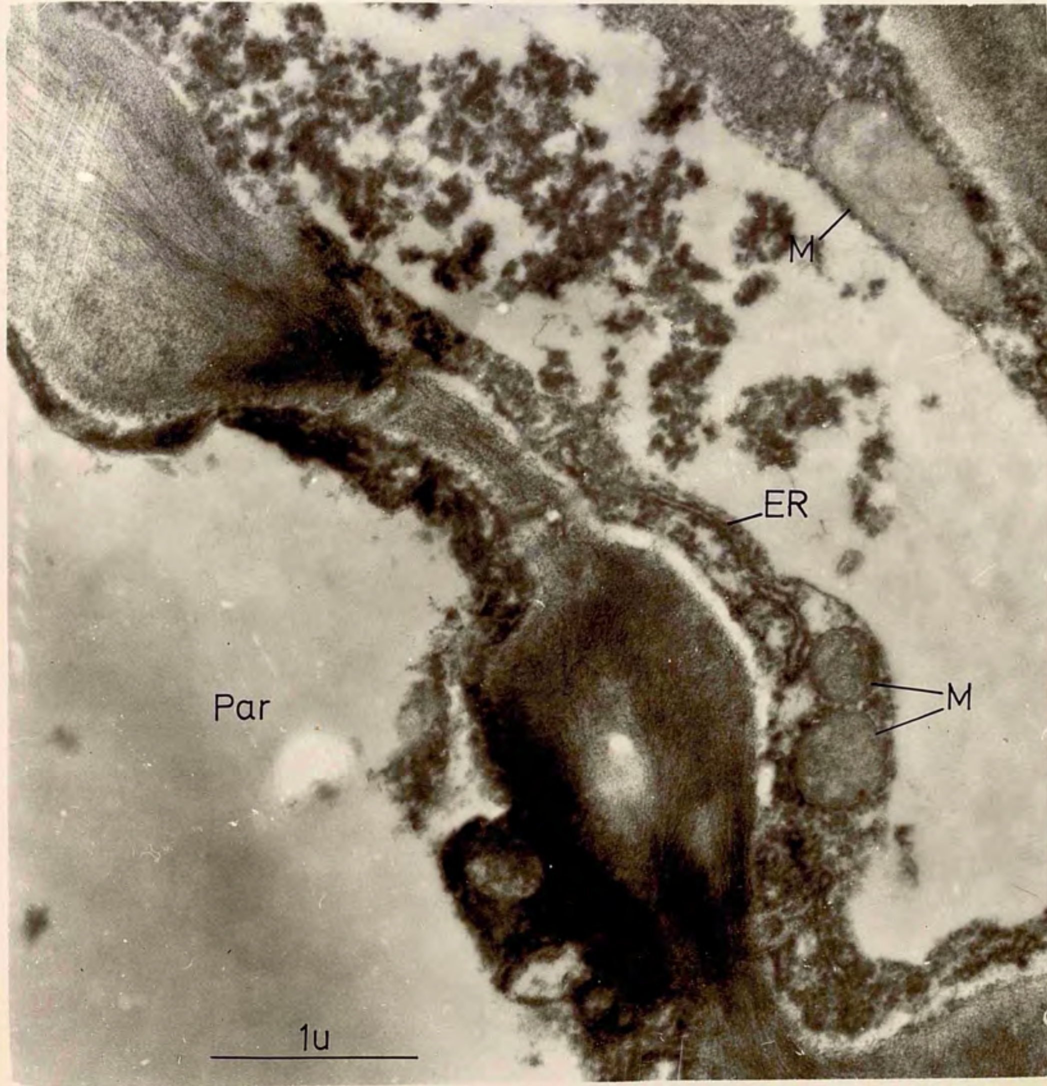
Transverse section showing
a parenchyma cell with
vacuoles, chloroplast and
mitochondria. Stained
with lead citrate. X30,000.



94

PLATE 95

Longitudinal section through two parenchyma cells. Note the ER and mitochondria in the parenchyma cell. Stained with lead citrate. X30,000.


M
ER
M
Par
1u

COMPANION CELL

Cytologic details were not discussed fully in the chapter dealing with optical microscopy. The companion cells of *Salix* have the usual remarkably dense protoplasts with a relatively small volume occupied by a vacuole or vacuoles (Plates 51, 90, 91). But in older cells larger vacuoles have been observed (Plates 41 & 93). The density of protoplasts is due to the abundance of organelles and membranous components (Plates 41, 51, 90, 92 and 93 The companion cells contain nuclei (Plates 35,59,92,93) with a two layered envelop. The nucleus is usually elongated, sometimes lobed (Plate 92).

Numerous mitochondria are found in companion cells. These look quite normal unlike the sieve tube mitochondria. Dictyosomes and endoplasmic reticulum are well represented. Some companion cells observed contained what appeared to be plastids which had lost their usual appearance probably due to bad fixation. They had very few internal membrane system (Plates 51 & 91).

Considerable shrinkage occurs in some of the companion cell cytoplasm (Plates 90 - 93). This is certainly a fixation artefact.

PLATE 96

Longitudinal section through two tannin-filled parenchyma cells. In one two egg-shaped plastids can be seen. Stained with lead citrate. X30,000.

264
268

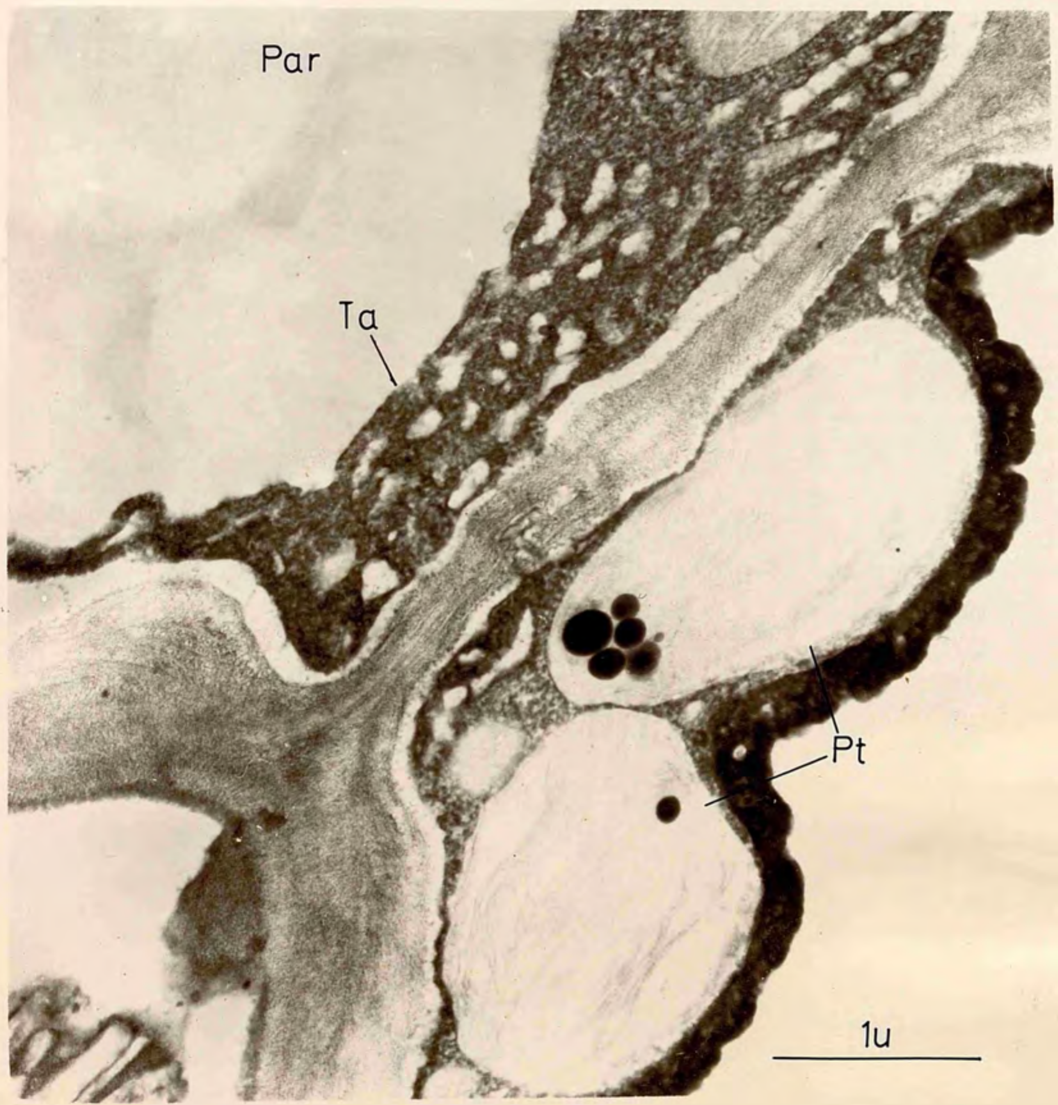
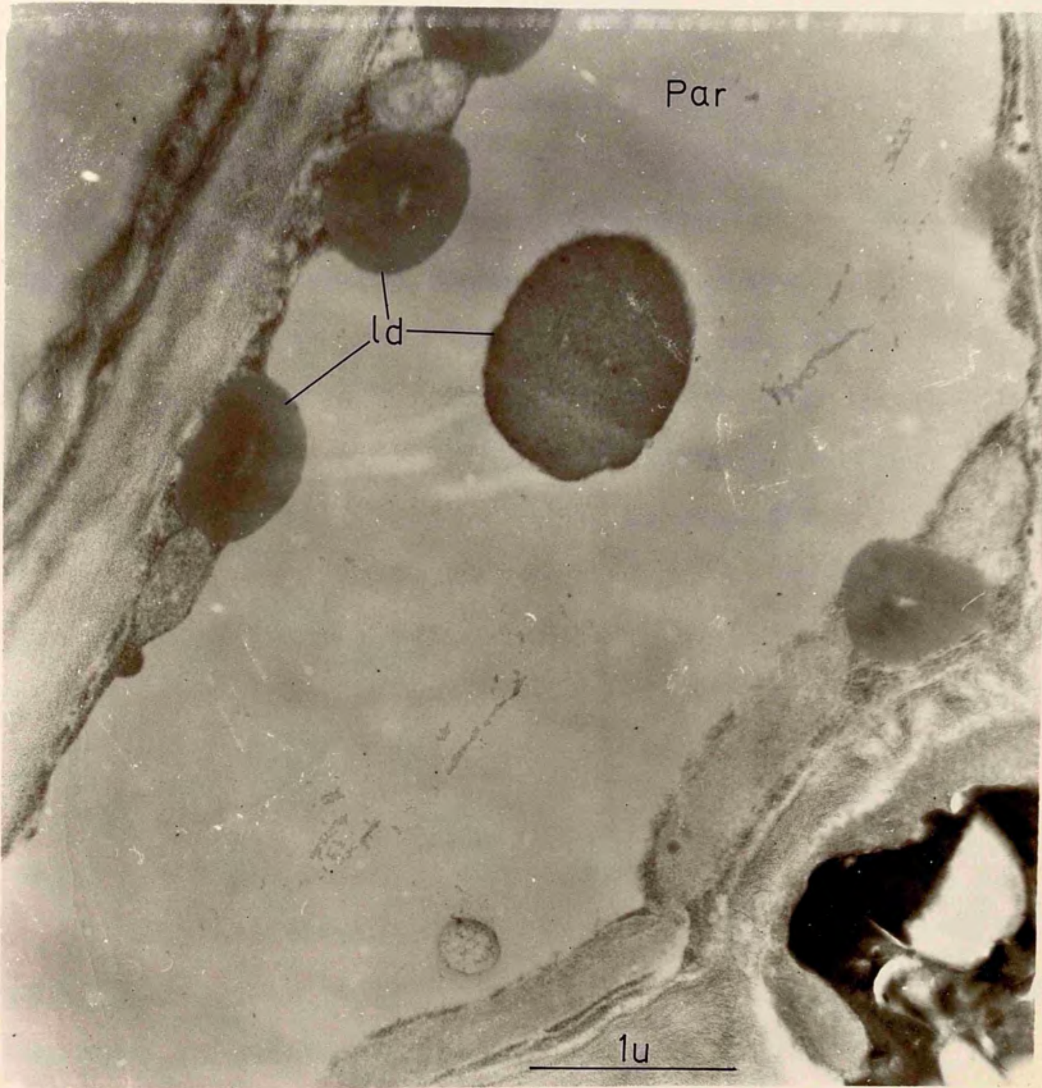


PLATE 97

Longitudinal section of a parenchyma cell containing possibly lipid. Stained with lead citrate. X30,000.


Par
ld
1u

PHLOEM PARENCHYMA

The parenchyma cells of Salix phloem are nucleate, highly vacuolated cells. As mentioned earlier in the chapter dealing with optical microscopy, they contain tannin, starch or crystals. It was very difficult to get any good sections of tannin or crystal containing cells. Plates (63,90) show tannin containing phloem parenchyma. Their high degree of vacuolation and presence of chloroplasts (Plates 94,98,99) help to distinguish them from companion cells. In the Plate 99 distinct starch grains are seen in the plastids. The chloroplasts have conspicuous stacks of grana (Plates 94, 98); elsewhere they appear atypical (Plate 96).

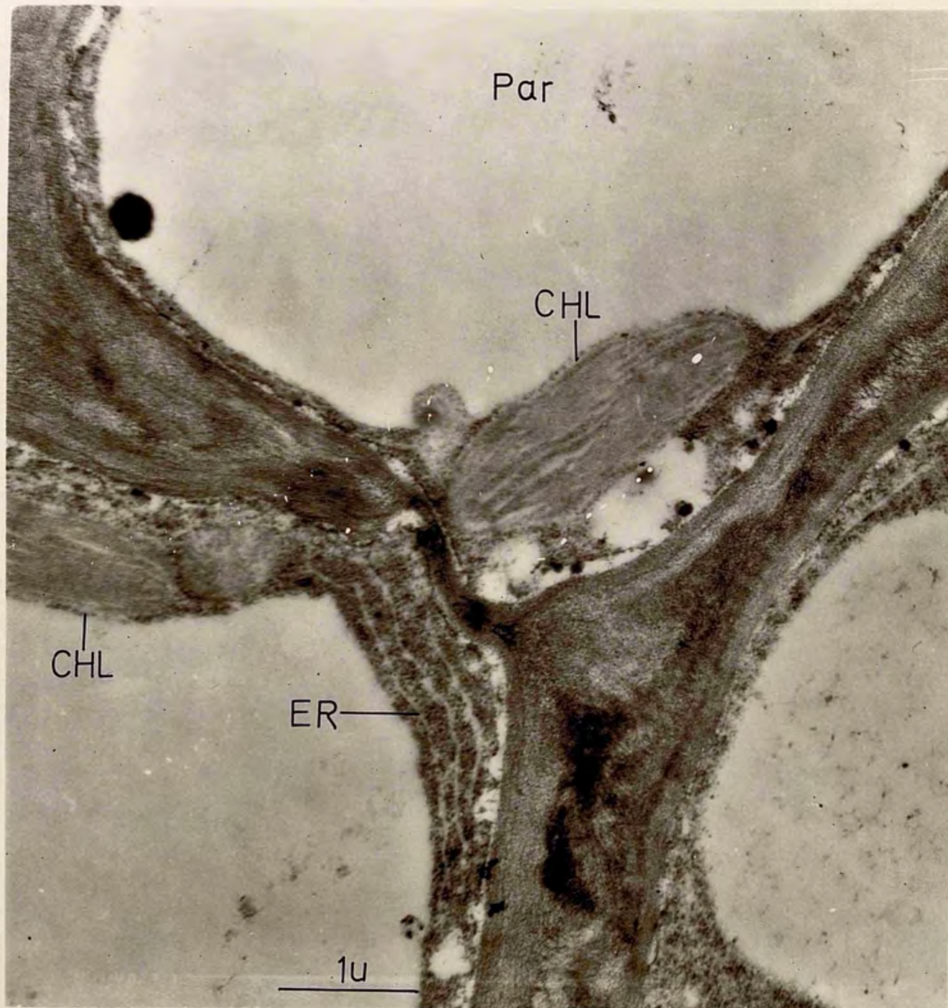
Mitochondria are well preserved with double outer layer and inner membrane structure (Plates 94,95).

Endoplasmic reticulum is a prominent component of parenchyma cells (Plates 92,95,98). In the latter the rough membrane is covered with abundant ribosomes.

Apart from the other cellular inclusions what have been interpreted as lipid drops have been observed in some parenchyma cells (Plate 97).

PLATE 98

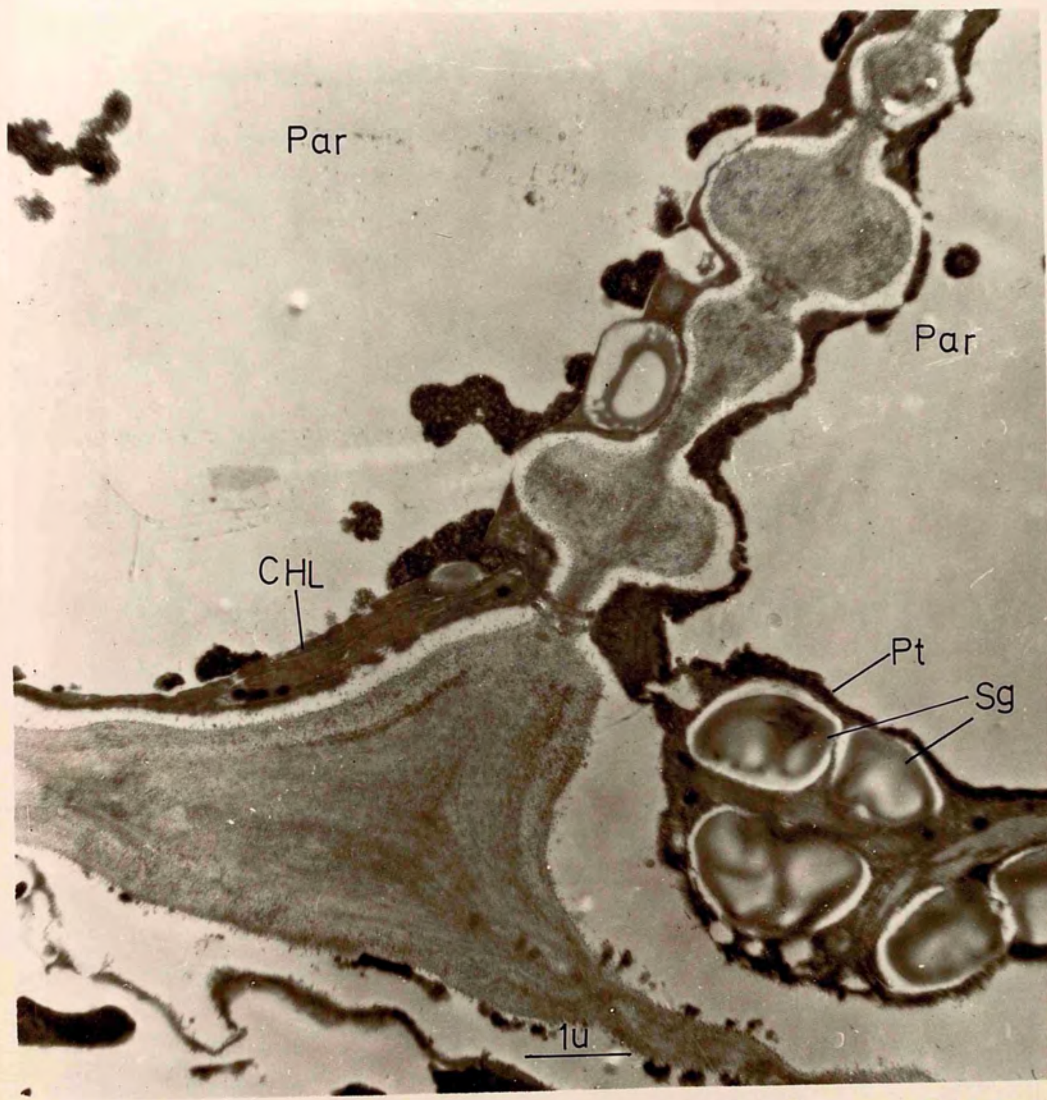
Transverse section showing
three parenchyma cells.
Note the big vacuoles, chloro-
plast and rough ER. Stained
with lead citrate. X20,000.



98

PLATE 99

Transverse section showing two parenchyma cells. Note the wall structure, chloroplast, and plastid with starch grains. Stained with lead citrate. X15,000.


Par
Par
CHL
Pt
Sg
1u

C H A P T E R V

MICROAUTORADIOGRAPHY
OF
CAMBIAL ACTIVITY

INTRODUCTION

During an interruption in the work due to the unavailability of electron microscope facilities an attempt was made, under non-ideal conditions, to apply the method of Waisel and Fahn (1965) to the phloem. These workers were concerned with the problem of determining when the cambium is active in the formation of xylem tissue. They placed potted plants in an atmosphere of CO_2^{14}, allowed them to fully assimilate it, waited a week and then sectioned, washed and autoradiographed the stems. Where the cambium had been active during the experimental time the cell walls of the young xylem cells were radioactive: where the plants had been dormant no darkening of the emulsion was discernible.

It was felt that it would be useful to apply this method to the problem of phloem formation even though the time of the year (the experiments were done in June, 1966) was probably on the late side.

Administration of CO_2^{14}

A healthy 2-3 year old branch on a young tree of *Salix capraea* was selected in June and the terminal portion, about

25 cm. long, was enclosed in a sleeve of medium-gauge polythene layflat tubing, the apical 5 cm having first been removed. In addition, the stem below the sleeve was stripped of leaves for a length of about 10 cm. This surgery was done to encourage a basipetal flow of labelled assimilate, and to reduce the amount of unlabelled assimilate in the region immediately below the sleeve. Within the sleeve a small glass tube containing about 200 μC of sodium carbonate was fixed to the branch, a few drops of lactic acid were added and the ends of the sleeve were tightly tied with string around the stem. After forty-eight hours the sleeve was removed. A week later the branch was harvested and sections 10 μ thick were cut after ester wax embedding. The sections were taken from the portion of the stem immediately below the sleeve.

Radio-autographic technique

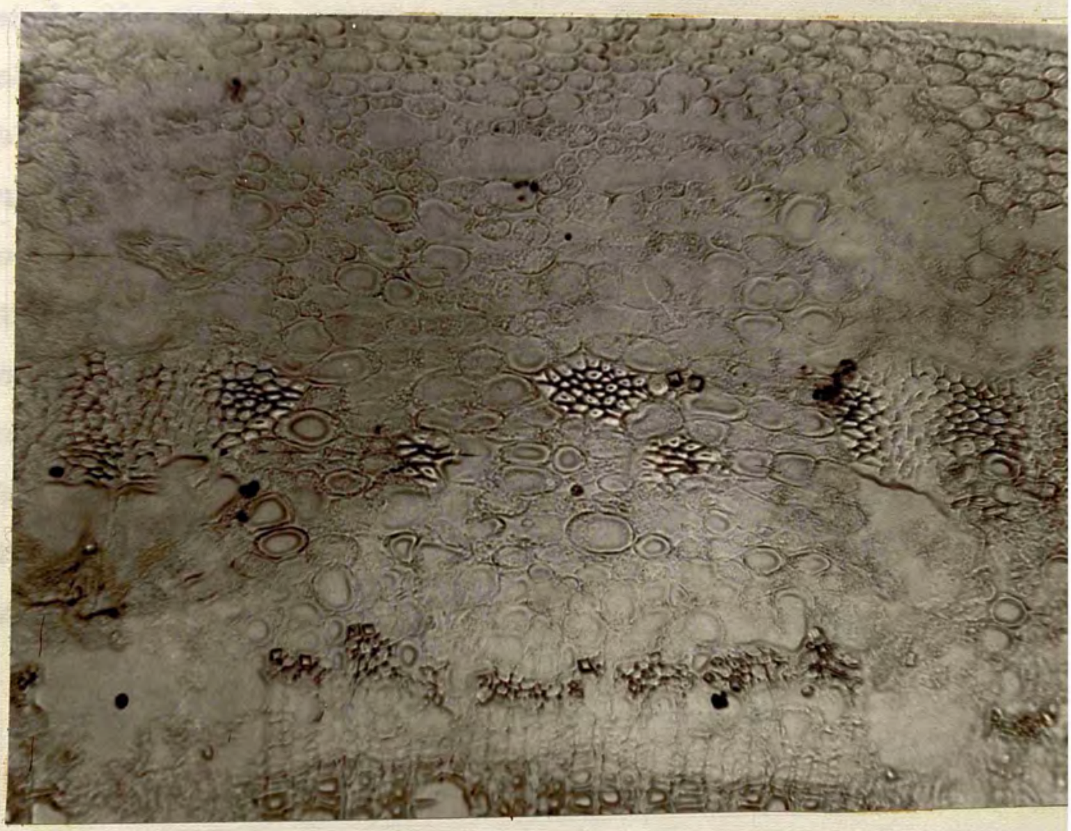
This followed well established practice. Slides were cleaned in a sulphuric acid-dichromate solution and then dipped in a solution made up as follows:

Gelatine	5 g
Chrome alum	0.5 g
Water	1000 ml

They were then drained and set aside to dry. When thoroughly dry the sections were fastened to the slide with Haupt's adhesive, the ester wax removed with xylene, and the mounted slides submerged and raised carefully under a floating rectangle of emulsion cut from Kodak AR 10 stripping plates. After drying the slides were kept in the dark at laboratory temperature for three weeks for exposure. They were then developed in ID36 phenidone and fixed in Kodafix.

Results

The results of the experiment were entirely negative. Plate 100 shows a micrograph of a section at the level of the specimen. It was stained weakly with light green in clove oil. Although the photograph as presented is not entirely conclusive it was quite obvious under the microscope that the emulsion showed no trace of blackening in either xylem or phloem. Unfortunately time did not allow the matter to be pursued further; and it can only be suggested that the surgery to which the shoot was subjected failed to ensure a downward transport of assimilates (perhaps even halting movement through callose formation), and that instead all the radioactive sugars moved into the



X200

axillary buds. Waisel and Fahn did incidentally also sample the axis below the labelled branch, the main difference in their treatment being that their plants were probably much smaller (since they were potted) and that no surgery of lower leaves or stem apex was practised. It is not surprising perhaps that the phloem was not labelled since it is probable that as late as June little phloem is being laid down. The method indeed is probably of less value for this tissue since the phloem is formed early in the year and presumably over-wintering starch rather than fresh assimilate is drawn upon. The problem is an interesting one and would repay further study.

C H A P T E R VI

DISCUSSION

DISCUSSION

The purpose of this investigation was to throw light particularly on the adequacy or otherwise of the electro kinetic theory of translocation for the phloem system of *Salix capraea*. It remains to summarise the findings in this connection.

In the first place it must be recognised that it is not yet known definitely at what stage in the course of their development the sieve tubes are functional; consequently it is not certain that the sieve tubes reported on here are the ones physiologically of interest. However, there seems every reason to believe that they are so. Secondly, owing to the difficulties experienced with the material no sustained attempt was possible in the time available to trace the ontogeny of the sieve tubes, and this inevitably makes the interpretation of some of the structures observed rather more tentative.

The optical work was undertaken partly as of importance in itself, and partly as a background to the electron microscope work. With regard to the former aspect it is necessary to remember that one requirement

of the electro kinetic theory is that there should be a return pathway through the living cells for the flux of potassium ions, and evidence for this is primarily a matter for optical investigation.

The analysis of the tiers and columns does not immediately suggest that a return path for potassium ions through living cells is provided. The analysis of tiers shows principally how the cambial initials divide to form sieve tube elements, companion cells and parenchyma; in particular it appears that companion cells nearly always seem to have a fair area of contact laterally with adjacent tiers. This may be important, for sieve tubes are connected by their plates to others disposed tangentially, not radially, to themselves. It is in the column analyses therefore, that the significant relationships are to be sought. These show that sometimes, but perhaps not in the majority of cases, companion cells do cover the sieve plate region longitudinally, an example of this being sieve tubes Z and Y of figure 6 (Column analysis II). This would accommodate the requirement. In other

cases a companion cell may be between two sieve elements tangentially disposed and in contact with them both (same figure, sieve tube X at section 49 and 51). In such cases a return flow would be possible into a tube of an adjacent column. However in other cases the companion cells do not seem to be helpfully disposed; for instance in column analysis III (Figure 7) where in sections 35 and 37 the companion cells belonging to elements X and Y are in contact with tannin filled and ray cells respectively and widely separated from each other.

Probably a great deal more work on these lines would be needed before it could be shown that the anatomy of the phloem of Salix would sustain the requirements of the electro kinetic theory.

A second requirement of the electro kinetic theory is that the flow should traverse a course membrane structure with pores wide enough - but not too wide - for large electro kinetic forces to be developed. It is natural to look for such a structure in the sieve plate and to expect that the pores of the latter would be loosely occupied by colloidal material to reduce the

effective pore size to the required dimensions. It is in this connection that the evidence of the present work is of interest. In common with many other investigations this suggests that the functioning sieve tubes contain as a prominent constituent a fine network of fibrils of a very interesting nature. They measure about 70-80 A in diameter and appear banded along their length with alternate light and dark bands, giving a period of about 140-150 A. Fibrils of up to 150-200 microns in length have been measured in the present work. Similar material has been found in plants whose taxonomic relationships are quite diverse - *Nymphoides* (*Gentianaceae*), *Acer* (*Sapindaceae*), *Phaseolus* (*Leguminosae*) and *Salix* (*Salicaceae*) and quite a number of published photographs of other plants - *Dioscorea* and *Cucumis* (Behnke and Dörr, 1967); *Cucurbita* (Eschrich, 1963), *Impatiens* (Engleman, 1963); *Tetragonia* (Falk, 1964) - lend themselves very readily to the same interpretation. Where the banded structure has not been found this may well be due to the fact that the fixation and staining techniques were unsuitable for it can hardly itself be an artefact (it has also been found in freeze etched material). Further

it appears to be conspicuous in Johnson's material only in mature sieve tubes where the coarser tubular fibrils of an earlier stage have frayed out into the finer ones. This may suggest why banded fibrils have not been found in cases such as Primula (Tamulevich and Evert, 1966) where tubular slime has been seen.

Everything considered therefore it seems that dispersed slime of "banded fibrils" is a very widespread and important constituent of mature sieve tubes. In the present work, as in most others where glutaraldehyde and osmium rather than potassium permanganate have been used as fixatives, the slime fibrils appear as traversing the sieve plate pores. The denseness with which they pack the pores has probably been artificially increased by callose formation consequent on manipulation, but it might be suggested that what the present work indicates is that the pores are normally traversed by fibrils about 70-80 A in diameter and at a guess about 200 A apart. Weatherley and Johnson (1967) have calculated on the basis of an unpublished formula of Spanner's that with fibrils 100 A in diameter lying 200 A clear apart flow across a

sieve plate of thickness 1 μ at a pore velocity of 200 cm per hour would involve a pressure drop of 0.14 atmosphere. These dimensions seem to fit the present case fairly well too, though the aggregate pore area relative to the cross section of the lumen (taken as 50%) is very tentative; and the plate thickness might be nearer 0.5 μ. The length of the sieve elements of Salix is about 150-200 μ. Thus for the present case the pressure drop per metre works out at about

$$0.14 \times 0.5 \times \frac{100 \times 10^4}{150} = 470 \text{ atm. per metre}$$

which would seem to rule out a simple Münch mechanism, though not an electro kinetic one.

The difficulties faced by the electro kinetic theory have been mainly two: the evidence for a satisfactory return path for the potassium ions (discussed earlier), and the magnitude of the electrical current which their movement seems to imply. The latter is in doubt mainly because it is not known how many water molecules are conveyed per potassium ion. Previous data (Fensom, unpublished) seems to indicate about 100. However with suitable membranes it is conceivable that this might

well be much greater, perhaps even 1000 or more. It is here that the present evidence is suggestive. The fibrillar slime has been variously regarded as protein (Engleman, 1963); lipoprotein (Eschrich, 1963 and Lafleche, 1966) or RNA - containing (Buvat, 1963 c) in nature. It would seem, from its wide occurrence, to be of some special significance in its chemical nature; and especially if it incorporates much RNA or other phosphate-containing substances it would probably have marked electrical properties. If charged groups are associated with the bands, and if as seems likely from the micrographs these come together (band to band of adjacent fibrils) in the pores of the sieve plate the electro kinetic properties might well be enhanced. There is the other possibility, of course, that the fibrils might be muscular in function; but that they are adapted to secure strong electro-osmosis is at least, on the present evidence, equally likely. Further work clearly need* to be on their chemical nature.

It may be concluded, therefore, that the present work provides further evidence against the adequacy of a pressure-flow mechanism in _Salix capraea_, and lends modest support to an electro kinetic one.

SUMMARY

The present thesis starts with a brief review of the problem of the channel concerned in translocation followed by a short discussion of the prevailing theories of transport. In this connection the ultra-structure of the sieve plate and the slime fibrils as investigated by other workers have been fairly fully reviewed. The structure of the phloem has been analysed from optical sections in the light of the requirements of the electro kinetic theory. The analysis, however has not thrown much light on the adequacy or otherwise of the theory.

Different approaches towards the preparation of tissue have not been possible due to shortage of time and limited availability of the microscope. All the electron micrographs produced were of material fixed with glutaraldehyde followed by Osmium. This fixative has proved to preserve the fibrillar slime very well. A triple staining technique (lead citrate, uranyl acetate and lead citrate) has shown the banded nature of the fibrils quite satisfactorily.

It has been shown that the banded slime fibril is an important component of sieve tube cytoplasm. These fibrils have always been found packed in the sieve plate pores in this investigation and they appear to run longitudinally in them. Evidences have been found of their stationary nature.

A number of inclusions of definite identity have been found in the sieve tubes, both near the sieve plates and further away from them and these are briefly described. They include plastids, mitochondria and starch grains.

The sieve tube of *Salix* shows a remarkable extruded nucleolus which persists for quite a long period. The fine structure of the extruded nucleolus has been described fairly fully. It has an urchin-like contour with radiating tubular structure. The tubes bear no resemblance to the slime fibrils.

Deposition of callose on the sieve plates has been shown both optically as well as with the electron microscope.

Some of the electron micrographs presented, have

shown "lamellar" structures which resemble the well known endoplasmic reticulum of previous workers.

Lastly the structural characteristic of the sieve plate and the slime fibrils have been discussed in relation to the prevailing theories of translocation, especially the electro kinetic theory. It is concluded that it lends a modest support to the latter.

REFERENCES

1 - Beer, M. (1959) - Fine structure of phloem of *Cucurbita* as revealed by electronmicroscope (Abstract).

Proc.IX Int. Bot. Congr: Montreal 2, 26.

2 - Behnke, H. (1965a) - Uber den Feinbau Gitt erartig "Aufgebauter plasmaeinschlüsse" in den Siebe elementen von *Dioscorea reticulata*. Planta - 66, 106-112.

3 - Behnke, H. (1965 b) - Uber des phloem der *Discoreaceen* unter besonderer Berücksichtigung ihrer phloembecken.

II. Elektron enoptische untersuchungen zun feinstruktur des phloembeckens.

Z. pflanzen physiol. 53, 214-244.

4 - Behnke, H. & Dorr I. (1967) - Zur Herkunft und Struktur Der plasma filamente in assimilatleitbahnen.

Planta - 74, 18-44.

5 - Biddulph, O. & Cory, R. (1957) - An analysis of translocation in the phloem of bean plant using THO, P^{32}, $C^{14}O_2$.
Plant Physiol. 32, 608-619.

6 - Biddulph, O. & Cory, R. (1960) - Demonstration of two translocation mechanisms in studies of bidirectional movement.
Plant. Physiol. 35, 689-695.

7 - Bisalputra, T. & Esau, K. (1964) - Polarized light study of phloem differentiation in embryo of Chenopodium album.
Bot. Gaz. 125, 1-7.

8 - Bouck, G.B. & Cronshaw, J. (1965) - The fine structure of differentiating sieve tube elements.
Jr. of cell Biology. 25, 79-93.

9 - Buvat, R. M. (1960) - Observations sur les infrastructure du cytoplasme au cours de la differentiation des cellules criblees de Cucurbita pepo.
L. Acad. Sci. Compt. Rend. 250, 1528-1530

10 - Buvat, R. M. (1963)a - Infra structure et differenciation des cellules criblees de *Cucurbita pepo* evolution du contenu cellulaire final.
C.R. Acad. Sci. 250, 1528-1530.

11 - Buvat, R. M. (1963)b - Infra structure et differenciation des cellules criblees du *Cucurbita pepo*. Relation entre la membranes plasmiques du cytoplasme.
C.R. Acad. Sci. 257, 221-224.

12 - Buvat, R. M. (1963)c - Sur la presence D'Acide Ribonucleique dans les corpuscles Muqueux des cellules criblees de *Cucurbita pepo*.
C.R. Acad. Sci. 257, 733-735.

13 - Buvat, R. M. (1963)d - Les infra structures et la differenciation des cellules criblees de *Cucurbita pepo L.*
Portugalie acta biologica. 7(3-4) 249-299.

14 - Canny, M. J. (1962) - The mechanism of translocation.
Ann.Bot. N.S., 26, 603-617.

15 - Cheadle, V. I. & Esau, K. (1958) - Secondary phloem of Calycanthaceae.
University of California publications in Botany. Vol. 29, 397-510.

16 - Cocking, E. C. Personal communication.

17 - Crafts, A. S. (1961) - Translocation in plants. Holt, Rinehart & Winston, Inc. - New York.

18 - Dixon, H. H. (1922) - Transport of organic substances in plants.
Nature, London, 110, 547-551.

19 - Dougherty, W. J. (1967) - Perforated BEEM capsules for precipitate-free transfer of ultra thin sections through staining solutions.
Stain Technology, 42, 104-105.

20 - Duloy, M., Mercer, F. V., Rathgeber, N. (1961). Studies in translocation.
II (A Submicroscopic Anatomy of the phloem)
Australian Journal of Biological Studies - 14, 506-518.

21 - Engleman, E. M. (1963) - Fine structure of proteinaceous substance in sieve tubes. Planta. 56, 420-426.

22 - Engleman, E. M. (1965)a - Sieve elements of *Impatiens Sultanii* 1. wound reaction. Annals of Bot. 29, 83-101.

23 - Engleman, E. M. (1965)b - Sieve elements of *Impatiens Sultanii* II. Developmental aspects. Annals of Bot. 29, 103-118.

24 - Esau, K. (1947) - A study of sieve tube inclusions. Amer. Jr. Bot. 34, 224-233.

25 - see Esau, K. (1961) ..... For Malpighe (1675-1679) - Plants Viruses and Insects. Havard University Press - Cambridge, Massachusetts.

26 - Esau, K. (1965)a - Fixation image of Sieve element plastids in *Beta.* Proceedings of the National Academy of Sciences, 54, 429-437.

27 - Esau, K. (1965)b - Anatomy & Cytology of Vitis phloem.
Hilgardia - 37, 17-72.

28 - Esau, K. & Cheadle, V. I. (1955) - Significance of cell divisions in differentiating secondary phloems.
Acta Botanica Neerlandica 4 346-357

29 - Esau, K. & Cheadle V. I. (1961) - An evaluation of studies on ultra structure of sieve plates.
Proceedings of the National Academy of Sciences 47, 1716-1726.

30 - Esau, K. & Cheadle, V. I. (1962)a - An evaluation of studies on ultra structure of tonoplast in sieve elements.
Proceedings of the National Academy of Science 48, 1-8.

31 - Esau, K. & Cheadle, V. I. (1962)b - Mitochondria in phloem of Cucurbita.
Bot. Gaz. 123, 233-243.

32 - Esau, K. & Cheadle, V. I. (1965) - Cytologic studies in phloem.
University of California publications in Botany 36, 253-344.

33 - Esau, K. & Cheadle, V. I. & Risely, E. B. (1962) - Development of sieve plate pores.
Bot. Gaz. 123, 233-243.

34 - Esau, K., Cronshaw, J. & Hoefort (1967) - Relation of Beet yellow virus to the phloem and to movement in the sieve tubes.
J. Cell. Biol. 32, 71-87.

35 - Escherich, W. (1956) - Kallose - Protoplasma 47, 487-530.

36 - Escherich, W. (1961) - Unter Suchungen über den Ab & Aufban der callose.
III über callose.
Ztchr. f. Bot. 49, 153-218.

37 - Escherich, W. (1962) - Elektronenmikrokopische untersuchungen und pollen mutterzellen callose
IV Callose
Protoplasma - 55, 153-218.

38 - Escherich, W. (1963)a - Beziehungen zwischen dem Auftreten von callose der fein struktur des primaren phloems bei Cucurbita ficifolia. Planta, 59, 243-261.

39 - Escherich, W. (1963)b - Der phloem saft von Cucurbita ficifolia Planta - 60, 216-224.

40 - Escherich, W. & Currier, H.B. (1964) - Identification of callose by its diachrome reactions. Stain Technology - 39, 303 - 307.

41 - Evert, R.F. (1960) - Phloem structure in Pyrus communis and its seasonal changes. Univ. Calif. Publications - Bit.32, 127-194.

42 - Evert, R.F. (1963)a - Ontogeny & structure of secondary phloem in Pyrus malus. Amer. J. Bot. 50, 8-37.

43 - Evert, R.F. (1963)b - The cambium and seasonal development of the phloem in Pyrus malus. Amer. J. Bot. 50, 149-1591

44 - Evert,R.F. & Derr, W.F. (1964) - Callose substance in sieve elements. Amer. J. Bot. 51, 552-559.

45 - Evert, R.F. & Murmanis, L. (1965) - Ultra structure of sec. phloem of Tilia Americana Amer. J. Bot. 52, 95-106.

46 - Evert, R.F. Murmanis, L. & Sachs, I.B. (1966) - Another view of the ultra structure of Cucurbita phloem.
Annals of Botany, N.S. 30, 563-585

47 - Falk, H. (1964) - Zur Herkunft Des Siebröhren-schleimes bie Tetragonia expansa MURR.
Planta - 60, 558-567.

48 - Fensom, D.S. (1957) - The bio-electric potentials of plants and their functional significance.
An electrokinetic theory of transport.
Can. J. Bot. 35, 573-582.

49 - Frasca, J.M. & Parks, V.R. (1965) - A routine technique for double-staining ultra thin sections using Uranyl and lead salts.
J. Cell. Biol. 25, 157-161.

50 - Frey-Wyssling, A. & Muller, H.R. (1957) -
Submicroscopic differentiation of plasmodesmata and sieve plates in Cucurbita.
J. of Ultrastructure research. 1, 38-48.

51 - Hales, S. (1769) - "Statical Essays containing vegetable statics or an account of some statical experiments on the Sap in vegetation". W. Innys, London - 1727.

52 - Hepton, C.E.L., Preston, R.D. & Ripley, G.W. (1955) Electronmicroscope observations on the structure of the sieve plates in Cucurbita. Nature 176 868-870.

53 - Hepton, C.E.L. & Preston, R.D. (1960) - Electronmicroscopic observation of the structure of sieve connexions in phloem of Angiosperms and Gymosperms. Jr. of Exptl. Bot. 11, 381-394.

54 - Hohl, H.R. (1960) - Uber die Submikroskopische Struktur normaler & hyperflasticher Gewebe Von Datura Stramonium. Ber. dt. Bot. Ges. 70, 395-439.

55 - Honert, T. H. V. (1932) - on the mechanism of the transport of organic materials in plants. Proc. K. ned. Akad. Wet. 35, 1104-1111

56 - Hüber, B. & Kolbe, R.W. (1948) - Elektronen mikroskopische untersuchungen an siebröhren. Svensk Bot. Tidskr 42, 364-371.

57 - Johansen, D.A. (1940) - Plant microtechnique. McGraw-Hill Book Company Inc. New York and London.

58 - Johnson, R.P.C. (1966) - Potassium Permanganate fixative and the electron microscopy of sieve tube contexts. Planta - 68, 36-43.

59 - Johnson, R.P.C. (1967) - Structure of the sieve tube in relation to its function. Thesis - Aberdeen University.

60 - Kay, D.H. (1965) - Technique for electron microscopy. 2nd Edition. Blackwell Scientific Publications - Oxford.

61 - Kollmann, R. (1960) - Untersuchungen über das protoplasma der siebröhren von Passiflora coerulea. II Elektronenoptische untersuchengen. Planta - 55, 67-107

62 - Kollmann, R. (1964) - on the fine structure of the sieve element protoplast. Phytomorphology 14, 247-264.

63 - Kollmann, R. & Schumacher, W. (1961) - Uber die Feinstruktur des phloem von Metasequoia glyptostroboides und seine jahreszeit.
I Das ruhephloem.
Planta - 57, 583-607.

64 - Kollmann, R. & Schumacher, W. (1962)a - Uber die Feinstruktur des phloems von Metasequoia glyptostroboides und seine jahreszeitlichen-veranderungen.
II Vergleichende untersuchungen der plasmatischen verbindungsbrücken in phloem parenchyma-zellen und siebzellen.
Planta - 58, 266-386.

65 - Kollmann, R. & Schumacher, W. (1962)b - Uber die Feinstruktur des phloems von Metasequoia glyptostroboides und seine jahreszeitlichen veranderungen.
III Die Reaktivierung der phloemzellen in fruhjahr.
Planta - 59, 195-221.

66 - Kollmann, R. & Schumacher, W. (1963) - Uber die Feinstruktur des phloems von Metasequoia glyptostroboides und seine jahreszeitlichen veränderungen.
IV. Weiters Beobachtungen zum feinbau der plasmabrücken in den siebzellen.
Planta - 60 360-389.

67 - Kollmann, R. & Schumacher, W. (1964) - Uber die Feinstruktur des phloems von Metasequoia glyptostroboides und seine jahreszeitlichen veränderungen.
V. die differenzierung der Siebzellen im verlaufe einer vegetationperiode.
Planta - 63, 155-190.

68 - Kursanov, A.L. (1956) - Recent advances in plant physiology in the U.S.S.R.
Annual review of plant physiology 7, 401-436.

69 - Küster, E. (1951) - Uber die siebplatten und siebfelder der Cucurbita - siebröhren und ihren kallus.
Ztchr. f. Plikros, 60, 157-162.

70 - La Fleche, D. (1966) - Ultrastructure et cytochimic des inclusions flagellees des cellules criblees de Phaseolus vulgaris. J. Microscopie. 5, 493-510.

71 - Ledbetter, M.C. & Porter, K.R. (1963) - A microtubule in plant cell fine structure. Jr. of cell biology. 19, 239-250.

72 - Luft, J. H. (1961) - Improvements in epoxy resin embedding methods. Jr. of Biophysics & Biochem. cytol.-9, 409.

73 - Mason, T.G. & Maskell, E.J. (1928) - A study of diurnal variation in the carbohydrates of leaf, bark and wood and of the effects of ringing. Ann. Bot. 42, 1-65.

74 - Mehta, A.S. & Spanner, D.C. (1962) - The fine structure of the sieve tubes of the petiole of Nymphoides peltatum (Gmel) O. Kunze. Annals of Bot. 26, 291-299.

75 - Munch, E. (1930) - Die stoffbewegungen in der pflanze.
1-1234, Gustav Fischer Jena.

76 - Murmanis, L. & Evert, R.F. (1966) - Some aspects of sieve cell ultrastructure in *Pinus strobus*.
Amer. J. of Bot. *53*, 1065-1078.

77 - Murmanis, L. & Evert, R.F. (1967) - Parenchyma cells of secondary phloem in *Pinus strobus*
Planta - *73*, 301-318.

78 - Northcote, D.H. & Wooding, F.B.P. (1966) - Development of sieve tubes in *Acer pseudoplatanus*.
Proc. Roy. Soc. B. - *163*, 524-537.

79 - Overbeek, J.V. (1956) - Absorption and translocation of plant regulators.
Annual Rev. of Plant Physiology. *7*, 355-372.

80 - Palmiquist (1938). - The simultaneous movement of carbohydrates and fluoresceine in oppositie directions in the phloem.
Amer. J. Bot. *25*, 97-105.

81 - Parker, J. & Philpott, D.E. (1961) - The ultra-structure of sieve plates of Macrocystis pyrifera.
Bulletin of the Torrey Botanical Club, 88 85-90.

82 - Parker, B.C. & Huber, J. (1965) - Translocation in Macrocystis II Fine structure of the sieve tubes.
Journal of phycology. 1, 172, 179.

83 - Pease, D.C. (1966) - Histological techniques for electronmicroscopy - 2nd Edition.
Academic Press, New York, London.

84 - Schumacher, W. & Kollmann, R. (1959). - zur Anatomie des Siebrohrenplasmas bei Passiflora coerulea.
Berichten der deutschen Botanischen Gasellschaft 4 176-179.

85 - Sharman, B.C. (1943) - Tannic Acid, Iron alum with saffranin & O.G. in studies of shoot apex.
Stain technology. 18, 105-111.

86 - Spanner, D.C. (1958) - The translocation of Sugar in sieve tubes.
Jr. of experimental Bot. 9, 332-342.

87 - Steedman, H.F. (1960) - Section cutting in microscopy.
Blackwell Scientific Publications - Oxford.

88 - Srivastava, L.M. & O'Brien, T.P. (1966) - on the ultrastructure of Cambium and its Vascular derivatives.
Protoplasma - 61, 277-293.

89 - Thaine, R. (1964) - Protoplasmic streaming theory of phloem transport.
Jr. of Exptl. Bot. 15, 470-480.

90 - Thaine, R., Probine, M.C. & Dyer, P.Y. (1967) - The existence of transcellular strands in mature sieve elements.
J. Exptl. Bot. - 18, 110-127.

91 - Tamulevich, S.R. & Evert, R.F. (1966) - Aspects of sieve element ultrastructure in Primula obconica.
Planta - 69, 319-337.

92 - Volz, G. (1952) - Elektronmikroskopische untersuchungen über die porengrössen pflanzlicher zellwände.
Mikroskopie. 7, 251-266.

93 - Waisel, Y & Fahn, A. (1965) - A radiological method for the determination of Cambial activity.
Physiologia plantarum - 18, 44-46.

94 - Wark, C.M. (1965) - Fine structure of the phloem of Pisum sativum II - The companion cell and phloem parenchyma.
Aust. J. Bot. 13, 185-193.

95 - Wark, C.M. & Chambers, T.C. - (1965) - Fine structure of the phloem of Pisum sativum 1 - Sieve element ontogeny.
Aust. J. Bot. 13, 171-183.

96 - Weatherley & Johnson (1967) - Form and function of sieve tube problem in reconciliation.
Internal Review of cytology (in the press).

97 - Wooding, F.B.P. & Northcote, D.H. (1965)a - The fine structure and development of the companion cells of the phloem of Acer pseudoplatanus. Jr. of Cell Biology 24, 117-128.

98 - Wooding, F.B.P. & Northcote, D.H. (1965)b - Association of the endoplasmic reticulum and the plastids in Acer and Pinus. Amer. J. Bot. 52, 526-531.

99 - Wooding, F.B.P. (1966) - The development of sieve elements of Pinus pinea. Planta - 69, 230-243.

100 - Ziegler, H. (1960) - Untersuchungen über die feinstruktur des phloems. I. Die Siebplatten bei Heracleum mantegazzianum. Somm, et Lev. Planta - 55, 1-12.

101 - Ziegler, H. (1963) - Untersuchungen über die feinstruktur des phloems. II Die Siebplatten bei der braunalge Macrocystis pyrifera. Protoplasma - 57, 786-799.

102 - Ziegler, H. & Ruck, I. (1967) - Investigations on the fine structure of the phloem.
III. The "Trumpet cell" of *Laminaria* species.
Planta - 73, 62-73.