ORIENTATION OF CELLULOSE FIBRILS IN THE CELL WALL OF GROWING COTTON HAIRS AND ITS BEARING ON THE PHYSIOLOGY OF CELL WALL GROWTH

by

P. A. ROELOFSEN

Laboratory for Technical Botany, Delft (Netherlands)

INTRODUCTION

Although cotton is a very important textile fibre and the structure of its surface must have an important influence on the friction between cotton hairs and thus on the strength of cotton threads, our knowledge of the structure of this surface in natural and alkali treated fibres is very meagre. In our opinion, one reason is that textile microscopists usually study the structure of full-grown fibres only. It stands to reason that the extremely thin primary cell wall is optically overshadowed by the secondary cell wall. Another reason is that with the electron microscope the surface is only accessible when replicas are made of it, which, however, is not very easy.

Young cotton hairs, which up to the 15th–20th day after flowering grow in length only, seem to offer a good opportunity to study the surface of cotton fibres, since there is no secondary thickening of the cell wall at all and therefore they might be thin enough to be photographed with the electron microscope as they are.

In studying the structure of these hairs we also expected to find some interesting facts concerning the physiology of cell wall growth. Since these unicellular hairs are easily obtainable and grow rapidly, attaining a length of 2½ cm in 20 days, they merit the attention of physiologists studying growth, but did not receive any so far.

Our investigation was carried out with the aid of the polarization microscope and the electron microscope.

I. FALSE IMPRESSION OF THE PRESENCE OF SPIRAL STRUCTURES IN THE PRIMARY CELL WALL

In another publication (ROELOFSEN 1950 a) we called attention to the fact that BALLS (1923) described two sets of striations in the primary cell wall of growing cotton hairs stained with congo red to enhance the birefringence and viewed between crossed nicols. Both striations made an angle of about 70° with the cell axis. ANDERSON AND KERR (1938), HOCK *et al.* (1941) and KERR (1946) described a third, transverse striation in addition to the two already seen by BALLS. It was believed that all these striations were due to oriented fibrillar structures. However, in our opinion, suspicion should have been aroused by BALLS' statement that the striations change their direction when the

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stage of the microscope is turned and by the statement by HOCK *et al.* that the three different systems are only visible in three different positions of the cell in relation to the vibration planes of the crossed nicol prisms.

FREY-WYSSLING (1941) is of opinion that the striations seen in young cotton hairs might be caused by obliquely oriented microfibrils, which will occur in a transverse network structure, if this is extended in axial direction. We must reject this explanation since single fibrils of about 200 Å thickness are too small to cause striations. Therefore a cell wall consisting of fibrils superimposed in different directions will give the impression of an optically homogeneous layer. Otherwise an isotropic cell wall with fibrils running in all directions would always show striations parallel to the diagonals, if such a cell wall would be examined in surface view between crossed nicols.

Exactly the same phenomena were seen by us in the primary cell wall of *Phycomyces* sporangiophores (ROELOFSEN 1950 a) and we advanced the same explanation for both cases, *viz.* the presence of slight undulations and wrinkles in the cell wall caused by shrinkage, the latter being due to loss of turgor and subsequent cleaning of the wall with alkali. Because shrinkage is mainly axial, these wrinkles have a preference for transverse orientation, but they may deviate considerably from this direction.

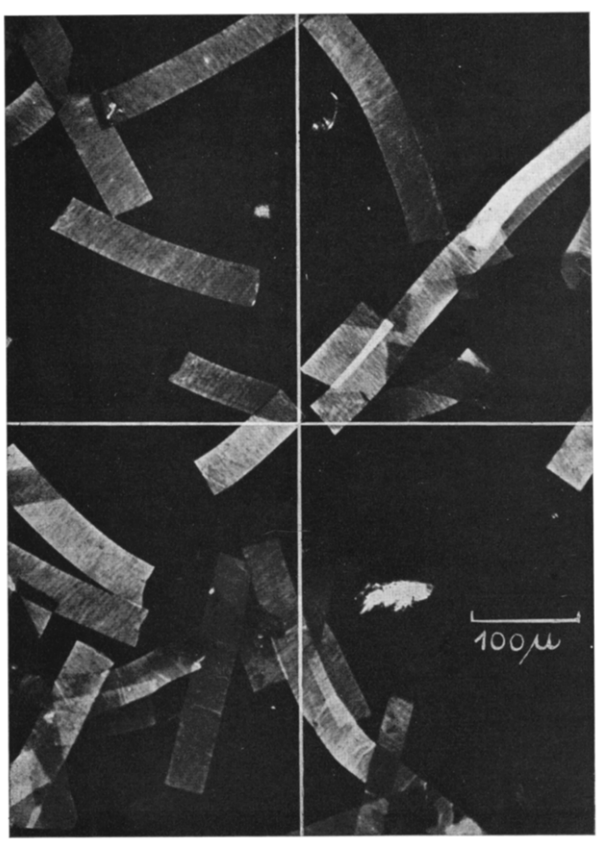

Fig. 1. Parts of young cotton hairs, stained with congo red and viewed between crossed nicols (vibration planes indicated)

As is well known, young cotton hairs, like all growing cells, show negative birefringence with reference to the cell axis. The highest refractive index ($n\gamma$) in the cell wall is transverse, $n\beta$ is axial, the smallest ($n\alpha$) radial. Therefore the double refraction is much stronger when the cell wall is viewed in tangential direction than when it is seen in radial direction, which means that wrinkles will show up when viewed between crossed nicols. Due to the negative birefringence of the cell wall, transverse wrinkles will be brighter than axial ones.

It is obvious that the wrinkles which are oriented diagonally to the planes of vibration of the nicols, or have the nearest approach to that position, will be brightest, giving the impression of striations. In other words, it will entirely depend on the positions of the vibrating planes whether certain sets of striations will be seen.

Although this explanation

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was obvious in view of the similarity of the descriptions and photomicrographs of the phenomena in young cotton hairs and in *Phycomyces* sporangiophores, it seemed desirable to study it in young cotton hairs also.

Our preparations were made from the hairs of cotton bolls, picked 15 days after flowering. Secondary thickening never occurred sooner than 20 days after flowering. These hairs were placed in 70% alcohol and cut into pieces of about 0.5 mm length by a blendor, which was allowed to rotate for about 20 minutes. After centrifuging and decanting the alcohol, they were dried on a slide, heated to 100° C in 5% NaOH during 20 minutes, washed with water, stained with Congo red, washed again for a short time and dried. After this cleaning and staining enough pieces still adhered to the slide. They were mounted in methylbenzoate when the observations started.

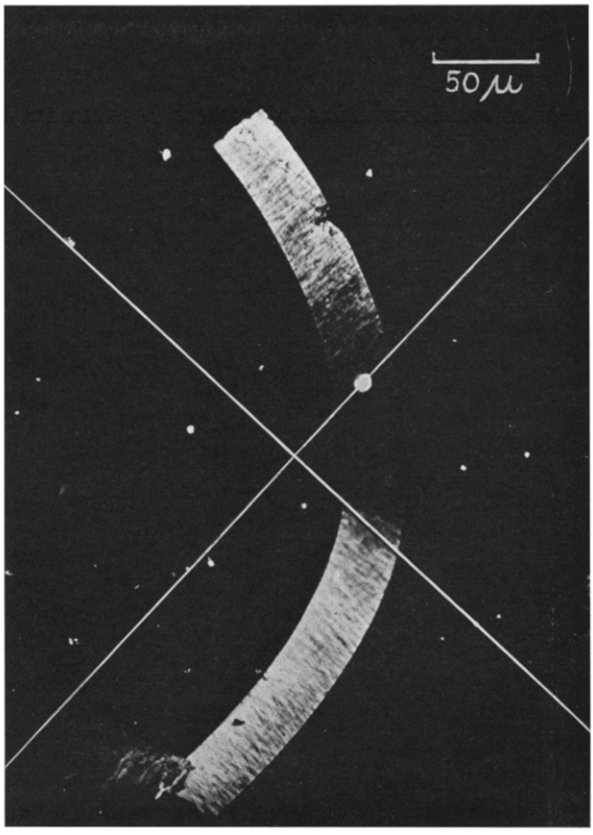

Fig. 2. Curved piece of young cotton hair, stained with congo red and viewed between crossed nicols (diagonals indicated). The striations tend to be parallel to the diagonals, but cannot deviate much from the transverse direction

In a polarizing microscope one then obtains pictures like the one given in Fig. 1. In Fig. 2 a curved piece of a hair is photographed as an example of the false impression one gets regarding the presence of striations. Different striations are clearly visible in the same cell; their direction is almost parallel to the diagonals indicated, but apparently they cannot deviate much from the transverse direction of the cell. Of course, the direction of the striation changes gradually with reference to the cell axis in the lower, curved, part of the cell. The same striations can be seen in Fig. 14–17 of BALLS (*l.c.*) and Fig. 1 of HOCK *et al.* (*l.c.*). They do not differ from those visible in the primary cell walls of *Phycomyces* (ROELOFSEN 1950 a, Fig. 13) and, as we observed recently, they are also to be found in the cellulose cell wall layer of staminal hairs of *Tradescantia virginica* (1951 b).

Since so far the presence of wrinkles was only deduced but not shown by direct observation, photomicrographs were also made using oblique illumination from above with non-polarized light. A special Reichert microscope with Epilum objective oil imm. 60 × was used, the object being immersed in methylbenzoate without cover glass. In Fig. 3 two crossed hairs are shown; the direction of the incident light is indicated by an arrow; the hair numbered 1 is lying on top of the other. This photomicrograph proves the presence of wrinkles and moreover demonstrates that there are more transverse than axial ones.

In conclusion, we may state, that the alleged presence of spiral structures in the

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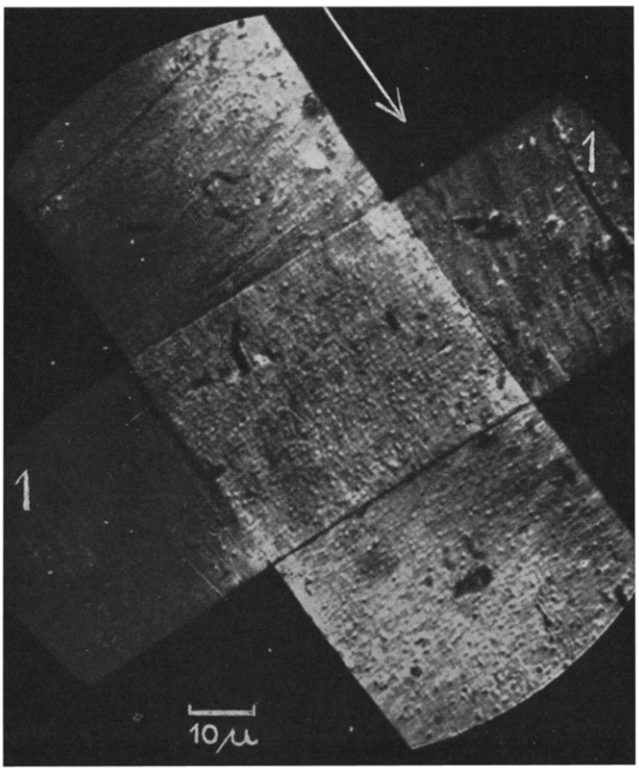

Fig. 3. A pair of young cotton hairs photographed with oblique illumination from above (direction of light indicated). The hair marked 1, is lying on top. The presence of wrinkles is shown, transverse wrinkles are more numerous than axial ones.

primary cell wall of cotton hairs is not proven; the striations are caused by wrinkles in the cell wall. These are oriented in all directions, but have a preference for the transverse direction.

II. MEASUREMENT OF EXTINCTION ANGLE IN ORDER TO DISCOVER A SPIRAL STRUCTURE

Recently (ROELOFSEN 1950 b) we have proposed a theory explaining the origin of spiral structures in the secondary cell wall, which requires the occurrence of spiral growth of the cell when still in the period of lengthening. This spiral growth might be due to a spiral structure in the primary cell wall or to a twist during growth imparted by external forces.

FREY-WYSSLING (1950) suggests that spiral growth might be due to "a circular wandering of the place where the woven texture (of the growing primary cell wall—*present author*) is loosened". However, such a process would not cause spiral growth as characterized by rotation of the top of the cell around the cell axis, but a movement of this top along a spiral, which is called circumnutation.

Since the secondary cell wall of cotton hairs has a spiral structure, we proceeded to investigate whether the presence of a spiral structure in the primary cell wall could

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be demonstrated by an oblique extinction (with reference to the cell axis) of the single cell wall of pieces of young cotton hairs when viewed between crossed nicols.

These observations were carried out on the same pieces of young cotton hairs as used for the observations described in section I. In many cases small portions of single cell wall (either the upper or the lower cell wall) protruded at the cut ends. This is demonstrated by Fig. 4. A very bright light source (Philips tungsten bandlamp for projection purposes, 6 V, 17 A) was used, since the birefringence was only slight notwithstanding the increase attained by staining with congo red. We also used cells cleaned with hot 2% HCl in addition to hot alkali before staining. This made no difference.

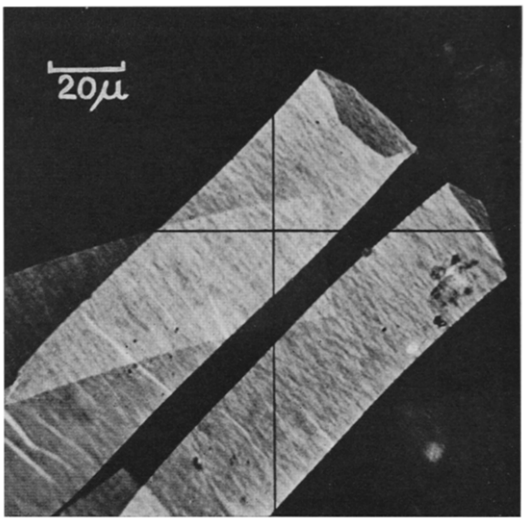

Fig. 4. Pieces of young cotton hair, stained with congo red and viewed between crossed nicols (crosshairs indicate vibration planes). Pieces of single cell wall protrude a tthe cut ends.

As a rule the extinction of the single cell wall was parallel to the extinction in the double wall and both were parallel to the cell axis. Since the double refraction was very slight, especially in the single wall, extinction positions differing less than 4° with the cell axis could not possibly be detected. Therefore the presence of a very flat spiral structure cannot be disproved.

Since according to the presence of many wrinkles, the cell wall has apparently shrunk considerably, especially in axial direction, an eventual spiral structure will have been flattened somewhat during preparation. This situation has also been proved to occur in *Phycomyces* (ROELOFSEN 1951 a). There, an oblique extinction angle was only found in some cases, the majority showing "transverse" extinction, although in all cases a very flat spiral structure must have been present.

We must conclude, that in young cotton hairs the average fibril orientation is either transverse or follows a very flat spiral, which is not detectable by the method used.

III. ELECTRON MICROGRAPHS

Electron micrographs of the outer surface of full-grown cotton hairs or of young cotton hairs are rather scarce. BARNES AND BURTON (1943) photographed young hairs without previous cleaning or disintegration. No structure can be seen. Another photograph shows that by disintegrating the hairs in a blendor many fibrils are formed, both in old and young hairs.

KINSINGER AND HOCK (1948) made replicas of full-grown hairs with Parlodion and published an electron micrograph indicating an irregular fibrillate structure.

MÜHLETHALER (1949) disintegrated a suspension in water of full-grown cotton hairs in a blendor and used the fine material which settled slowly. Among these he found pieces with isotropic fibrillar structure as in felt and he rightly recognized these as pieces

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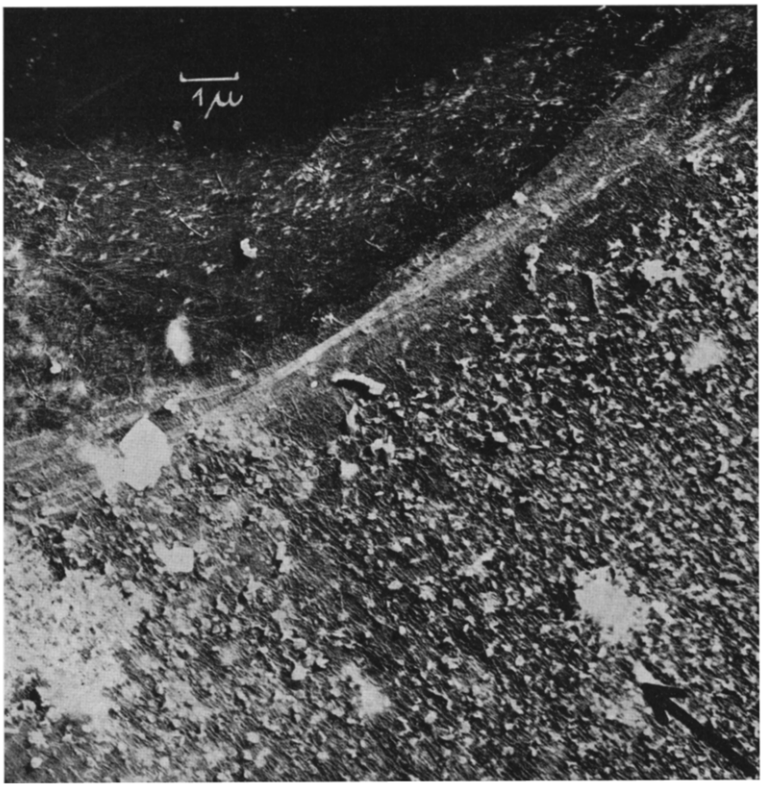

Fig. 5. Electron micrograph of cut end of young cotton hair. The lower cell wall is protruding. Cell axis indicated. Dirt is still adhering to the cell wall.

of the primary cell wall. Of course it is not known whether the micrograph published depicts the outside or the inside of the primary cell wall. It might even have been only a layer of this wall, although this is very unlikely. The direction of the cell axis cannot be indicated.

For our preparations we used hairs from bolls, picked 15 days after flowering and disintegrated with a blendor while suspended in 70% alcohol. The suspension was centrifuged, the alcohol was removed by suction and 5% NaOH was brought into the centrifuge tube, which was then heated in boiling water during 20 minutes. After centrifuging and removing the alkali, the tube was filled with distilled water, heated, centrifuged and this washing process was repeated several times. A small drop of the final concentrated suspension was dried on the Parlodion covered mounting grids, shadowed with chromium and photographed by means of the Philips electron microscope by the Institute for Electron Microscopy in Delft.

Fig. 5–8 all show cut ends of hairs, where pieces of the lower cell wall are protruding. So these pieces are seen from the inside, while the surface texture can be seen where both walls are present. Because the direction of shadowing will certainly show up the fibrils running more or less perpendicular to this direction, one has to be very careful in deducing average fibril directions from electron micrographs. This might be the reason for the difference in apparent average fibril orientation on the inside of the wall in Fig. 8

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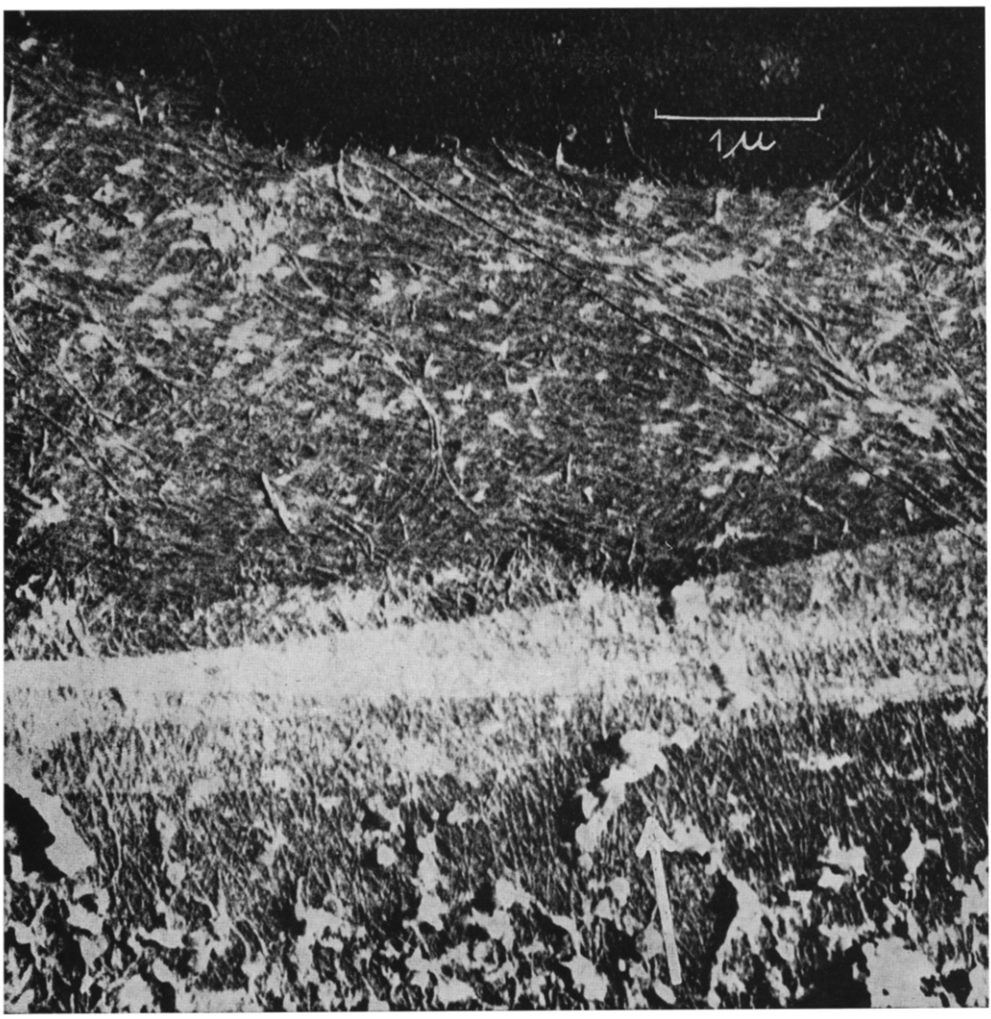

Fig. 6. Part of Fig. 5 enlarged, showing axial structure on the outside and more or less transverse structure on the inside. Direction of shadowing is transverse

as compared with Figs. 6 and 7. In the first case the direction of shadowing had been axial, in the second cases transverse.

So the only thing we can conclude is, that the direction of the fibrils on the inside is on the average more or less transverse, but that many fibrils deviate from this to a considerable degree. It is impossible to say whether the average direction on the inside is exactly transverse or runs according to a very flat spiral. It is clear that the outside of the primary cell wall consists of fibrils running axially. The fibril thickness is about 200 Å units.

The structure on the outside is rather coarse and this surface does not seem to be quite clean. We suppose that the waxy or cutinous material of the cuticle had not been removed completely.

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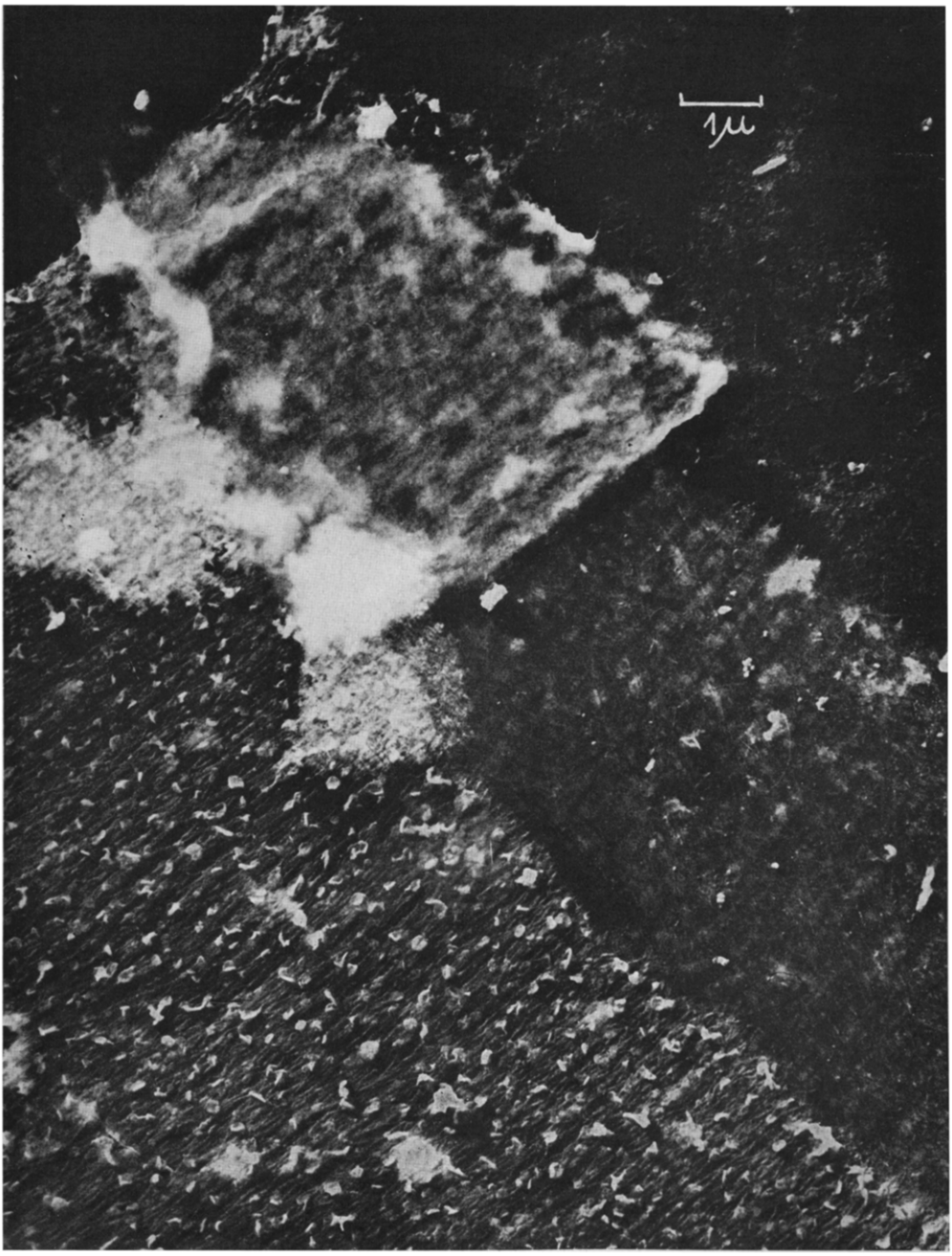

Fig. 7. Electron micrograph of cut end of young cotton hair, The lower cell wall is protruding. The direction of the cell axis is obvious. Axial structure on the outside, more or less transverse structure on the inside. Direction of shadowing is transverse.

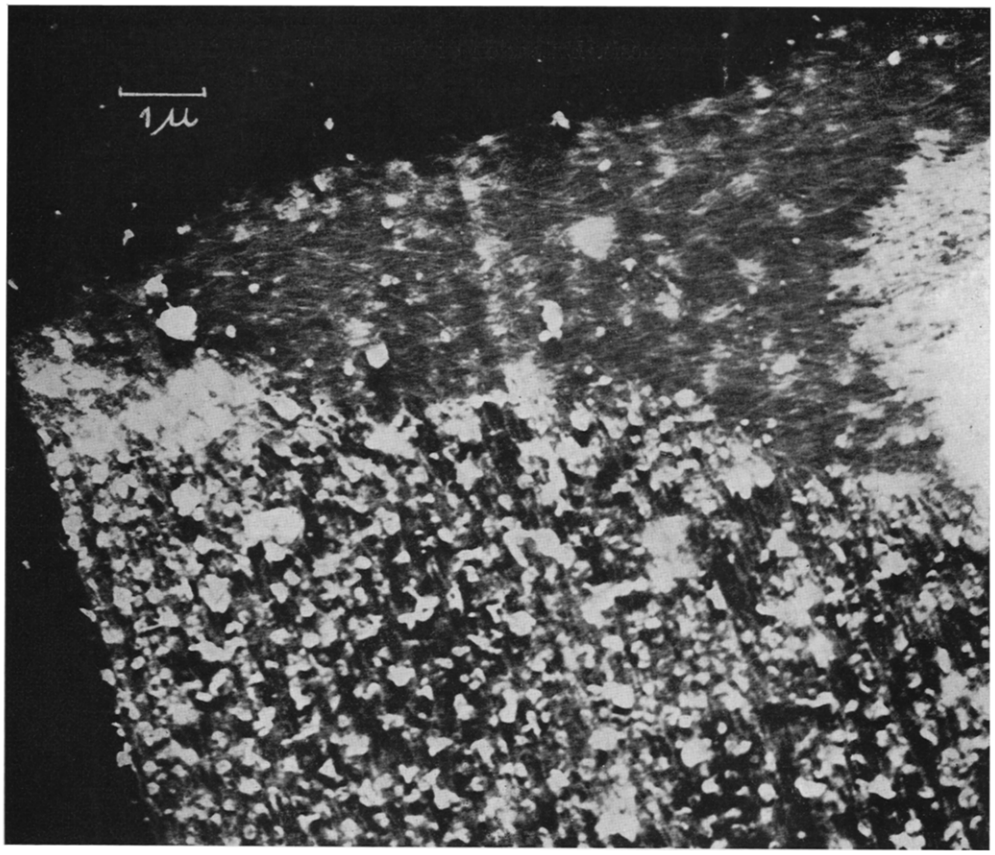

Fig. 8. Electron micrograph of cut end of young cotton hair. The lower cell wall is protruding. The direction of the cell axis is obvious. Direction of shadowing is axial. Axial structure on the outside and conspicuous transverse structure on the inside.

We have taken much trouble in trying to obtain good micrographs of the outside of young cotton hairs still covered with their cuticle. Here the cut hairs were not cleaned with alkali but by immersion in 1% pancreatin at 35° C during one night. However, such hairs remained dirty and caused the Parlodion membranes to burst, due to the heat generated and the contraction of the hairs. We did not obtain reproducible micrographs, but we got the positive impression that the surface of such hairs is without structure.

IV. DISCUSSION OF THE SIGNIFICANCE OF THE RESULTS FOR OUR KNOWLEDGE OF CELL WALL GROWTH

In the primary cell wall of *Phycomyces* sporangiophores FREY-WYSSLING AND MÜHLETHALER (1950) and ROELOFSEN (1951 a) have shown the presence of an isotropic fibrillar structure on the outside and an almost transverse structure on the inside. (Recently the same has been found by us in *Tradescantia* hairs, 1951b). Now, in cotton hairs, we meet with another case of a cell growing in length, where an analogous difference in orientation is seen on the outside and on the inside of the growing cell wall.

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As an explanation of this phenomenon we (ROELOFSEN 1950 b, page 97) have proposed the following hypothesis. Extension of the cell in length in general is made possible by intussusception in the wall of fibrils which are oriented transversely. The inner layer of the wall, lying nearest to the protoplasm, will be in a more favourable position for this intussusception than the outer part of the wall. It is rather difficult to imagine how a long cellulose fibril might find its way from the protoplasm to the outside of the wall. The wall might be interwoven with protoplasm strands which might produce fibrils *in situ*, but this seems very unlikely. Even more so while the outer parts probably are already incrustated with some waxy or cutinous material.

Therefore it is rather self-evident that thanks to intussusception, the inner layer can preserve its transverse orientation notwithstanding heavy axial extension, while the outer layer cannot do this and consequently is passively reversed in orientation. First an isotropic structure will be formed (as seen in *Phycomyces*) and with further extension an axially oriented structure (as seen in cotton). This is merely a quantitative, not an essential difference.

As we stated previously (1950 b), a reversal of orientation of fibrils in the whole wall of tubular cells that are increasing in length as a consequence of internal pressure, is impossible, because the stress in the wall in transverse direction always exceeds the stress in axial direction. Only with partial reversal, *viz.* ot one or more layers of the wall. one may understand how turgor pressure can change the sign of birefringence of the cell wall from negative to positive, as described by FREY-WYSSLING AND SCHOCH-BODMER (1938) in *Anthoxanthum* stamens. The electron micrographs of *Phycomyces* and of cotton primary cell walls confirm our explanation of this phenomenon.

Since the spiral structure in the secondary cell wall of cotton is very pronounced, we had expected also to find a conspicuous spiral structure in the primary cell wall. This has not been found and, although the existence of a very flat spiral structure is not disproved, one may alternatively suppose that in cotton hairs the origin of the spiral structure of the secondary cell wall is to be seen in the second possibility mentioned at the beginning of section 2, *viz.* a twist of the young hair during growth caused by external forces. The young hairs indeed grow to a considerable length in a very limited space and are therefore forced to turn, twist and bend many times. Perhaps the well-known reversion points in the spiral structure coincide with reversions in these turns.

ACKNOWLEDGEMENT

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SUMMARY

Under certain conditions young cotton hairs, viewed between crossed nicols, falsely create the impression that transverse and spiral structures are present (Figs. 2, 3). This is caused by wrinkles in the shrunken cell wall, which are not oriented in spirals at all.

The extinction angle of the birefringence in pieces of single cell wall protruding from cut ends of disintegrated young cotton hairs (Fig. 4) is almost transverse, which proves that the average fibril direction is either transverse or follows a very flat spiral, making an angle of less than 4° with the transverse.

Electron micrographs (Figs. 5–8) show that the outer layer of the primary cell wall has an axial

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structure, while the inner layer has a more or less transverse structure. This is explained by passive extension of the outer part of the wall due to inadequate intussusception of fibrils therein. Into the inner part of the cell wall, however, new fibrils will be easily intussuscepted if the cell lengthens, permitting this part to retain a more or less transverse structure. The negative birefringence shows that this inner part is the predominating one.

RÉSUMÉ

Sous certaines conditions, de jeunes poils de coton, observés entre nicols croisés, donnent la fausse impression que des structures transversales et en spirale sont présentes (Figs. 2, 3). Cette impression est causée par des rides dans la paroi cellulaire ratatinée, rides qui ne sont pas du tout orientées en spirale.

L'angle d'extinction de la biréfringence de morceaux de paroi cellulaire simple, dépassant les bouts coupés de jeunes poils de coton désintégrés (Fig. 4), est presque transversale, ce qui prouve que la direction moyenne des fibrilles est soit transversale, soit suivant une spirale très plate, formant un angle de moins de 4° avec la transversale.

Des micrographies électroniques (Figs. 5–8) montrent que la couche extérieure de la paroi cellulaire primaire a une structure axiale, tandis que la couche intérieure a une structure plus ou moins transversale. Ceci s'explique par une extension passive de la partie extérieure de la paroi, due à une intussusception inadéquate des fibrilles dans cette dernière. Par contre, dans la partie intérieure de la paroi cellulaire de nouvelles fibrilles seront intussusceptées aisément lorsque la cellule s'allonge, permettant à cette partie de garder une structure plus ou moins transversale. La biréfringence négative montre que c'est cette partie intérieure qui prédomine.

ZUSAMMENFASSUNG

Unter gewissen Bedingungen geben junge Baumwollhaare zwischen gekreuzten Nicols fälschlich den Eindruck, dass Transversal- und Spiralstrukturen vorhanden sind (Fig. 2, 3). Dies wird durch Runzeln in der geschrumpften Zellwand, welche gar nicht spiralförmig gerichtet sind, verursacht.

Der Extinktionswinkel der Doppelbrechung in Stücken von einfacher Zellwand, welche an den abgeschnittenen Enden von jungen zerrissenen Baumwollhaaren vorstehen (Fig. 4), ist beinahe transversal; dies beweist, dass die durchschnittliche Fibrillenrichtung entweder transversal ist oder einer sehr flachen Spirale folgt, welche mit der Transversalen einen Winkel von weniger als 4° bildet.

Elektronenmikroskopische Aufnahmen (Fig. 5–8) zeigen, dass die äussere Schichte der primären Zellwand eine axiale Struktur besitzt, während die innere Schichte eine mehr oder weniger transversale Struktur aufweist. Dies wird durch passive Ausdehnung des äusseren Teiles der Wand erklärt, welche auf ungenügende Intussuszeption der Fibrillen zurückzuführen ist. In die innere Schichte der Zellwand aber, werden neue Fibrillen leicht eingefügt wenn die Zelle länger wird, sodass dieser Teil eine mehr oder weniger transversale Struktur behalten kann. Die negative Doppelbrechung zeigt, dass die innere Schichte vorherscht.

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