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ARCHITECTURE AND GROWTH OF THE PRIMARY CELL WALL IN SOME PLANT HAIRS AND IN THE PHYCOMYCES SPORANGIOPHORE

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INTRODUCTION

Electron micrographs of the primary cell wall in the growth-zone of *Phycomyces* sporangiophores show an isotropic network of chitin fibrils on the outside and an almost transverse structure on the inside (3, 8). The same applies to the cellulose fibrils in the cell wall of staminal hairs of *Tradescantia* (10). Axial orientation of the outer fibrils is apparent in the primary cell wall of growing cotton hairs, which on the inside again show a more or less transverse structure (5, 9).

The present paper reports the finding of a similar architecture in the growing hairs of two more plant species, viz. *Ceiba pentandra var. caribaea* and *Asclepias cornuti*. Furthermore some new E.M.-graphs of cotton hairs are presented, since those published earlier were of a low standard. Obviously because the present E.M.-graphs revealed more details, we were struck by a particular common feature in the fibrillar structure which led to some deductions regarding the process of cell wall extension in plant hairs and in the *Phycomyces* sporangiophore. These will be discussed in the last section.

METHODS

Fruits, gathered at definite intervals after flowering, were preserved in alcohol *.

If the hairs of young fruits were viewed in radial direction between crossed nicols, a negative double refraction with reference to the cell axis was found. This always occurs in growing tubular cells, at least in growing regions of such cells. Of course, hairs of nearly full-grown fruits were always entirely positive.

With *Ceiba*, negative double refraction was found in the sample of 25 days and in all younger ones, the next sample being 35 days old.

* We are greatly indebted to the manager of Siloewok Sawangan estate, Java, who furnished the *Ceiba* material. *Asclepias* and cotton were grown in our botanical garden.

In order to be sure that the sample to be used for the electronmicroscopy would contain primary cell walls only, fruits gathered ca 20 days after flowering were taken. The mean hair length was roughly 12 mm as against 25 mm in the 35 days sample.

The *Asclepias* hairs were negatively birefringent up to 35 days, though less distinctly than after 28 days. Hairs of 21 days old were used for the E.M.-work. Like those of *Ceiba* they were negative from the extreme tip down to base. Their length was 8 mm as against 30 mm in the 35 days sample.

The cotton hairs used, were 15 days old and were from the same fruits as used in earlier work (9). Of these hairs too, the extreme tips were negatively birefringent.

The hairs were cut off, suspended in 70 % ethanol and cut into pieces of 30–60 μ length by means of a small electric blendor. The ethanol and all other liquids used, had previously been freed from dust, etc. by centrifuging.

The fragmented hairs were centrifuged and then cleaned by the following successive treatments, alternated with centrifuging in water:

a. 30 min. in perhydrol-glacial acetic acid 1:1 at 100° C,
b. 20 min. in 2 % sulfuric acid at 100° C,
c. 20 min, in 2 % NaOH at 100° C,
d. neutralisation and frequent washing with water.

The cleaned fragmented hairs were mounted on collodion covered E.M. grids and shadowed with Pt.

Results

The similarity of the architecture of the primary cell wall of hairs from *Ceiba* (figs 1, 2), *Asclepias* (figs 3, 4, 5, 6) and *Gossypium* (figs 7, 8) is very striking indeed. There is, in fact, no difference whatsoever.

Especially with a more or less transverse direction of shadowing, as in figs 2, 4 and 8, it is clear that the outermost fibrils are roughly axial. Even if the direction of shadowing is also nearly axially, this is apparent (figs 1 and 7). Since the hairs very probably shorten during the chemical treatment, the axial orientation will be more marked in vivo. Often several fibrils are combined to bundles (fig. 8). In all the E.M.-graphs, but in particular in figs 2, 7 and 8, one can see that in a deeper layer the fibrils run in an oblique direction and that in a still deeper layer a transverse direction predominates.

Accordingly, a transverse structure is very conspicuous on the inside of the primary cell wall, as is clearly demonstrated by figs 1, 3, 5 and 7. This stratum is of considerable thickness and the fibrillar structure is more compact than on the outside. Hence, one rarely sees the obliquely or axially oriented fibrils underneath, e.g. in the frayed-out cell wall edge in fig. 6.

Obviously, there is a gradual change from a compact transverse structure on the inside to a loose, more or less axial structure on the outside of the growing cell wall.

Not only the fibril orientations, also the interfibrillar spaces differ on the two sides. Figs 3, 5 and 7 show numerous transversely oriented tiny meshes on the inside of the cell wall. Hence, the fibrils, or bundles of these, show a wavy structure, which is very conspicuous in fig. 7. There are no broken fibrils and apparently, the meshes have originated by a pushing or drawing apart of the fibrils rather than by tearing the cell wall or breaking the fibrils. The internal surface of the cell wall resembles a submicroscopic fishing-net with transversely elongated meshes. These are roughly 200–500 Å wide and 1000–1500 Å long. Their depth varies even more considerably, but in many cases it will be more than the total thickness of several fibrils. Usually one can see one or more fibrils at the "bottom" of the meshes, running in oblique or axial direction, which demonstrates that the meshes do not perforate the cell wall but are confined to a stratum of it.

After having observed the meshes on the inside of the cell wall, a look at the outside (figs 2, 4, 7 and 8) makes clear that here as well one might speak of meshes, though larger and axially elongated ones. Moreover, they are confined to a very thin superficial stratum. Their mean width corresponds roughly with the mean length of the transversely elongated meshes on the interior surface of the cell wall, but their lengths vary considerably.

Discussion

If the architecture of the growing hairs of *Ceiba*, *Asclepias* and *Gossypium*, is compared with those of *Tradescantia* staminal hairs (10) and of the primary cell wall of the *Phycomyces* sporangiophore (8), the only difference seems to be that in the latter two cases the outermost fibrils are oriented at random instead of axially. However, since the sporangiophore shortens considerably on loss of turgor and during the chemical treatment, it is probable that in vivo a small preference for axial orientation occurs. Quite apart from that, the structural difference between the inside and the outside of the cell wall is "analogous", viz. likewise indicating an extension of the transverse structure of the inside.

Meshes, similar to those described here, are also visible on the E.M. graphs of *Tradescantia* and *Phycomyces*. They are less conspicuous, probably merely because the E.M. graphs show less details.

This similarity of the fibrillar architecture of the growing cell walls of all these cells, belonging to widely different plant families, must have a common cause, in all probability: the manner of cell wall extension. Thus, the question arises how these cell walls grow.

First we should discuss whether the manner of cell growth as has recently been described for other plant cells, might possibly occur in the cells under consideration.

In parenchyma cells and epidermal cells of *Avena* coleoptiles, tip growth has been demonstrated (6). This was also found in cambium cells (1) and in *Spirogyra* (2, p. 283). The authors state that the tips of the cells are opened and that the cytoplasm apparently oozes out of the cell. It weaves its wall. First, more or less longitudinal fibrils

are deposited. These act as a kind of warp and are interwoven with a weft of mainly transverse fibrils, which soon outnumber the longitudinal ones, thus producing the negative birefringence of the fully grown primary cell wall. The unfinished cell wall, however, is either isotropic or positively birefringent with reference to the cell axis. In view of the following discussion we wish to emphasize this optical behaviour.

In other areas of the same parenchyma and cambium cells, growth is not such an addition of new cell wall areas, but it is a true cell wall extension, presumably due to a pushing apart of fibrils by local plasmatic growth. In these spots the cellulose structure is completely perforated temporarily. These perforations have dimensions of $\frac{1}{4}$–1 μ. Soon new fibrils are inserted. This kind of intussusception growth was also discovered by FREY-WYSSLING and his collaborators and named mosaic-growth (1, 4).

Does tip growth occur in the *Phycomyces* sporangiophore and the plant hairs under consideration?

Obviously not in the sporangiophore when the latter carries a sporangium. Neither can there be any question of similar addition of large pieces of cell wall in the growing regions below the sporangium. Firstly the electron microscope nor the studies on the distribution of growth in the growth-zone ever revealed such local differences. Secondly, the whole growth-zone shows a negative double refraction of a very uniform intensity. Thirdly, a perforation of the cell wall of such a turgid cell, surrounded by air, is quite inconceivable. The same conclusion applies to the sporangium-free growth stage of this sporangiophore. Admittedly, its apex grows, but in the same manner as the rest of the growth-zone and not in the manner of typical tip-growth, for the whole growth-zone including the extreme tip is negatively birefringent (3).

Neither can there be tip-growth or any other similar addition of new pieces of cell wall in growing *Tradescantia* staminal hairs. Firstly, the hair is a chain of cells and the cells cannot have growing tips. Secondly, the cells are negatively birefringent throughout, even immediately after cell division (10). Here too, perforation of the cell wall by protoplasm obviously is out of the question.

Whether the hairs of cotton, *Ceiba* and *Asclepias* grow at their apex only, or over their full length, seems uncertain. Generally with cotton, the latter type of growth is taken for granted. Some authors assume tip growth, but as far as we know, nothing has been proven. Anyway, if solely the apex grows in these cases, the sequence of deposition of a "warp" and — later on — the interweaving of a "weft", as is typical for tip-growth, does not occur here either. Firstly, we have observed that the extreme cell apices in all these cases are negatively birefringent like the rest of the cell wall of the growing cells. Secondly, the axial and the transverse fibrils are not interwoven, but occur in different layers.

Typical mosaic-growth, characterized by rather few, but large perforations, as found in the cell wall of parenchyma- and cambium

cells, apparently does not occur in hairs, nor in the *Phycomyces* sporangiophore, for such conspicuous perforations would not have escaped notice on the E.M.-graphs.

Therefore, the conclusion is inescapable that the complicated fibrillar structure of the cell wall as revealed in the E.M.-graphs of the plant hairs and the *Phycomyces* sporangiophore, was in the act of extending at the moment of fixation. This clearly means that we have come across a third type of cell wall extension, which is characterized by the occurrence of a great number of shallow submicroscopic tears or meshes, uniformly distributed in the cell wall in all growing regions of the cell and varying in shape and area in the different layers of the cell wall, the latter therefore never being perforated. Unlike the perforations occurring during mosaic-growth, these meshes never seem to be filled in with fibrils (at least not completely), but to persist and extend. Probably they are filled in with non-fibrillar material and the very small holes visible in the cell wall, e.g. in fig. 8, will not be true perforations.

To attain a dynamic picture of this type of cell wall extension, an assumption must be made. We think that although plasm threads probably will occur in deeper layers of the cell wall, the majority of new fibrils will be deposited on the inside, adjacent to the outer protoplasm layer. Since the outer layers of the cell wall will be incrustated with cutin and wax, one can hardly expect fibrils growing or being inserted here. Apparently these new fibrils are deposited in a nearly transverse direction. Most probably, the fibrils are never strictly parallel, but interwoven or rather intertwined with their "contemporaries". In the growth zone of the *Phycomyces* sporangiophore the mean direction is according to a flat spiral.

Our conception of the manner of extension of this cell wall is as follows. Owing to cell wall tension and, may be, assisted by local plasm growth into the cell wall, the small bundles of newly deposited, intertwined fibrils occurring on the inside of the cell wall will be split locally, thus producing the transversely oriented superficial meshes. Since these meshes are not filled in with new fibrils, axial cell wall extension elongates them more and more in axial direction. Meanwhile new fibrils are deposited on the inside and the extending mesh is therefore gradually shifting to the outside of the cell wall, probably being more and more filled in with incrustating materials. The greater the ultimate extension, the more axial the fibril orientation on the outside will be. Since the hairs of the samples used, had not yet acquired their full length, the axial orientation probably will be even more conspicuous in older hairs. Even hairs of one fruit may differ in age, hence will show minor structural differences. As will be evident, the occurrence of random orientation on the outside of the *Tradescantia* staminal hairs and the *Phycomyces* sporangiophore is to be considered as a quantitative and hence irrelevant difference.

Fig. 9 (left part) very schematically illustrates the essential part of our theory.

Our conception readily explains some additional structural details,

already mentioned. The diameter of the cells under consideration, keeps practically constant during growth. So, if the meshes visible on the outside, have originated from meshes similar to those occurring on the inside, the transverse dimension of both should be roughly equal. This lines up with the facts. However, the mean circumference

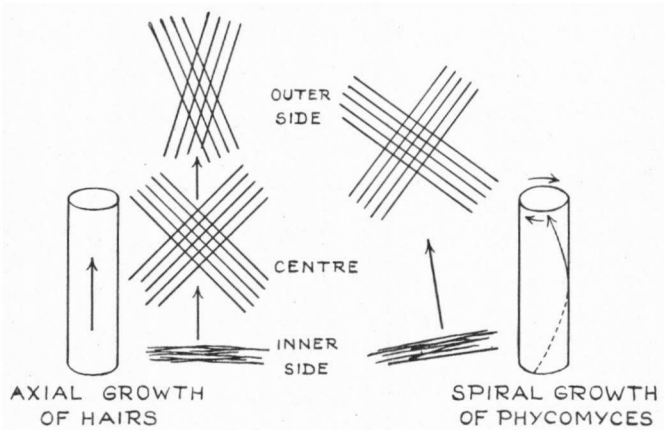

Fig. 9. Scheme of multi-net-growth in the upper cell wall of a tubular cell in connection with growth-direction and rotation of the apex.

of the meshes greatly increases. This means that the fibrils occurring in one bundle, are not fixed to each other, but may shift somewhat. What appears as one mesh on the inside, is subdivided in several axially elongated meshes on the outside. As a matter of course these will be more shallow than those on the inside and the fibrillar structure will moreover be much looser than on the inside. This is clearly visible.

We propose for this type of growth the name multi-net-growth, since the cell wall may be compared with a sheaf of fishing-nets which gradually change in mesh and in direction of the twine. In reality, of course, the "nets" are not separated, but extensively interwoven. There are no distinct layers, but a gradual change in structure.

If our conception is right, this is apposition-growth if one considers the fibrillar material only, but it is intussusception-growth if one considers the incrustating material which fills in the meshes.

We are aware that the name multi-net-growth simplifies the reality, but it has the merit of stressing the most characteristic feature. In our previous publications on cell wall structure of growing cells the reversion of the fibrillar orientation was explained in a similar manner, except that the occurrence and behaviour of the meshes had not yet been noted. It was assumed that these were filled in with fibrillar instead of non-fibrillar material. The principle of reversion of fibrillar orientation on cell wall extension is by no means new, since it was postulated by J. BONNER as early as 1936.

We wish to point out that multi-net-growth in a tubular cell with

a flat spiral structure, as occurs in *Phycomyces* sporangiophores (8), will produce rotation of the free top (spiral growth) as a matter of course. This will be evident after examination of fig. 9 (right part).

Several years ago, one of us (7), has advanced evidence for the view that this spiral growth is partly due to the purely mechanical effect of the stretching of a flat spiral structure and partly to a process of "active intussusception" of fibrils, governed by protoplasm. We now have a better notion of the latter process. It is not an intussusception of fibrils, but the creation of the meshes and perhaps the deposition of non-fibrillar material into these.

In explaining the spiral growth of *Phycomyces* sporangiophores, FREY-WYSSLING (2, p. 305), postulates a "circular traveling of the intercalary growth" in the growth zone. However, as was already stated, there are no localized growth spots. Moreover, if there were, their supposed circular traveling would merely produce circumnutation instead of rotation of the apex.

The suitability of multi-net-growth and the unsuitability of "mosaic-growth" and "tip-growth" for cells with aerial growth, is obvious. In *Phycomyces* sporangiophores a turgor pressure of 2 atm. has been found (7). This pressure must be exerted by the growing cell wall. Is'nt this quite incompatible with large perforations of its fibrillar structure such as occur with "mosaic growth" as well as with "tip growth"? The latter two types of growth will very probably be confined to tissues where the cells mutually compensate their turgor pressures and where intercellular spaces are practically lacking, e.g. in primary meristems. Here, only the thick outer epidermal cell wall exerts a pressure. Admittedly, tip growth does occur in epidermal cells too (6). How this is possible without the occurrence of perforations in the outer cell wall, is not yet quite clear. Maybe, perforations only occur in the radial and the inner-tangential cell walls, the outer cell wall probably showing multi-net-growth.

In young xylem cells BOSSHARD (1) has seen mosaic-growth and tip-growth, but he also remarks (p. 494): "In Abb. 19 ist eine junge Xylemfaser abgebildet, in der die Wachstumsbezirke nicht scharf gegeneinander abgegrenzt sind, sondern es scheint, wie wenn in diesem Falle eine gröszere Fläche gleichzeitig am Wachstum teilnehme". He also describes primary cell walls as having several interwoven layers with different orientation (p. 489). Possibly, multi-net-growth or some analogous type of growth also occurs in young phloeem and xylem cells.

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SUMMARY

The cellulose fibrils on the interior surface of the primary cell wall of growing hairs of *Ceiba*, *Asclepias* and *Gossypium* are oriented more

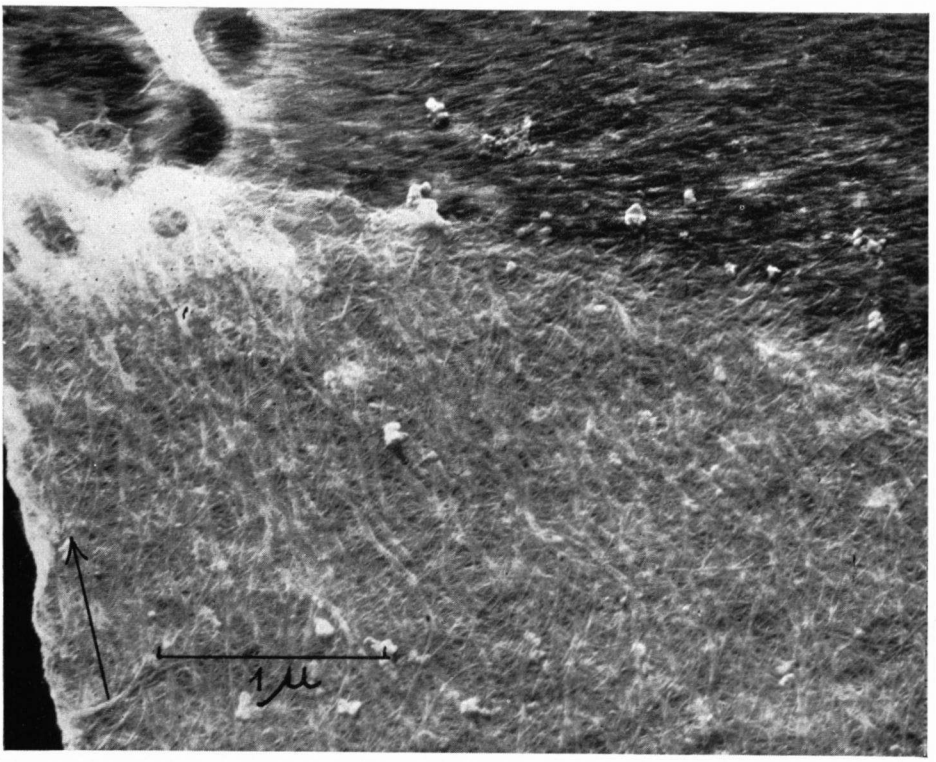

Fig. 1. *Ceiba pentandra* hair, outer and inner side top) of primary cell wall.

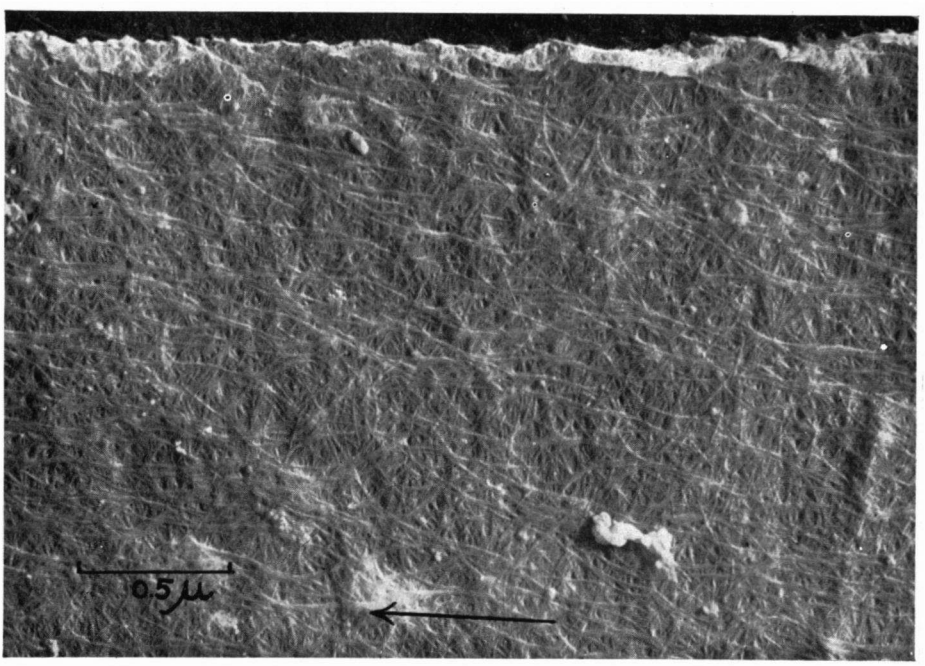

Fig. 2. *Ceiba pentandra* hair, outer side of primary cell wall.

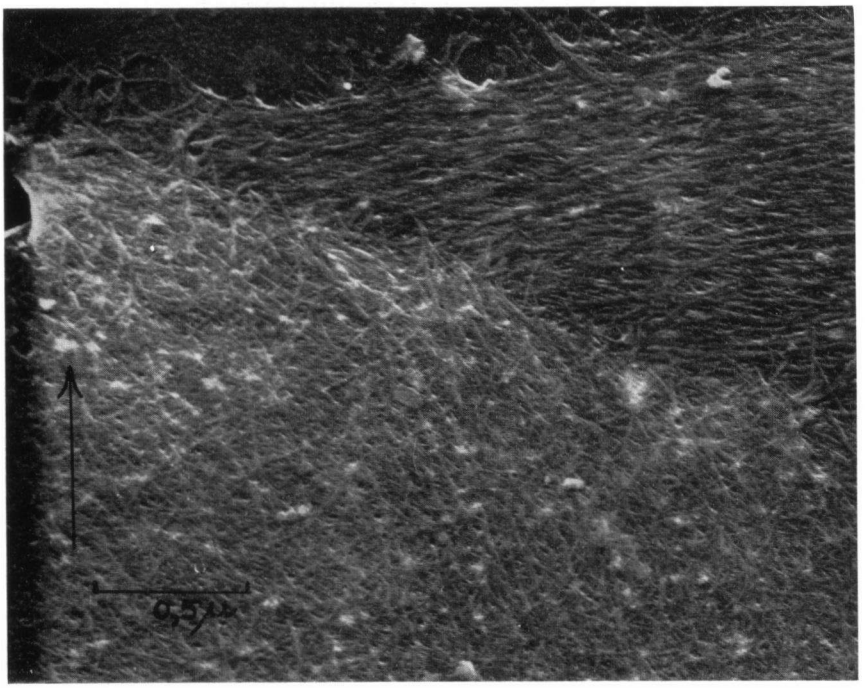

Fig. 3. *Asclepias cornuti* hair, outer and inner side (top) of primary cell wall.

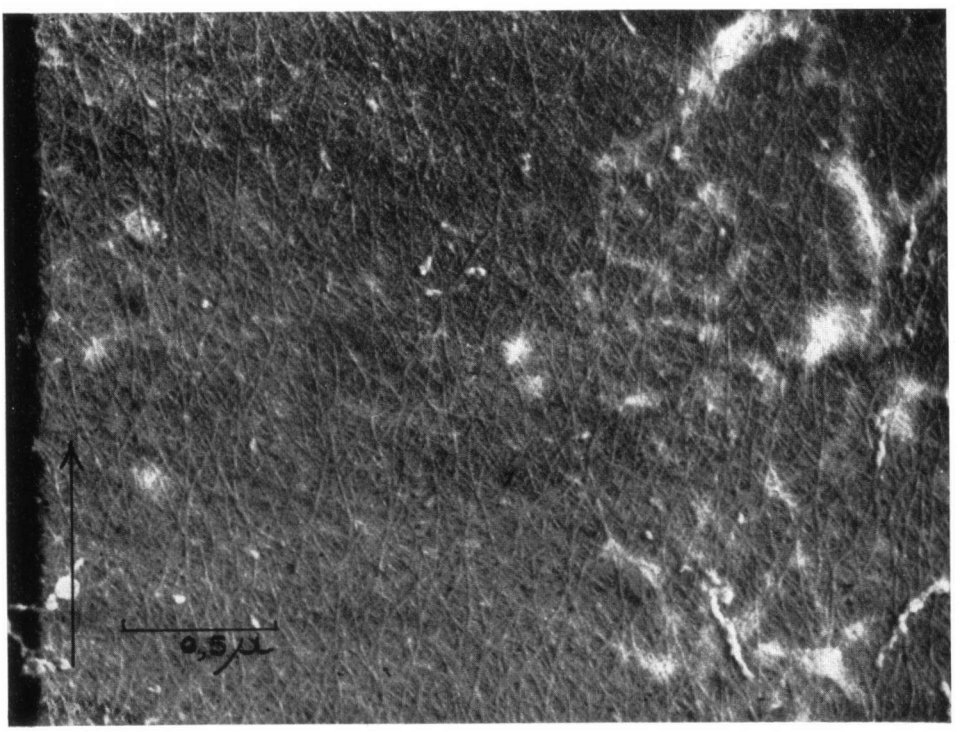

Fig. 4. *Asclepias cornuti* hair, outer side of primary cell wall.

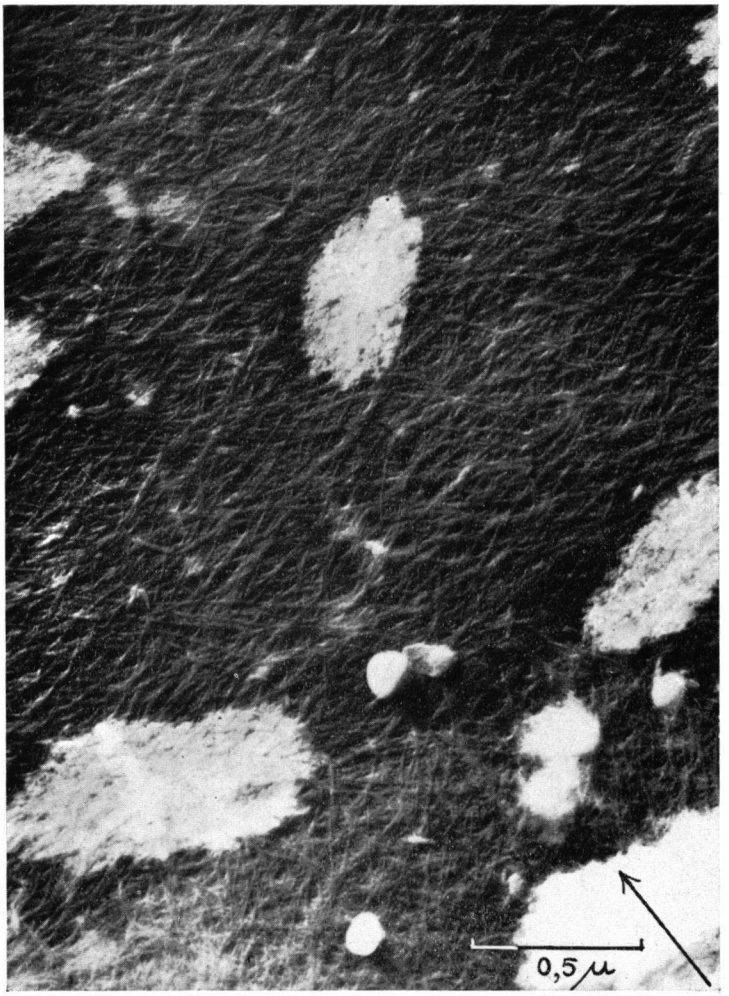

Fig. 5. *Asclepias cornuti* hair, inner side (top) and outer side (bottom) of the primary cell wall. Cell axis indicated.

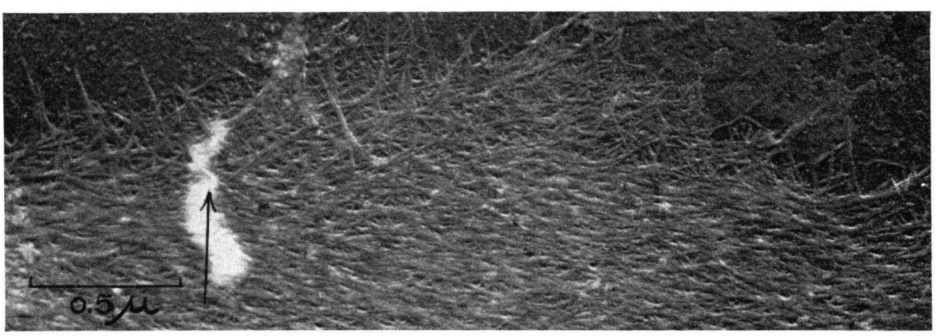

Fig. 6. *Asclepias cornuti* hair, inner side of primary cell wall. Cell axis indicated.

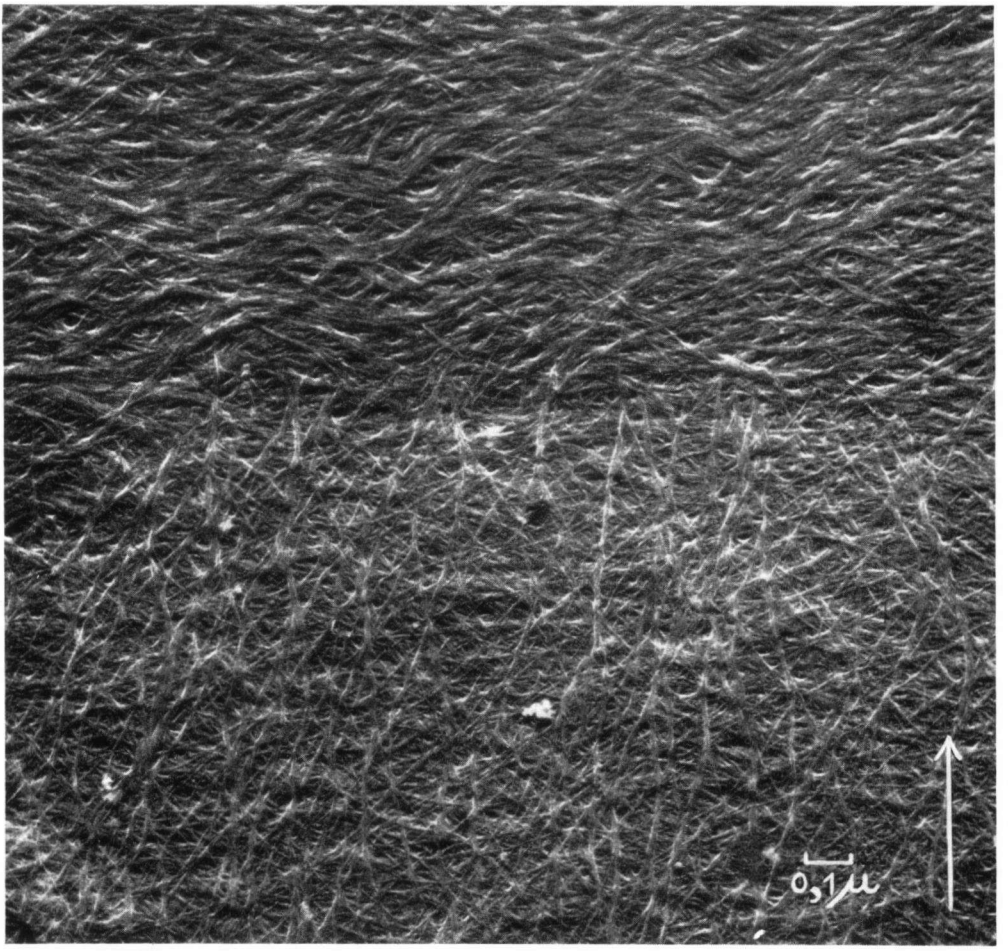

Fig. 7. *Gossypium* hair, inner side (top) and outer side (bottom) of primary cell wall. Cell axis indicated.

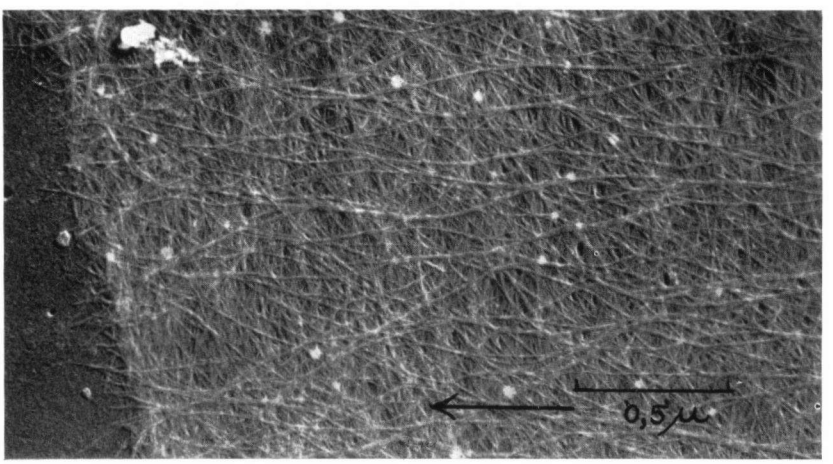

Fig. 8 *Gossypium* hair, outer side of primary cell wall. Cell axis indicated.

or less transversely. In more peripheric layers their direction changes gradually untill they are mainly axial in the outermost layer. Numerous small shallow meshes in the cell wall layers, the shapes, areas and orientation of which vary from the interior to the exterior of the cell wall suggest a new type of cell wall growth, for which the name *multi-net-growth* is proposed. In *Tradescantia* staminal hairs and in *Phycomyces* sporangiophores, an analogous architecture and obviously the same type of growth occur.

References

1. Boshard, H. H., Thesis Zürich, (1952).
2. Frey-Wyssling, A., Submicroscopic morphology of protoplasm. 1953.
3. Frey-Wyssling, A., K. Mühlethaler, Vierteljahresschr. Naturforsch. Ges. Zürich *95*: 45. 1950.
4. Frey-Wyssling, A. and H. Stecher, Experientia *7*: 420. 1951.
5. Kling, W. and H. Mahl, Melliand Textilberichte *33*: 829. 1952.
6. Mühlethaler, K., Ber. Schw. Bot. Ges. *60*: 614. 1950.
7. Roelofsen, P. A., Rec. trav. botan. Néerl. *42*: 72. 1950.
8. Roelofsen, P. A., Bioch. bioph. acta *6*: 340, 357. 1951.
9. Roelofsen, P. A., Bioch. bioph. acta *7*: 43. 1951.
10. Roelofsen, P. A., and A. L. Houwink, Protoplasma *40*: 1. 1951.