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FIBRILLAR ARCHITECTURE OF GROWING PLANT CELL WALLS

BY

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With Plates 17—23

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INTRODUCTION

In an earlier communication (13) the present authors published electron micrographs of the cellulose texture in the primary cell wall of growing hairs of *Gossypium*, *Ceiba* and *Asclepias*. The micrographs showed that, in general features, the structure of these hairs resembled the structure previously found in the growing cell walls of *Tradescantia* staminal hairs (12) and *Phycomyces* sporangiophores (11). Common to all was a compact transverse fibrillar structure on the inner side. The outer side, with cotton hairs, had an axial orientation of the fibrils and in between the two a more or less random orientation seemed to occur. With *Phycomyces* and *Tradescantia*, however, the outer fibril layer was isotropic.

As an explanation of this architecture a theory, named *multi-net-growth theory*, was proposed (13). It necessitated three assumptions,*viz.*:

1. The cell walls studied were growing until the moment of fixation and hence, all cell wall layers were extending axially until fixation.
2. New fibrils are deposited onto the inner face of the cell wall. This involves that every layer of fibrils of the same age is gradually shifting outwards.
3. On being extended axially each of the transversely oriented layers on the inner side of the cell wall behaves like a network consisting of intertwined fibrils.

Extension of the cell wall, as assumed in 1 and 3 involves reversion of fibril orientation, *viz.*, in the outer layers, from isotropic to more nearly axial, and in the inner layers, from transverse to more nearly isotropic and eventually also to more nearly axial. In this respect every layer of fibrils behaves like a net.

The twines of this net, however, should not be imagined to be tied together with fixed knots but merely to overcross, hence able to slip past one another. FREY-WYSSLING has pointed out that reversion of fibril orientation from transverse to axial involves a reduction of cell

diameter, except if the fibrils slip past one another. Since the growing cells examined either widen or retain their original width, reversion of fibril orientation involves slipping of the fibrils.

In this paper we studied growing tips and evidence will be presented of the orientation of the outermost fibrils being actually changed in the course of cell wall extension. The diameter of the cells examined does not decrease, so the fibrils do slip past one another.

The three assumptions also involve that the transverse orientation on the inner face of the cell wall must gradually merge via an isotropic into a more nearly axial orientation on the outer face.

According to 2. the fibrils of the outermost layer, like those of the other layers, have been deposited onto the inner face of the cell wall at an earlier stage. So this layer is the oldest one in any part of the cell wall at any moment. In cells with a growing tip, as presumably the cotton hair, this layer must have originated on the inner side of the tip; in cells lacking a growing tip, *e.g.*, *Phycomyces* sporangiophores, however, it must have originated at the apical end of the growth zone.

As regards assumption 1.: with *Phycomyces* sporangiophores and with young *Tradescantia* cells we were certain that the electron micrographs actually represented growing cell walls, but the other hairs might grow at their apex only, whereas the micrographs represented a portion of the tubular part. Only from the negative double refraction, wich implies that a secondary cell wall had not yet developed, it was deduced that the tubular part had not stopped elongating. Although, as far as we know, there are no exceptions to the rule, it offers no proof [1]). At present, however, we can point to the results of O'KELLEY (8), which make very probable that the cotton hair grows over its entire length. He wrote us (9): "If cotton fiber size is plotted against time .... the (growth) curve is a typical S-curve. This .... indicates that more of the fiber is concerned with elongation as the fiber gets larger, until growth is slowed down for other reasons. This is not compatible with tip growth".

METHODS

In this work we used the same methods of cleaning, mounting and shadowing of the cell walls as have been described in our previous paper (13).

RESULTS

A. COTTON HAIRS

The hairs examined belonged to the sample used before (13). A hair tip, enlarged 30.000 x, is shown in plate I. A full grown cotton hair of 2,5 cm would have a lenght of 750m when enlarged to this scale. The

[1]) Wide tubular cells like vessels stay negatively birefringent since the secondary cell wall is transversely oriented. *Tradescantia* hairs, also with rather wide cells, likewise retain their negative double refraction. The correlation only holds for cells with an axially oriented secondary wall; the other hairs examined belong to this category.

network of cellulose fibrils completely envelops the tip; apparently it is nowhere perforated. Plates 18a and b show 40.000 x enlargements of two areas of Plate 17. On the semispherical tip the fibrils are oriented at random, but as soon as the tip merges into the tubular part axial orientation prevails, becoming more evident along the next 10 μ down.

The same features are shown in plate 19.

To our mind, the apparent thickness of the cell wall and the fact that it is surrounded by air are incompatible with new fibrils being deposited on its outer face. This implies that the axially oriented fibrils of the tubular part were situated at the tip of the hair at an earlier stage and then were oriented at random.

Why should the change of fibril orientation from isotropic into axial coincide with the merging of the semi-spherical tip into the cylindrical part of the hair? Obviously, because in the tip, cell wall extension is not confined to the axial direction. This is visualized by a schematic drawing (fig.1). Sectors have been drawn in what represents the tip, and in the right part of the figure the extension of an area originally situated near the extreme tip, is illustrated. Axial extension prevails, but the width of the area also increases.

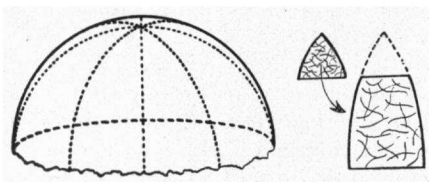


Fig. 1. Schematic illustration of multilateral cell wall extension in the tip of a growing cotton hair.

According to polarisation microscopical observations the semi-circular part of the flattened tip is not isotropic as is the randomly oriented fibril texture on the outer face, but it is negatively birefringent with reference to the cell axis. Therefore, transversely oriented fibrils like those found on the inner side of the wall in the tubular part (13) must occur in the tip as well. Probably only the central area of the extreme tip is isotropic both on the outer and on the inner side. (fig. 4). The newly deposited fibrils will rapidly shift outwards, since the extension of the cell wall requires a continuous generation of new fibrils all of which will be deposited onto the inner face of the cell wall. Meanwhile the older layers will acquire random orientation.

This concept is illustrated in fig. 2A. Here, as well as in B and C, distinct layer boundaries are drawn, but this has been done for illustrative purposes only. In reality, however, every layer merges into its neighbours (13, fig. 7 and 8). In fig. 2B the mean orientation of the fibrils of the outermost layer is supposed to have become axial. Evidently, the elongation of the part of the cell wall they belong to, causes the layer to grow thinner. According to our hypothesis the inner layer in

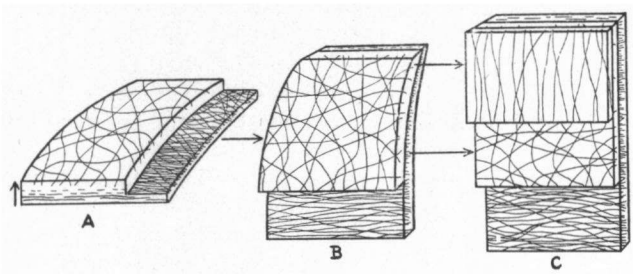


Fig. 2. The fibrillar structure in the growing cotton hair. A: near the top, B: where the tip merges into the tubular part, C: in the tubular part. Layer boundaries are drawn for illustrative purposes only; the transition is gradual.

B will gradually shift to the periphery and will acquire a random, or perhaps even a more nearly axial, orientation.

B. Root hairs of *Zea mays*

Corn seeds were germinated in moist air. The root hairs were cut off and prepared as usual.

Plate 20a shows the outer faces of the tip of a root hair; plate 20b shows both the inner and outer faces of the cell wall at the cut end somewhere below the tip.

The outside layer of the root hair, unlike that of cotton hairs, retains an isotropic structure when getting older and more distant from the tip. Onto the inner face of this layer a layer of axially oriented fibrils is deposited. On looking at the inner face of the cell wall on plate 20b one will observe that the fissures in the latter layer reveal the lack of transverse fibrils as would have been comparable with those found in cotton hairs.

Most root hairs are known to grow at their tips only. The axially oriented inner layer obviously is the secondary cell wall. Polarisation microscopy shows that it is deposited from just below the tip downwards. This is in line with growth being limited to the tip. The isotropic structure of the tip is retained downwards because the tubular part does not grow. The latter fact is also in line with transversely oriented fibrils lacking.

On plate 20a some loose fibrils and several thick fibril bundles may be noticed at the tip. However, we did not find the 1 μ thick felt-like layer of fibrils, wich was reported by Frey-Wyssling and Mühlethaler (3), to cover the cell wall proper at the tip. The method of cleaning of the hairs as used by these authors (10 % HCl, boiling 15 % NaOH) may have been too drastic. In other respects their results are corroborated by us.

C. Stellate cells of *Juncus effusus*

Multi-net-growth being established in free growing epidermal hairs of cotton and the like, a similar architecture of the cell wall was looked for in parenchymatous cells. If in any, it might be found in the wall of

cells bounding on intercellular space, for here conditions are comparable to those cotton hairs are subject to. In both cases the cell walls should be thick enough to withstand turgor pressure in contrast with cell walls between adjacent meristematic cells.

The stellate pith cells in the meristematic bases of the leaves (so-called stems) of *Juncus effussus* were chosen because the arms of these cells are in similar conditions as young cotton hairs. Both of them grow free in an air-filled space, the difference being that the tip of each cell arm of the *Juncus* cell meets the tip of the corresponding arm of an adjacent cell.

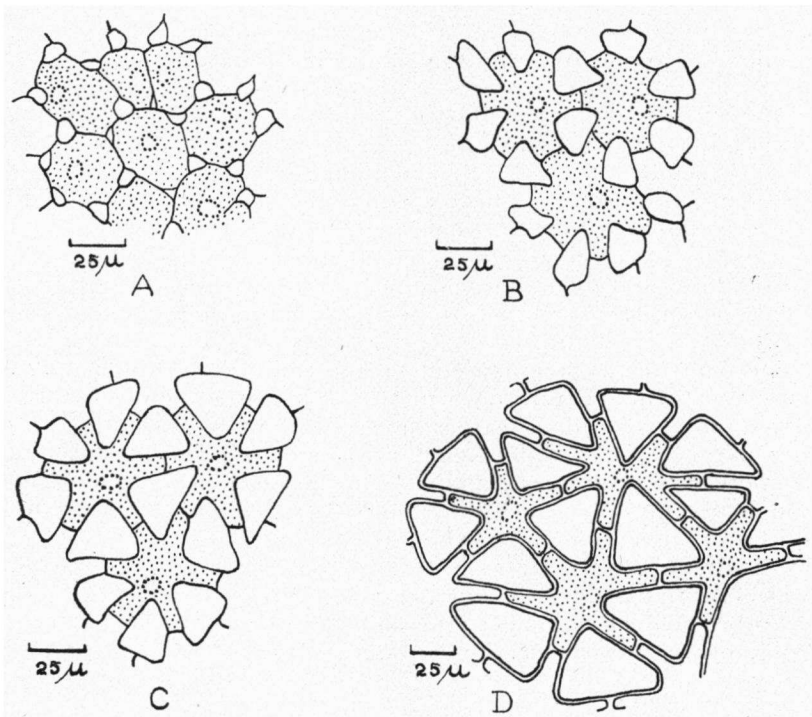


Fig. 3. Redrawn from MAAS GEESTERANUS (6). A, B and C: growing stellate pith cells of *Juncus* leaves. D: full grown cells.

MAAS GEESTERANUS (6) described the genesis of the stellate cells and the big intercellular space between them. Fig. 3 has been redrawn from this writer's paper. Cells like those examined in the present work are represented by fig. 3B and C. The arms of the cells are lengthening and, accordingly, prove to be negatively birefringent, as also are growing tubular cells like hairs. Arms of full grown cells, however, are positively birefringent because of the secondary wall which is oriented parallel to the arm axis.

Cells at stages B and C are found only in a zone of *ca.* 1 cm length at the base of growing leaves. The basal end of this zone was located

by examining between crossed nicols cross-sections of the leaf, starting with a section through the meristematic zone, and proceeding in apical direction until a section with cells at stage B had been obtained. Then, the upper end of the zone was found by examining a cross section *ca* 1,5 cm from the basal end and proceeding downwards until cells at stage C had been found. Thereupon the pith was removed, frozen, and cut into 15 μ sections with a freeze microtome. The sections were cleaned as usual. By this process many cells got free.

In plate 21 one arm and a portion of the central part of the cell are shown; the arm of the adjacent cell has broken off from the latter at its base. Plate 22a represents the joint of two corresponding arms. The cells must be at stage B, for the arms are rather short.

Plate 22b represents a similar joint of cells at stage C.

Clearly the fibrillar network of the (longer) arms of older cells is axially oriented as contrasted with the isotropic layer on the outer side of the (shorter) arms of younger cells. The altered orientation can only be ascribed to cell wall extension, unless it is supposed that fibril deposition is not confined to the inner side of the cell wall and that new fibrils have been deposited on the outer side. This supposition, however, seems to us untenable because of the cell wall being rather thick and bounding on the intercellular space. Moreover, judging from HÄUSERMANN's results (5) with similar cell walls, it should be slightly cutinized.

The joints of the rounded ends of the cell arms are enveloped by many fibrils linking up one arm with the other. The micrographs suggest that this fibrillar sheath is detached from the fibril layers of the cell wall proper. Probably these fibrils originate from the cell wall of the mother cell, but this explanation is not entirely satisfactory. Although several free fibril ends appear in the micrographs, nothing indicates that cellulose fibrils should have been dissolved (see FREY-WYSSLING and MÜHLETHALER (4).

The negative double refraction of growing arms is caused by a transverse fibril layer on the inner side of the cell wall, as is shown on plate 23a Randomly oriented fibrils show up between the axial outer, and the transverse inner layers.

Plate 23b shows peculiar fibril bundles which have often been found on the outer side of the central part of the pith cells. These bundles cannot be supposed to have originated by disconnection of arm joints. Though lying flat on the cell wall in the desiccated preparation they are completely free from the wall except at their bases. They may be artefacts formed by aggregation of loosened fibrils. If not, these funnel-shaped structures may have been formed by plasmodesms. We have not found an explanation satisfying both of us.

Both the outer side and the inner side of the cell wall of the central part of the cell are isotropic. This is in line with its multilateral growth.

DISCUSSION

The multi-net-growth hypothesis, aiming as it does at explaining by unilateral growth, the different cellulose fibril textures on the inner as compared with the outer side of the walls of many plant cells, should be based on observations on extending cell walls. In our previous work on the cell walls of cotton and other hairs (13) we missed the tips of the hairs and so we had to *assume* that the texture we studied was a product of cell wall extension, but now, with young *i.e.* growing cotton hairs, the tips as well have been examined.

Cotton hair. The outermost fibril layer is isotropic at the tip proper, but it is axially oriented further down along the cylindrical part of the hair, with a gradual transition from isotropic to axial orientation. Since deposition of fibrils onto the outer face of the cell wall is unlikely, a gradual re-orientation of these fibrils, while drifting away from the tip, seems well established. It has been argued already (p. 386) that reversion of fibril orientation should involve a change in cell diameter unless the fibrils are free to slip past one another. So they must be, for reversion of fibril orientation was proved to happen in a cell of constant diameter. Thus the cellulose texture in the cell wall is not as firm and inextensible as it appeared to FREY-WYSSLING and collaborators when they rejected the idea of cell wall extension as a major factor in growth, retaining it merely for exceptional cases, *e.g.*, the onset of a local outgrowth of a cell or the widening of vessels (1). Trylu the primary cell wall looks a very entangled network, but it should be recalled to mind that FREY-WYSSLING (2) again, pointed out that the structure is very loose, the cellulose fibrils representing only 2,5 % by weight of the primary cell wall. Undoubtedly hemicellulose, pectin and water fill up the space between the fibrils. Recently RÅNBY (10) found that the adhesion of the cellulose fibrils is very much reduced by hemicellulose. These considerations may help to imagine fibrils slipping past one another.

In order to visualize the behavior of the fibrils in the cellulose network we may compare the latter with a tangle of fresh, filiform algae spread on a glass plate. The algal threads, though frequently intertwined, will freely slip past one another. The tangle can be extended at will and the threads will become oriented accordingly.

As a consequence of the second one of the three assumptions mentioned in the Introduction, *viz.*, that new fibrils are deposited onto the inner layer of the cell wall, the origin of the outermost fibril layer should be discussed. Confining ourselves for the present to cotton hairs, we may state that these fibrils must have been deposited in the central area at the extreme apex of the semispherical tip of the hair. Here growth is multilateral, and fibrils in the inner layer of the cell wall probably are oriented at random as are those in the outer layer. On shifting outwards any area will stay isotropic until, getting farther and farther away from the centre, and in regions where growth is mainly axial, the fibril layer becomes more and more axially oriented.

Returning to the inner side of the cell wall at the tip, we may expect

that here as well, *growth* in any area is the more axially directed the farther from the tip the area is situated. Now, the cytoplasm is known to react to unilateral — here axial — growth, by depositing the fibrils transversely with respect to the direction of growth. The negative double refraction at the tip shows that this also applies with cotton. If the inner face of the cell wall at the tip of the hair could be examined, a gradual, but maybe rather fast transition from isotropic to transverse apposition of fibrils would be found. Fig. 4 may serve to illustrate our views on the fibril orientation at the extreme tip.

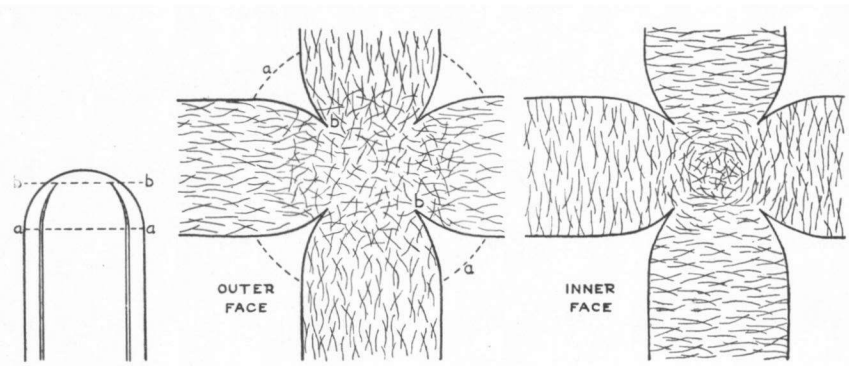


Fig. 4. Fibrillar architecture of the outer and inner faces of the tip of the cotton hair.

Assumption 3 reads: "On being extended axially each of the transversely oriented layers on the inner side of the cell wall behaves like a network consisting of intertwined fibrils".

Whereas any fibril layer, if isotropic, on being extended unilaterally, must show a change of the orientation of the fibrils, this needs not occur in case the fibrils run parallel to one another and transversely with respect to the direction of growth. The micrographs of the inner side of cotton hairs (13) and of *Juncus* cell arms (plate 23a), however, show that the fibrils are not exactly parallel. Instead, the inner layer looks like a net with transverse meshes. Every fibril will be intertwined, though very loosely, with several fellow fibrils of the same generation.

No reason can be devised why slipping of the fibrils past one another should not happen in the inner layer of the cell wall like it has been shown to occur in the outermost layer. Incrustation with cutine and lipoids is less likely to have occurred in the inner part of the cell wall than in the outer part. So, no objection on account of the fibrils having to slip, can be raised against our assumption 3.

Hence the fibrillar texture of the cell wall of the cotton hair can be completely explained by the multi-net-growth hypothesis.

Juncus stellate cell arms have no growing tips. Growth along an annular zone should be responsible for cell arms coming into being. From the very first, growth is unilateral. The growth zone lengthens considerably; in fact the whole of the cell all of the arm is nothing but

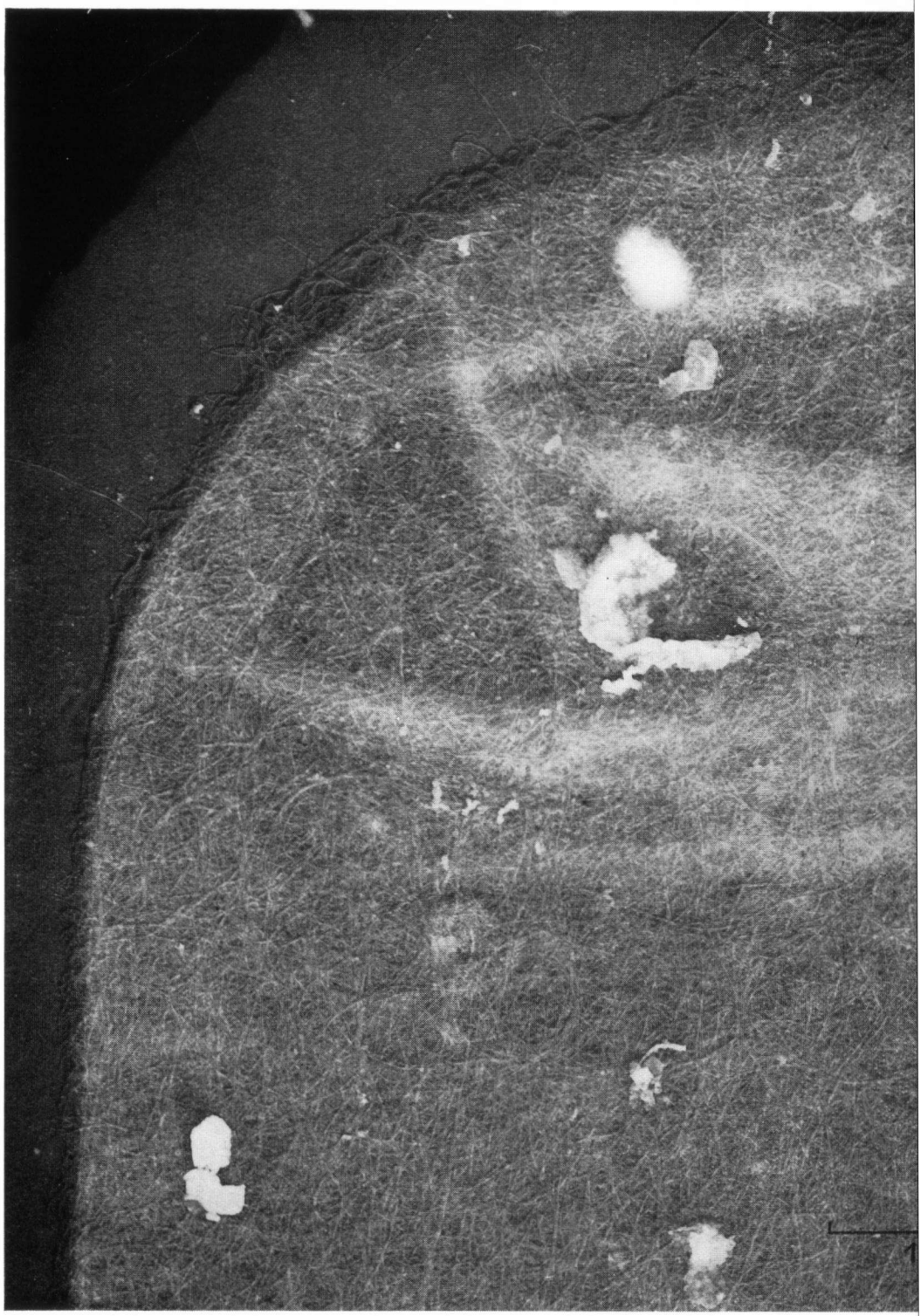
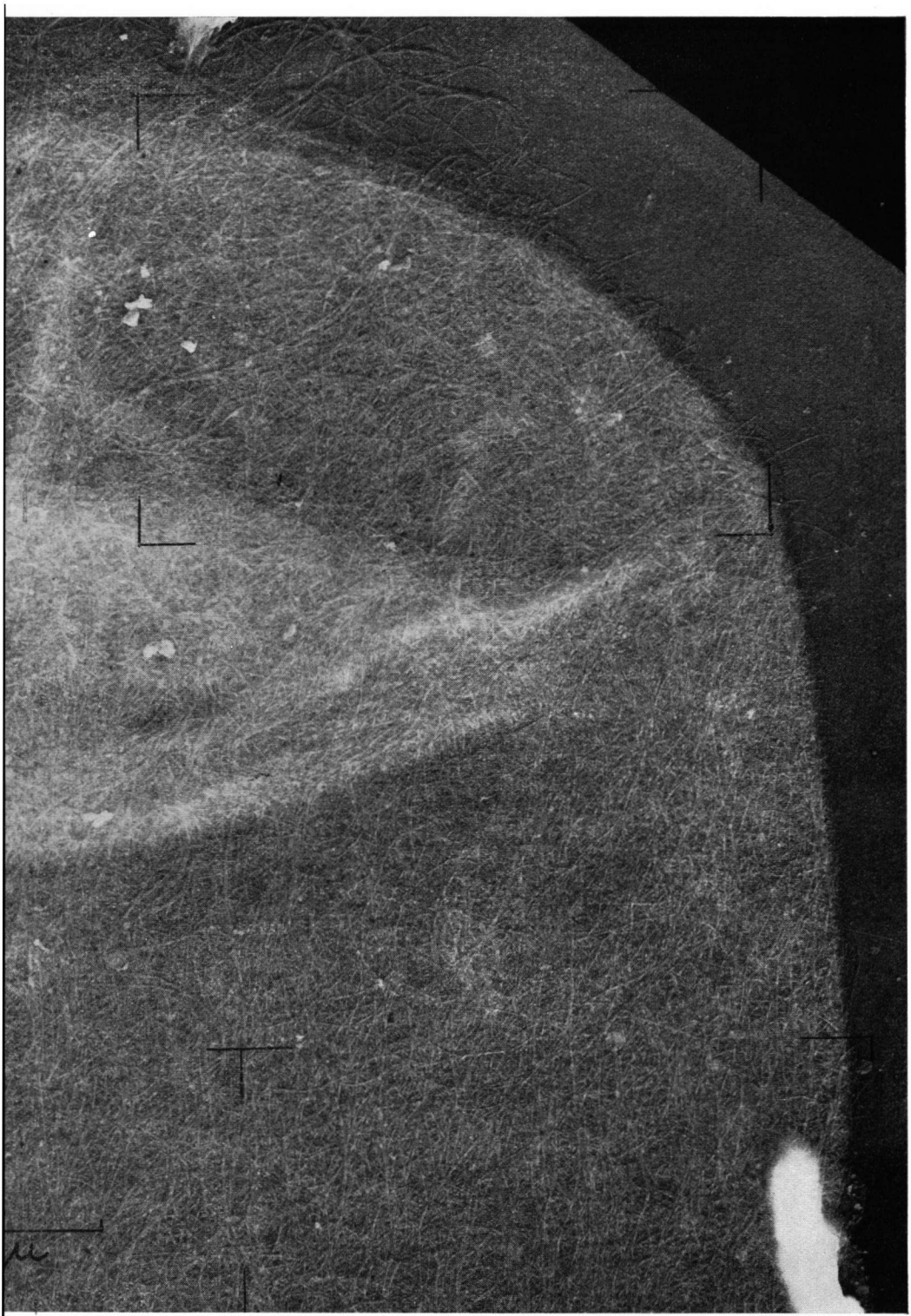


Plate 17. Outer face of the tip of a young cotton hair, showing the transition of fibril orienta



ion from isotropic at the tip to nearly axial in the cylindrical part. See also Plate 18a and b.

a lengthening growth zone. All over its length transverse fibrils will be deposited onto the inner face of the cell wall. On shifting outwards the fibrils will become isotropically and eventually, axially oriented.

In the root hairs of *Zea mays*, maybe exemplary for the root hairs of most plants growing in soil, neither birefringence nor electron microscopy indicate the presence of transverse fibrils on the inner side of the cell wall. This would indicate that the manner of growth in the tip — the tubular part of the root hair does not grow at all — is such that axial extension does not in any area prevail over transverse extension. Fig. 5, I may serve to illustrate our concept[1].

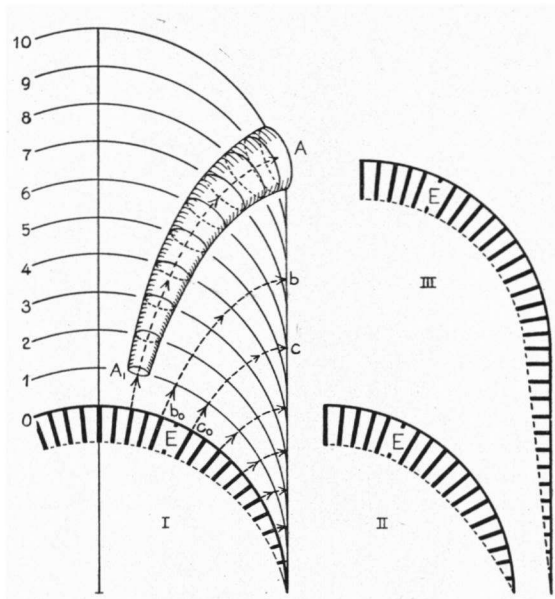


Fig. 5.

I. Schematic representation of the growth of the cell wall of a root hair.
0, 1, 2, etc. borderlines at subsequent time intervals.
b_0b, c_0c; paths of points on the cell wall.
A_1A: path and extension of an area of the cell wall.
E: graphic representation of the amount of cell wall material deposited in the unit of time per unit area onto the inner face of the cell wall.

II and III: Distribution of cell wall material leading to predominant axial extension: II different distribution of E in the tip, III additional growth in the tubular part.

The way the tip of a cell grows, is correlated with the distribution, by the cytoplasm, of cell wall materials — cellulose, pectin, hemicellulose etc. — over the inner face of the cell wall at the tip. If these substances are deposited according to the concept visualized in fig. 5 I, the primary cell wall will stay isotropic throughout. A different distribution of materials as, *e. g.*, in fig. 5 II and 5 III will lead to prevailing axial growth and this will involve deposition of transverse fibrils onto the inner side of the cell wall and reorientation of the fibrils on the outer side. *Vide* DE WOLFF end HOUWINK (14)

[1] Compare REINHARDT, M.O. 1892 Jahrb. wiss. Bot. 23:479.

In the *Tradescantia* staminal hairs the inner layer of cellulose fibrils is transversely oriented. This would indicate that in any area axial extension of the cell wall prevailed over transverse. The outer layer, however, is not axially oriented. The latter observation proves that the prevalence of the axial extension is only slight. This makes us assume that the way these hairs grow, is intermediate between the ways root hairs on the one hand and cotton hairs on the other, do grow. Growth in this case is not confined to the tip, as it is in the root hair. The cylindrical cells of the hair, however, on elongating, also widen, and this will keep the fibrils from becoming axially oriented.

Phycomyces sporangiophores too, have an isotropic outer and a transversely oriented inner fibril layer. With young sporangiophores we may assume the cells to grow in a way intermediate between root hairs and cotton hairs. After a sporangium has been formed, however, the sporangiophore has an annular growth zone, with maximum growth slightly below the sporangium. Hence the fibrils will be transversely oriented from the start. A transverse layer has to be extended much more than an isotropic layer if it is to be turned into an axially oriented one. Moreover, in both cases the growth zone is slightly conical, widening towards its base.

If fibril reversion and hence, slipping of fibrils, happens in epidermal cell walls, in fungal hyphae and in cell walls of pith cells bordering on the intercellular space, then this is even more likely to occur in cell walls separating meristematic cells, for the latter walls are much thinner and moreover they are not cutinized. In a previous paper (13) attention was drawn to some facts pointing to the probability of multi-net-growth occuring in wood. Also the EM-graphs of cell walls of meristematic cells published by MÜHLETHALER (7) are compatible with the concept of multi-net-growth.

The Zürich school offers quite a different explanation for the architecture of these cell walls and for the growth of these cells. It involves several assumptions, *viz.*, intrusive growth of cell tips and tip perforation by the protoplasm. This would weave a new cell wall and in this way add a new tip to the cell. However, since extension of a fibrillar network has been proved to occur, our explanation of tip growth seems more likely and the possibility of symplastic growth should not be disregarded.

SUMMARY

The architecture of the extending cell walls of cotton hairs, *Juncus* stellate pith cells, and maize root hairs has been studied. The observed facts could be explained on the basis of the multi-net-growth hypothesis.

The cells investigated were found to be completely enveloped by a continuous network of cellulose fibrils. Every area of the growing part of the cell wall is extending in one or in all directions. The cellulose fibrils in the cell wall must slip past one another. The cell wall does not grow thinner since new fibrils are continuously deposited onto its inner face. In the apex of the root hair, extension is the same in every direction, so that the orientation of the fibrils does not change. In cotton hairs (the extreme tip excepted) and *Juncus* stellate cell arms, however, a change in fibril orientation has

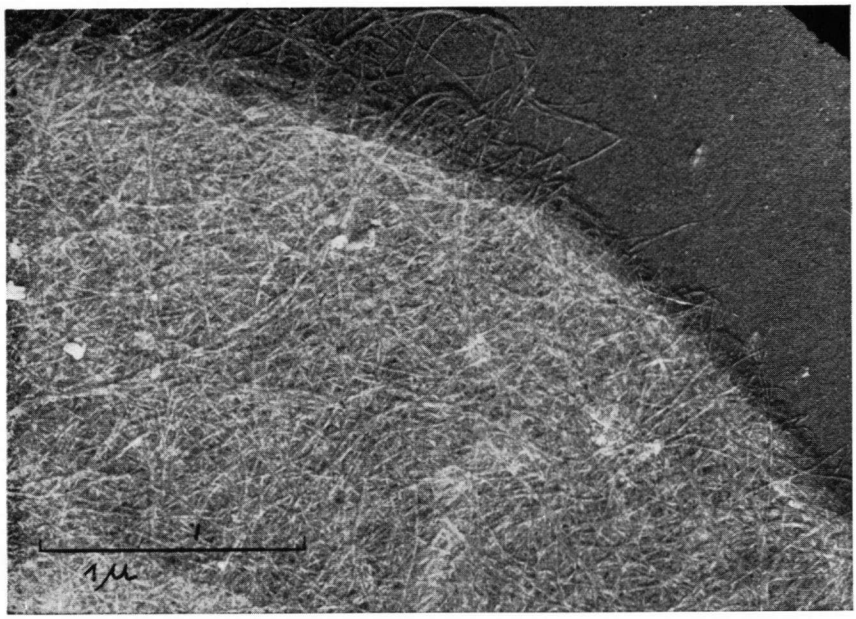


Plate 18a. Detail of plate 17, apical part.

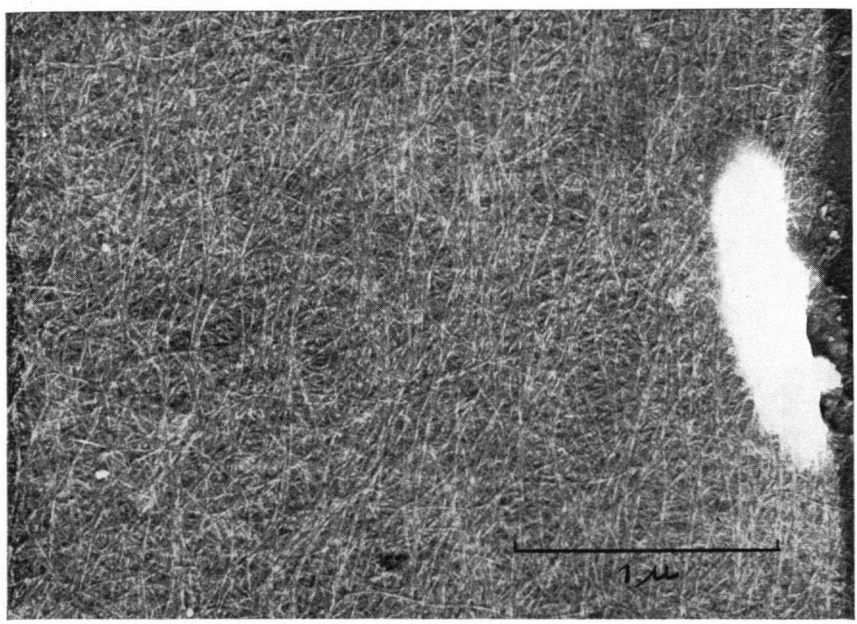


Plate 18b. Detail of plate 17, basal part.

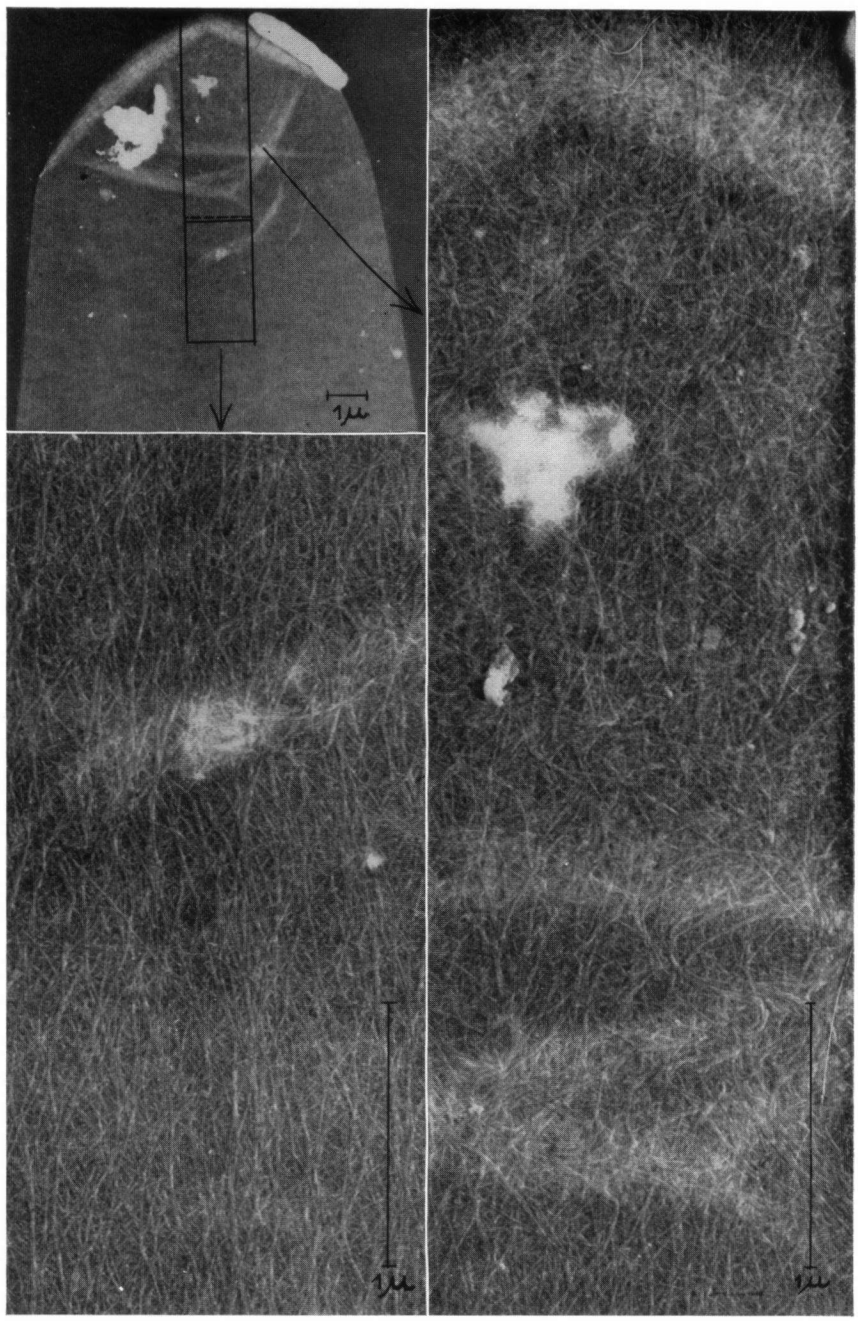


Plate 19. The tip of another young cotton hair, compare plates 17 and 18.

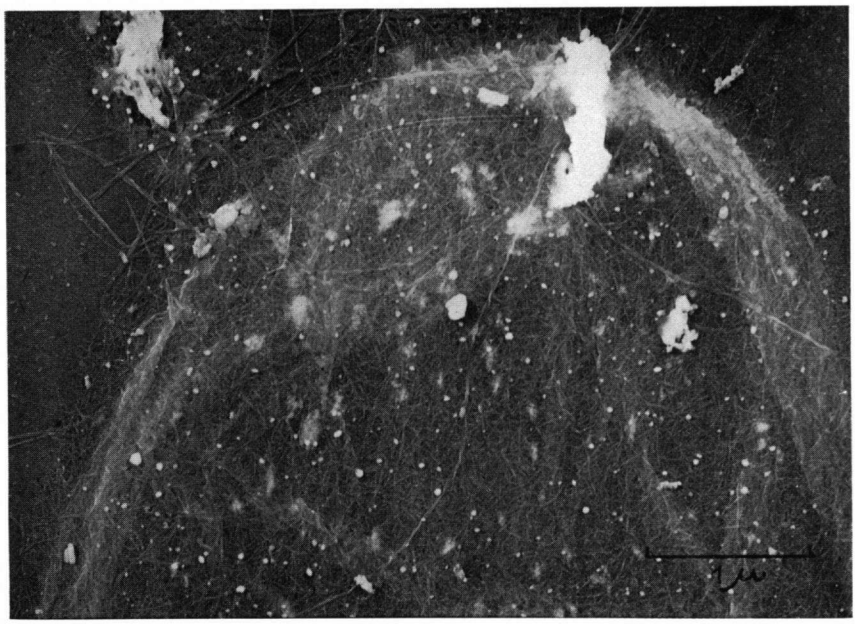


Plate 20a. *Zea mays*; Outer face of the tip of a root hair

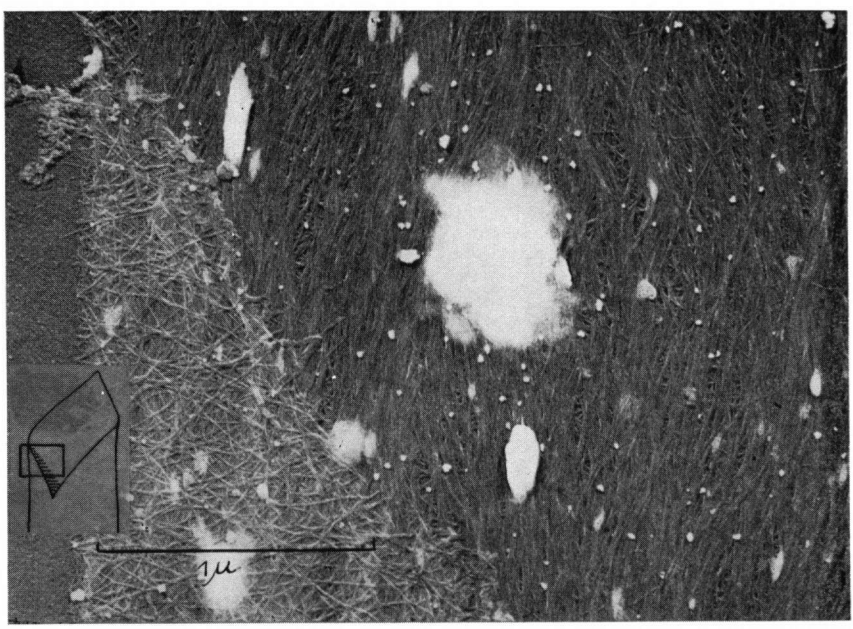


Plate 20b. *Zea mays*; obliquely cut end of a root hair, showing the isotropic outer face as well as the axially oriented inner face.

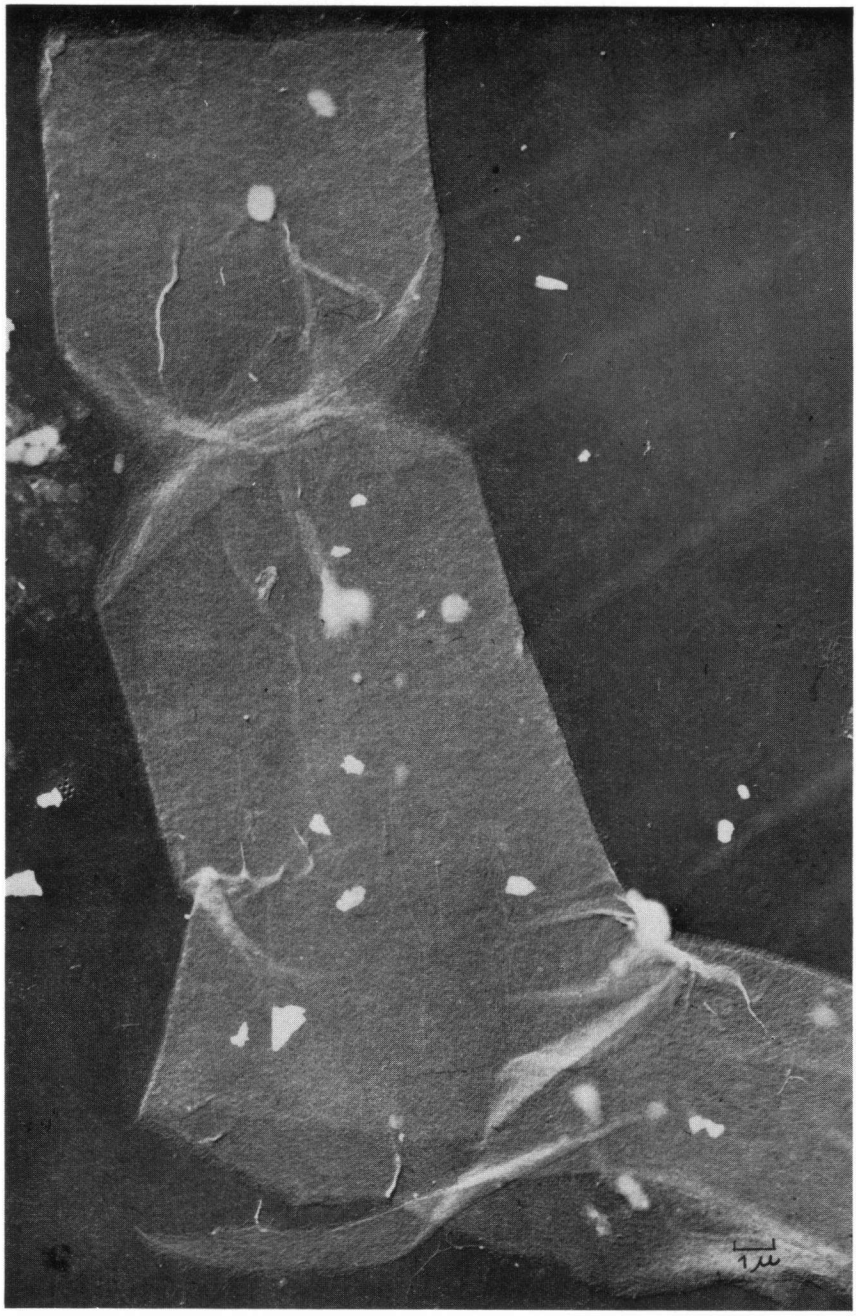


Plate 21. *Juncus effusus*: an arm of a stellate cell from the pith of a leaf base. Bottom: a portion of the central part of the cell. Top: the corresponding arm of an adjacent cell, from which it is detached.

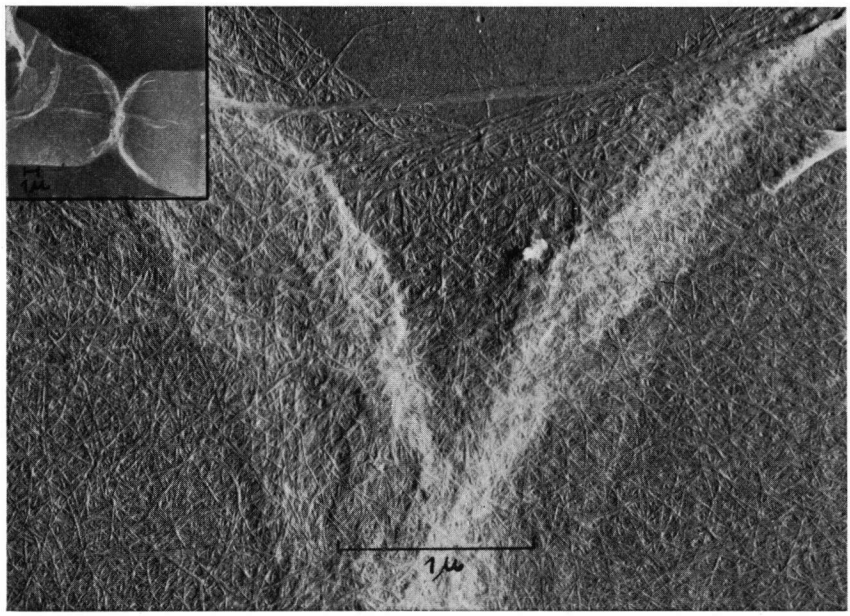


Plate 22a. *Juncus effusus*; detail of a joint of two corresponding cell arms; inset: gene ral view. Cells at stage B.

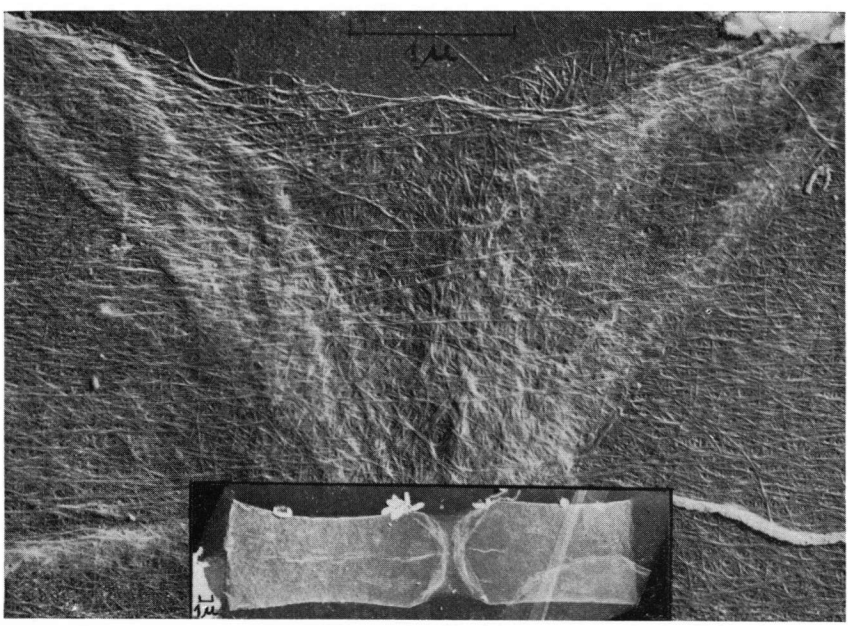


Plate 22b. *Juncus effusus*; as plate 22a. Cells at stage C.

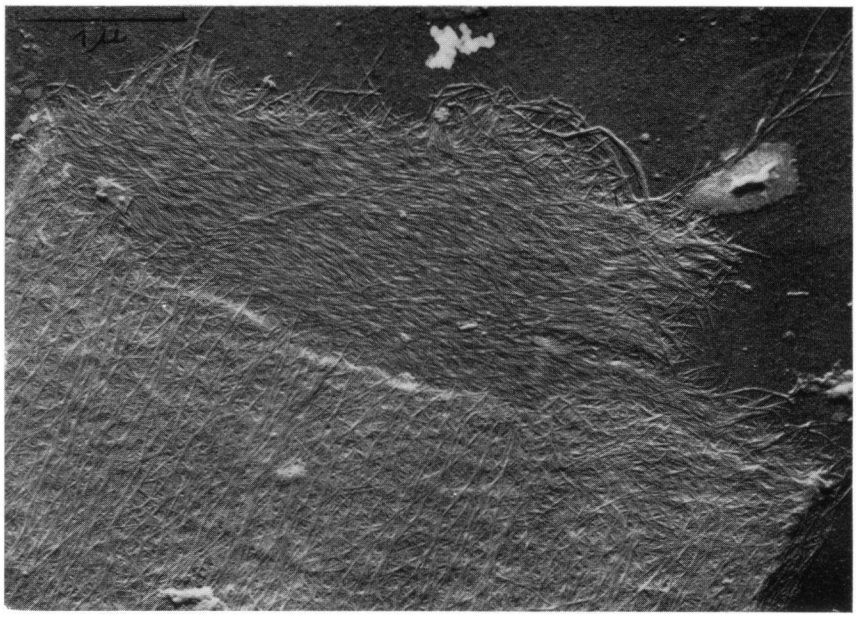


Plate 23a. *Juncus effusus*; a cell arm, cut nearly transversely. The transversely oriented inner face and the axially oriented outer face of the cell wall are shown.

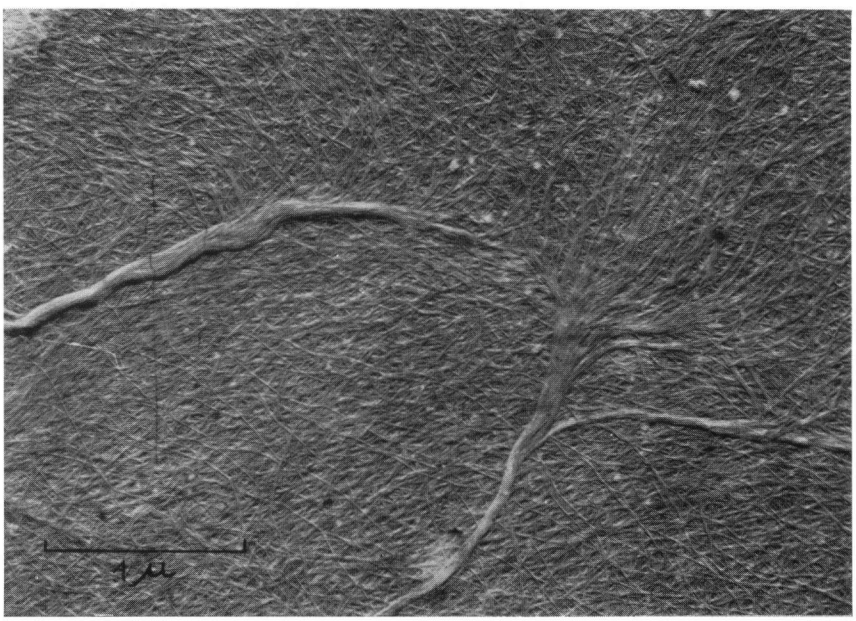


Plate 23b. *Juncus effusus*; fibril bundles found on the outside of the central part of a stellate cell. Isotropic cell wall structure.

been observed. Here axial extension prevails over transverse. This results in the orientation of the fibrils altering from the moment they are deposited until the area gets out of the growth zone.

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