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Glossary of Some Cytological (Cyt.), Histological (Hist.) and Crystallographic (Cryst.) Terms Used in this Review

Collenchyma (Hist.) is a plant tissue composed of elongated cells whose walls are locally thickened along the longitudinal edges.

Golgi vesicles (Cyt.). The Golgi apparatus is a cell organelle (visible in the electron microscope) which buds off submicroscopic vesicles concerned with cell metabolism (Golgi activity).

Hemimorphic (Cryst.) describes bi-terminated crystals which exhibit distinctly different forms at their two ends.

Intussusception (Cyt.). Growth in area of membranes by the intercalation of submicroscopic particles between those already present, in contrast to growth by apposition.

Matrix (Cyt.). Homogeneous and, therefore, isotropic ground mass of the cell wall.

Meristem (Hist.) is a plant tissue composed of undifferentiated cells capable of repeated divisions.

Multi-net growth (Cyt.). The wall of expanding cells consists of many superposed submicroscopic lamellae of interwoven fibrils; the texture of these networks varies in the superposed lamellae as they are differently extended during cell elongation.

Parenchyma (Hist.) is a plant tissue composed of cells with thin unlignified walls.

Pile (Engineering). A column which is sunk into the ground to support vertical loading.

Plasmalemma (Cyt.). The living protoplasm is coated by a submicroscopic membrane called plasma membrane or plasmalemma.

Plasmodesmata (Cyt.). Plasma strands penetrating submicroscopic perforations in the cell wall connecting neighbouring cells.

Primary wall (Cyt.). The first wall layer produced by a young cell; it is rich in highly hydrophilic matrix and poor in reinforcing cellulose fibrils so that it is more plastic than elastic.

Sclerenchyma (Hist.) is a plant tissue composed of cells with thick lignified walls.

Slip planes (Cryst.). Planes along which slipping or gliding takes place in crystals.

Tracheids (Hist.). Cellular elements of the water conducting tissue in wood.

Since the publication in 1951 of the report on "The Fine Structure of Cellulose" in this Series (*25*), several then unsolved problems have been cleared up and new questions have arisen, so that a presentation of the progress in cellulose research during the last 20 years seems desirable. The following discussion will be based on the article in Volume 8 of this Series and restricted to native cellulose (cellulose I, α-cellulose).

I. Elementary Fibrils

1. Evidence of Subunits in Microfibrils

Until 1960 it was thought that the fibrillar elements of native cellulose fibres and other plant cell walls were the microfibrils, with a diameter of around 200 A, discovered in the electron microscope (*23, 28, 68*). However, MÜHLETHALER (*56*) showed with the method of negative staining that there are much narrower fibrillar elements with a constant diameter of 35 A *(Fig. 1)* which have been termed *Elementary fibrils* (*26*). This term had been anticipated in 1953 (*15, 19*) and used for flat fibrillar elements of 100 A (*15*) or 70–90 A width (*16*) whose thickness was found to measure only about 30 A (*78*). It was shown as early as in 1951 that the fibrillar elements have a tendency to aggregate by fasciation (*14*), and MÜHLETHALER proved that the 100 A and broader ribbons are merely triplets *(Fig. 10a)* or other multiple aggregates of such strands with a more or less isodiametric 35 A cross-section. Since these cellulose strands represent a type of general occurrence, the term *Elementary fibril* must be reserved for the 35 A fibrillar elements.

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The elementary fibrils are identical with the so-called "micellar strands", which were postulated because the microfibrils, indirectly demonstrated (*13*) or directly observed, are much broader than the rod-shaped cellulose crystallites (micelles) that are identified by X-ray diffraction and incomplete hydrolysis (*68a*). Originally, a diameter of 50–60 A was attributed to those rods (*32*) and as their length could not be evaluated precisely, the term micellar strands, i. e. crystallites with

Fig. 1. Elementary fibrils from macerated onion root cells. Negative staining with phosphotungstate, according to MÜHLETHALER (*56*). These strands show kinks (angular bends) (arrow). 95 000 : 1. [From: Beih. Z. Schweiz. Forstver. Nr. 30, 55 (1960).]

undetermined length, was chosen. The diameter 60 A was also found on sonic disintegration of ramie fibres and cotton hairs (*24*). More accurate measurements with X-ray diffraction were performed by HEYN (*36*) which showed crystalline strands of 28 A width for jute or flax and 43 A for ramie.

2. General Occurrence of Elementary Fibrils

OHAD et al. (*63*) estimated the diameter of shadow cast fibrils of bacterial cellulose *(Acetobacter xylinum)* around 30 A. This result has been refuted by COLVIN (*6*) who insisted that the smallest fibrils are as broad as 150–200 A. The same author also criticizes the method of negative staining, claiming that the largest part of the fibril would be

covered by the electron-dense phosphotungstate, so that only a narrow strip of the embedded fibril would be visible in the electron microscope. This criticism has been successfully rejected (*62*), so that it can be taken for granted that the bacterial cellulose consists of 35 A elementary fibrils (*26*).

The microfibrils of the cell wall of the green alga *Valonia* are another well-known object for native cellulose research (*68*). PRESTON (*2*) found

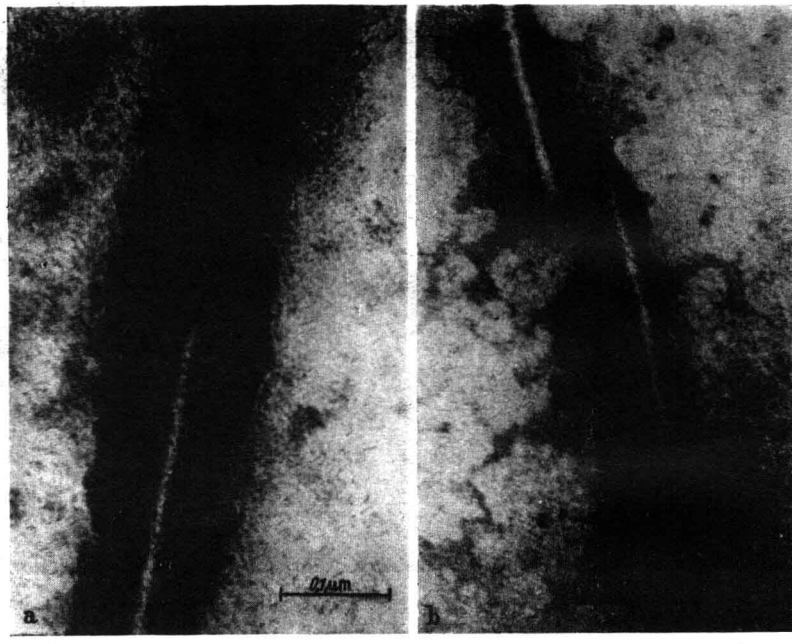

Fig. 2 a and b. Microfibrils of *Valonia* cellulose with slip planes produced by sonication. The microfibril has disintegrated into elementary fibrils. Negative staining with phosphotungstate. According to FREY-WYSSLING et al. (*27*). 150 000 : 1. [From: Holz als Roh- u. Werkst. **24**, 443 (1966).]

them to measure 200 A in diameter, and from similar results in other types of fibrillar high polymers (silk, actomyosine, vanadium pentoxide), he sponsored the erroneous theory that the 200 A fibril was a general "thermodynamic" unit of special stability. However, so-called slip-planes can be produced in *Valonia* microfibrils *(Fig. 2)* by sonic treatment, which show that these 200 A fibrils consist of subunits with 35 A diameter (*27*). The microfibrils also decay into narrower units when treated enzymatically (*10*).

MÜHLETHALER (*56, 58*) has detected the elementary fibrils in the primary cell wall of the onion root parenchyma. There these elements

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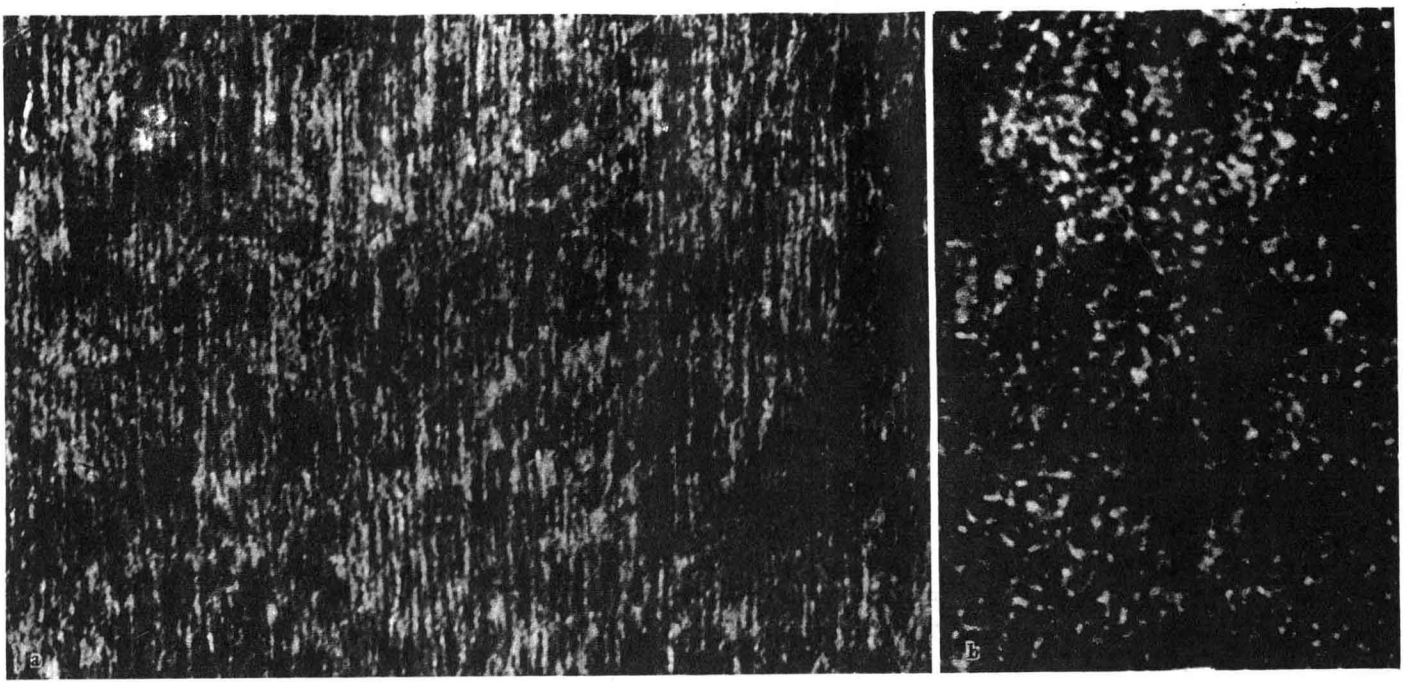

Fig. 3. Elementary fibrils of cellulose demonstrated in situ by HEYN (37). a) Longitudinal section of ramie. 300000 : 1. b) Cross-section of ramie. The elementary fibrils with a rectangular cross-section of 35 A diameter have a tendency of forming clusters (microfibrils). 350000 : 1. [From: J. Cell Biol. **29**, 181 (1966).]

are dispersed and individualized, whilst in the secondary cell wall with its pronounced parallel texture they aggregate forming fasciated microfibrils. HEYN (*37*) succeeded in preventing this aggregation which seems to occur when the cell wall is naturally or artificially dehydrated and dried. He introduces the contrast medium into the fully hydrated fibres and then sections these objects, whereupon electron micrographs as reproduced in *Fig. 3* are obtained.

Evidently, there are only fibrillar elements of 35 A diameter with a more or less rectangular cross-section. The individual elementary fibrils are separated by narrow interfibrillar spaces which vary in width. In the cross-section the number of elementary fibrils per unit area changes. It must be expected that the more densely packed clusters aggregate to form microfibrils when the cell wall is dried. From HEYN's pictures it must be concluded that the secondary wall of plant fibres and wood tracheids have no heterocapillary system of voids in the functioning living tissues; seemingly this heterocapillarity (*25*, there Fig. 7) as postulated in 1937 (*13*) originates only when the fibres are dried and aggregated microfibrils are formed in a process which is highly furthered when the matrix (pp. 1, 10) and incrusting lignin between the elementary fibrils is chemically removed by eau de Javelle, chlorine or sulphites. It is characteristic that after such a treatment the fibres which are transparent in the natural state, turn white as a sign of the formation of coarser light diffracting voids in the purified cellulose fibres.

Since 35 A strands have been found in primary wall cellulose (*56*), in secondary fibre walls [jute, flax, ramie, cotton (*37*)], in wood tracheids (*38*), bacterial cellulose (*26*, *62*) and *Valonia* cellulose (*27*), the elementary fibril must be considered as a basic element of crystalline cellulose.

3. Crystal Lattice

The crystal lattice of the β-D-1,4-glucose chains as devised by MEYER and MISCH (*51*) has been modified according to HERMANS (*33*), who proposed that the glucosidic C_1—O—C_4 bonding would not be straigth but crooked. This conformation could be verified by infrared spectral research (*43*). As a consequence of a bond angle approaching the tetrahedral angle 110°, an intramolecular hydrogen bond between O_5 and O_3' *(Fig. 4)* is formed which stabilizes the ribbon shape of the cellulose chain molecule.

Besides this intramolecular bond, *intermolecular* hydrogen bonding is important for the formation of the elementary fibrils and their tendency to aggregate along the (101) plane of the crystal lattice *(Fig. 5)*. It can be shown that the primary alcoholic group O_6 (O_V in Fig. 5) develops a hydrogen bond towards the oxygen of the glucosidic bridge O_1. If in

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the model of MEYER and MISCH (*51*) the primary hydroxyl is rotated around the axis C_5—C_6, it can approach the bridge oxygen O_1 by $d =$ $= 2.54$ A (*17*). This intermolecular bond, active in the (101) plane, is therefore stronger than the intramolecular hydrogen bond of Fig. 4 which bridges a distance of 2.68 A. Another similar bond is working in the (10$\bar{1}$) plane (Fig. 5a); it measures $d' = 2.80$ A and is therefore weaker than the intramolecular hydrogen bond. The distances for the intermolecular bonds have been calculated from the MEYER-MISCH model

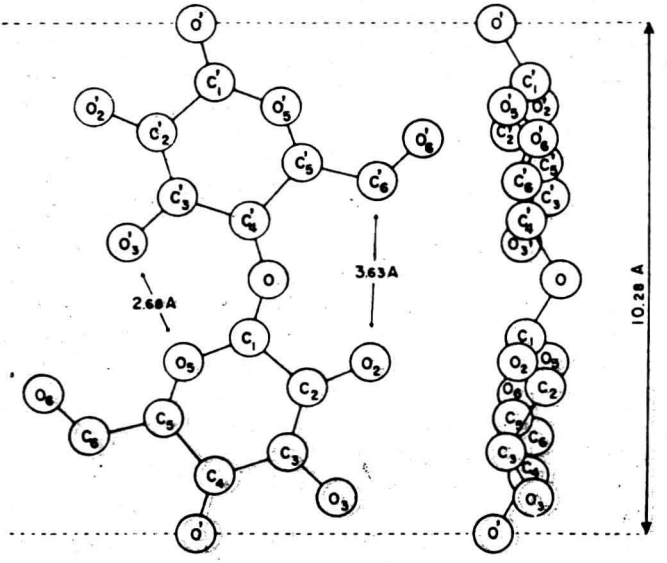

Fig. 4. Cellobiose member with bent C_1—O—C_4 glucosidic bridge, according to LIANG and MARCHESSAULT (*43*) showing the intramolecular $O_6 \ldots H \ldots O_3$ hydrogen bonding 2.68 A which stabilizes the ribbon-like shape of the cellulose chain. [From: J. Polymer Sci. *37*, 385 (1959).]

but they are only slightly different from those in the LIANG-MARCHESSAULT model (*43*). The similar strength of the intermolecular hydrogen bonds in two almost perpendicular planes seems to be responsible for the formation of a nearly isodiametric cross-section of the elementary fibrils; and the preponderance of the d-bond in Fig. 5b determines the (101) plane as a plane of aggregation and growth for the microfibrils (*60*, *61*).

4. Antiparallelism and Folding of the Cellulose Chains

In the current models of crystalline native cellulose the two chains with the coordinates ooo and $\frac{1}{2}\frac{1}{4}\frac{1}{2}$ run antiparallel (*18*). Although

this feature cannot be proved by the available X-ray diffraction evidence, the antiparallelism has been postulated for crystallographic reasons (*51*) or for the sake of the intermolecular hydrogen bonding (*43*). Fig. 5c demonstrates how, in the plane (101), the intermolecular hydrogen bonds display a more balanced distribution with antiparallel than with unidirected chains. It may also be suggested by analogy to other crystalline biopolymers such as chitin (*5*), silk fibroin (*46*), deoxyribosenucleic acid (*80*), etc., where in all cases the antiparallelism favours the establishment of interchain hydrogen bonds. Other polymeric chains, for instance collagen (*71a*) and chitin (*70*), are known in both parallel and antiparallel configurations.

ROELOFSEN (*69*, there p. 30) and COLVIN (*7a*) oppose the concept of antiparallelism in native cellulose because in this case the tip growth of fibrils would need two enzymes: one for the addition of monomers to the C_1 and a second one for the attachment to the C_4 atoms. However, a unidirectional orientation of the cellulose molecules would yield a hemimorphic structure, that is, the elementary fibril would have a head and a tail with the different properties of an aldehydic reducing and a nonaldehydic nonreducing end, respectively. Until now no such hemimorphic properties have been detected in plant fibres. Not even piezoelectric research which is the classical method for finding hemimorphic structures in crystals has yielded any polarity in wood (*29*). Of course, such shortcomings can be explained by assuming that, although the chain molecules are arranged unidirectionally, the elementary or microfibrils are deposited in an antiparallel manner by some unknown morphogenetic principle of the growing cell wall.

In recent times a discussion has arisen, whether the cellulose chains in native cellulose are straight or folded. Although folded polymeric chains have a tendency to crystallize in sheets as shown for xylan (e. g., *45*, there Fig. 19)–a feature which has long been known from molecular films of folded α,ω-dicarbonic acids floating on water–, one tries to apply this lamellar principle also for fibrillar structures. It is thought that a crystal lattice with folded chains would settle the question of the antiparallel chain orientation. Although the lattice inhomogeneities can easily be interpreted as chain bends over 180° with a reversal of the chain direction, there is no strict proof for the existence of folds in the chain lattice of native fibres. Likewise, the interpretation of the crystal structure of the antiparallel α-chitin as a system of loops (*45*, *70*) is for the present only a hypothesis. The fact that W. T. ASTBURY's well-known theory of folded keratin chains has proved to be wrong and that on the strength of electron microscopic evidence the folding theory for actomyosin in the muscle had to be abandoned, forces the textile chemistry to handle the folding question cautiously and with special care.

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