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CHANGES IN MICROFIBRIL ORIENTATION IN THE WALLS OF ELONGATING PLANT CELLS

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SUMMARY

The cell wall organization of sub-epidermal parenchyma of coleoptiles of *Avena* and the collenchyma of *Apium* has been investigated using staining techniques, freeze etching and the examination of shadowcast sections from which the embedding medium was removed. In the coleoptile parenchyma of *Avena* it has been confirmed that in the cell wall adjacent to the plasmalemma the microfibrils are transversely oriented and at the outer surface of the wall a dispersed arrangement of microfibrils is present. Longitudinally oriented microfibrils are present in the corner thickenings of the walls. There was no evidence of the crossed polylamellate structure described as being present in collenchyma; epidermal parenchyna; and subepidermal parenchyma of some roots and hypocotyls.

The collenchyma of *Apium* showed the presence of wall layers in which the microfibrils were alternately of transverse and longitudinal orientation. In some layers microfibrils with a different orientation were present.

The wall organization of the subepidermal parenchyma of *Avena* and the crossed polylamellate organization of *Apium* collenchyma are considered to be structural extremes of wall organization in elongating cells. The observations are discussed in relation to the 'multi-net growth' hypothesis and the 'ordered fibril' hypothesis. It is concluded that the observed changes in microfibril orientation in the walls of elongating cells of collenchyma and subepidermal parenchyma can be best explained in terms of the concept of multi-net growth.

1. INTRODUCTION

Of the various attempts to describe changes in microfibril orientation in the walls of elongating plant cells, the most widely accedpted view has been that contained in the 'multi-net growth' hypothesis (Roelofsen & Houwink 1953). This was based on the observations of Roelofsen (1951) made mainly on macerated material. It was observed that microfibrils adjacent to the plasmalemma have an orientation approximately transverse to the longitudinal cell axis, whereas their orientation on the outside of the wall differed from the transverse direction. Subsequently, this observation was made on many types of cells (Roelofsen 1958, 1965). It was proposed that transversely oriented microfibrils were deposited continuously adjacent to the plasmalemma, and, during cell elongation, were progressively changed in orientation to a degree which depended on the extent and polarity of the wall growth which took place.

The concept of multi-net growth would require relative movement between

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the microfibrils. This would appear feasible, since in growing walls the cellulose framework has been calculated to occupy between 14 per cent (*Avena* coleoptiles – FREY-WYSSLING 1936) and 8 per cent (cambium – PRESTON & WARDROP 1949) of the wall volume. The observed orientation of the microfibrils is consistent with calculated distribution of strain in the cell wall (PROBINE & PRESTON 1961).

However, in collenchyma of various genera (ROLAND 1961) and of *Eryngium* (WARDROP 1969) it was shown that the wall consists of layers of microfibrils which are alternately of transverse and longitudinal orientation (crossed polylamellate structure). Subsequently it was shown that this structure was present in all types of collenchyma (CHAFE 1970) in epidermal parenchyma (CHAFE & WARDROP 1972) and the sub-epidermal parenchyma of roots of *Pisum sativum* and the hypocotyl of *Phaseolus aureus* (ROLAND et al. 1975, 1977).

The wall structure seen in the above investigations did not appear to be in accord with the concept of multi-net growth. At first it was proposed that some modified form of multi-net growth was operative involving the reorientation of layers of microfibrils during elongation (WARDROP 1969). However, the demonstration by ROLAND et al. (1975) of the deposition of microfibrils adjacent the plasmalemma, either transverse or parallel to the longitudinal cell axis appeared to exclude this possibility.

It was pointed out by ROLAND et al. (1975, 1977) that the walls in which the crossed polylamellate type of organization was present had been prepared by histochemical or staining techniques or by the examination of shadow-cast sections from which the embedding medium had been removed, or at least partly so. In contrast, the observations on which the multi-net growth hypothesis was based, were made mainly on macerated material, the preparation of which involved the removal of matrix components from the wall. Thus it was shown by ROLAND et al. (1977) that removal of the matrix components from sections of walls caused the disorientation of the microfibrils and it was considered that this could be the reason why the crossed polylamellate type of organization was not observed in early studies on the walls of elongating cells. In view of these considerations, and the widespread presence of the crossed polylamellate structure, ROLAND et al. (1975), proposed the 'ordered fibril hypothesis'. Microfibrils were considered to be deposited in layers, alternately of transverse and longitudinal orientation, this orientation being maintained during elongation and during wall thickening. It was recognised that during elongation the already deposited layers would decrease in thickness because of the relative movement between the microfibrils.

Although the ordered fibril hypothesis is in accord with observations on many types of cells, it was noted (WARDROP 1969) that the capacity of collenchyma to elongate was related to the number of wall layers present. It was also observed that in addition to the layers of microfibrils of longitudinal and transverse orientation, at the outer surface of the wall the microfibrils exhibited a disordered arrangement. It was suggested that this outermost region constituted a wall which was formed during cell division and that the crossed polylamellate wall was deposited during cell elongation. Since the crossed polylamellate struc-

ture has also been observed in macerated cells (DESHPANDE 1976a, 1976b, 1976c) it would not seem probable that the dispersed arrangement of microfibrils on the outside of the cell wall could arise by disorientation during the removal of matrix components. This gives rise to the question as to whether the disordered arrangement of microfibrils represents a wall formed at cell division, and which may become further disordered during elongation, or whether it resulted from a period of multi-net growth of the first-formed wall, before a period of growth in which the formation of the crossed polylamellate structure was developed.

In view of these considerations, it was decided to re-examine the cell wall of *Avena* coleoptiles which had been studied previously (WARDROP 1955, 1956; BÖHMER 1958; VEGA 1972) to determine if in these rapidly elongating cells the crossed polylamellate structure is present and to determine if the structure is related to the rate or the extent of cell elongation which takes place. For comparison, further observations were made on the cell wall organization of collenchyma of *Apium graveolens*.

2. MATERIALS AND METHODS

2.1. Material

Parenchyma: Seeds of *Avena sativa* (var. Purchase) were soaked in water for 1 hr before being placed on filter paper in petri dishes and exposed to light from an incandescent lamp for 16 hours. They were then planted in vermiculite and allowed to grow at 25°C until the coleoptiles had reached the required degree of elongation. In addition to the above procedure, coleoptiles were grown to a length of 10–15 mm or 20–25 mm. In each case the terminal 5 mm was removed and discarded and the subterminal 5 mm was used for experimental purposes. After removal of the leaf segment contained within them, the subterminal 5 mm segments were allowed to elongate in each of the following five media: (1) Water; (2) 10^{-6} M 3-indole-acetic acid (IAA); (3) 0.3 M sucrose in 10^{-6} M IAA; (4) 10^{-6} M IAA at 4°C; (5) 0.35 M mannitol in 10^{-6} M IAA. The required number of coleoptile segments was suspended in 10 ml of the appropriate medium and incubated at 25°C in a water bath for 12 hours.

Collenchyma: The collenchyma of *Apium graveolens* which was examined for comparative purposes was dissected from both immature and mature petioles from plants grown in the field.

2.2. Methods

Fixation: Small segments of coleoptiles or petioles were cut under a solution of 6.25 per cent gluteraldehyde in 0.2 M cacodylate buffer, pH 7.2 and fixed for 2 hrs at room temperature. After fixation, the specimens were extracted at 25°C for 2–14 hrs in 0.03 M EDTA. When using the staining method of THIÉRY (1967) the material was then post-fixed in 2 per cent aqueous osmium tetroxide, for 1 hr following the procedure of ROLAND et al. (1975). In control specimens the extraction with EDTA was omitted.

Embedding: Following fixation the specimens were dehydrated in the graded

series of ethanol prior to embedding in Spurr's resin.

Staining: 1. Uranyl acetate/lead citrate staining: Sections of the control or extracted materials were stained in 2 per cent aqueous uranyl acetate followed by staining with lead citrate (REYNOLDS 1963).

2. Thiocarbohydrazide-silver proteinate method of Thiéry (1967): The procedure as described by ROLAND et al. (1975) was followed using sections of both controls and sections which had been extracted with EDTA and postfixed with osmium tetroxide.

Shadow casting of thin sections: Thin sections of control or EDTA extracted material were mounted on gold grids and immersed in sodium methoxide according to the procedure of MAYOR et al. (1961). After this treatment the sections were shadow cast with platinum-palladium at an angle of 15°.

Freeze-etching: Avena coleoptile parenchyma and the collenchyma of *Apium* were treated with glycerol for 14 hours and were then examined using the freeze-etching procedure described by MOOR (1964). Examination of the specimens prepared by the above procedures was made using a Philips 300 or a Siemens 102 electron microscope.

3. RESULTS

3.1. Coleoptiles at different stages of elongation

3.1.1. Stained sections

In coleoptiles of 1.5, 2.5 and 3.5 cm in length the organization of the cell wall at a distance 5 mm from the coleoptile tip appeared to be the same. In transverse sections, the microfibrils of the cell wall appeared quite uniform in orientation (*figs. 1* and *2*) and there was no evidence of any heterogeneity of organization such as observed in the crossed polylamellate structure. It may be noted that in sections prepared by the Thiéry method the deposition of silver is discontinuous (*figs. 3* and *4*). This is especially apparent where microfibrils are detached from the wall (*fig. 4*). In tangential longitudinal section, the stained sections showed regions of dispersed micro-fibrillar components (*figs. 5* and *7*) or exhibited longitudinal bands of microfibrils corresponding to the corner thickenings (*figs. 6* and *7*). Microfibrils oriented approximately transverse to the cell axis were present adjacent to the plasmalemma (*fig. 6*). Microfibrils of intermediate orientation can be seen in *fig. 7*. This structure was observed using both the staining procedures employed and was present at all stages of growth of the coleoptiles. These characteristic features of the cell wall organization were apparent also in all of the coleoptile segments allowed to extend under the experimental conditions 1–5 described under Methods and no relation could be detected between the cell wall organization which was present and the degree of extension of the segments. These features are illustrated in *figs. 5–7*. It should be emphasized that the cell wall of the sub-epidermal parenchyma is extremely thin, measuring c. 0.1 μm in thickness, so that any curvature out of the plane of section results in the presence of microfibrils differing in orientation within the one section. However,

the longitudinal band of corner thickenings serves as a means of determining the orientation of the cells in the microscope.

3.1.2. Shadow cast sub-epidermal parenchyma

In tangential longitudinal sections the walls should show features similar to those illustrated in the stained sections. Thus in *fig. 8* microfibrils with both transverse and a dispersed orientation can be seen. Where the section presumably passed through the inner part of the wall adjacent to the cytoplasm, transversely oriented microfibrils were observed, while the dispersed microfibrils are assumed to be in the outer region of the cell wall.

3.1.3. Freeze-etched preparations

Freeze etched replicas of coleoptiles 1 cm and 2.5 cm in length showed the same features described above. The presence of predominantly transverse microfibrils can be seen in *figs. 9* and *10*. Part of a corner thickening with longitudinally oriented microfibrils can be seen in *fig. 9*. The outer regions with a dispersed texture are apparent in *fig. 10*.

3.1.4. Epidermal parenchyma

In transverse sections of coleoptiles both epidermal and sub-epidermal parenchyma were present in some sections. In these sections the uniform structure of the cell wall of the sub-epidermal parenchyma described above, contrasted strongly with the crossed polylamellate structure of the epidermal parenchyma described previously by CHAFE & WARDROP (1972).

3.2. Collenchyma of *Apium*

In transverse section the cell walls of collenchyma of *Apium* showed the typical polylamellate structure (*fig. 11*) described earlier (ROLAND 1966; WARDROP 1969). Parenchyma adjacent to the collenchyma did not show the crossed polylamellate structure (*fig. 12*). In tangential longitudinal section both transverse and longitudinal microfibrils could be seen (*fig. 13*). However, in young collenchyma in successive sections through the thickening of the cell wall, microfibrils of an orientation which was neither transverse nor longitudinal could be seen (*figs. 14* and *15*). In freeze etched preparations the existence of such orientations was confirmed (*fig. 16*).

Further evidence for the presence of layers of microfibrils in which the orientation was neither truly transverse nor longitudinal is shown in *fig. 17*. This *fig. 17* is a montage of a slightly oblique longitudinal section of the wall of collenchyma of *Apium* from which the embedding medium was removed. Four layers of longitudinal orientation are apparent but the intervening layers showed considerable variation from the transverse orientation. Between Tn and Ln the microfibrils are oriented at an angle of c. 45° to the cell axis while the microfibrils of Tn2 apparently curve away from the transverse to a more longitudinal orientation.

4. DISCUSSION

Evidence obtained using the staining with uranyl acetate – lead citrate, the THIÉRY stain (1967) as used by ROLAND et al. (1975) by shadow casting and the examination of replicas prepared by the freeze-etching technique, demonstrate the presence of a simple organization of the cell wall in the sub-epidermal parenchyma of *Avena* coleoptile. It can be seen in *figs. 6, 8, 9* and *10* that the microfibrils lying adjacent to the plasmalemma are oriented in a direction transverse to a longitudinal axis of the cells, but some microfibrils with a dispersed arrangement lie at the outside of the cell wall (*figs. 7* and *10*). Longitudinally oriented microfibrils of the corner thickenings were also apparent (*figs. 6* and *9*). This organization of the wall was present in all of the sub-epidermal parenchyma of coleoptile segments allowed to extend under various growing conditions listed above.

These observations agree with the earlier observations of shadow cast (WARDROP 1955, 1956; BÖHMER 1958) and stained sub-epidermal cells of *Avena* (VEGA 1972) and would be consistent with the view that the changes in microfibril orientation in the cell wall which accompany extension growth are similar to those envisaged in the multi-net growth hypothesis.

However, the presence of the crossed polylamellate structure in elongating collenchyma cells (ROLAND 1966; WARDROP 1969) and also in elongating cells of epidermal parenchyma (CHAFE & WARDROP 1972) and sub-epidermal parenchyma (ROLAND et al. 1975, 1977) raises the possibility that the wall organization here observed in coleoptile sub-epidermal parenchyma may represent the beginning of the development of the crossed polylamellate organization in which only the first transversely oriented layer of microfibrils were formed. If this was so, and growth does proceed as proposed in the ordered fibril hypothesis, the question arises as to the significance of microfibrils in a dispersed array which are present at the outside of the cell wall (*figs. 7, 8* and *10*) and which were observed in earlier studies (WARDROP 1955, 1956; BÖHMER 1958). The contention that the dispersed arrangement may arise during extraction of matrix components is not supported by the observation (WARDROP 1969) of a layer with dispersed microfibril orientation at the outside of thick-walled collenchyma with a crossed polylamellate organization of the cell wall and in which also a thick secondary cell wall with helical organization was present. It was suggested therefore, that the layer of dispersed microfibrils was formed at cytokinesis and might be further changed in orientation during the growth of a wall with crossed polylamellate structure. This could also apply to the present observations, but equally the dispersed texture could arise from multi-net growth. This point is considered further below.

A further factor to be considered in formulating any model of changes in microfibril organization in extending walls concerns the problem of whether the microfibrils are interwoven or are present in lamellae. That microfibrils of growing cell walls are interwoven was first suggested by FREY-WYSSLING et al. (1948) and in early studies on *Avena* parenchyma using macerated material the

microfibrils appeared to be interwoven (WARDROP 1955, 1956; BÖHMER 1958). Interweaving of microfibrils has been observed, even in the highly organized cell walls of algae such as *Chaetomorpha* (PRESTON 1964). In contrast to the earlier observations on shadow cast parenchyma the microfibrils in the wall layers of collenchyma and of parenchyma appear to be aligned parallel to the cell surface. This is especially apparent in preparations stained by the Thiéry procedure (*fig. 2*). In the observations of ROLAND et al. (1975, 1977) the microfibrils did not appear to be interwoven and were depicted diagramatically as being parallel to each other (by ROLAND et al. 1977). In the view of these authors, the microfibrils are not to be considered as present in distinct lamellae. However, if the preparations stained by the Thiéry procedure are examined at higher magnification, it can be seen that the silver from the silver proteinate is deposited in spherical deposits ca 3 nm in diameter and are separated by an average interval of ca 7.5 nm so that the 'fibrils' appear as a series of aligned spots like the beads of a necklace (*fig. 3*). The microfibrils themselves however, are not visible. This effect can be seen clearly when the microfibrils are apparently detached from the cell wall (*fig. 4*). The reason for this apparent discontinuity of staining is not known. It is difficult to see what chemical variation could be present at a distance of c. 7.5 nm, since this distance corresponds to only 15 glucose residues. Alternatively, the discontinuous spacing of the silver deposits may result from purely physical forces such as surface tension. Thus, if a cylinder of liquid is formed over a thread it becomes unstable and then assumes an undulatory form and finally breaks to form evenly spaced droplets (THOMPSON 1942). This phenomenon is seen when the outer less viscous secretion dries on the inner more viscous radial threads of the web of spiders (THOMPSON 1959; EISNER et al. 1964). A similar discontinuous staining of microfibrils is observed using uranyl acetate and lead citrate (*fig. 1*) but the metal deposits are less regular in their spacing from each other. Further, when sections of cell walls of collenchyma stained by the Thiéry method are examined at high magnification it is not possible to follow a single line of silver deposits for more than c. 10 particles (*figs. 3* and *4*) and it cannot be seen whether the microfibrils on which the silver was deposited are present parallel to the surface of the cell or may in fact interweave. It would thus seem possible that the appearance of parallelism of microfibrils with the wall surface may result because particles on one microfibril may be aligned with particles on one adjacent to it. The discontinuity of staining of the microfibrils gives rise to the possibility that it may not be possible to distinguish a woven fibrillar texture from a parallel ordered fibrillar texture. Thus the apparent ordered arrangement of silver particles revealed by the Thiéry staining procedure could under some circumstances be misleading, especially in walls examined in transverse section.

It has been argued (ROLAND et al. 1977) that the woven appearance of microfibrils, apparent in the cell walls prepared by maceration, results from disorientation of the microfibrils following the removal of matrix components. However, as pointed out above, in collenchyma with secondary walls (WARDROP 1969) it is difficult to see how such a disorientation of the outer microfibrils could come about as a result of the removal of these cell wall components.

Thus, for subepidermal parenchyma of *Avena* coleoptiles it is suggested that, although in transverse section the microfibrils appear after staining to be parallel to the wall surface, in fact a woven texture could be present because of the discontinuity of staining of the microfibrils both by the Thiéry method and by staining with uranyl acetate and lead citrate.

In tangential sections the microfibrils appear to be transverse to the longitudinal cell axis ajacent to the plasmalemma irrespective of the method of staining. This was also observed in preparations prepared by shadow casting after removal of the embedding medium and after freeze etching. The only longitudinal microfibrils observed were at cell corners and it was therefore concluded that in *Avena* coleoptile parenchyma the crossed polylamellate structure is absent and that the microfibril arrangement observed is consistent with the operation of a mechanism such as that envisaged in the multi-net growth hypothesis.

It may also be noted that although collenchyma of *Apium* showed the crossed polylamellate wall organization (*fig. 11*) this was not present in adjacent parenchyma cells (*fig. 12*).

It is clear however, that the crossed polylamellate structure seen in collenchyma, epidermal parenchyma, and the subepidermal parenchyma of roots of *Pisum sativum* and the hypocotyl of *Phaseolus aureus* (ROLAND et al. 1975, 1977) cannot be explained simply in the above terms. However, an explanation would seem possible if it is assumed: (1) that the cells of these tissues have the capacity to deposit microfibrils in both the transverse and longitudinal orientation, as is apparent from published electron micrographs; (2) that the microfibrils in each layer present in a particular wall are interwoven irrespective of their orientation and (3) that the change in orientation of microfibrils during elongation of all wall layers follows a pattern as envisaged in the multinet mechanism.

On these assumptions the growth of the wall of a collenchyma cell may be considered. The wall consists of layers of microfibrils deposited alternately in the longitudinal and transverse direction. At least some of these layers must have been present during the phase of active elongation of the cell, i.e. those towards the outside of the cell wall. It may be noted that the thickness of these layers is approximately the same, or somewhat greater, than that of the whole cell wall of the sub-epidermal parenchyma of *Avena* coleoptiles. During elongation the microfibrils in those layers with longitudinal microfibril orientation may be considered to move relative to each other as in the corner thickenings of *Avena* parenchyma. In the layers with transverse orientation, the change in each layer would follow the pattern assumed to take place by multi-net growth so that it would be anticipated that the degree of change in orientation in the layers with transverse orientation would be less for layers near the plasmalemma than for layers near the surface of the cell wall. This view would be consistent with the postulated distribution of strain in the cell wall of elongating thickening cells proposed by PROBINE & PRESTON (1961). Further, the layers would be expected to decrease in thickness during extension and it has already been reported for epidermal parenchyma that the outer layers are thinner than those of the inner layers of the wall, irrespective of their orientation (CHAFE & WARDROP 1972).

Again, if the microfibrils in all layers of the wall are interwoven, it would be expected that during any changes in orientation resulting from extension growth the integrity of the layers would be retained. It can be seen that this concept simply extends the application of the multi-net growth hypothesis but the concept is applied to each successive layer of the cell wall. It will be apparent that this model differs from that envisaged in the ordered fibril hypothesis of ROLAND et al. (1975, 1977). However, if by structural studies it were possible to determine the orientation of microfibrils in a series of tangential sections cut from the innermost region of the wall adjacent to the plasmalemma through the wall of the intercellular layer it should be possible to distinguish between the two possibilities. Assuming the ordered fibril hypothesis to apply, then in all tangential longitudinal sections of a particular wall layer the microfibrils should show only transverse or longitudinal orientation.

On the basis of the hypothesis advanced above, however, somewhat different observations would be anticipated. Sections of layers with longitudinally oriented microfibrils would show longitudinally oriented microfibrils, irrespective of the degree of elongation of the cell or of the position of the layer in the wall. Sections through layers with transverse orientation in the most recently formed layers, i.e. those nearest the lumen, should show transverse orientation. However, in sections through layers originally of transverse orientation but in the outer part of the cell wall, i.e. the region subjected to greatest strain during growth, the microfibrils should show an orientation differing from the transverse arrangement proposed.

It may be noted that within each layer of transverse orientation the innermost region would have a more nearly transverse arrangement of microfibrils than the outer region – in accordance with the multi-net concept.

Some preliminary observations made in this study appear to be in accord with this view. *Figs. 13–15* show sections through the cell wall of collenchyma of *Apium*. Near the lumen of the cell the microfibrils can be seen to be either longitudinal or transverse (*fig. 13*). Towards the outer part of the cell, however, microfibrils are present which are neither longitudinally nor transversely oriented and indeed lie at an angle of 40–60° to the longitudinal cell axis (*figs. 14* and *15*). In frozen-etched images orientation of microfibrils other than those in the tangential or longitudinal orientation can be seen (*fig. 16*).

For *Apium* collenchyma additional evidence of a change of microfibril orientation in the layers initially of transverse orientation is contained in *fig. 17*. This is a montage showing three layers of longitudinal oriented microfibrils labelled Ln, Ln1, Ln2 and three layers of transverse microfibrils labelled Tn, Tn1 Tn2. It could not be determined which layers were nearer the plasmalemma. The area called Tn represents an interface. Clearly the orientation is neither transverse nor longitudinal, but at an angle of ca 50° to the longitudinal cell axis. At the interface between Ln1 Ln2 the microfibrils of the layer Tn2 appear to curve towards a more longitudinal orientation. These observations suggest that the microfibrils of the layers of initially transverse orientation change orientation during cell elongation. Although this evidence is not conclusive, the occurrence

of microfibrils oriented in neither the transverse or the longitudinal direction shown in *figs. 14–17* would seem indicative of the operation of a mechanism other than that envisaged in the ordered fibril hypothesis. The concept proposed above of the operation of the multi-net mechanism in all layers would seem at this stage to be sustained.

It would be expected that in growing walls with a crossed polylamellate structure the cell wall layers near the lumen would exhibit the type of crossed polylamellate structure which constituted the basis of the ordered fibril hypothesis proposed by ROLAND et al. (1975).

The hypothesis as proposed would also be consistent with the observation (WARDROP 1969) that in radial longitudinal sections of collenchyma the difference in appearance of layers with transverse microfibril orientation and those with longitudinal microfibril orientation is less apparent in the outer parts of the wall as compared with regions adjacent to the lumen. This observation was also made during the course of the present investigation.

In more general terms it will also be appreciated that the expansion of a wall involves changes in the properties of its matrix components throughout the wall thickness even in thick-walled growing cells such as collenchyma. This would seem reasonable since it is known that deposition of encrusting components such as lignin in greatly thickened secondary walls may take place in regions of the wall spatially remote from the plasmalemma (WARDROP 1976). In view of this, if the wall expansion involves a change in matrix components it is reasonable to assume that any such change could be controlled through the whole thickness of the wall so that although changes in microfibril orientation reflect changes in growth and form of the cell, the basic morphogenetic processes which allow these changes, result from the programmed genetic factors which determine the pattern of differentiation of the cell.

In conclusion some attention may be given to problems of terminology. The important fact which emerges from recent cell wall studies is that the microfibrils in the walls of elongating cells are considerably ordered, whether with a predominantly transverse orientation or with some more complex organization as in cells with the crossed polylamellate organization. Further it may be that this organization becomes apparent only during elongation, i.e. the wall may thicken during elongation (ROLAND et al. 1975). Again the outermost region of mature walls usually consists of microfibrils with a dispersed arrangement and which appear to be formed at cytokinesis, and this structure persists during the elaboration of the wall which occurs during elongation (WARDROP 1969). Customarily, the term 'primary wall' has been applied to the structure enclosing the protoplast during surface growth, and the 'secondary wall' is the term applied to those structures which are formed after the cessation of surface growth.

It would seem desirable, however, to recognise the presence of a 'primary wall' in the sense of meristematic wall, a 'growth wall' present during elongation and a 'secondary wall' formed after cessation of wall growth either locally or over the whole cell surface. It would seem preferable not to confuse the terminology by the reference to the physical organization or chemical nature of the primary wall; growth wall or secondary wall.

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FIGURES

Unless specifically stated the illustrations refer to the subepidermal parenchyma of coleoptiles of Avena sativa. Specimens were extracted with EDTA prior to staining as described under 'Methods'. Arrows indicate the longitudinal axis of the cells.

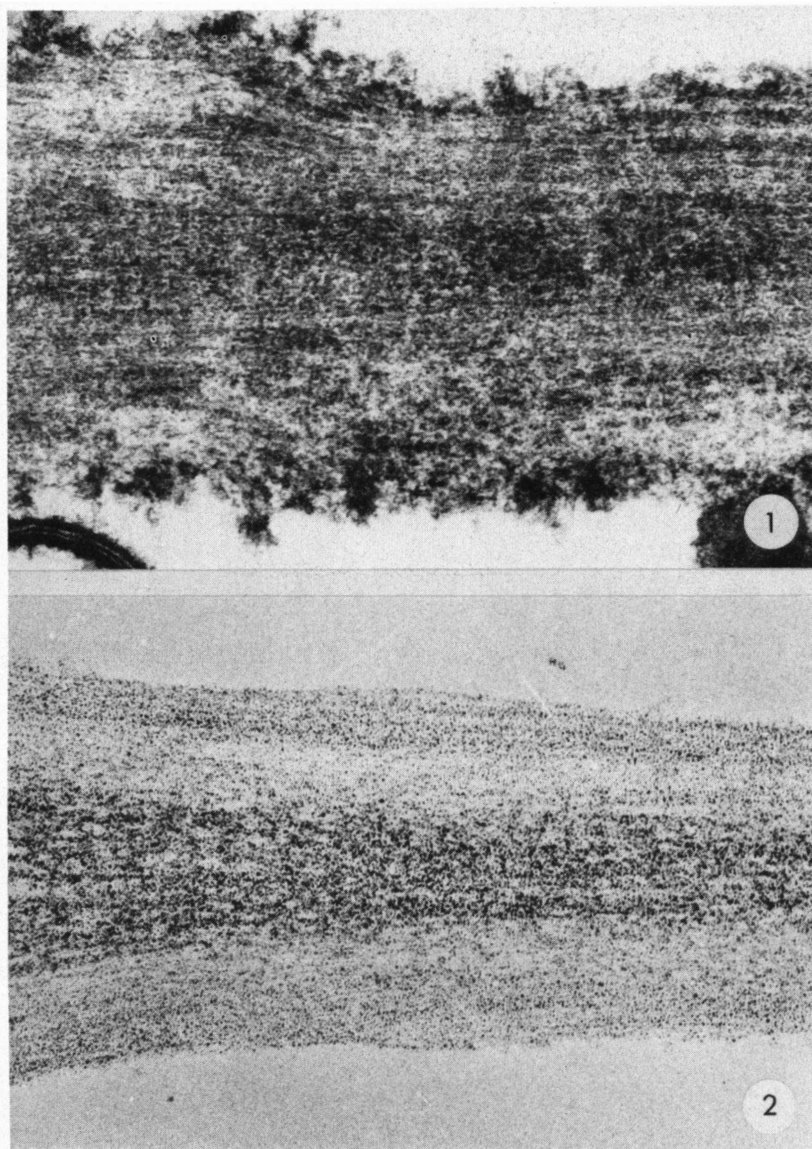
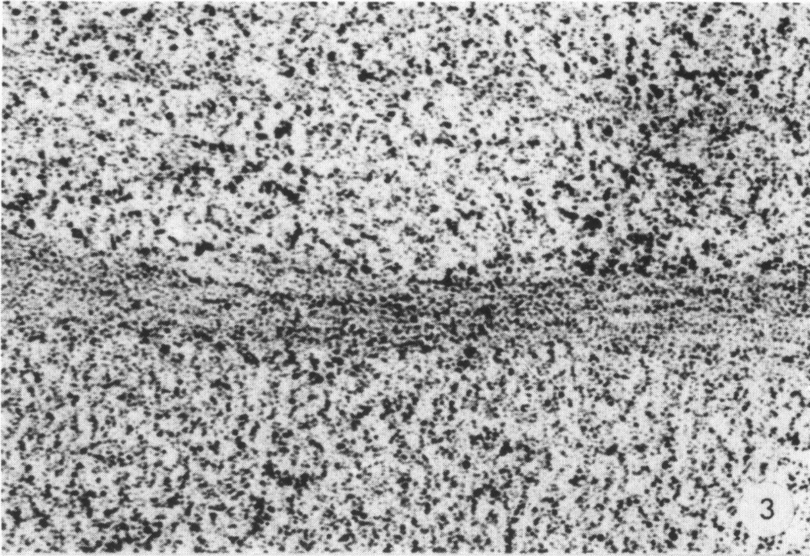


Fig. 1. Transverse section from a coleoptile 1 cm in length and 3 mm from the cell tip. Glutaraldehyde fixation, staining: phosphotungstic acid, uranyl acetate, and lead citrate. The wall appears to be of uniform organization with fibrillar elements parallel to the wall surface. There is no evidence of the crossed polylamellate structure. (× 85,000)
Fig. 2. A transverse section taken 5 mm from the tip of a coleoptile 2.5 cm in length. Prepared using the Thiery (1967) procedure described by ROLAND et al. (1975). The wall appears similar in organization to that shown in *fig. 1*. (× 81,000)



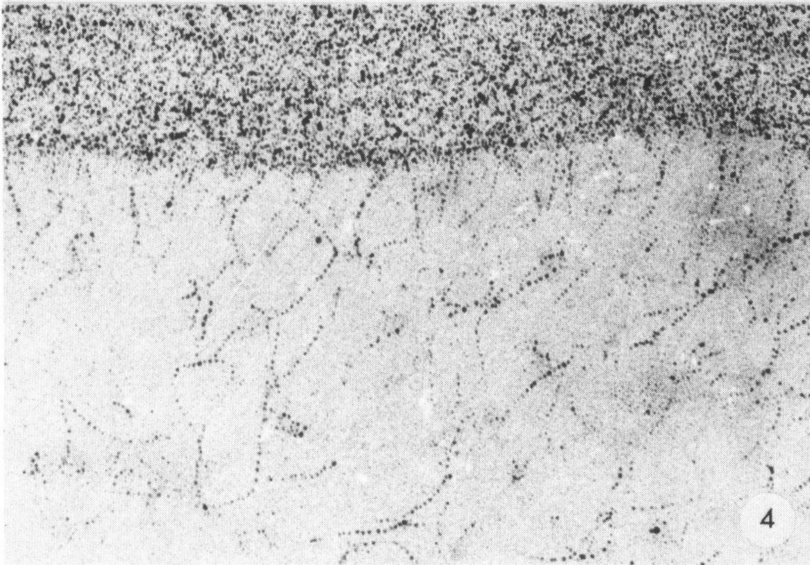


Fig. 3. Part of a transverse section of collenchyma of *Apium graveolens* stained by Thiéry (1967) procedure. Note the discontinuous staining in the bulk of the wall. (× 137,000)
Fig. 4. As *fig. 3*, showing the discontinuous staining of microfibrils apparently detached from the cell wall. (× 142,000)

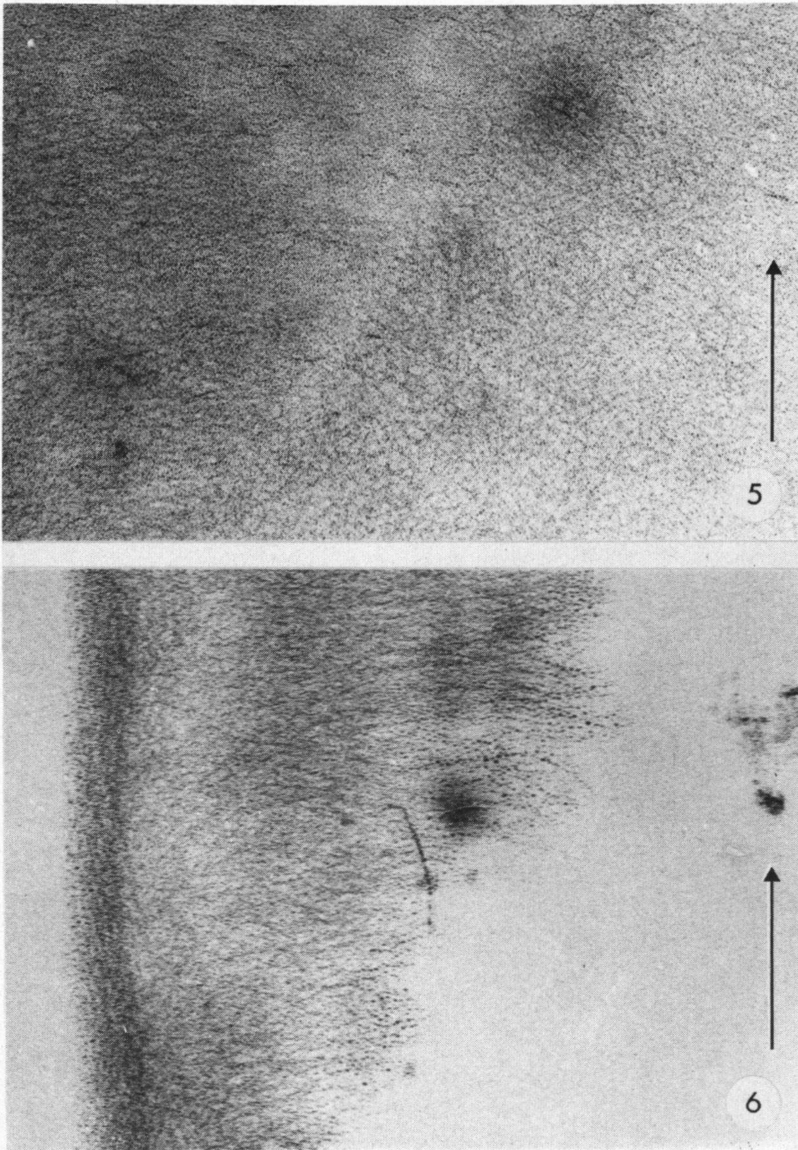


Fig. 5. Tangential longitudinal section from a segment taken 5 mm from the tip of a coleoptile 2.5 cm in length, and showing microfibrils both in a dispersed arrangement and with an approximately transverse orientation to the cell axis. (× 82,000)

Fig. 6. A tangential longitudinal section from a coleoptile segment treated as in (2) 'Methods' above. Longitudinally oriented microfibrils of a corner thickening can be seen as well as those with a transverse orientation. (× 68,000)

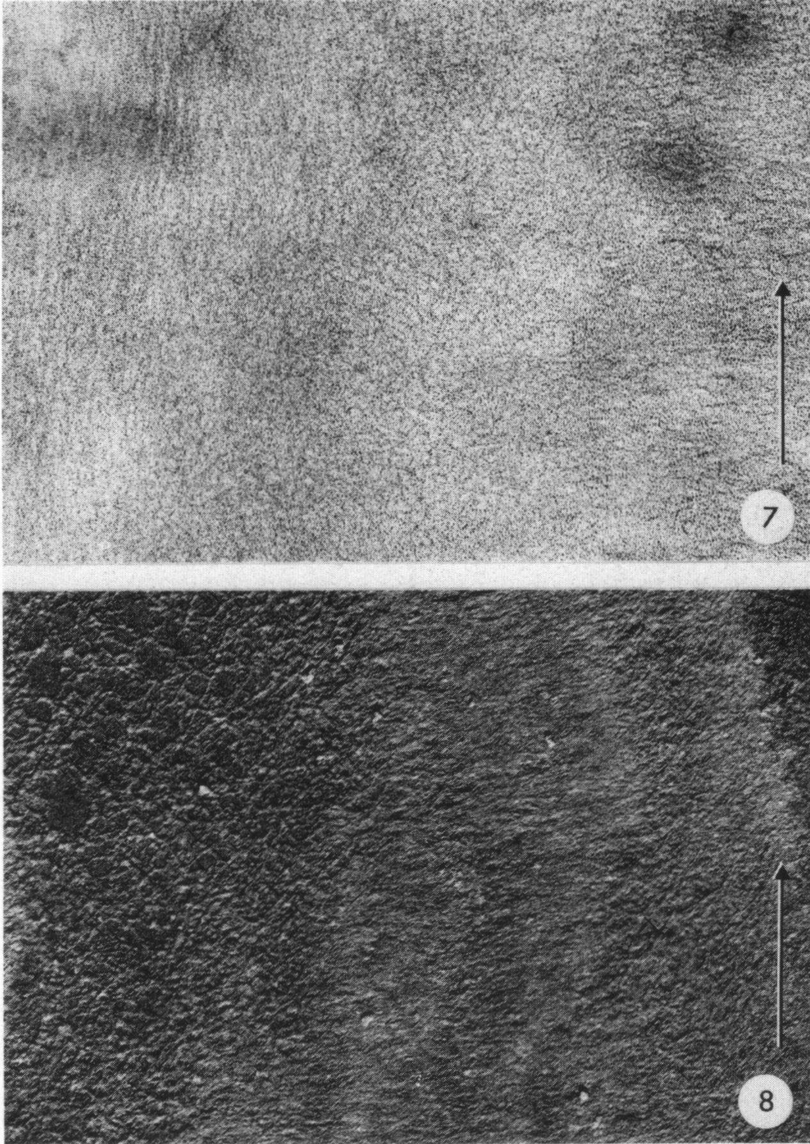


Fig. 7. Tangential longitudinal section from a coleoptile segment obtained after treatment as in (3) 'Methods' showing a transition of microfibril orientation from longitudinal (corner thickening) at left, dispersed arrangement at centre, to transverse orientation at right. (× 65,400)

Fig. 8. A tangential longitudinal section cut from a segment taken 5 mm from the tip of a coleoptile 4 cm in length. The embedding medium was removed and the section was then shadowcast with platinum palladium. Microfibrils with both a dispersed arrangement and those transversely oriented can be seen. (× 32,000).

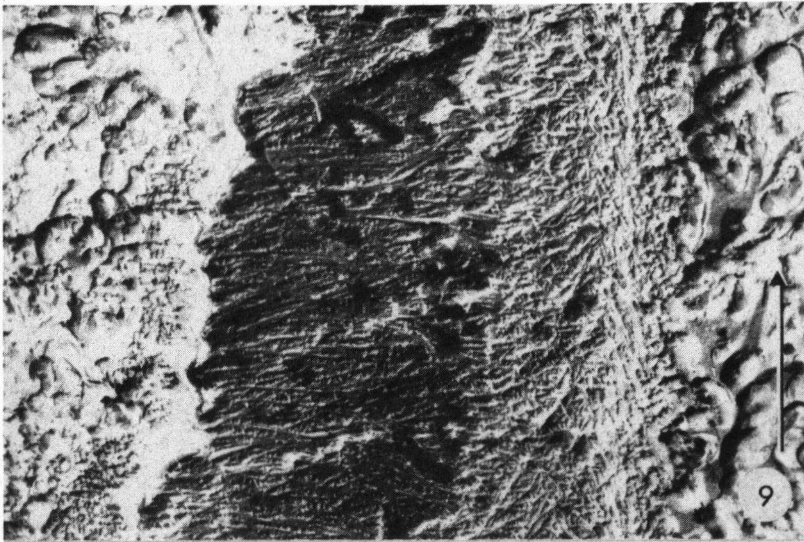


Fig. 9. A replica prepared by the freeze etching technique, showing transversely arranged microfibrils (centre) and longitudinally oriented microfibrils of a corner thickening. (× 53,900).

Fig. 10. Similar to *fig. 9*. Microfibrils with a transverse orientation can be seen (left) and those with a dispersed texture are apparent (right). (× 76,200).

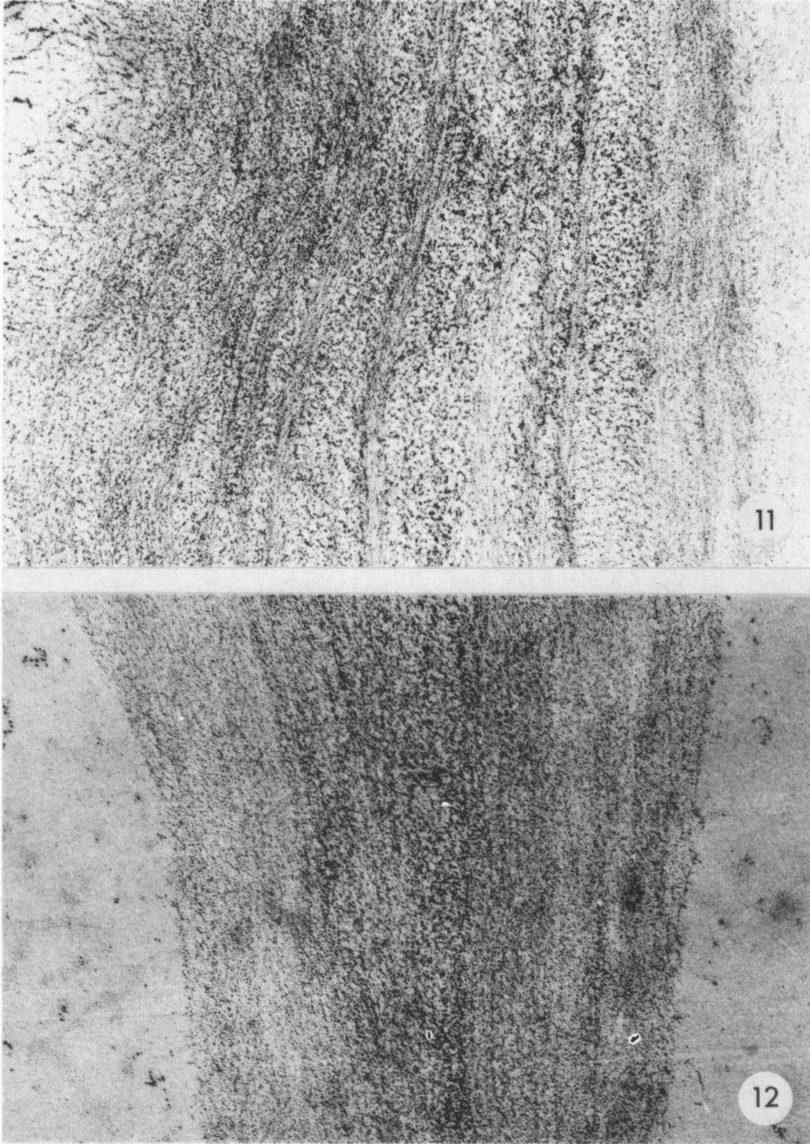


Fig. 11. A transverse section of collenchyma of *Apium graveolens* stained by the procedure of THIÉRY (1967) as described by ROLAND et al. (1975) showing a typical crossed polylamellate structure. (× 50,000).

Fig. 12. A transverse section of parenchyma of *Apium graveolens* adjacent to the collenchyma shown in *fig. 10*. There is no evidence of a crossed polylamellate organization. (× 71,000).

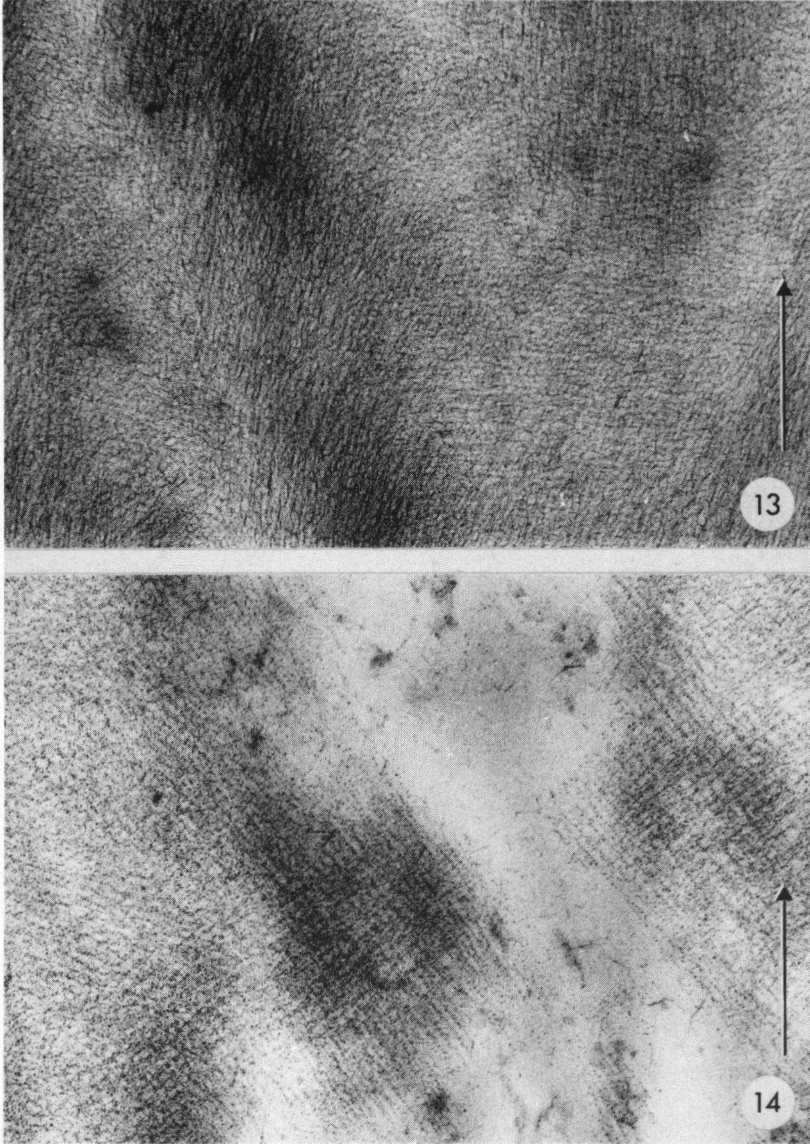
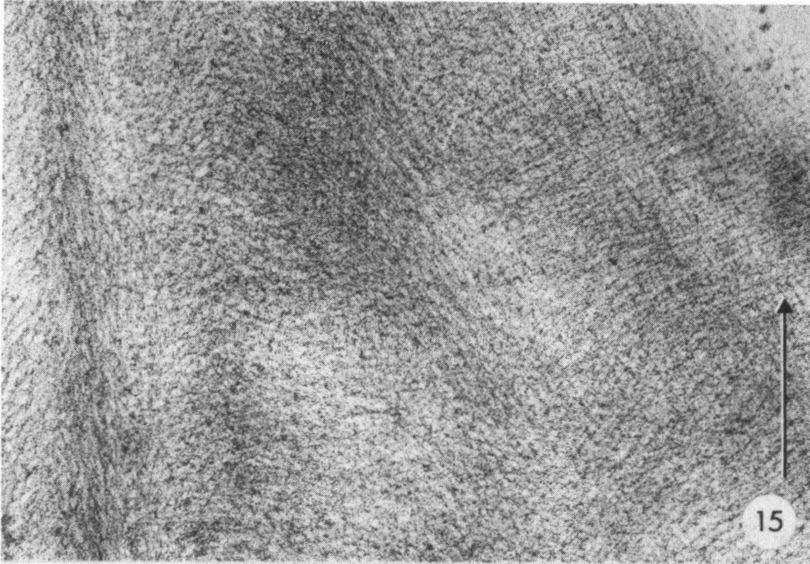


Fig. 13. An oblique tangential longitudinal section of collenchyma of *Apium graveolens* with uranyl acetate and lead citrate showing both transverse and longitudinally oriented microfibrils. (× 41,000).

Fig. 14. As *fig. 13*, showing obliquely oriented microfibrils. (× 67,000).



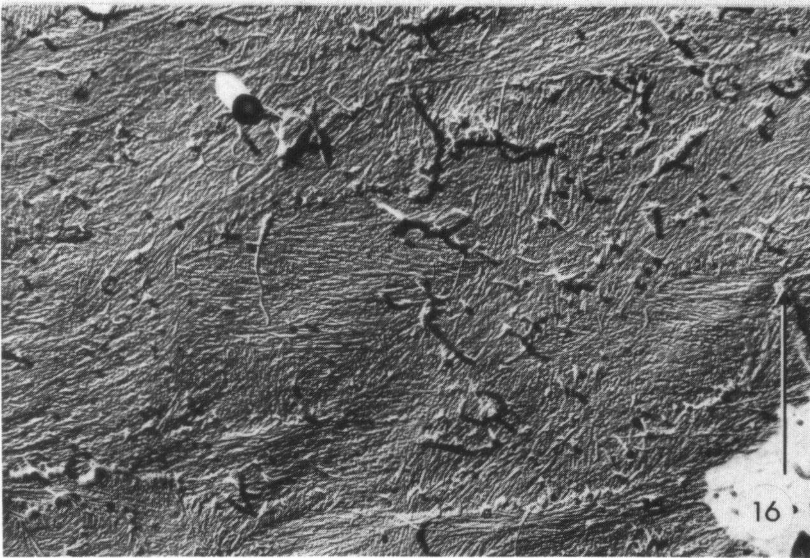


Fig. 15. As *fig. 14.* (× 55,000).
Fig. 16. A freeze-etched replica of an oblique tangential fracture face of collenchyma of *Apium* showing the differing microfibril orientation in the cell wall. (× 21,000).

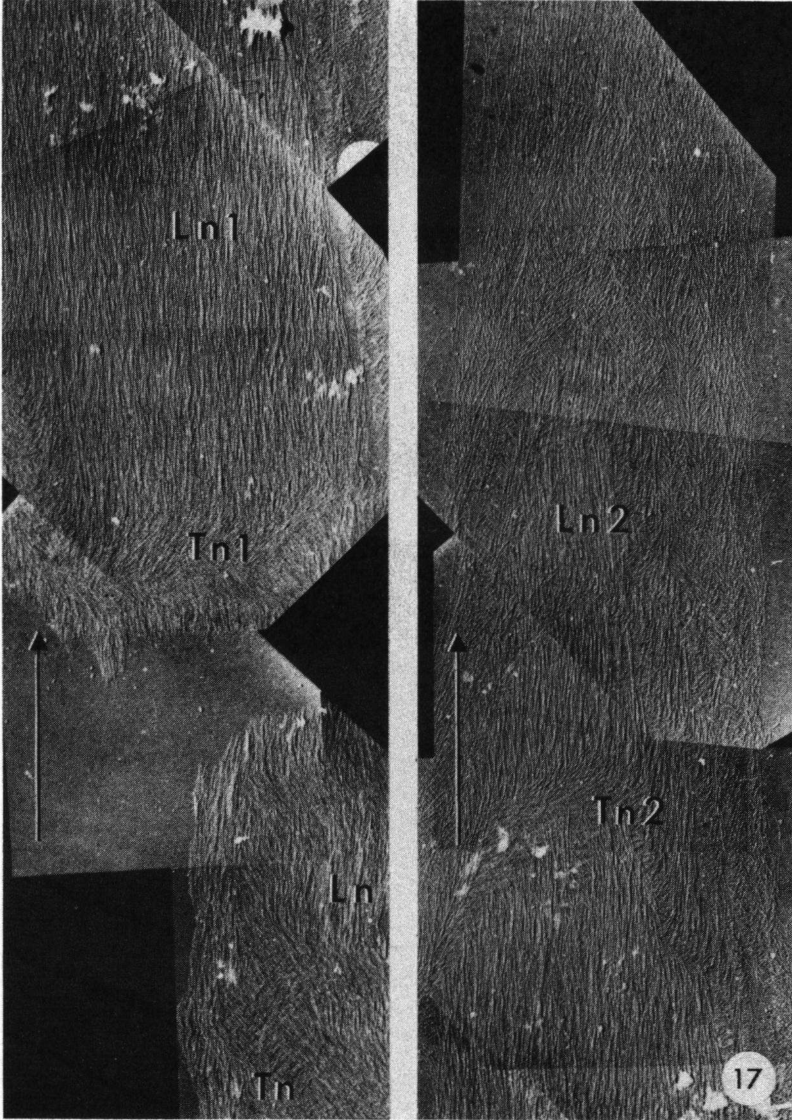


Fig. 17. Part of the cell wall of collenchyma of *Apium* in slightly oblique tangential longitudinal section. Parts of at least three layers with longitudinally oriented microfibrils (Ln, Ln1, Ln2) and of three layers with 'transversed' oriented microfibrils (Tn, Tn1, Tn2) can be seen. (× 7,800).