THE DISAPPEARING PEEL TECHNIQUE: AN IMPROVED METHOD FOR STUDYING PERMINERALIZED PLANT TISSUES

by JOHN HOLMES *and* JOËLLE LOPEZ

ABSTRACT. A new technique is described for the preparation of sections of anatomically preserved plant fossils starting from the simple and well-known cellulose acetate peel method. Improvements of the results obtained by the peel method are described whereby use of very dilute acid allows extraction and observation in planar view of cell walls that are almost always destroyed by traditional methods. The Disappearing Peel Technique has been especially developed for histological investigations of the delicate cell walls of extra-xylary vascular tissue. Fern phloem from a Carboniferous coal ball is used to demonstrate this new method. It includes transfer of the peel to Araldite, dissolving the peel in acetone, complete demineralization of the plant cells remaining on the Araldite and allows optical examination of cell walls under immersion oil. Artefacts in coverslip and peel as well as residual carbonate content of the peel that may simulate cell wall sculpturing are thereby eliminated. Furthermore SEM and TEM observation of the same cell walls previously examined by the light microscope is possible. This technique will be applied to evolutionary studies of vascular tissues in Palaeozoic ferns.

THE techniques described here were developed while the authors were initiating studies on the vascular tissues of Palaeozoic ferns (Anachoropteridaceae, Botryopteridaceae, Psalixochlaenaceae) from British coal balls with a view to elucidating their phylogeny. Fertile structures of these essentially Carboniferous ferns are extremely rare and the number of taxa known to date, although confirming the leptosporangiate character of these ferns, is too small to fill in the gaps in our knowledge of their phylogeny. Morphological studies have revealed a wide range in anatomy, branching patterns, and habit (see references in Taylor 1981, pp. 525–527; Stewart 1983, pp. 193–194). Even so, phylogenetic trends within the group are not clear and their possible ancestry in Devonian plants and the extent of their connection with modern ferns remains unknown. These ferns are generally simple protostelic plants and possess only a small number of variable characters compared with gymnosperms or angiosperms. In the absence of fertile parts classification tends to be based on the xylem anatomy of leaves. One variable and widely available character that has only recently begun to be studied in detail and that may be of phylogenetic value in fossil ferns is the sculpturing of the cell walls of the vascular tissue, notably the phloem (Smoot 1979, 1985; Smoot and Taylor 1978, 1984).

The techniques presented in this paper were designed as a result of repeated failures by the authors to view successfully the phloem tissues in the stem of an anatomically preserved (coal ball) Palaeozoic filicalean fern in optical microscopy (OM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The morphology of this fern, *Psalixochlaena cylindrica* (Will) Holden, is known (Holmes 1977, 1981) but recently attempts to characterize the phloem tissues were fraught with artefacts in all three modes of observation, i.e. OM, SEM, and TEM. The word artefact is used here in the sense of phenomena that are not of biological origin or that are created during the preparation of the material. Additionally it was discovered that the use of traditional strengths of hydrochloric acid (1–6 %) used in the preparation of cellulose acetate peels (Joy *et al.* 1956) is almost totally destructive of delicate cell walls lying parallel to the coal ball surface. During optical microscopy examination of peels prepared using 6 % HCl and containing thin walled phloem cells, this destruction led to false observations caused by cell wall debris and

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the presence of calcite embedded within the peel. The new Disappearing Peel Technique has been devised not only to eliminate artefacts, but to totally isolate delicate cell walls from their mineral matrix with very dilute HCl whilst constantly providing them with a support to prevent collapse. Starting from the cellulose acetate peel method, this technique also enables the rapid preparation and transfer of the same group of cells to either of the three types of study, OM, SEM or TEM, with ease and a minimum of destruction.

METHODS

Initial methods of investigation, the peel method, and artefacts

(*a*) Optical microscopy. The traditional peel technique (Joy *et al.* 1956) consists in etching with HCl a cut and polished surface of a carbonate rock containing plant fossils (text-fig. 1A). HF is used for silica-preserved fossils. The cell walls then stand proud of the surface which is wetted with acetone. A sheet of cellulose acetate is applied which is softened by the acetone and embeds the cell walls. When dry, the sheet is peeled off (text-fig. 1C), hence the name 'peel' and, after washing in HCl to remove adhering crystals (text-fig. 1D, E), the peel is mounted in canada balsam on a slide under a coverslip.

There are many disadvantages to this traditional method. Observation of the acid-etching process under the binocular microscope revealed that HCl as dilute as 1 % was extremely destructive of delicate cell walls lying parallel to the coal ball surface. Face view observation of the sculpturing of these walls in OM and SEM is essential to the type of study being undertaken. Those cell walls lying at the surface are destroyed by effervescence as the acid penetrates beneath them and reacts with the calcite crystals filling the cell (text-fig. 1A, B). If the lower wall from the opposite side of the cell is pulled out by the peel (text-fig. 1C) then it is destroyed by the effervescence of its own calcite content as the peel is demineralized (text-fig. 1D, E). Therefore, only the cell outline remains to be seen in the peel. The result, in the stelar tissues of *P. cylindrica*, is that only a few tracheid walls will be visible in face view because their scalariform thickening confers the mechanical strength necessary to resist the effervescence. This destructive process was observed on large xylem cells; most phloem

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EXPLANATION OF PLATE 66

Fig. 1. SEM. Circular to oval configurations 1·5–6·0 μm in cellulose acetate peel produced by wetting with acetone, × 1,000.

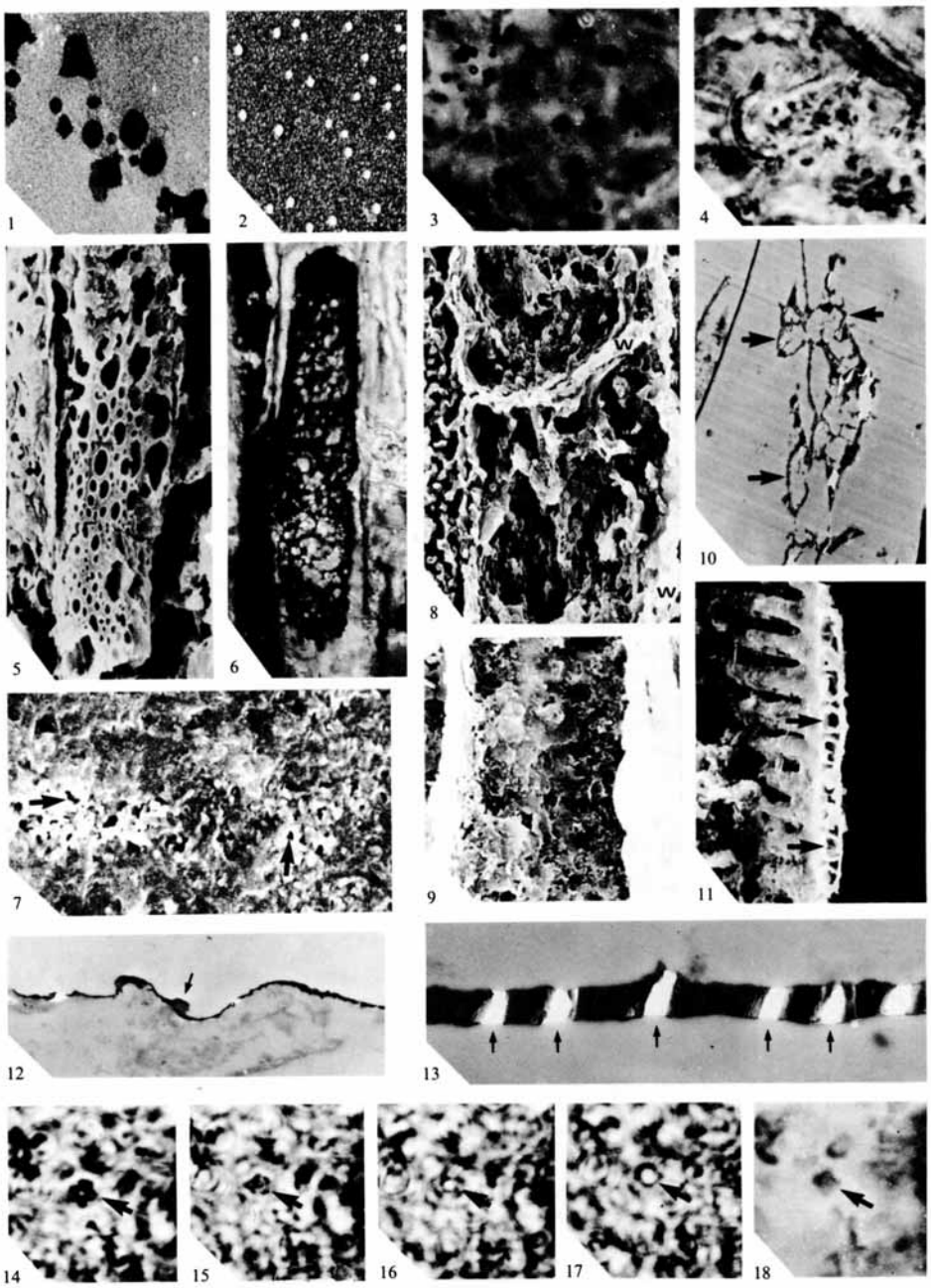
Fig. 2. SEM. Regular scattering of 0·1 μm spots on coverslip, × 14,400.

Figs. 3–11. *Psalixochlaena cylindrica*. 3, OM, phase contrast. Apparent pores 0·5–1·5 μm in wall of phloem cell, × 2,000. 4, OM, normal light. Apparent pores forming sieve areas in phloem cell wall. Effect probably produced by calcite in peel, × 2,000. 5, SEM. Contents of phloem parenchyma cell, × 7,200. 6, OM. Contents of phloem parenchyma cell. Note resemblance to sieve areas in cell wall, × 400. 7, SEM. Etched surface of calcite viewed in fault in xylem zone. Note numerous pits (arrows) in surface, × 3,600. 8, SEM. Phloem parenchyma cell viewed on etched surface of coal ball. Apart from outline of cell wall (w), distinction between organic matter and residual calcite is not clear, × 1,080. 9, SEM. View inside sieve cell on etched surface of coal ball. Pitted wall in face view cannot be identified with certainty as calcite or as cell wall, × 1,200. 10, TEM. Vertical section through xylem wall showing empty bars (arrows) of scalariform thickening, × 2,700. 11, SEM, idem fig. 10, × 1,200.

Fig. 12. TEM. Section through gold layer put on Araldite by sputter coater showing lump of gold (arrow) 0·4 μm across, × 6,800.

Fig. 13. TEM. Section through wall of phloem cell showing false pores (arrows) of 0·4 μm probably caused by stretching of Araldite. Note their barrel shape which recalls that of plasmodesma-derived pores, × 10,000.

Figs. 14–18. OM, normal light. Photographs taken inside a phloem cell. The arrowed area appears as a particle or group of particles (figs. 14, 15, 18) or as a hole (figs. 16 and 17) depending on the plane of focus. Opening (fig. 18) or closing (fig. 14) of the diaphragm totally alters the aspect of the field of view, × 3,000.



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cells are too small to be observed under the binocular microscope but the almost total lack of any of their walls lying in face view in the peel suggests that they are destroyed due to their more fragile nature.

As is demonstrated by Plate 70, fig. 5, a good deal of cell wall is ripped from the matrix by the peel and projects unprotected, but held in place by its calcite content, from the lower surface of the peel. The habit of some palaeobotanists of scrubbing their peels in acid is therefore highly destructive of longitudinally orientated walls. If the peel is not demineralized the lower cell wall is masked by its own calcite content in OM.

This cell wall destruction, combined with artificial optical phenomena described here caused the authors to observe and photograph false phloem cell walls for some considerable time.

Peels containing longitudinal sections of *P. cylindrica* phloem were mounted in canada balsam under a cover slip and viewed with a Leitz Orthoplan microscope in both normal and polarized light and a Leitz Ortholux for phase contrast. Plate 66, fig. 3 is typical of some of the pictures of 'cell wall' obtained in phase contrast and Plate 66, fig. 4 in normal light, where a scattering of circular pores about 0·75–2·0 μm in diameter appears to occur in the wall in planar view.

Several obstacles arose to the interpretation of pores within cell walls: some circular objects proved to be dust particles in the microscope since they occurred in the same position in every picture. SEM examination of surfaces of coverslips and of cellulose acetate sheet that had been previously wetted with acetone revealed a regular scattering of 0·1 μm particles on the glass surface (Pl. 66, fig. 2) and an irregular occurrence of circular patterns from 1·5 to 6·0 μm in the cellulose acetate (Pl. 66, fig. 1).

Further photographs from peels mounted in canada balsam are shown in Plate 66, figs. 14–18 and Plate 67, figs. 2, 3, 5, 7. They illustrate several other artefacts likely to be interpreted as sculpturing of cell walls. These artificial phenomena often occur where sieve cells are expected to be found. Plate 67, fig. 2 shows an apparent wall pattern viewed under polarized light that closely resembles that of the leptoid walls of *Polytrichum commune* viewed under the same conditions (Pl. 67, fig. 1). In the photograph (Pl. 67, fig. 2) there is in fact no cell wall present in face view and the 'sculpturing' effect may result from diffraction and interference patterns around calcite particles in the peel accentuated by over-use of the diaphragm. Plate 67, fig. 3 illustrates what was initially interpreted as a tangential sieve cell wall with a sieve area and other isolated pores. When these two examples were re-photographed under varying optical conditions it proved impossible to reproduce an identical negative. These artificial images are due not only to calcite embedded in the peel, but also to combinations of the many variable conditions of optical photography, e.g. condenser level, light intensity, polarization, diaphragm, etc. The peel shown in Plate 67, fig. 3 proved to be

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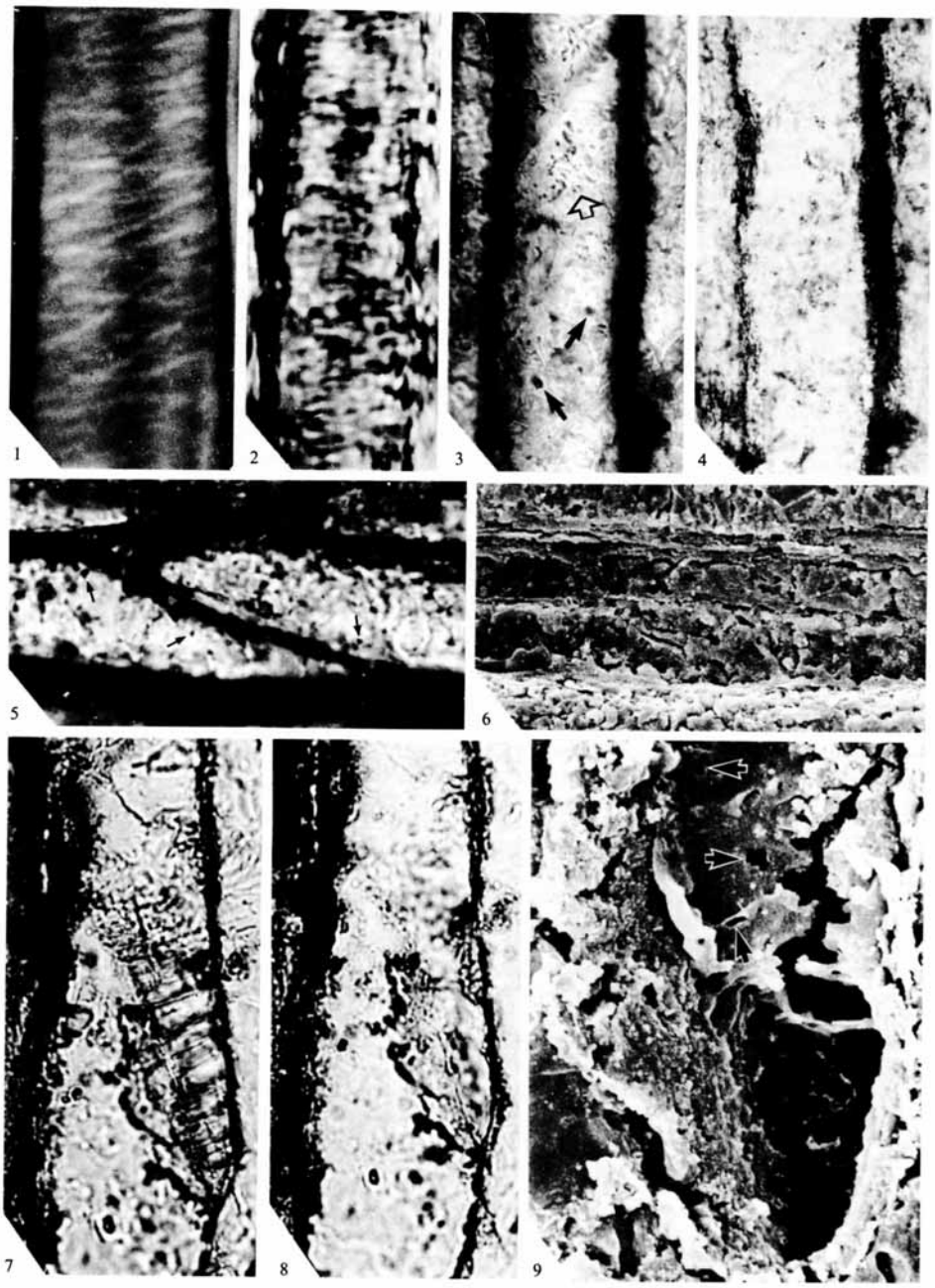
EXPLANATION OF PLATE 67

Fig. 1. OM, polarized light. *Polytrichum commune* leptoid wall. Re-photographed from specimen figured by Hébant (1964, fig. 6), × 2,000.

Figs. 2–9. *Psalixochlaena cylindrica*. 2, OM, polarized light. View inside sieve cell in longitudinal section. No cell wall is present here, note similar aspect to fig. 1, × 2,000. 3, OM. Tangential longitudinal section through sieve cell. Appearance of sieve area (large arrow) and other isolated pores (arrows) is due to calcite content of peel, × 600. 4, OM. Same peel as fig. 3 after 24 hrs. in 1 % HCl. Note disappearance of apparent perforated wall, × 600. 5, OM. Contact area between two sieve cells, note apparent perforated nature of lateral walls (arrows), × 600. 6, SEM. Exactly the same field of view as fig. 5 after preparation of section by Disappearing Peel Technique but without demineralization. The calcite content of the cells masks details of cell walls and creates in fig. 5 the impression of perforated lateral walls, × 600. 7, OM. Contact zone between two sieve cells with apparent wall sculpturing imitating sieve areas, × 1,000. 8, OM. Same field as fig. 7 after treatment by Disappearing Peel Technique, apparent wall sculpturing was due to calcite and has been removed by HCl. Note that although cell wall is shown to be present by SEM in fig. 9, it is too thin to be observable here, × 1,000. 9, SEM. View of area where false wall sculpturing due to calcite has disappeared in fig. 8. Note delicate cell wall with pores (arrows) about 1·0 μm in diameter, × 3,000.



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incompletely demineralized. It was removed from the slide and left overnight in 1 % HCl. When re-photographed (Pl. 67, fig. 4), the 'perforated wall' had disappeared, demonstrating that the illusion of a cell wall had been produced by calcite filling the intracellular space.

One factor probably responsible for the production of artefacts by diffraction and interference in the etch-peel technique is the white powder of some 300–500 μm depth that forms on the coal ball surface after etching with HCl. Despite placing peels in HCl to remove the adhering crystals pulled off with the peel, some of this powder is actually incorporated within the peel (text-fig. 1E) and is protected when the peel is demineralized in acid. This was demonstrated by scraping the undersurface of a demineralized peel, and treating the scrapings with HCl where they were seen to effervesce. The formation of this powder seems to be accompanied by an increase in volume as it is often level with or above cell walls exposed by etching prior to making a peel. Comparison of Plate 67, figs. 5, 6 shows the extent to which this powder masks details of cell walls. Analysis by X-ray diffraction showed that this white powder retains a crystalline structure and may simply be partially dissolved $CaCO_3$. Its composition is not clear, a small calcium peak occurred with a larger peak for an unidentified component. Tests for chlorides were negative. *A priori* this powder is another source of diffraction in light microscopy and may create the impression of pores and mask details of true cell walls. Plate 67, figs. 5, 7 illustrate phloem end walls which, in OM, appear to have some kind of sculpturing. Application of the Disappearing Peel Technique to these specimens revealed the artificial nature of these wall patterns.

Experiments in 'peeling' the surfaces of extant leaves showed that cellulose acetate perfectly moulds the smallest detail of cells and stomata and that this is best seen in phase contrast microscopy. It is possible, therefore, that when a peel is removed from a coal ball surface it has moulded not only the carbonate surface but cell walls that have remained in the matrix. The apparent perforations seen in phase contrast in Plate 66, fig. 3 and in normal light in Plate 66, fig. 4 may be due not only to patterns in the coverslip (Pl. 66, fig. 2) and circular areas (Pl. 66, fig. 1) or calcite powder in the peel but also to moulding of the carbonate surface or cell wall by the acetate sheet.

Work on extant Filicales (Esau 1969; Hébant 1969; Lamoureux 1961; Liberman-Maxe 1968, 1978; Maxe 1964) has shown that these ferns have wall perforations of 0·2–1·5 μm or more rarely up to 4·0 μm in their metaphloem cell walls. One logically expects to find similar perforations in the phloem walls of a Palaeozoic filicalean. However, this size range of pores is exactly that of the potentially interfering objects in peels and coverslips described above. The permanent calcite content of the peel is another factor that may create the appearance of pores by optical effects. Around the 0·5 μm order of magnitude and below (the limit of resolution of OM) a particle and a hole of the same size give the same diffraction pattern and turn from dark to light on changing focus. Additionally, use of the diaphragm can totally change the image at high magnification. Plate 66, figs. 14–18 are five photographs of the same field within a phloem cell showing an area which may appear as a particle (Pl. 66, figs. 14, 15, 18) or a hole (Pl. 66, figs. 16, 17) depending on the level of focus. This area totally changes in appearance with different diaphragm values (compare Pl. 66, figs. 14 and 18) and it was impossible to judge, at such high magnification, whether the area represented a pore or whether indeed cell wall was present.

Several interfering factors (dust, particles on coverslip, patterns and calcite in peel) are present in traditional preparations of longitudinal peel sections through permineralized tissues. We have found that these are likely to create interference and imitate a pitted cell wall, specifically in areas where cell wall is not, in fact, present in planar view. Cellulose acetate sheet, previously wetted with acetone, develops circular areas which do not appear to be bubbles (Pl. 66, fig. 1). All these factors, and the alteration of image obtained by changes in diaphragm closure and focusing, caused the authors to feel that little faith could be placed in high-magnification optical micrographs such as those referenced in this section where apparently perforations are present in a cell wall. It was concluded that not only is it difficult to detect if cell wall is really present but cellulose acetate sheet is not a good medium through which to detect minute pores in thin cell walls seen in planar view.

(*b*) Scanning electron microscopy. Stems of the fern *P. cylindrica* were examined on both broken and sawn etched surfaces in longitudinal section. The specimens figured here received 250–300 Å of gold in a Polaron E5000 sputter coater and were examined with a Jeol JSM 35 Scanning Electron Microscope. Although the phloem zone is easy to locate external to the xylem, the photographs proved difficult to interpret for the following reasons. First, the different cell layers are not as easy to identify as they are in a series of peel sections viewed in OM; secondly, with the JSM 35 used by the authors there is no method of knowing whether one is viewing carbonate or organic matter in the image provided by the secondary emission of electrons. The phloem cells under investigation generally contained a mass of irregularities and projections which probably correspond to the white powder mentioned above which remains after etching (Pl. 66, fig. 8). Although this powder dissolves in HCl, further etching to reach a stage where empty cells were visible caused the collapse of these cells.

Smooth areas of calcite in a fault in the coal ball where no powder was present proved to contain a scattering of pits 0·2–0·8 μm (Pl. 66, fig. 7) much as one would expect to find in a phloem cell wall! After development of the Disappearing Peel Technique described below which allows isolation of organic matter from its carbonate matrix, the authors returned to SEM examination of etched surfaces, and in fields such as Plate 66, fig. 9 were not able to decide if they were viewing calcite surfaces or perforated walls inside a phloem cell.

(*c*) Transmission electron microscopy. Portions of stem 5–10 mm long were isolated with a microsaw and left in a bath of 1 % HCl for 48 hrs. with one change of acid. Any movement of the isolated stems caused them to break into small fragments often losing their cortex and phloem. The remaining stelar portions were embedded in a standard acetone–Araldite sequence and cut with a glass knife on a SORVALL MT1 ultramicrotome. Ultra-thin sections of 600 Å were examined with a JEM 200C microscope. Due to the fragility and small size of the stem fragments, their precise longitudinal or transverse orientation on the stubs proved impossible and the resulting micrographs were difficult to interpret.

A number of phloem cell walls appeared to have many pores averaging 0·4 μm diameter whose barrel-like shape corresponds to that of certain types of plasmodesmata-derived pores in extant plants, but they were apparently unpenetrated by the Araldite (Pl. 66, fig. 13). Much longer embedding baths were given to further specimens and subjected to vacuum at the pure acetone and pure Araldite stages, the whole embedding process lasting one week. The same results were produced. To determine whether or not these 'pores' were filled by an electron lucid substance, ultra-thin sections on grids were examined by SEM. This showed that the 'pores' were empty and low power transmission micrographs revealed their regular orientation perpendicular to knife marks. It was thus concluded that these 'pores' were artificial and resulted from stretching of the Araldite during sectioning causing rupture of the cell wall. However, in view of their very regular occurrence, the possibility of rupture having occurred at the site of an original plasmodesma is not dismissed.

Using these methods, several more convincing pictures of small pores 0·1–0·2 μm were obtained in both transverse (Pl. 70, fig. 4) and longitudinal sections (Pl. 70, fig. 8). These pores were correctly penetrated by the Araldite but no accurate identification of the tissue these walls occurred in was possible, due to the specimens being fragmentary and sectioned in an unknown plane.

The first specimens prepared by this method sectioned correctly but further preparations still contained mineral matter that blunted the knives. Any other treatment to remove dolomite, pyrite, and perhaps silica would have resulted in further fragmentation of the specimens. This type of preparation was therefore considered inadequate and abandoned.

Efforts at sectioning portions of embedded peel, in which tissue positions had been previously identified, revealed that, despite attempted demineralization, they still contained mineral matter and confirmed the permanent powder content of the peels already referred to above. A few semi-thin microtome sections by this method were successful but the thin acetate sections curled up in contact with chloroform vapour used to disperse the sections on the glass knife. Both these obstacles rendered this method unsatisfactory.

The initial investigations in OM, SEM, and TEM described above, although using inadequate techniques, led us to suspect the existence of perforations 0·1–2·0 μm in the phloem cell walls of *P. cylindrica*. Paradoxically this later turned out to be true, but at this stage our conclusions were based mainly on observation of artefacts. After the discovery of these the need to refine the techniques became evident.

Improved method of peel preparation

The destructive effect on cell walls of using HCl concentrations higher than 1 % has been described (text-fig. 1A–E). By etching longitudinally orientated fern stems with HCl solutions ranging from 1·0 % down to 0·01 %, we found that with very weak acid, a greater amount of cell contents, and also cell wall was exposed undamaged parallel to the surface in the peel. In fact there was little observable difference in results between 0·1 % and 0·01 %; therefore, a compromise strength of 0·05 % HCl was used.

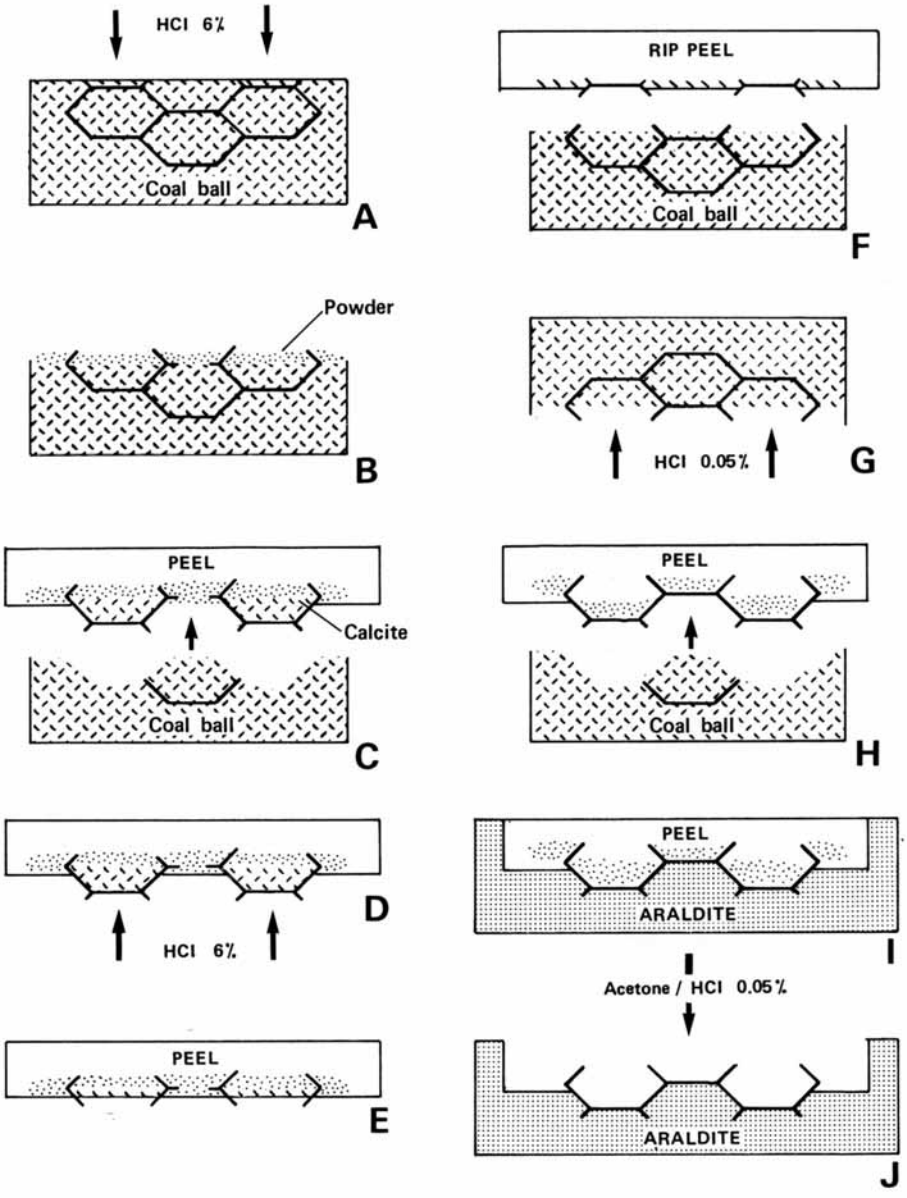
The ground surface to be etched is placed upside down (text-fig. 1G) to let debris fall away, on convenient supports (a knob of Plasticine in each corner) in 1 litre of the dilute acid with a magnetic stirrer. Other surfaces of the block can be protected with grease, paraffin, etc. The depth of etch depends on the cell type to be examined and should correspond to a half to three-quarters of the cell diameter. A deeper etch renders the collapse of the cell more likely. On non-dolomitic coal balls from England, 30–60 sec. in 0·05 % HCl gives an approximately 1·0 μm deep etch. The peel is then placed over the etched surface which has been wetted with acetone. Normally, therefore, only the lower surface of the peel is in contact with the acetone but the authors have acquired the habit of also wetting the upper surface to render the acetate completely liquid and ensure a maximum embedding of the organic matter.

After drying, the peel is removed by separating it from the rock with as little bending as possible and in a direction perpendicular to the stem to avoid shattering of delicate cell walls. Some phloem parenchyma walls are only 0·2 μm thick. A razor blade is not used because this is likely to shear off cell walls projecting beneath the peel (text-fig. 1D). Demineralization is not performed at this stage because, as stated above, cell walls embedded in the lower surface of the peel will be destroyed by the reaction of their calcite content with the acid (text-fig. 1D, E).

Following this first peel of the fern stem, another peel is placed on the surface without prior etching. When removed after drying, those cell walls flush with the surface that would normally be deteriorated by effervescence are ripped out (text-fig. 1F). This is termed a rip peel. Good examples of results obtained by this technique are seen in Plate 68, figs. 1–5.

The authors have also employed the technique of etching for a depth of 1·0 μm or less after highly polishing the coal-ball surface, first with fine carborundum powder on a glass plate, then with aluminium silicate powder on a revolving felt pad. The surface must shine. This gives a thin longitudinal section (a one micron peel) through a vertical cell wall where pores as small as 0·3 μm are visible under a ×40 oil immersion lens and the pitting of the cell wall over a large area can be rapidly determined (Pl. 70, fig. 6). For cell walls to be examined in planar view this type of peel may also have the same, if not better result than the rip peel. Portions of cell wall are removed undestroyed from the coal-ball surface having been slightly freed from the carbonate matrix by the delicate etching (Pl. 70, fig. 7).

TEXT-FIG. 1. A–E. Destructive effect of traditional peel method on cell walls as seen in section view. A, HCl etch of coal ball surface. B, cells exposed, upper cell walls destroyed. C, peel removed from coal ball, calcite content in cell walls projecting from lower surface. D, demineralization of peel with HCl. E, lower cell walls destroyed by reaction of HCl with their calcite content. Note permanent calcite content of cells and peel. F–J, new method. F, extraction of cell walls at surface by rip peel, no etching involved. G, dilute HCl etch to avoid damage to cell walls. H, removal of peel from coal ball. I, transfer of undemineralized peel to Araldite. J, after dissolving of peel with acetone and of calcite with HCl, cells are left exposed on Araldite support. See text-fig. 2 for applications to OM, SEM, and TEM.


HCl 6%
Coal ball
A
Powder
B
PEEL
Calcite
Coal ball
C
D
HCl 6%
PEEL
E
RIP PEEL
Coal ball
F
HCl 0.05%
G
PEEL
Coal ball
H
PEEL
ARALDITE
I
Acetone / HCl 0.05%
ARALDITE
J

The investigator must use rip peels, one micron peels, or deeper etch peels in an order that obtains the maximum amount of information from cells exposed at any one moment.

The Disappearing Peel Technique

Slides must be prepared in the following manner. A portion of cellulose acetate sheet, slightly larger than the peel that is to be mounted, is placed on a glass slide wetted with acetone. When the acetate sheet dries, it adheres to the slide merely by the exclusion of air. The acetate is then covered with a thin layer of commercial Araldite (manufactured by Ciba-Geigy and sold in two tubes, epoxy-resin and hardener) which must spread beyond the edges of the acetate sheet on to the glass. The glue is left to harden for at least 12 hrs. at 55 °C (text-fig. 2A).

The acetate sheet, which is simply in contact with and does not firmly adhere to the glass, serves to liberate chosen portions of the final preparation when cut out for TEM. The layer of Araldite provides a rigid support for the organic matter during the following stages.

A peel containing the fossil section is glued, with the same type of Araldite, rough side down on to a slide prepared as above (text-figs. 1I, 2B). It is first placed smooth side down on a heavily greased glass plate. A small amount of Araldite is spread over the rough, now uppermost, surface of the peel where the organic matter is embedded, and also over the prepared area of the slide. Both are heated for 5 min. at 55 °C. This renders the Araldite quite fluid. The slide surface with the liquid Araldite on it is turned over on to the peel on the glass plate and a lead weight placed on top. The preparation is polymerized at 55 °C for 45 min. after which time the slide is easily removed from the glass plate. The timing is critical as after longer periods the slide may stick to the glass despite the grease. It is therefore advisable to predetermine the minimum time taken for the glue to become hard but of a rubbery consistency. Excess grease is removed from the peel with xylol and polymerization is continued overnight at 55 °C.

This type of mounting is no more arduous than mounting under a coverslip in balsam and the peel can be examined by transmitted light at this stage. Any blurring caused by irregularities in the peel surface can be eliminated by applying a smear of immersion oil.

In the above preparation, commercial Araldite is used for its resistance to the acetone and acid treatments that now follow and provides a suitably transparent support for OM by transmitted light.

The slide is placed, peel side sloping downwards, in an acetone bath with a magnetic stirrer for 15 min. and during this time the cellulose acetate dissolves completely (text-fig. 2C). The organic matter remains undisturbed and is now exposed on the Araldite which supports its lower surface (text-figs. 1J, 2D).

Demineralization is now effected to remove the calcite powder previously imprisoned in the peel. The disappearance of a white hue from the section indicates this change. For longitudinal sections where small cells filled with calcite are likely to have been removed from the coal ball, a 1–2 hrs. bath of 0·05 % HCl is recommended to avoid breakage of cells and their contents by effervescence. The exact demineralization time can be determined by an examination under polarized light. Treatment with HCl seems sufficient to remove the mineral matter for coal ball plants.

(*a*) Optical microscopy. The hollow left by the dissolving of the acetate peel is filled with a drop of immersion oil (text-fig. 2E). A slight vacuum may be necessary for a few minutes if air bubbles

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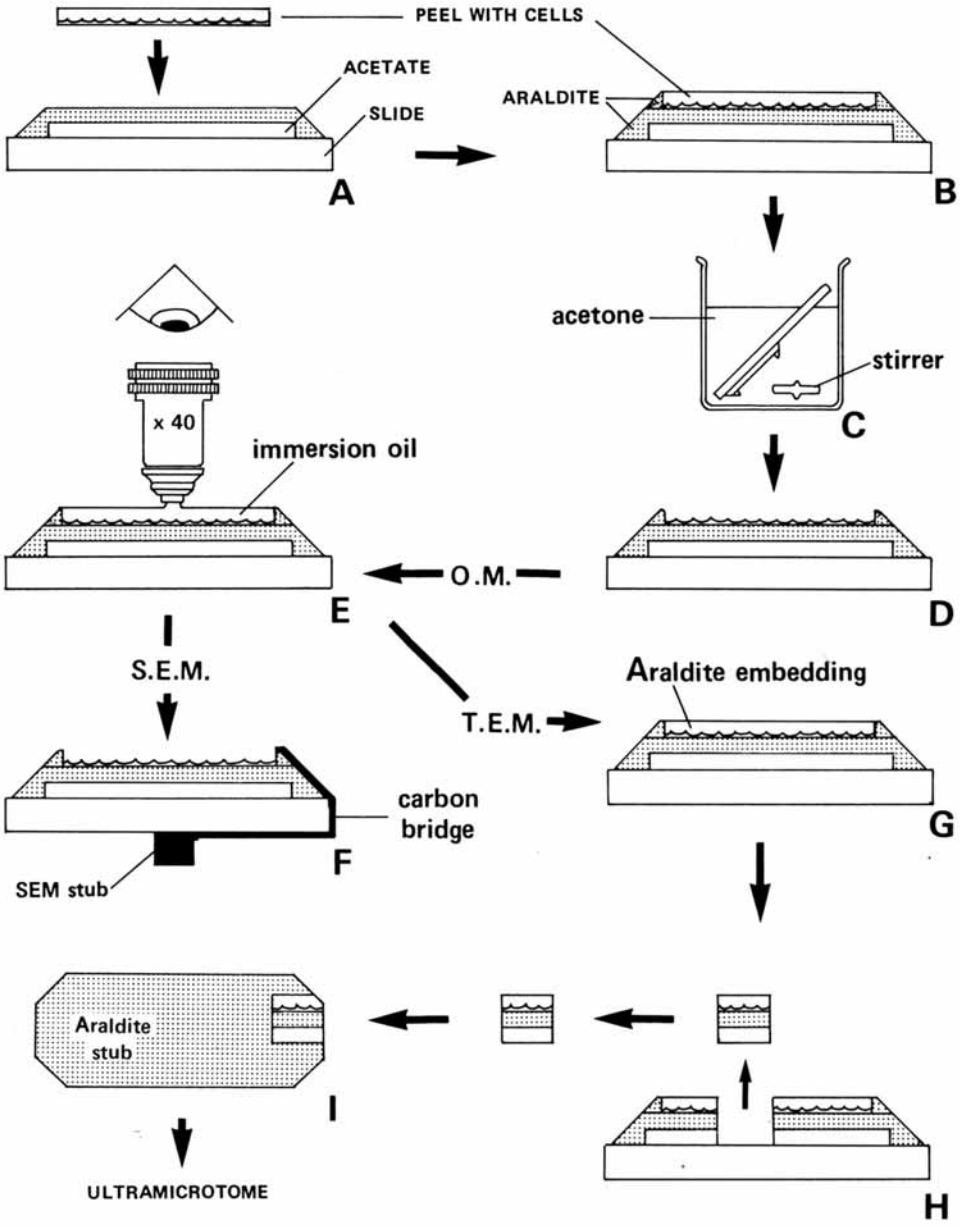
TEXT-FIG. 2. Applications of the Disappearing Peel Technique to OM, SEM, and TEM. A, B, peel with cells is glued with Araldite on to a slide prepared as described in text. C, peel is dissolved by 15 min. agitated bath in acetone. D, the dissolution of the peel leaves the cells exposed in a hollow in the Araldite. E, optical microscopy is performed after filling the hollow with immersion oil. Immersion objectives dip directly into the oil. F, after removal of immersion oil by acetone (as in C) the slide is placed on SEM stub and gold coated. Note the carbon bridge. G, in preparation for TEM studies, the cells are embedded by filling the hollow with Araldite. H and I, a selected portion of the slide is dissected out and glued on to an Araldite stub for sectioning with ultramicrotome.


PEEL WITH CELLS
ACETATE
SLIDE
A
ARALDITE
B
acetone
stirrer
C
x 40
immersion oil
E
O.M.
D
S.E.M.
T.E.M.
Araldite embedding
carbon
bridge
F
SEM stub
G
Araldite
stub
I
ULTRAMICROTOME
H

remain inside entire cells. Wall sculpturing of stelar cells may now be photographed without interference from peel or coverslip. Oil immersion objectives may dip directly into the oil covering the preparation (text-fig. 2E). This is particularly useful as it solves the often-encountered problem where the combined thickness of coverslip and peel prevents focusing of some × 100 objectives.

At this stage of the preparation the investigator has the choice of performing either SEM or TEM on groups of cells that appear of interest under the optical microscope.

(*b*) Scanning electron microscopy. The immersion oil is removed from the slide by washing in acetone for 2 min. (as in text-fig. 2C) and the end of the slide bearing the specimen is broken off after scoring with a diamond and mounted on an SEM stub with double-sided tape. The observation chamber of the JSM 35 conveniently housed the 25 × 25 mm slide bearing the preparation. Two bridges of conducting carbon glue must be made between the upper surface of the slide and the stub before metal coating (text-fig. 2F).

The greatest advantage of this type of preparation is obtained by giving the coal ball surface a very high polish (as for a one micron peel) before etching and making the peel. In observing longitudinal sections not only can one see cell walls in planar view but the vertical cell walls are seen neatly sectioned by the polishing with their pores cut at different levels (Pl. 69, figs. 5, 6). The SEM picture thus combines the advantages of depth of field with a section view of walls rising towards the observer.

As already pointed out, when etched pieces of coal ball containing fossil tissue are examined by SEM, it is difficult, if not impossible, to distinguish organic matter from its mineral support (Pl. 66, figs. 8, 9). The same problem exists when the organic matter from a peel is transferred to its Araldite support which also contains many pits. This is probably because the liquid acetate sheet moulds the calcite surface where pits occur (Pl. 66, fig. 7) and in our new technique the fluid Araldite then moulds the peel. A control SEM observation of the Araldite surface before and after acetone and acid treatment revealed it to be perfectly smooth in both cases. The transfer of these pits to the Araldite by moulding is one confusing artefact that we have not been able to eliminate. This highlights the fact that a careful observation of the tissues is first necessary by a series of light micrographs at different levels of focus (Pl. 68, figs. 3, 4). This not only ascertains the extent of cell walls but an optical map of overlapping photographs helps rapidly locate a given area under SEM. Arrows can also be engraved in the Araldite as an aid to location.

In a radial section through a stem it is almost impossible to obtain a face view of walls of all cells comprising the phloem zone in order to compare the sculpturing of the different types of cell wall. This information can be more easily obtained by peeling well-preserved axes sectioned transverse-obliquely at 20–30° and etched to a depth of 15–20 μm after fine polishing. After transfer to

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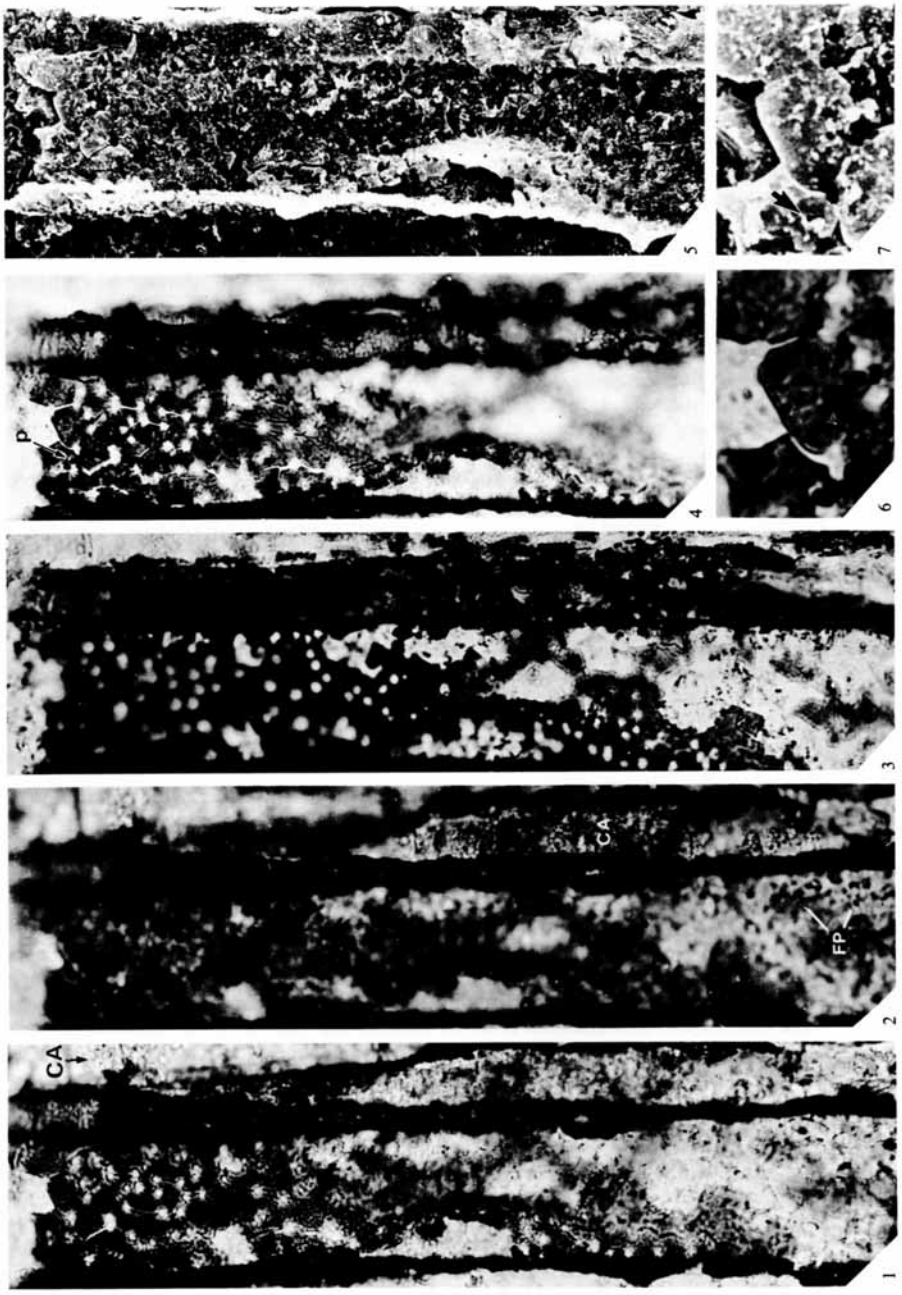
EXPLANATION OF PLATE 68

Figs. 1–7. Radial wall of sieve cell of *Psalixochlaena cylindrica* with numerous pores. Rip peel prepared by the Disappearing Peel Technique. 1, OM. Areas where cell wall are present appear dark. Plane of focus is low causing pores at top and bottom of picture to appear white. CA = calcite, × 700. 2, OM. Higher plane of focus causing all pores to appear dark. Note false pores (FP) at bottom of picture where no wall is present. Figs. 1 and 2 are not demineralized, note zones of calcite (CA) imitating perforated wall, × 700. 3, OM. Demineralized section. Full extent of perforation is visible as plane of focus, intermediate between figs. 1 and 2, causes almost all pores to appear as white patches, × 700. 4, OM. Demineralized section in same plane of focus as fig. 1. Arrow shows 0·5 μm pore (magnified under SEM in fig. 7) in focus as white patch, × 700. 5, SEM of same cell shows up relief, notably bulging part of wall in lower part of picture which is probably part of end contact wall, × 700. 6, OM. Detail of uppermost portion of cell showing dark patches on wall. These are not focusable as white and without SEM confirmation (fig. 7) cannot be identified as minute pores or particles, × 2,000. 7, SEM. Same field as fig. 6. Some but not all of dark patches correspond to particles on the cell wall. Note 0·5 μm pore (arrow) focused as white patch at top of fig. 4, × 2,000.



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Araldite and complete demineralization, the stem is viewed under SEM where the walls are seen rising obliquely from the support.

By this preparation one can observe almost in face view, on one side of the stem the inner, and on the other side of the stem the outer tangential walls of all tissues with the rising radial walls seen in section view (Pl. 69, fig. 6). At 90° to this axis both sides of radial walls can be examined in face view with tangential walls in section view. This type of preparation is perhaps the richest in information with the limitation that only about 40–50 μm lengths of wall are visible and any possible patterns of alternating smooth and pitted wall can only be viewed over greater lengths in longitudinal section. The thickness of this type of preparation renders prior optical examination uninformative.

(*c*) Transmission electron microscopy. After optical examination and photography of areas of interest, the immersion oil is washed away from the slide with acetone and replaced, after drying, by a drop of embedding quality Araldite (M Araldite) containing hardener and accelerator (text-fig. 2G; composition: Araldite M–10 ml, Hardener HY 964–10 ml, Dibutyl phthalate–1 ml. Well mix all three before adding Accelerator DY 064–0·6 ml). The slide is put under a −1 bar vacuum overnight and then hardened at 55 °C for two and a half days. The embedding of a thickness of only one or two cell walls is rapid, and eliminates the need for several baths of progressively less dilute Araldite solutions.

If no further use is immediately made of the slide, this stage represents a good permanent preparation of a demineralized section on which optical photography can be performed.

Small portions of the preparation about 1 mm × 2 mm containing cell wall to be microtomed are dissected out. The layer of cellulose acetate sheet in contact with the slide (text-fig. 2H) immediately liberates the dissected portion which is then glued on to the end of an Araldite stub with the required orientation (text-fig. 2I).

If the slide contains a tangential section, then several cells of the same tissue can fairly easily be dissected out. For radial sections as in the case of the sieve cell shown Plate 70, fig. 2, it may be desired to section one cell only. A microscope is then set up horizontally to view the stub in the microtome vice and to compare the cells in the dissected portion with optical micrographs taken previously. It is thus possible to spot the tissue from which the ultra-thin sections originate when the knife passes through the chosen cell.

RESULTS

A lesson in artefacts

The authors' conclusion is that, contrary to xylem walls where well-defined scalariform pitting is present, the identification of a thin phloem cell wall perforated by minute pores is highly problematical. When treated with the Disappearing Peel Technique and demineralized, the end wall in Plate 67, fig. 7 loses its artificial sculpturing (Pl. 67, fig. 8), and examination of the same field by SEM reveals a partly intact cell wall with scattered perforations to the order of 1·0 μm in diameter (Pl. 67, fig. 9). It is important to point out here that this peel was not washed in HCl following removal from the coal ball surface: had this been done the delicate cell wall rendered visible under SEM by the Disappearing Peel Technique would have been destroyed by the effervescence of the same calcite content causing artificial wall sculpturing. These transverse crystalline structures across the end walls have been observed several times and only in the sieve cell position. It has been suggested to us (J. Moret, *pers. comm.*) that this may be due to surface tension or gravitational effects. The cell in Plate 67, fig. 3 was not treated by our new technique but was merely washed in 1 % HCl for 24 hrs. It is seen to have no tangential wall after acid treatment (Pl. 67, fig. 4), and the entire aspect of a pitted wall in this figure was created by a calcite infill. By washing the peel in acid, any cell wall that might have been present behind the calcite has been destroyed by effervescence of the latter.

Thus an important aspect of the Disappearing Peel Technique is to protect these cell walls that project from the lower surface of the peel by providing them with an Araldite support on a slide as soon as they are removed from the coal ball (text-fig. 1I).

Plate 67, fig. 5 shows a terminal contact wall between two phloem cells. The side walls appear to contain scattered circular 1·0 μm perforations. This field, viewed by SEM after the Disappearing Peel Technique but without demineralization, shows the upper layer of calcite powder picked up by the peel (Pl. 67, fig. 6). Many depressions occur in the carbonate which are responsible for the apparently perforate wall seen in optical view (Pl. 67, fig. 5).

In view of these results, optical micrographs (Pl. 66, figs. 3, 4; Pl. 67, figs. 2, 3, 5, 7) from mounted peels and scanning electron micrographs from etched coal ball blocks (Pl. 66, fig. 9), where groups of pores or sieve areas appear to be present in a cell wall, must be accepted with great caution. There is doubt not only as to whether real pores are being viewed but also as to whether organic cell wall is actually present. Finally, the first pores the authors viewed with TEM were very convincing indeed (Pl. 66, fig. 13), but also difficult to interpret as this type of preparation was being viewed for the first time.

It was only the successful viewing of real cell walls in planar view (Pl. 68, figs. 1–4; Pl. 70, fig. 7), either from rip peels or from peels made after very dilute acid treatment and their preparation by the Disappearing Peel Technique, that finally revealed the artificiality of the above examples.

Botanical results

Plate 68, figs. 1–5 shows in face view the radial wall of a sieve cell picked up by a rip peel. The wall appears dark in light microscopy and the 0·5–1·5 μm perforations are easily visible. However, when photographed before demineralization it can be seen that zones of calcite (Pl. 68, figs. 1 and 2) remarkably imitate a perforated cell wall. These zones disappear in Plate 68, figs. 3 and 4 following demineralization. Examination of the same wall by SEM (Pl. 68, fig. 5; Pl. 69, fig. 1) not only confirms the perforate nature of what is suspected to be a pitted wall but also shows up much smaller pores 0·2 μm across (Pl. 69, fig. 3) undetected by any plane of focus in light microscopy.

It is obvious that optical mapping in several planes of focus (Pl. 68, figs. 3 and 4) is absolutely necessary to establish the true extent of the cell wall before proceeding to an SEM examination. It was found that the only valid distinction of a 1·0 μm pore in OM is that it must be focusable as a white spot with low magnification objectives such as × 10 (Pl. 70, fig. 2). The false 0·5 μm pores in Plate 69, fig. 7 only became apparent with a × 100 objective and not at lower powers.

Focusing on the upper surface of the wall (Pl. 68, fig. 2) in the cone of light diffracted by the pores causes them to appear dark and indistinguishable from other dark patches in the field of view giving the impression of a more extensive pitted wall than really exists. Combined OM and SEM of areas where small dark patches occur in the cell wall such as that seen in Plate 68, fig. 6 and 7 reveals that they are particulate and not perforate. The importance of this lies in the fact that pores much smaller than the wavelength of light used will always appear dark. The authors have found, however, that pores as small as 0·5 μm (the wavelength of green light) can be focused as white patches (Pl. 68, figs. 4, 7)

The SEM micrograph in Plate 68, fig. 5 shows a bulging portion of the same cell wall whose shape is not clearly distinguishable in OM (Pl. 68, figs. 1–4). The size and curvature of this portion suggests it is part of a contact area between two sieve cells (compare with Pl. 67, fig. 5). It is perforate in the same manner as the lateral walls.

SEM examination of other sieve cell walls, either in longitudinal section or in polished oblique section (Pl. 69, fig. 6) reveals a scattering of pores 0·5–1·5 μm (rarely 2·0 μm) in diameter on both radial and tangential walls. Barrel-shaped pores have been seen in some cell walls (Pl. 69, fig. 5).

Plate 69, fig. 4 shows a wall fracture leading to a pore and the tapering of the wall towards the pore edge is seen in section. A striking feature of the pores is the very irregular nature of their periphery. Plate 69, fig. 2 shows one of a few examples found of an area of similar diameter (1·6 μm) but composed of a number of much smaller pores (0·2 μm). It is thought possible that the larger pores each result from the collapse of such an area leaving an irregular border. Whether this happened during the life of the plant, during fossilization, or during preparation of the fossil section, is unknown.

Contrary to the above example, combined OM and SEM study of the same cell reveal that its

wall may convincingly appear to be pitted in transmitted light but appears smooth viewed by SEM (Pl. 69, figs. 7 and 8). This illustrates the fallibility of high power optical micrographs.

These correlated OM and SEM observations also reveal that sponge-like contents, which in *P. cylindrica* occur in all cells of the phloem tissue except for sieve cells, may appear very similar to walls with pores or sieve areas in OM (Pl. 66, fig. 6). These contents are preserved in the peel all the more frequently when weak acid is used for etching. Scanning electron micrographs reveal the volume and sponge-like nature of the cell content which contains a mass of pores from 1·0–4·0 μm wide upwards (Pl. 66, fig. 5). There is positional evidence compared with modern plants that these cell contents do in fact represent remains of cytoplasm. The sieve elements of modern ferns and the leptoids of bryophytes are almost devoid of cytoplasm whereas the parenchyma cells that surround them retain their contents. Cell contents occur in the phloem zone of *P. cylindrica* (Pl. 70, fig. 1) in homologous positions.

The method of preparation for TEM described here by the Disappearing Peel Technique has the advantage, as in SEM preparation, of enabling the precise identification, by prior optical examination, of the tissue to be microtomed. To our knowledge this is the first time fossil phloem has been viewed in ultra-thin section by TEM. Breaking of the cell walls occurs frequently. This is believed to be due to the elasticity of the Araldite and the brittle nature of the fossil material because the Araldite embedding and glass knife sectioning techniques are those used by the late Professor C. Hébant in this laboratory and provide excellent results on extant material. Additionally, trials on fusainized Lower Carboniferous material from Loch Humphrey Burn showed no shattering. Slightly better results have been obtained recently using a diamond knife on fossil material (Pl. 70, fig. 5). Another advantage of this type of preparation is that the thin sections of the fossil cell wall occur at the junction of the support and embedding Araldite. The former is much darker than the latter (Pl. 70, figs. 5, 9) and allows easy location of this zone at low magnification.

The multiple pitted sieve cell wall seen in radial section in Plate 70, fig. 2 is seen in ultra-thin section in Plate 70, figs. 3 and 5. These micrographs confirm in TEM the presence of 1·0–2·0 μm pores and reveal the presence, on one side of the wall, of larger depressions similar to those of some bryophytes (Burr *et al.* 1974, fig. 8). A portion of the same wall is seen totally embedded in the support Araldite (Pl. 70, fig. 5). No vacuum is applied when the peel is glued to the slide. The ease with which one cell wall is embedded and the manner in which fragile cell walls project from beneath the peel is thereby illustrated.

A transmission electron micrograph of a section through the tangential wall of a phloem parenchyma cell with pores 0·2–0·02 μm in diameter is seen in Plate 70, fig. 9. The smallest pores are likely to be invisible by SEM where the layer of gold is at least as thick as the width of a pore. No contrasting was used in the preparation in this figure. Staining with $KMnO_4$ only served to mask the

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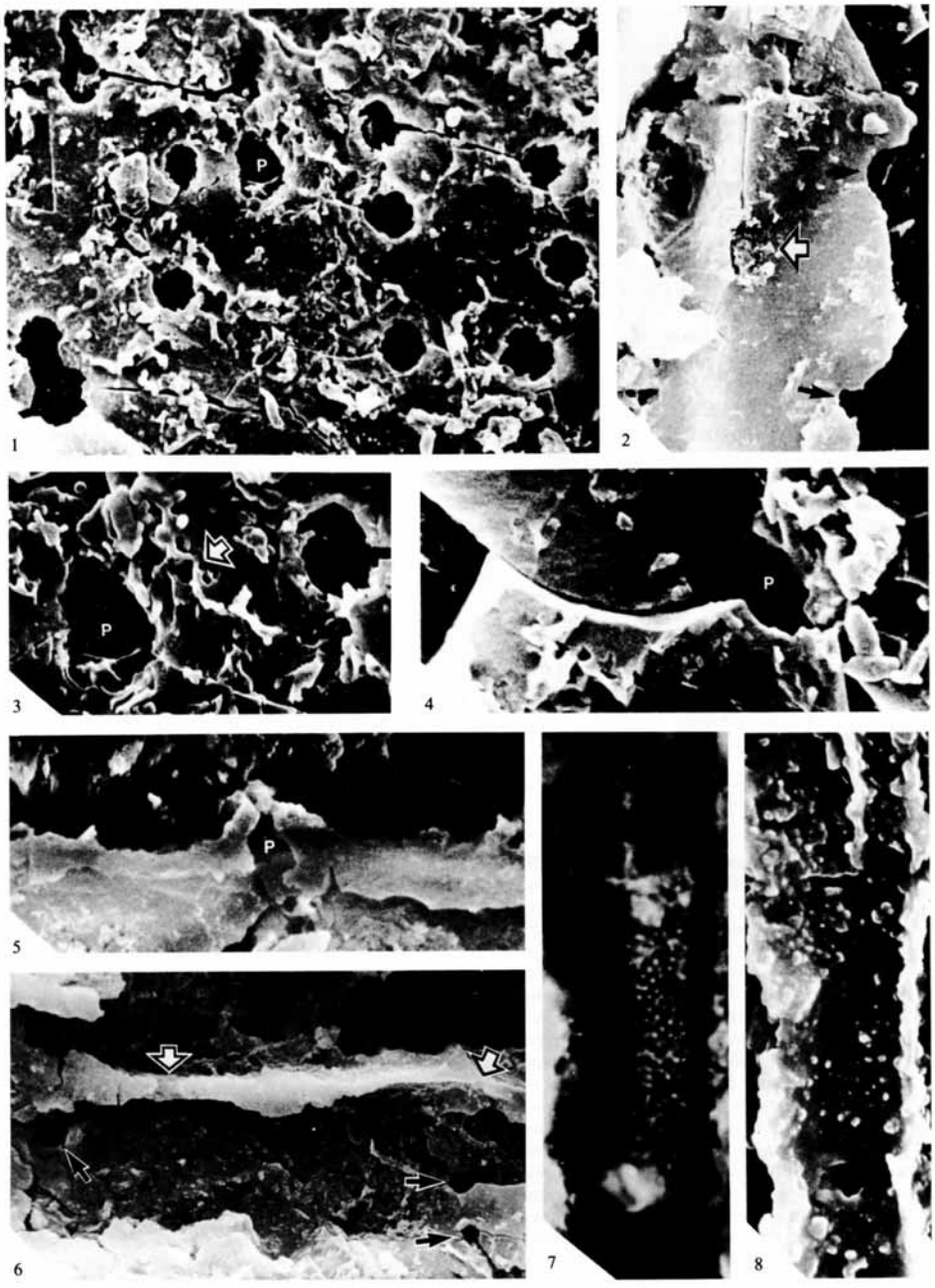
EXPLANATION OF PLATE 69

Figs. 1–6. SEM micrographs of *Psalixochlaena cylindrica*. Preparation by Disappearing Peel Technique. 1, Enlarged area from Plate 68, fig. 5 showing details of pores (P) averaging 1·25 μm in diameter with irregular borders, ×4,800. 2, Portion of wall broken across pores (arrows). Area in centre of field (large arrow) is composed of many small pores about 0·2 μm in diameter, ×4,400. 3, Enlargement of pore 'P' in fig. 1. Note next to this pore smaller perforations 0·2 μm in diameter (arrow), ×9,000. 4, Crack shows tapering of cell wall towards edge of pore (P), ×10,000. Figs. 5 and 6. Section views of pores revealed by fine polishing of coal ball surface prior to Disappearing Peel Technique. 5, Section view of barrel-shaped pore (P) measuring 1·25 μm in its widest part, uppermost neck opening 0·4 μm across, ×4,800. 6, Sieve cell with pores seen in face view on tangential wall (arrows) and in section view on radial wall (large arrows), ×1,600.

Figs. 7 and 8. *Anachoropteris gillotii*. Comparative views of same phloem cell wall viewed by OM and SEM. 7, OM. Cell wall appears perforated by a mass of closely spaced 0·5 μm pores in transmitted light, ×2,000. 8, SEM. Same cell wall is seen not to be pitted. The area in the centre of fig. 7 is in fact smooth, ×2,000.



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difference between inner light and outer dark wall areas. This section was obtained from a peel made and mounted in balsam in 1973. The peel had not been demineralized and contained intact cell walls. Old peel preparations containing intact cell walls in face view can therefore be examined by these techniques if they can be liberated from their slides by soaking in xylol.

Our results on the largest phloem cells of *P. cylindrica* (Pl. 70, fig. 1) indicate that their side walls, both radial and tangential, and end walls are perforated in a similar manner, with pores that average 1·0 μm in diameter. They therefore correspond to the definition of sieve cells and clearly resemble the descriptions of Hébant (1969) of sieve cells of many extant filicalean taxa. It is expected that further studies on this and other coal ball ferns will include description of the cell morphology and wall sculpturing of all the cells comprising the phloem zone.

TEM and SEM observation of the xylem revealed that, in *P. cylindrica*, the centre portion of the scalariform bars is hollow (Pl. 66, figs. 10 and 11). Hollow xylem bars were noted in the early Devonian *Psilophyton dawsonii* by Hartman and Banks (1980) who observed tracheids by SEM and by 2·0 μm microtome sections in OM. Liberman-Maxe (1982) in her ultrastructural studies of the extant filicalean *Polypodium vulgare*, showed that it is the equivalent of this hollow area that stains for lignin by ClEtAg testing while the outermost portion of the bar (the 'inner margin' of Liberman-Maxe and that which is preserved in *Psalixochlaena*) stains for polysaccharides with Ruthenium Red (Liberman-Maxe 1982, figs. 29 and 30). If one can validly compare plants so different in age, our results, and those of Hartman and Banks (1980), suggest that after death the lignified portions of the plant were removed, with the cellulosic portions, which include the walls of all phloem cells studied here, remaining well preserved. No investigation has yet been undertaken to determine at what stage this removal might have occurred.

DISCUSSION

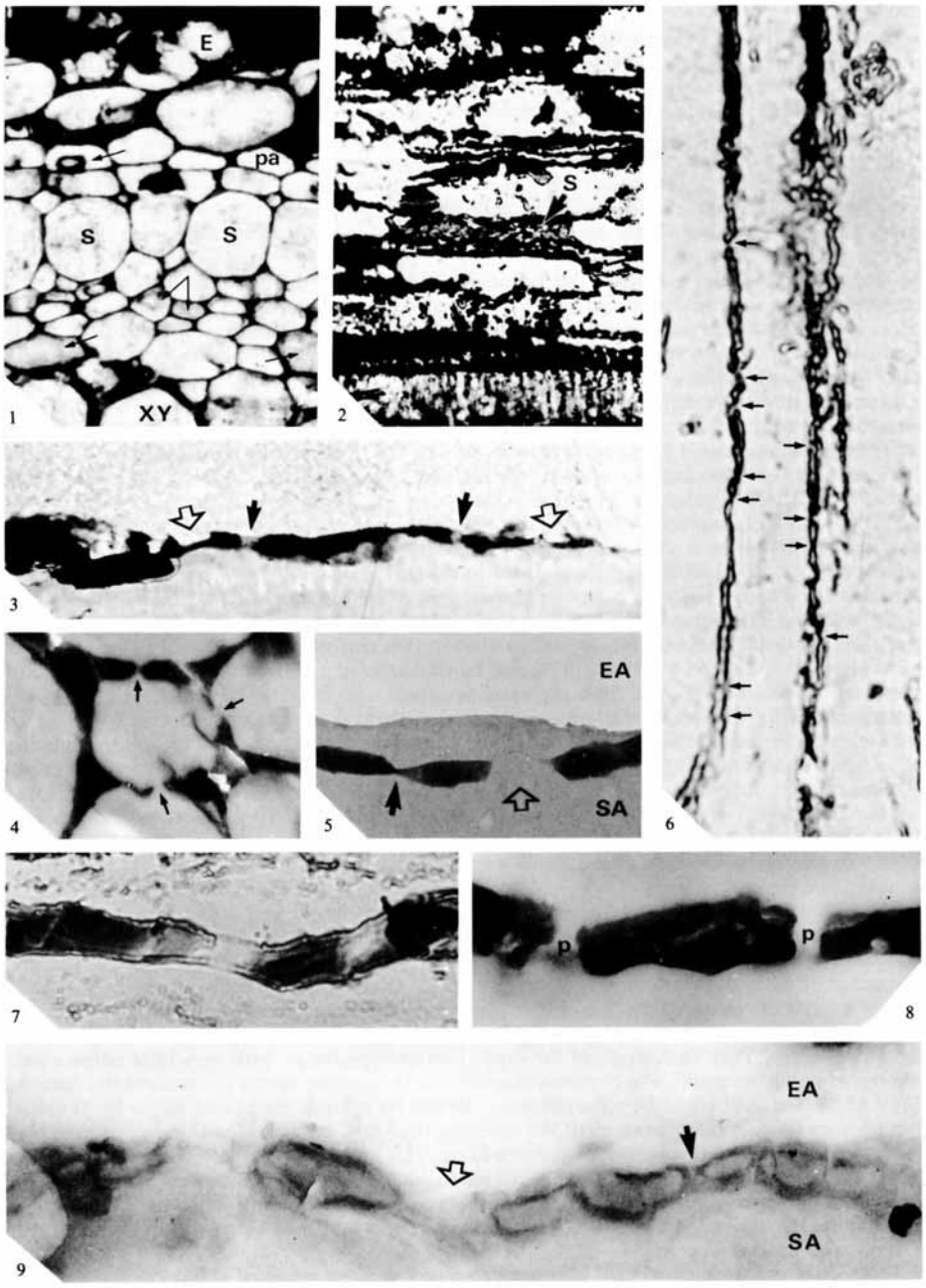
In their introduction Eggert and Kanemoto (1977) give an interesting history of the difficulties encountered by various authors in interpreting extra-xylary stelar tissues in *Lepidodendron*. Obviously the problem of their identification has never been an easy one in fossil plants and the authors have had the same experience in this study of a fern.

EXPLANATION OF PLATE 70

Figs. 1–5. *Psalixochlaena cylindrica*. 1, OM. Phloem zone in transverse section from thin slide. Note large sieve cells (S) and cell contents (arrows) in the parenchyma (pa). E = endodermis, XY = xylem, ×400. 2, OM. Radial section through phloem zone positioned so that tissues correspond in position to fig. 1. The sieve cell wall (S) contains many pores and is seen in TEM in fig. 3, ×400. 3, TEM. Ultra-thin section through sieve cell in fig. 2. Two pores about 1·0 μm in diameter are seen (arrows) as well as depressions (large arrows) in one side of the wall, ×2,700. 4, TEM. Transverse section through small cell in phloem zone showing pores 0·1 μm or less (arrows) in three of the four walls, ×14,000. 5, TEM. Section made with diamond knife in sieve cell in fig. 2. One pore 2·5 μm wide (large arrow) and another sectioned near its edge (small arrow) are visible. Note the difference in density between the embedding Araldite (EA) and the support Araldite (SA) and that the cell wall has sunk into the latter and was thus projecting from the bottom of the peel, ×4,100.

Figs. 6, 7. *Botryopteris hirsuta*. 6, One micron peel technique in longitudinal section applied to phloem. A number of minutes pores (arrows) are discernible in the cell walls, ×2,000. 7, Portion of smooth parenchyma wall seen in face view obtained by one micron peel technique, ×1,500.

Figs. 8 and 9, TEMs of *Psalixochlaena cylindrica*. 8, Longitudinal section through phloem cell wall showing pores (p) about 0·2 μm in diameter. The maceration techniques employed to obtain this micrograph and that in fig. 4 do not allow precise identification of the tissue, ×20,000. 9, Micrograph through tangential wall of parenchyma cell external to sieve cells (pa in fig. 1) obtained from section prepared by the Disappearing Peel Technique. Note pores from 0·02 μm (small arrow) to 0·2 μm (large arrow) and the difference in density between the support (SA) and the embedding Araldite (EA), ×54,000.



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Having examined etched calcite surfaces where no cell wall or residual powder is present under SEM, the authors have been able to recognize some characters which may distinguish calcite from cell walls. Although calcite is full of pits, its surface is otherwise smooth but angular. Phloem cell walls, in addition to being pitted, are generally covered with minute projections and irregularities (Pl. 69, figs. 1, 3) but may also be quite smooth (Pl. 69, fig. 2).

Pictures interpreted as sieve areas with pores in the phloem of *Etapteris leclercqii* figured by Smoot (1979, figs. 7, 8, 11) as much resemble calcite surfaces as they do cell walls. We suggest that the appearance of a sieve area may be created by the rupture, during effervescence, of a line of fibres of calcite crystals forming the coal ball matrix. This is likely because HCl will preferentially etch between the fibres and cause breakage. The 'pores' of the sieve area figured by Smoot (1979, fig. 11) are very angular and recall the rhombohedral section of calcite fibres. In her fig. 12 where she interprets a paratracheal parenchyma cell as showing a smooth wall, the rhombs of the calcite fibres are clearly visible and correspond in cross-section dimension to those observed from English coal balls (G. Rex, *pers. comm.*). Smoot's fig. 10 (1979) is similar to the pitted surfaces of etched calcite we have observed.

Lepidodendrid sieve cell walls figured by Eggert and Kanemoto (1977, figs. 18, 23) strongly resemble cell contents that we have observed in our fern. The comparison with a lateral sieve cell wall of the extent lycopod *Lycopodium tristachyum* figured by these authors (Eggert and Kanemoto 1979, fig. 24) is convincing, but their figures 21 and 22 are also very similar to false sieve areas caused by calcite illustrated here (Pl. 67, fig. 3).

It is not our wish to discredit observations previously made by other workers whose figures have here been compared with what we have proved to be artefacts in *Psalixochlaena cylindrica*. We feel, however, that little credibility can be afforded to either OM or *in situ* SEM observations alone. We consider that a combination of both on the same field of view is absolutely necessary (Pl. 69, figs. 7 and 8) and that TEM observations of the same tissue should confirm the existence of organic walls and the sculpturing they exhibit, as well as showing up perforations too small to be seen under SEM. The method used by Smoot (1979) and by Cichan *et al.* (1981) which involves first an SEM examination, removal of gold from the specimen (Sela and Boyd 1977) and then peeling, only embeds in the peel that material in which effervescence damage has already occurred.

In trials on British and Belgian coal balls the authors have found no advantage in using ethylene-diaminetetra-acetic acid (EDTA). After use of this acid a long wash is necessary to eliminate crystals of the acid itself. Additionally an EDTA etch of the coal ball surface leaves behind a layer of white powder as does HCl, and treatment with EDTA following an HCl etch does not remove this powder. Indeed the use of 0·05 % HCl leaves behind very much less powder than stronger concentrations. For unknown reasons, peels made after a shallow etch of 20·0 μm with EDTA are extremely difficult to remove from the rock surface.

The techniques described here improve the extraction of fragile cell walls lying parallel to the surface, give artefact-free preparations that derive from the well-tried and rapid cellulose acetate peel technique and permit optical, SEM, and TEM observations on the same slides. The great advantage of the peel technique is to allow close serial sections and, in extra-xylary stelar tissues it is only a series of close sections from radial through to tangential orientation that allows accurate identification of the different cell layers. Examination of serial sections by SEM is thus possible by the Disappearing Peel Technique. In the case of unique specimens with very little phloem tissue preserved, it may be possible to perform observations in all three modes on one and the same cell. After SEM, the gold could be removed from the cell by cyanide (Sella and Boyd 1977) and the specimen embedded and microtomed. We have not tried this, but have found that it is possible to microtome a gold-plated specimen after embedding. This metal is very soft and offers no resistance to a glass knife. In particular it is possible to see to what extent fine features may be deformed by the layer of gold. In Plate 66, fig. 12 the gold occurs as a dark layer at the junction of the two types of Araldite. Particles of gold up to 0·4 μm in height occur and may be partly responsible for the irregular cell wall surfaces in Plate 69, figs. 1, 3.

The problem of artefacts in light microscopy on peel sections mounted in balsam under a cover-

slip arises partly due to the destruction, in longitudinal section, of cell walls lying parallel to the surface by the effervescence of HCl. Thus it is not surprising that Eggert and Kanemoto (1977) noted that longitudinal sections of *Lepidodendron* showed phloem much less well preserved than was expected from transverse sections. The apparently more detailed light micrographs of phloem walls in face view in peels from silicified plants such as *Rhynia* (Satterthwait and Schopf 1972), an aneurophytalean progymnosperm (Wight and Beck 1984), and *Calamopitys* (Galtier and Hébant 1973) may be due to the fact that cell walls are not destroyed by liberated gas during the reaction of HF with silica.

The work described in this paper involved totally rethinking the way in which organic matter is exposed and partially destroyed by acid treatment and how it is picked up in the cellulose acetate peel. The problems posed by longitudinal sections are very different from transverse sections where the CO_2 can escape along the cell lumen without destroying the walls. Cell contents, however, are often destroyed even in transverse section and can only be seen in ground sections (Pl. 70, fig. 1).

The use of very weak acid solutions and the making of rip peels has partially solved this problem, but in view of the difficulty of extracting cell walls in planar view the authors wish to emphasize the value, both in transverse and longitudinal section, of the one micron peel which allows a rapid examination of the sculpturing of a cell wall in longitudinal section.

Techniques presented here allow confirmation of wall sculpture of permineralized tissues in three different modes. The exposure of cell walls on an inert support such as Araldite will perhaps in future enable staining of delicate cell walls to improve optical micrographs.

Further refining of extraction methods may reveal that some carbonate permineralized tissues are better preserved than previously thought. Work is in progress to apply these techniques to plant fossils preserved in silica and also to *in situ* spores.

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