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CELL-WALL STRUCTURE OF SOME MONOCOTYLEDONES

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1. Introduction

Numerous studies have been reported about the swelling of cellulose walls of fibres and hair and the accompanying phenomena. A historical survey of these investigations was published by Mangenots and Raison (1951).

When the "Querhaut-Theorie" of Lüdtke (1928) had been refuted, it was generally held that the formation of bead-strings in swelling fibres and hair is caused by a swelling anisotropy of two successive layers in the cell wall. It was thought that a layer which swells strongly in transverse direction (to the cell axis) is surrounded by a layer swelling less, or not at all, in that direction. Further it was supposed that a bead is formed if, because of a fissure in the outer layer, the strongly swelling inner layer pours out through this fissure.

The difference in swelling of two layers of a cell wall can be caused by a different chemical composition as well as, in the case of a cellulose wall, by a difference in orientation direction of the cellulose chains. A cellulose wall (or wall layer) whose main direction of orientation is approximately parallel to the axial direction of the cell will swell strongly in transverse direction; a wall (or wall layer) in which the chains are mainly oriented transversely to the cell axis will not swell in transverse direction or only very little so.

The non-swelling or little-swelling outer layer of fibres and hair can be:

a) the primary wall in which a transverse orientation predominates (Frey-Wyssling, 1941; Roelofsen, 1951; a.o.);
b) the outer part of the secondary wall (Bailey and Kerr, 1935);
c) an outer layer of different chemical composition;
d) a combination of two or more of these components.

With regard to the swelling of some monocotyledones, e.g. *Andropogon sorghum*, *Arundo donax*, *Pandanus*, and some species of bamboo, it has been observed that inside a bead-string a second bead-string can form, and again inside the latter sometimes a third. From this it follows that the wall of these fibres consists of several concentric layers, each corresponding with the wall of a "normal" fibre (Lüdtke, 1928 and 1935; Anna Schlottmann, 1933; Bailey and Kerr, 1935; Griffioen, 1935; Sachet, 1946; Mangenots and Raison, 1951).

Various investigators observed that in the swollen fibre the above-mentioned composed concentric layers in many places are lying loose and can slide along each other.

ANNA SCHLOTTMANN (1933) incidentally mentioned how on swelling of bamboo fibres the "tubes" which together form the cell wall telescoped out ("teleskopisch auseinander schoben").

Several workers observed that in a broken fibre the various layers at the surface of the fracture dissolve in the swelling medium (particularly in cuprammonium) with different speed, the cell assuming the form of a pulled-out telescope.

2. MATERIAL

The swelling phenomena of this study have been observed on pulps of some monocotyledones, particularly on those of sugar cane.

Pulp of a fibre-producing plant is a macerated, more or less delignified mass of the fibre-producing tissue of the plant, usually of the stalk or stem. In monocotyledones fibres mainly originate from the sclerenchyma sheath of vascular bundles.

Pulp is generally made by heating with NaOH, an alkaline sulphide, or a sulphite solution. After this process, the cell walls often still contain lignin which can be eliminated by a bleaching process. For this purpose chlorine is used in the form of chlorine gas, chloride of lime, or hypochlorite.

Cross-sections were made from alcoholic specimens of sugar cane and *Andropogon sorghum*, and the dry stalk parts of various kinds of bamboo.

As a swelling medium cuprammonium was used. Some comparing observations have been made with other swelling media which are mentioned below.

3. LIGHT MICROSCOPE STUDIES ON PULPS

a. *Pulp of sugar cane, with cuprammonium as a swelling medium*

On swelling bleached, lignin-free, pulp of sugar cane in cuprammonium, diluted with three to four parts of concentrated ammonia solution, it is seen that in the cell walls of many fibres there are layers that begin to separate. These layers loosen in many places and lie in curves and twists inside each other (Fig. 1). The fracture surface of a broken fibre soon changes into the well-known image of the pulled-out telescope (Fig. 2), as has been described for other monocotyledones.

Fig. 1. Swollen fibre of the short type

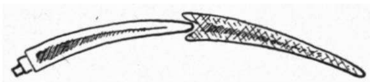

Fig. 2. The "inner tip" of a fibre has escaped from the "outer tip"; the fracture surface shows the form of a pulled-out telescope

If some drops of cuprammonium are drawn through a cover-slipped pulp specimen in water or ammonia solution, swelling proceeds less gradually, and the following phenomena are observed:

1. In a broken-off fibre tip the various concentric "tubes" which form the cell wall telescope out, some slowly, others quickly, so that after some time two or more fibre tips come to lie in line and level with each other (Fig. 2, 3 and 4). It appears as if the inner tubes are pushed with force out of the surrounding tube. This impression is even more striking in the following phenomenon:

2. If a bead has been formed in a fibre rather close to the tip, an "inner tip" can be pushed by the surrounding "outer tip" in the direction of the bead. In the bead this inner tube is pressed into curves until the tip of the inner tube has entered the bead. At that moment the inner tube breaks through the swelling cellulose which forms the wall of the bead, and stretches itself with a swing out of the fibre, so that this fibre has got two ends. Fig. 5 shows a fibre which, in that way, has got a double tip at both ends.

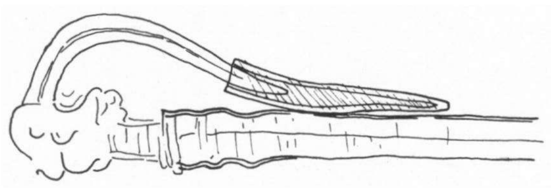

Fig. 4. The same stage as in Fig. 2

3. Not only in fibres but also in sclereids and parenchyma cells "inner and outer cells" telescope out. In these more or less isodiametric elements no beads are formed, but from time to time a whole wall collapses, and then two to four cell-wall layers can telescope out as intact cell surroundings, slowly or quickly, at the same time or one by one. Lüdtke (1928), although not giving an exact description, reported on a phenomenon in bamboo which may be identical herewith.

In sugar cane no sharp limits can be drawn between parenchyma cells, sclereids and fibres, but all intermediate forms are found. It is therefore not surprising that these cell types which are often said to bear little resemblance as to the structure of their cellulose wall, behave in an analogical way in this respect.

In bleached pulp of sugar cane, also if made by a process different from that described above, these phenomena are frequently seen. In exceptional cases they can also be observed in unbleached pulp.

On swelling in cuprammonium some fibres, almost all parenchyma cells, and few sclereids show a distinct fibrillisation striation. Each cell-wall layer, telescoped off or out, shows a similar striation. Almost without exception this striation has a Z-spiral orientation.

b. Pulp of other monocotyledones, with cuprammonium as a swelling medium

The phenomena described above can also be observed on pulp of *Bambusa arundinacea*, species of *Melocanna*, and *Andropogon sorghum* (*saccharatum*).

We did not succeed in obtaining these phenomena on pulp of cereal straw (probably *Triticum sativum*), *Oryza sativa*, *Pandanus* spec.

c. Pulp of sugar cane, with other swelling media

For obvious reasons, the swelling proceeds much more slowly in saturated $ZnCl_2$ solution, phosphoric acid 75 %, and NaOH 20 % than in cuprammonium. There is usually no bead formation. The telescoping-out also occurs infrequently, though the layers of the cell wall show a distinct loosening from each other. Only a few times a telescoping-out phenomenon has been observed in saturated $ZnCl_2$ solution and in phosphoric acid solution, both spontaneously or after exerting pressure on the cover-slip.

4. Polarization microscope studies on pulps

In polarized light between crossed nicols all fibres show a positive birefringence (to the longitudinal axis of the cell). Parenchyma cells and sclereids have a positive or negative double refraction, or are isotropic. One half of a sclereid or parenchyma cell may show a positive, the other half a negative birefringence.

In the orthogonal position of the fibres there is no extinction. Is a compensator plate, red 1st order, inserted between preparation and analyzer, then the fibres show addition colours in the orthogonal position parallel to the vibration plane of the polarizer; this is an indication of a Z-spiral orientation of the cellulose micelles (Naegeli and Schwendener, 1877; Frey-Wyssling, 1941).

Not only the intact wall but also walls missing one or more layers which have been telescoped off or out, always show the colour indicating a Z-spiral orientation. It was too difficult to determine the colour in the separate telescoped layers, probably because the part of the layer that is not yet swollen and is, therefore, still birefringent, is very thin.

5. Studies on cross-sections

Cross-sections through the stalk of sugar cane, bamboo, and *Sorghum* show that the wall of the fibres is layered. This layered structure is clearly seen in the wall that is swollen in H_2SO_4 solution 62% (Fig. 6). Also in the wall of non-treated parenchyma cells high magnification reveals a layered structure (Fig. 7).

In polarized light between crossed nicols, the cell walls of sugar cane (Fig. 8) and *Sorghum* show dark and light concentric rings in the cross-sections of sclerenchymatic cells and parenchyma cells. Bailey and Kerr (1935) described the same observation for the fibres of *Pandanus*, Preston and Singh (1951) for bamboo.

6. Electron microscope studies

For the study of cell walls with the electron microscope replicas from the cross-sections of sugar-cane stalk were made. From Fig. 9 it is clearly seen that the secondary wall of a fibre consists of at least three layers.

Fig. 10 illustrates the high degree of fibrillisation in the layer adjoining the lumen of the cell. The replica film that covered that wall layer is folded back here, and lies in the plane of the micrograph. The direction of fibrillization here too indicates a Z-spiral orientation.

The almost round pits in a strongly oriented wall, or at least in the wall layer may be incidentally mentioned.

An attempt to determine the absolute direction of orientation in the various layers by making oblique sections met with too many technical difficulties.

7. Discussion

From the observations described above it appears that the cell wall of the examined fibres, sclereids and parenchyma cells is built up of several layers. In a swollen condition of the bleached pulp these layers lie loose, and can easily slide along each other. Upon the periphery of these layers, except upon that of the outer one, a pressure is exerted then in the direction of the lumen. By this radial pressure the layers are squeezed out as soon as an opening occurs somewhere in the outer layer of the cell wall (formation of a bead). The radial pressure upon the whole periphery of a certain layer is exerted by the surrounding layer. Each layer swells towards the lumen. Because the outside of a layer does not swell, it does not distend, and thus the whole swelling pressure of the inner part is directed towards the lumen. During swelling and dissolving in cuprammonium the peripheral part of a layer maintains the form of the cell longest. Usually, the inner part of the layer soon becomes a formless mass — unless it is prevented from distending —, for instance, if a single layer has slipped off of a cell wall and lies free in the swelling medium. In a cell from which an inner layer of the cell wall is slipping out, it can be seen that immediately behind that layer the lumen is filled with swelling cellulose from the surrounding layer (Fig. 3).

Loose layers slipped-out of fibre tips often form beads immediately after their release.

On account of the picture of the cross-sections between crossed nicols, it may be concluded that the different properties of the two concentric parts of a cell-wall layer are due to a difference in orientation direction of the cellulose chains. The angle formed by the main orientation direction and the cell axis is greater in the outer part of the layer than in the inner part. The outer part produces the bright rings in the cross-section, the inner part the dark ones.

Preston and Singh (1950, 1952) using another method, reported the same cell-wall structure for the fibres of *Bambusa arundinacea*, *Melocanna bambusoides* and some species of the genus of *Dendrocalamus*.

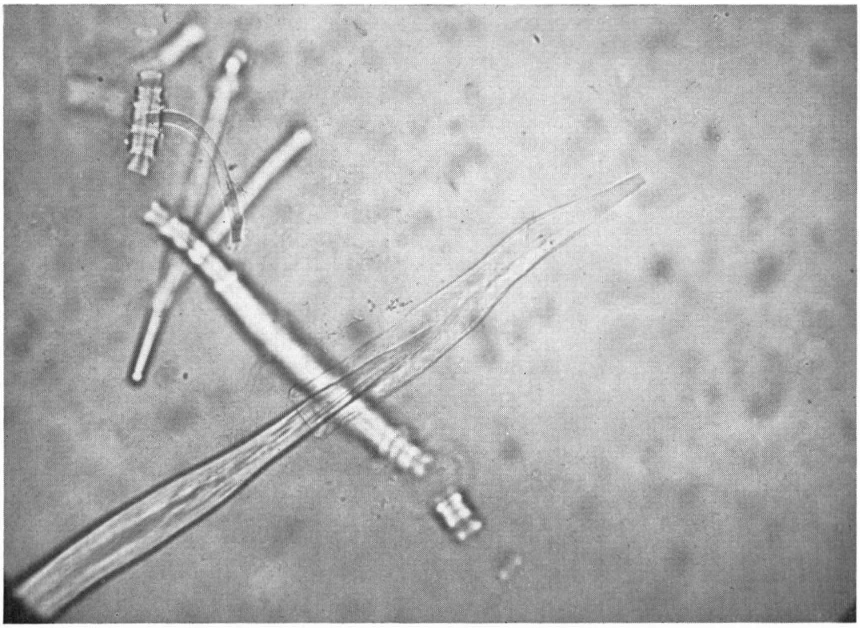

Fig. 3. The same stage as in Fig. 2; the fibre lies across another swelling, telescope-ended one

Fig. 5. Two beads have been formed in one fibre; two "inner tips" have slipped out of two "outer tips", that are still in connection with the fibre

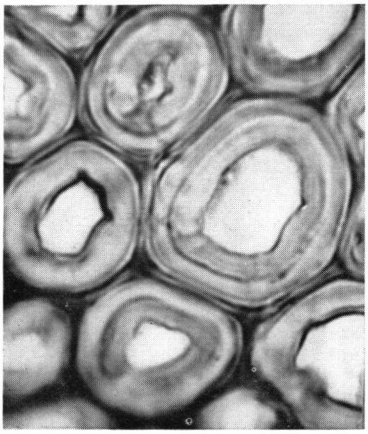

Fig. 6. Cross-section of fibres in the stalk of sugar cane swollen in H_2SO_4 62 %. Magnification 1150 ×

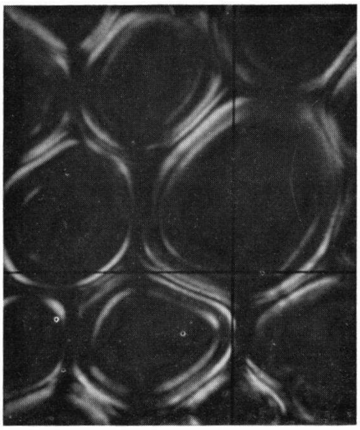

Fig. 8. The cross-section of Fig. 6 in polarized light between crossed nicols. Magnification 1150 ×

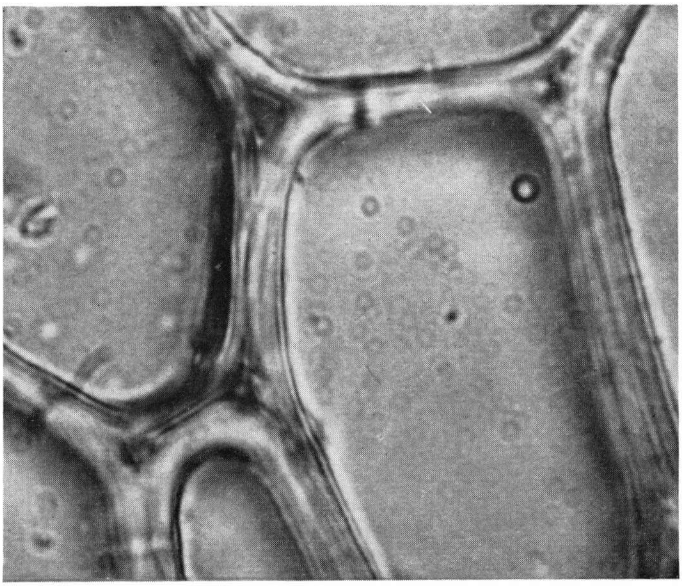

Fig. 7. Cross-section of parenchyma cells in the stalk of sugar cane (non-treated)

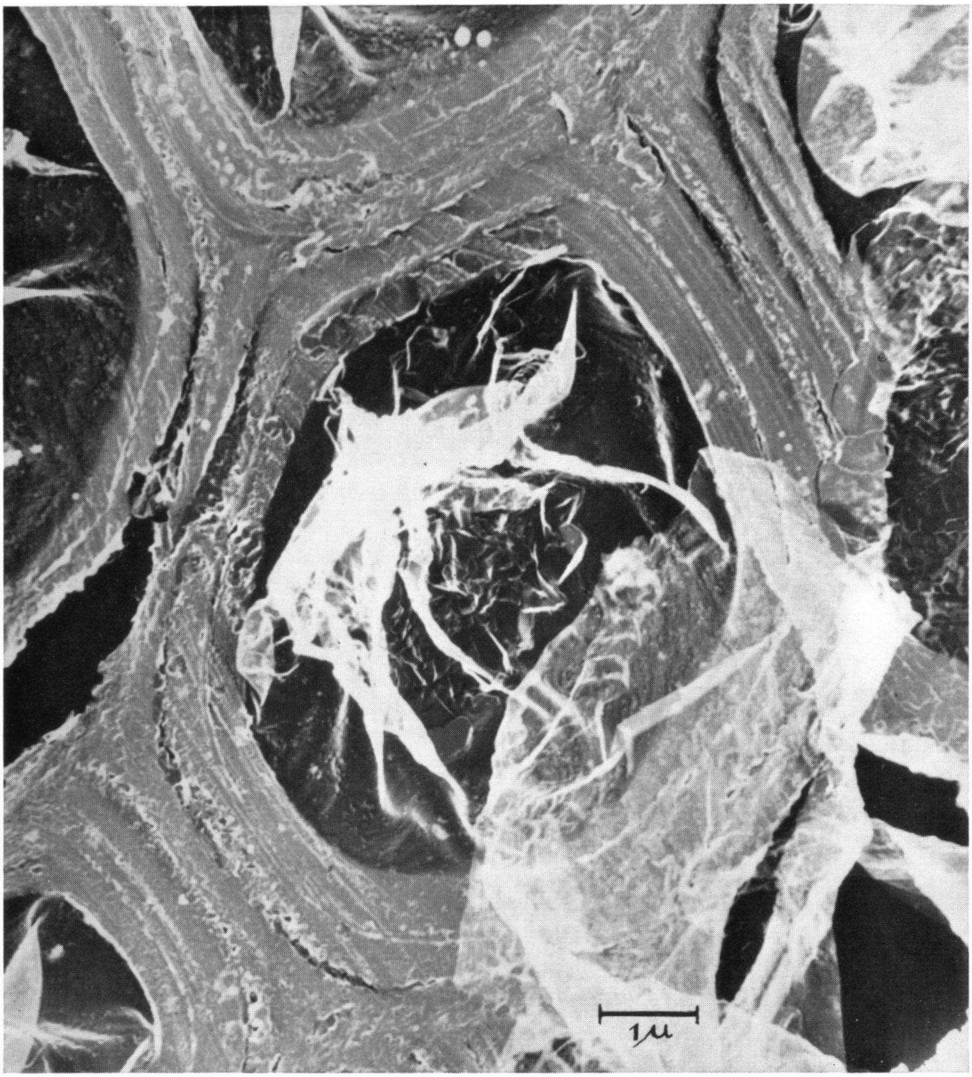

Fig. 9. Electron micrograph (replica) of a cross-section of a fibre (sugar cane). Magnification 12500 ×

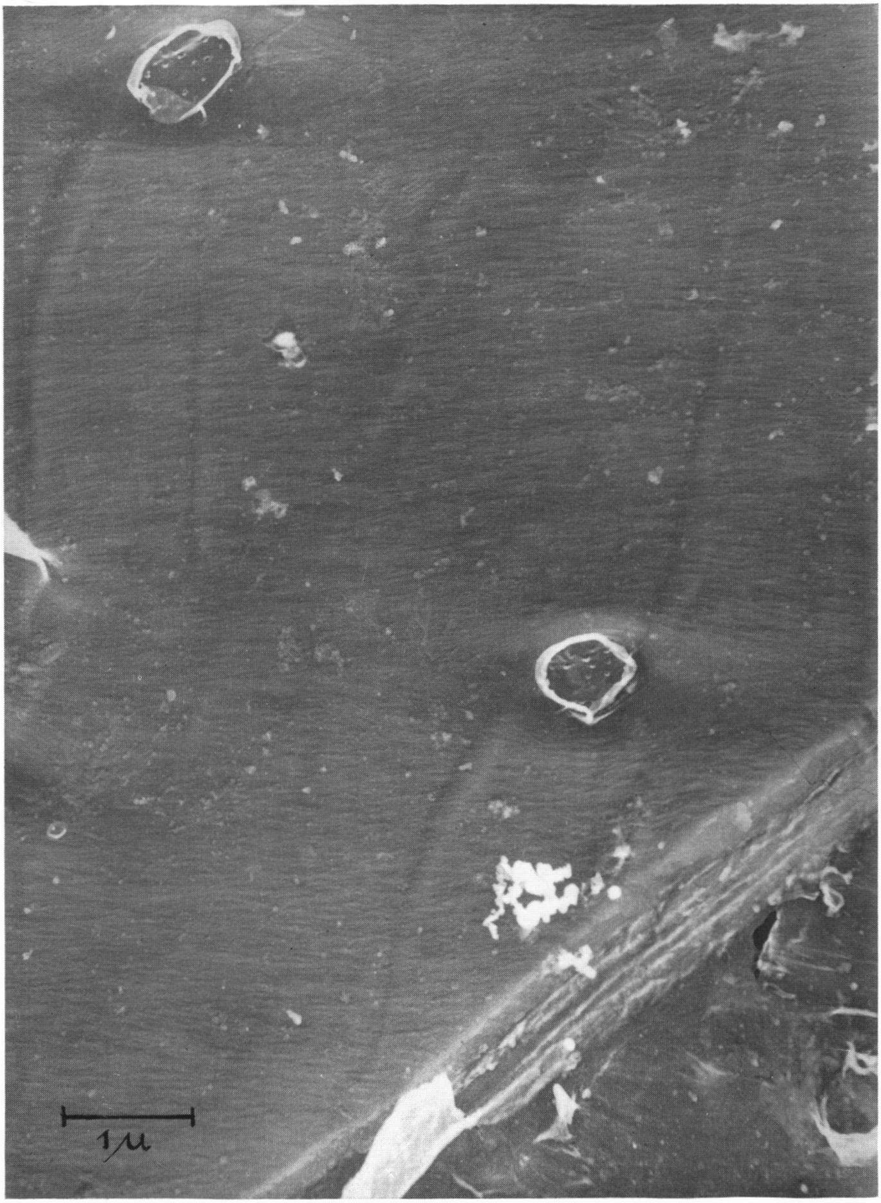

Fig. 10. Electron micrograph (replica) of a cross-section of a parenchyma cell. The replica film that covered the wall layer adjoining the lumen, is visible. Two pits with plasmodesm openings. Magnification 17000 ×

As mentioned above, telescoping phenomena have also been observed in the fibres of bamboo, e.g. of the genus of *Melocanna*.

PRESTON and SINGH (1950) stated that the spirals in which the cellulose chains are orientated, are generally steeper in the same ratio as the quotient $\frac{\text{cell length}}{\text{cell width}}$ is greater. Hence the swelling anisotropy of the successive layer parts is smaller in the fibres than in the sclereids and parenchyma cells. In accordance with this statement it appears from our investigations that in sugar cane the telescoping phenomena cannot be observed in the long, very thin fibre type, but in the long, thicker type. Most frequently the phenomenon can be seen in parenchyma cells and sclereids, and in the fibres of the short, spool-shaped type.

Summarizing, in sugar cane the structure of the cell wall of the described cells may be as follows: The secondary wall consists of layers which, as regards their network of cellulose, do not cohere, while the network of one wall layer forms a cohering whole. The chain molecules of all parts of the cell wall have a Z-spiral orientation. In the outer part (morphogenetically in the beginning) of each wall layer the spiral is more or less flat. Towards the centre (morphogenetically as the layer becomes thicker) the spiral becomes steeper.

The inner parts of the layers give the dark rings in the cross-sections between crossed nicols. In these parts the spiral of orientation, therefore, forms an angle with the cell axis of probably not more than about 10°.

The outer parts of the layers form the light rings in the cross-sections between crossed nicols. Here the angle in question must be considerably greater.

In the cells with a positive birefringence the beginning of a wall layer, i.e. the outer part of it, probably has an orientation spiral whose direction makes an angle with the cell axis smaller than 45°.

In the isotropic cells the angle referred to must be, in the beginning of a layer, greater than 45°. This spiral becomes gradually steeper.

In the cells with a negative birefringence the angle in question must be, in the beginning of a layer, considerably greater than 45°. This spiral becomes steeper but very slowly.

Whereas in the pulp the layers of the cell wall lie loose, these layers will cohere in the intact wall. The coherence may be caused by pectin, lignin or hemicelluloses. The latter do not come into account here, because they dissolve only partly during the pulping process. Pectin on the other hand dissolves completely during this process, and lignin for the greater part. By the following bleaching process lignin disappears almost entirely. In unbleached pulp telescoping phenomena occur sporadically, in non-treated tissues never. It is therefore concluded that the layers of the cell wall are connected with each other by pectin or lignin, or by both.

It might be taken into consideration that apart from a difference in orientation of the cellulose chains, the swelling anisotropy may be caused by alternating layers of cellulose and layers of a different chemical composition. This hypothesis cannot, however, be held for the following reasons.

If the dark rings in the cross-sections between crossed nicols were due to cellulose (with a steep spiral structure), the light rings would be caused by a strongly birefringent substance of a different chemical composition, which is more or less transversely oriented. Such a cell-wall substance is, however, not known. Pectin which sometimes shows a very small double refraction (ROELOFSEN and KREGER, 1951 and 1954), is no longer present in pulp.

The bright rings in the cross-sections should, therefore, be due to a cellulose layer (with a more or less transverse orientation). But then the dark rings may only be attributed to non-birefringent hemicelluloses which are present in the stalk of sugar cane in a rather large quantity (T.A.O. Forestry and Forest Products Study, 1953). This would not seem to be impossible, but then the telescoping phenomena should not occur after extraction of the hemicelluloses with 6,5 % NaOH according to Heuser, which apparently was not the case.

The swelling anisotropy of the inner and outer part of a wall layer may also be caused by a different degree of crystallization, this being high in the outer part and low in the inner. This supposition, the investigation of which is probably impossible, is contradicted by Fig. 10 showing an inner part of a layer in a highly fibrillized condition. Since it must be assumed that the fibrils consist of parallel oriented cellulose molecules, we hardly can think that the cellulose in certain fibrils may be non-crystalline and in identical ones crystalline.

If any given fibre swells in cuprammonium and forms beads as a result of a difference in orientation direction of concentric parts of the cell wall, the question arises of what may prevent the outer part of the cell wall from swelling in longitudinal direction. In swelling the cell does not elongate but, on the contrary, often shortens. This problem has hardly attracted attention but would perhaps be worth while further investigation.

The same applies to each cell-wall layer which forms part of the "composed wall" of fibres and of other elements in the stalk of sugar cane. The idea of the structure of that composed wall as developed above, may give a solution of the question. In the foregoing it has been suggested that the cellulose network of one cell-wall layer forms a cohering whole. This coherence of the network of cellulose chains will prevent the peripheral part of the wall layer from extending in longitudinal direction. The following phenomenon gives a nearer verification of this theory.

When in a fibre of sugar cane swelling in cuprammonium, beads have been formed and the beads are dissolved in the liquid, the intermediate "cuffs" keep floating for a considerable time. When these cuffs finally dissolve too, they always do so from inside and shockwise. It is seen that, all at once, one of the concentric cuffs extends to a high degree (like a compressed spring that is loosened) and then disappears, i.e. dissolves so suddenly. The outer part of a layer in a cuff tends to extend highly in longitudinal direction, but is prevented from doing so: its cellulose fibrils or micelles tend to swell and form separate hydrated chain molecules, but they are everywhere connected with the cellulose network of the inner part of the layer that "no longer gives way". As soon as, from the lumen, enough cellulose of the inner part of that layer is dissolved that the outer part is no longer restrained, the latter can give in to the tension in longitudinal direction caused by the swelling, and will suddenly extend to a high degree.

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SUMMARY

From the observations the following conclusions can be drawn:
1. The cell walls of the fibres, parenchyma cells, and sclereids in the stalk of sugar cane principally have the same structure.
2. The cell wall consists of several concentric layers.
3. The cellulose chains have a Z-spiral orientation in all layers.
4. In each layer of the cell wall the cellulose forms a cohering network.
5. The orientation direction of this network in the inner part of each layer is always steeper than that in the outer part.
6. The various layers are not connected by cellulose chains and, after the chemical pulping process, come to lie somewhat loose.
7. The cohesion of the cellulose network prevents the outer part of a layer in a swelling cell wall from extending in longitudinal direction as long as the inner part of that layer is still intact.

RÉSUMÉ

Les conclusions suivantes peuvent être déduites des observations décrites ci-dessus:
1. Les parois cellulaires des fibres, des cellules de parenchyme et des scléréides dans le tige de canne à sucre ont principiellement la même structure.
2. La paroi cellulaire se compose de plusieurs couches concentriques.
3. Dans toutes les couches les chaînes de cellulose ont une orientation en forme de spirale en Z.
4. Dans chaque couche de la paroi cellulaire la cellulose forme un treillis cohérent.
5. La direction d'orientation de ce treillis dans la partie intérieure de chaque couche est toujours plus raide que celle dans la partie extérieure.
6. Les couches diverses ne sont pas jointes par des chaînes de cellulose; après la lessivage du tissu ces couches sont détachées un peu.
7. La cohésion du treillis de cellulose empêche la partie extérieure d'une couche dans une paroi cellulaire dilatante de s'étendre dans la direction longitudinale aussi longtemps que la partie intérieure de cette couche est intacte.

ZUSAMMENFASSUNG

Aus den oben beschriebenen Wahrnehmungen können folgende Schlüsse gezogen werden:
1. Die Zellwände der Fasern, der Parenchymzellen und der Sklereide im Stengel von Zuckerrohr haben grundsätzlich die gleiche Struktur.

2. Die Zellwand besteht aus einigen konzentrischen Schichten.
3. Die Zelluloseketten sind in allen Schichten gemäss Z-Spirale orientiert.
4. In jeder Zellwandschicht bildet die Zellulose ein koherentes Netzwerk.
5. Die Orientierungsrichtung dieses Netzwerks ist im inneren Teil jeder Schicht durchwegs steiler als im äusseren Teil.
6. Die verschiedenen Schichten sind nicht durch Zelluloseketten verbunden und kommen nach dem Aufschliessen des Gewebes einigermassen gesondert zu liegen.
7. Der Zusammenhang des Zellulosenetzwerks verhindert den äusseren Teil einer Schicht in einer quellenden Zellwand sich in longitudinaler Richtung auszudehnen, solange der innere Teil dieser Schicht noch intakt ist.

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