Ultrastructural Behavior of Cell Wall Polysaccharides

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Abstract: Studies on the behavior of wood polysaccharides inside and outside the cell wall were carried out under varying conditions using the electron microscope in combination with physico-chemical methods to gain new information on the supermolecular structure of the polysaccharides. Several fractions containing different polyoses were obtained by fractionation of the alkali extract of holocellulose. Isolated polyoses are at least partially able to arrange themselves in fibrillar elements. After delignification, the cellulose is recognizable within the cell wall as microfibrils with an average diameter of about 250 Å. However, these fibrillar units seem to be rather unstable as they can easily be split into units 120 Å in diameter by chemical treatment. After partial hydrolysis, isolated cellulose shows more subunits about 30 Å in diameter at higher magnification. Moreover, the more sensitive portions of the fibrils in the longitudinal direction are attacked by partial hydrolysis, and these portions seem to grow with increasing duration of the hydrolysis. Fractionation of cellulose isolated from thermally treated wood shows that the chain fragments are multiples about 300 Å in length. A tentative model of the supermolecular arrangement of the cellulose in relation to the other cell wall components is presented.

Keywords: Softwoods · Hardwoods · *Picea abies* · *Fagus sylvatica* · Electron microscopy · Cell wall · Cell structure · Fibrils · Microfibrils* · Cellulose · Polysaccharides · Hemicelluloses · Carbohydrates · Xylan · Mannan · Degree of polymerization

CONSIDERABLE information on the ultrastructural organization of the plant cell wall and the supermolecular arrangement of the cell wall components, in particular of cellulose, has been obtained with the electron microscope. However, some ultrastructural features were clarified by indirect methods such as X-ray diffraction (*1*) and light birefringence (*2*) several years before the first electron microscope was constructed (*3*). The X-ray studies gave evidence that the cellulose chains are arranged in monoclinic lattices (*4*). In addition, it was found that the ordered regions build up higher-level systems in the longitudinal as well as in the lateral direction. Several such systems have already been seen in the electron microscope but it has not yet been possible to resolve single polysaccharide molecules although modern high-performance instruments have a resolution power down to 3 Å.

This paper reports results obtained during the study of polyoses and cellulose from wood, primarily by electron microscopical observation but supplemented by other chemical and physical methods. It is an attempt to combine a series of results, some of which have been published previously, others quite new, into a model of the supermolecular organization of the cell wall polysaccharides.

MATERIALS AND METHODS

The basic material for investigation was air-dried wood meal from spruce (*Picea abies* Karst.) and from beech (*Fagus sylvatica* L.). The samples were extracted for 6 hr with methanol-benzene and delignified with sodium chlorite at 70°C (*5*). The polyoses were extracted from holocellulose by treatment with 5 and 24% potassium hydroxide in two stages under a nitrogen atmosphere, followed by precipitation with ethanol and acetic acid (*5*). The two alkali-soluble fractions could each be further subdivided into three other fractions by treatment with water and ethanol according to the scheme of fractionation previously described (*6*).

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The partial hydrolysis of the cellulose was carried out by boiling the samples in an excess (about 100 times the weight) of 20% sulfuric acid for 1 or 5 hr.

In special cases, the wood meal was thermally pretreated by heating at different temperatures in the range of 100 to 200°C for 24 hr (*7*).

The samples used for metal shadowing were generally suspended in 80% ethanol; those used for negative staining were suspended in buffer solution (pH 7). The suspensions were precipitated by ultrasonic atomizing onto copper grids bearing a collodium film. The specimens were stained by shadowing with platinum at an angle of 20° at a high vacuum or by negative staining with a 1% phosphotungstic acid (PTA) solution or a 1% uranyl acetate solution for 15 min. The specimens were examined in an AEG-Zeiss EM 8/IV, in a Siemens Elmiskop 1, and in a Siemens Elmiskop 101.

For the determination of the average degree of polymerization, the viscosity of cellulose in cadoxene (*8*) was measured in a capillary viscosimeter.

For chain length fractionation, solutions of cellulose nitrate in acetone were precipitated in stages with acetone-water according to the method of Philipp and Linow (*9*). The degree of polymerization was determined by measuring the viscosity of each fraction in acetone solution in a capillary viscosimeter. The curves of chain length distribution were calculated by adding up the percentage weight of the single fractions according to Schulz (*10*).

RESULTS AND DISCUSSION

Polyoses

One of the most striking properties of the wood components, from the analytical point of view, is their intimate mixing with one another within the cell wall. Consequently, they cannot be separated quantitatively without incurring changes. Only a small amount of polyoses can be removed intact by means of water extraction, e.g., the galactans from larch wood (*11*) and part of the xylans in hardwoods (*12*). Even careful removal of lignin from finely milled wood by extraction with dioxane results in a decrease in the degree of polymerization (DP) (*13*). The main portion of the polyoses must be isolated from holocellulose by means of alkali extraction.

By the method applied in this study, two alkali-soluble fractions containing polyoses and one alkali-insoluble fraction containing alpha cellulose were obtained. The subsequent fractionation of the two polyose fractions resulted in six more fractions. The composition of each separate fraction was determined by paper chromatography of the totally hydrolyzed product.

Polyose Fraction A2 contained the highest percentage of the total alkali-soluble polysaccharides. Thus, Fraction A2 from beechwood contained about 73% and Fraction A2 from sprucewood about 52% of the total. Paper chromatographic identification of the constituents of these fractions revealed that Fraction A2 from beechwood consists of rather pure xylan while Fraction A2 from sprucewood con-

sists of a mixture of glucomannan and arabinoxylan (*6*). The same mixture is found in a different fraction from sprucewood, termed B2, which amounts to about 20% of the total alkali-soluble polysaccharides.

In the electron microscope, the abovementioned fractions show fibrillar elements (Figs. 1 and 2). Contrary to the fraction from beechwood, the fibrils in the two fractions of sprucewood polyoses are partially veiled by an amorphous-looking substance (Fig. 2). Here "amorphous" is used in the sense of "structureless," i.e., substances appearing amorphous in the electron microscope may show a lattice order when submitted to X-ray or electron diffraction. From the similarity of the shape to that of the polyose fraction from beechwood and from the changes taking place in the polyose fractions from sprucewood during heating of wood (*7*, *14*), it is concluded that the fibrils consist of arabinoxylan and the amorphous-looking substance consists of glucomannan. The amorphous shape of the glucomannan on the electron micrographs should not lead to the assumption that it is in fact without any structure. There is some evidence that the amorphous-looking substance in sprucewood

Fractions A2 and B2 consists of very fine fibrils (Fig. 2) which can barely or not at all be resolved under conditions prevailing in this study (metal-shadowed specimens, microscope of relatively low resolution power). This question will be solved by further studies with an instrument of high resolution power, as already used for some details in the observation of cellulose. A distinctly fibrillar mannan was isolated in one fraction from sprucewood which, however, amounted to only about 0.5% of the alkali-soluble polysaccharides (Fig. 3).

A highly interesting change of the

Fig. 1. Polyose Fraction A2 from beechwood containing fibrillar xylan. Platinum-shadowed.

Fig. 2. Polyose Fraction A2 from sprucewood containing fibrillar arabinoxylan and amorphous-looking glucomannan. In several places, the smooth areas are resolved into fine fibrillar structures. Platinum-shadowed.

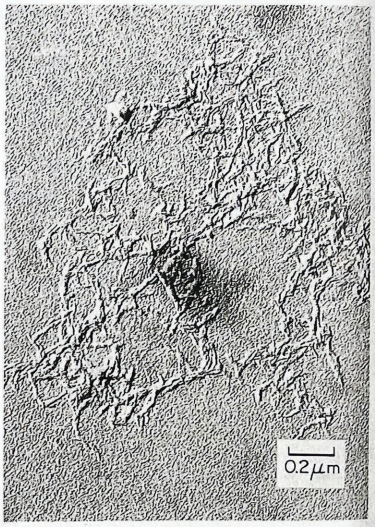

Fig. 3. Polyose Fraction B1 from sprucewood containing fibrillar glucomannan. Platinum-shadowed.

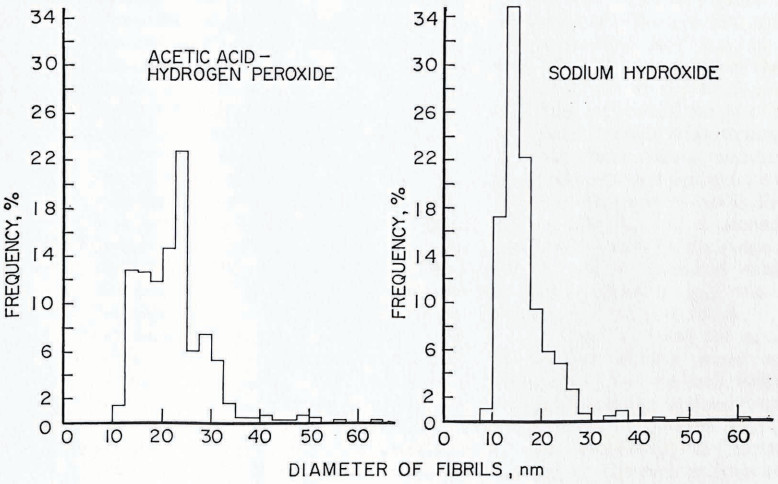

Fig. 4. Diameters of fibrils in the S1 of delignified sprucewood fibers after treatment with acetic acid-hydrogen peroxide (1:1, 60 min, 70°C) and with sodium hydroxide (5%, 10 min, 20°C).

Fig. 5. Cell wall fragment of sprucewood meal after delignification. Platinum-shadowed.

CITE THIS PAPER: Fengel, D., *Tappi* 53(3): 497(1970). https://doi.org/10.32964/TJ53.3.497.

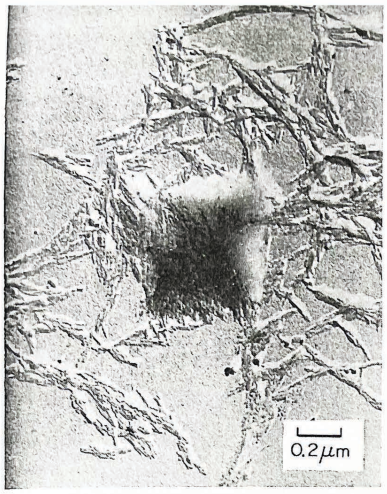

Fig. 6. Partially hydrolyzed cellulose (20% H_2SO_4, 5 hr, 100°C) from sprucewood. The fibrillar fragments are arranged in various lateral aggregates. Platinum-shadowed.

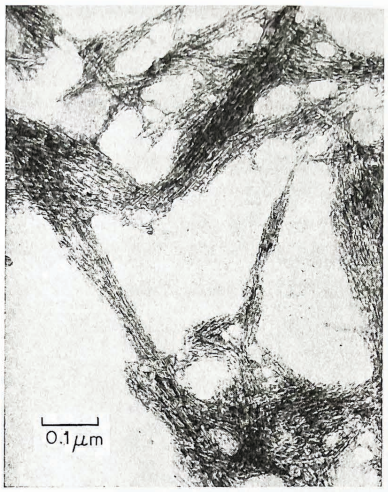

Fig. 7. Partially hydrolyzed cellulose (20% H_2SO_4, 5 hr, 100°C) from sprucewood. The light-colored lines are elementary fibrils of about 30 Å in diameter. Negative stained with uranyl acetate.

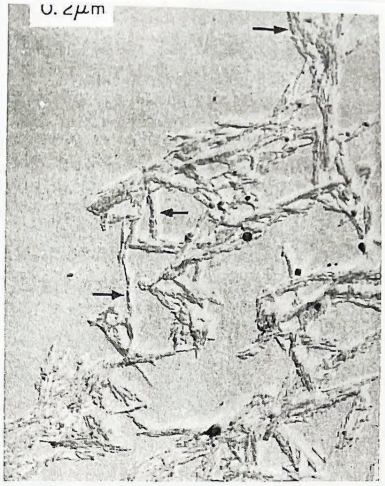

Fig. 8. Partially hydrolyzed cellulose (20% H_2SO_4, 5 hr, 100°C) from sprucewood. The fibrillar fragments are subdivided in longitudinal direction (arrows). Platinum-shadowed.

fraction from sprucewood containing fibrillar mannan can be observed if the wood is thermally treated prior to the isolation of the polyoses. After heating the wood for 24 hr at various temperatures, this fraction, termed B1, showed the most striking increase in percentage of all alkali-soluble polysaccharide fractions. While the percentages of the other fractions vary more or less with treatment temperatures between 120 and 200°C, there is a strong increase in fraction B1 to about 21% of the wood at temperatures between 150 and 200°C (*14*). The higher the treatment temperature, the higher is the content of glucan in Fraction B1. The glucan originates from the cellulose cleaved during the thermal treatment of wood. Thus, the main decomposition products of the cellulose are found in Fraction B1. From this, it may be concluded that the mannan fibrils in this fraction from untreated wood are parts of the mannan which cannot be separated from the alpha cellulose by alkali-extraction and which is known as "resistant" mannan.

From the fibril-forming behavior of the xylan and of at least a portion of the mannan after their isolation from wood, it is assumed that the molecules of these polyoses are mainly oriented in the direction of the cellulose fibrils within the cell wall. Moreover, the intimate association of part of the mannan with the cellulose, the behavior of the cellulose fibrils under the influence of different chemical treatments, and the partial chemical bonding between polyoses and lignin lead to the conclusion that the polyose molecules are predominantly arranged around the larger and between the smaller fibrillar units of the cellulose. This concept implies a combination of the ideas of Preston (*15*) and of Marchessault (*16*) concerning the arrangement of polyoses in relation to the cellulose fibrils. The various side groups of the polyose molecules probably prevent the same strict orientation as shown by the cellulose molecules. Meier and Welck (*17*) studied the association of the polyoses with the cellulose fibrils in the electron microscope, but their observations gave no definite information.

Cellulose

Within the plant cell wall, the molecular chains of cellulose are arranged in systems at various levels of magnitude. The cell wall itself can be regarded as the highest-level system. It is built up from fibrillar units which are called "microfibrils" and whose diameters measure about 250 Å. The microfibrils, however, do not seem to be very stable units since they can easily be split into smaller units within the fiber wall, e.g., by treatment with alkali. Figure 4 shows the result of measurements of the fibril units within ultra-thin sections of the S1 of delignified sprucewood fibers. After treatment of the cellulose fibers with a mixture of acetic acid and hydrogen peroxide, a maximum for fibril diameters occurs in the range of 225 to 250 Å, and a second, smaller maximum occurs in the range of 125 to 150 Å. After treatment with sodium hydroxide, there is only one maximum in the range of 125 to 150 Å.

The basic material for the isolation of cellulose during wood analysis is wood meal, i.e., the cell walls are disintegrated and the various reactions take place in these fragments (Fig. 5). In the electron microscope, the isolated cellulose can be observed as fragments of the cell wall. The fibrillar structure, however, is only clearly recognizable after an acid treatment since it is covered by amorphous cellulose material created in the various isolation steps. After partial hydrolysis, specimens of isolated cellulose show lateral fibrillar aggregates of various dimensions (Fig. 6). The diameter of a single fibril measures about 120 Å.

It is concluded from these observations that fibrils of about 120 Å in diameter are constant units within the cell wall and that they are subunits of the microfibrils.

Some years ago, cellulose fibrils about 30–35 Å in diameter were detected in meristematic tissue and bacteria slime by several authors (*18*). Frey-Wyssling and Mühlethaler (*19*) termed these smallest cellulose units "elementary fibrils." At higher magnification and higher resolution power in negative-stained specimens of partially hydrolyzed cellulose from wood, fibrillar elements about 30 Å in diameter also become visible (Fig. 7). This observation proves that the elementary fibrils are also order systems of the cellulose in wood (*20*).

On the other hand, electron micrographs of partially hydrolyzed cellulose also show a subdivision of the fibrillar elements in the longitudinal direction. This subdivision is recognizable in several places in Fig. 8. Since this picture is taken from a platinum-shadowed specimen, the interspaces between the longitudinal units seem to be holes or notches, i.e., the fibrillar units had been attacked at regular distances by the acid. After negative staining, the fibrils are visible in the form of white dotted lines (Fig. 9); consequently, the interspaces are more accessible to the staining material than the fibrillar units which show up as white. At high magnification, it is obvious that the bright units are often of a more or less rectangular shape and that these units as well as the more accessible interspaces are structured. In Fig. 10, there is a highly interesting area within the framed

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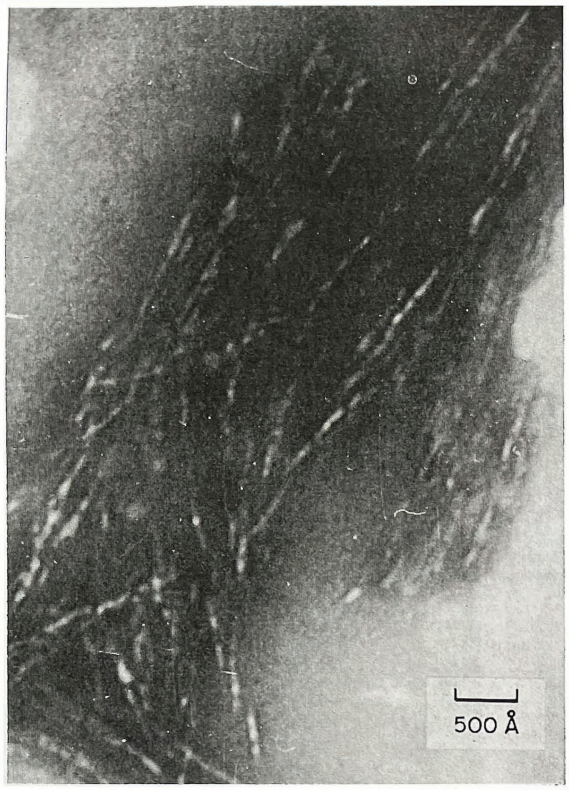

Fig. 9. Partially hydrolyzed cellulose (20% H_2SO_4, 5 hr, 100°C) from sprucewood. The fibrillar elements have a beaded appearance. Negative stained with phosphotungstic acid (PTA).

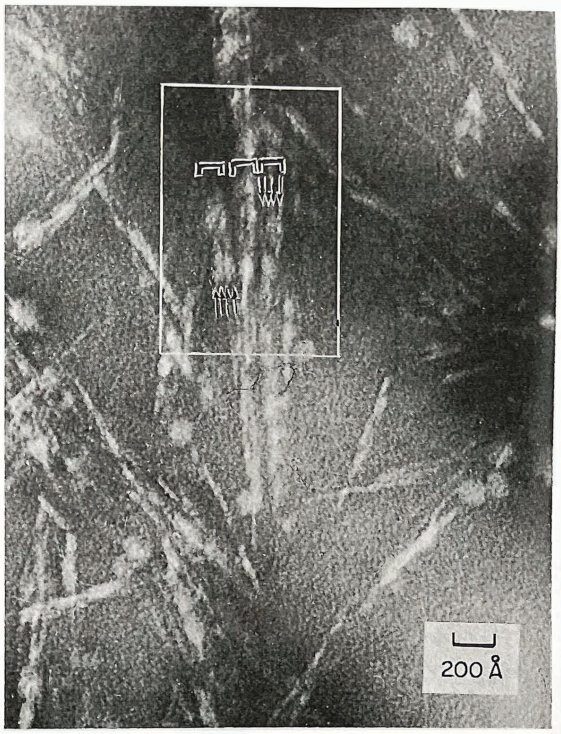

Fig. 10. Partially hydrolyzed cellulose (20% H_2SO_4, 5 hr, 100°C) from sprucewood. The high-magnification photo shows fibrils which are subdivided into darker and lighter zones. Within the framed field, there are three 120-Å units (in brackets) which are built up of four elementary fibrils in lateral direction (arrows). Negative stained with PTA.

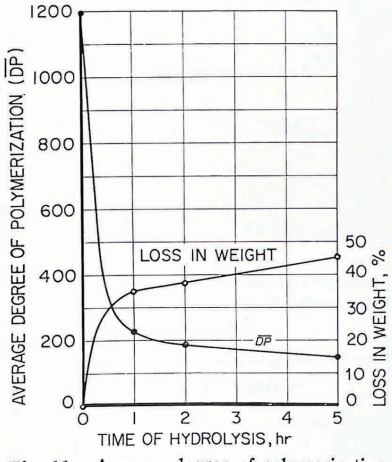

Fig. 11. Average degree of polymerization and loss in weight of cellulose from sprucewood as a function of time of hydrolysis.

field. The structural details are indicated by arrows. Three fibrillar fragments about 120 Å in diameter, each subdivided into four fibrillar units about 30 Å in diameter, are recognizable. Within the less accessible regions, the 30-Å subunits seem to be thicker and seem to contact each other so that they cannot be completely discerned. The lengths of the less accessible regions after 5 hr of hydrolysis with sulfuric acid measure about 150 Å.

Early in the thirties, a decay of the fibrillar elements into shorter pieces in chemically treated cellulose was observed with the aid of the light microscope (*21*). Later studies with the electron microscope and measurements of DP resulted in observation of different lengths of fibrillar units of partially hydrolyzed cellulose, depending on the origin of the specimen and the reaction conditions. The respective values in the literature range from 60 to 2500 Å (*22*).

During the present study, the "leveling-off DP" (*23*) was reached after 1 hr of acid treatment of the cellulose samples (Fig. 11). Between 1 and 5 hr of treatment, there is only a slight decrease in the $\overline{DP}$ value.

For more detailed information, chain-length fractionation was also performed. Figure 12 shows the integral distribution curve of the DP of cellulose treated with 20% sulfuric acid for 1 hr. The DP decreases continually from about 200 to about 40. The steepest part is within the range $\Sigma m_p = 40$ and $\Sigma m_p \approx 0$ with a DP value of about 40–50, i.e., there is a considerable portion of cellulose chains about 200–250 Å in length.

The fractionation of cellulose hydrolyzed for 5 hr with sulfuric acid resulted in the main portion of cellulose chains having a DP below 40, i.e., the chains are shorter than 200 Å. In this low DP range, however, the applied method of determination no longer gives exact values. Nevertheless, it may be assumed that there is a good agreement of these results and of the chain length measured in the electron micrographs at about 150 Å from the less accessible regions of cellulose hydrolyzed for 5 hr.

In several cases, the less accessible regions of the fibrillar fragments are separated from one another and are visible as grains on the micrographs (Fig. 13). As these particles are the result of fragmentation of cellulose fibrils, they would consist of short cellulose chains which are still oriented in a parallel arrangement. This consideration is further supported by the observation that these particles are able to rearrange themselves in new fibrillar units. The newly formed fibrils are oriented in parallel systems (Fig. 14) or grow in the shape of whiskers (Fig. 15).

In addition to the heterogenous acid hydrolysis, cellulose was decomposed by heating. The behavior of the cellulose was studied as part of an extensive study on the changes taking place in the wood during heating (*7*, *24*). The heating results in a strong decrease of the cellulose content between 120 and 200°C, determined as the alkali-insoluble fraction.

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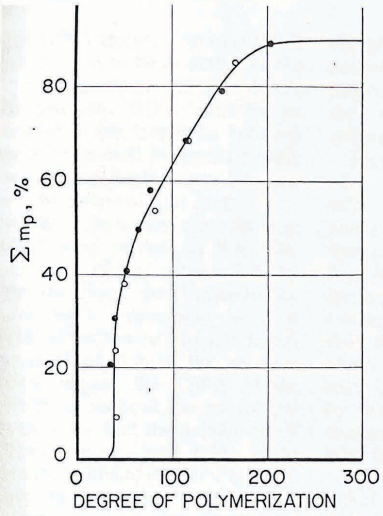

Fig. 12. Curve of chain length distribution of cellulose hydrolyzed for 1 hr with 20% H_2SO_4 at 100°C as a result of two different fractionations.

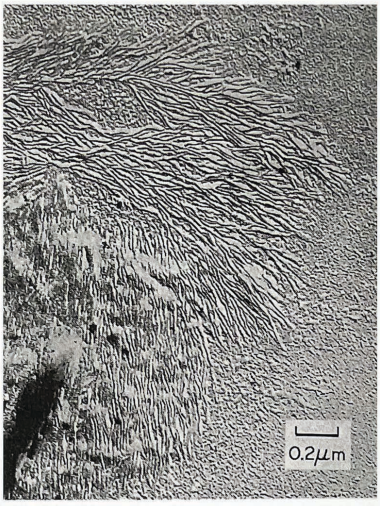

Fig. 13. Partially hydrolyzed cellulose (20% H_2SO_4, 1 hr, 100°C) from sprucewood. Very short fragments are deposited on the supporting films. Platinum-shadowed.

Fig. 14. Partially hydrolyzed cellulose (20% H_2SO_4, 1 hr, 100°C) from sprucewood. Newly formed fibrillar systems. Platinum-shadowed.

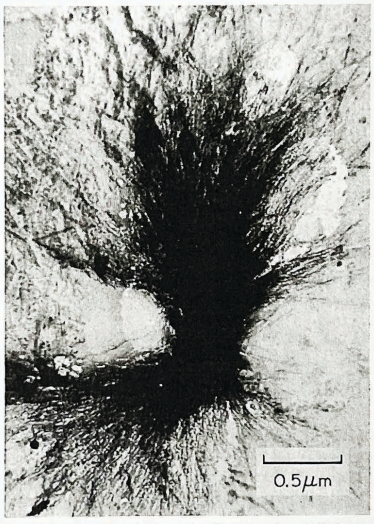

Fig. 15. Partially hydrolyzed cellulose (20% H_2SO_4, 5 hr, 100°C) from sprucewood; reaggregation of fibrillar fragments in the shape of a whisker. Platinum-shadowed.

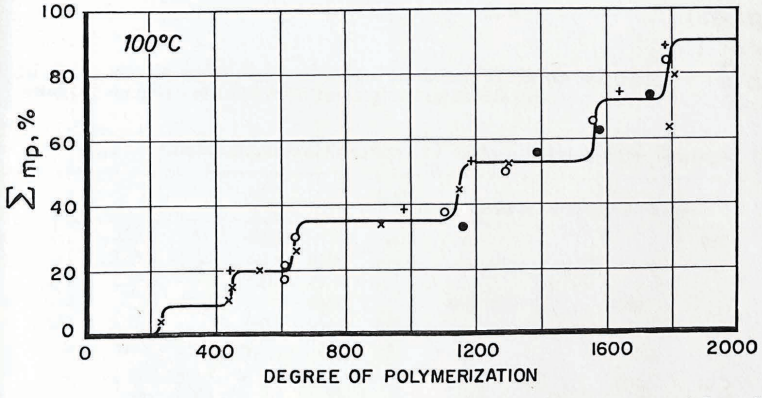

Fig. 16. Curve of chain length distribution of cellulose isolated from sprucewood, heated for 24 hr at 100°C, as a result of four different fractionations.

Within the temperature range of 120 to 200°C, there is also a decrease in the average DP. If there are sensitive regions equidistant from one another within the cellulose fibrils, one would assume that the thermally induced cleavages occur in these regions.

The cellulose isolated from wood which had been heated for 24 hr at 100, 120, 150, 180, and 200°C was submitted to a chain-length fractionation. As an example, Fig. 16 shows the chain-length distribution of cellulose isolated from wood heated at 100°C for 24 hr. The results of the fractionation of all tested celluloses are compiled in Table I. The DP values represent kinks in the distribution curves. In curves of three or four samples of different treatment temperature, there are various kinks in the same DP range. As shown in the bottom row, there is evidence that all DP values are multiples of about 60. This result leads to the conclusion that the thermally induced cleavage of the cellulose chains occurs at certain distances which are multiples of about 300 Å.

CONCLUSIONS

Although many points are still open to question, these findings can be projected in a model from which the observations may be interpreted satisfactorily. A model of this kind should not be considered final, but may be varied and supplemented if subsequent observations prove it necessary. It is on this basis that the tentative model of the supermolecular organization of the polysaccharides within the cell wall presented below should be considered.

The molecular chains of the polysaccharides are able to form fibrillar units or to arrange themselves in the direction of fibrillar units. The formation of well-ordered fibrils is a known property of the cellulose molecules. The smallest fibrillar units which were detected as constant elements 30–35 Å in diameter in various lower and higher plants are called "elementary fibrils." These elementary fibrils are densely packed into higher units 120 Å in diameter. There must be interfaces between the elementary fibrils, otherwise they could not be separated in such a uniform manner by chemical treatment. The recently published observations of Heyn (*25*) showed that in the natural swelling state of the wood cell wall, the elementary fibrils are visible even without chemical treatment. It is assumed that the interfaces between the elementary fibrils are formed by less-ordered cellulose chains and by polyose chains. From the fibril-forming properties of isolated polyoses, it is concluded that these molecules are oriented in the direction of the cellulose fibrils within the cell wall.

The next higher-level system is represented by the "microfibrils" which are

built up of 120-Å fibrils. The microfibril unit does not seem to be as stable as the two lower systems since the microfibrils are easily split into 120-Å units by an alkali treatment; the interfaces between these units are assumed to consist mainly of polyoses. The microfibrils in turn are surrounded by polyoses and lignin.

The graphic projection of these findings is shown in cross section in Fig. 17a. The elementary fibrils represented by framed square fields are bordered by narrow interfaces containing only a few polyose molecules within the 120-Å units. The main portion of the polyoses is deposited around the 120-Å fibrils, within as well as without the microfibril unit. It is assumed that the movement of water within the cell wall takes place mainly in the interfaces containing polyoses; processes such as shrinkage and swelling occur preferentially within these interfaces. At the surface of the microfibrils, the polyoses are in intimate contact with the lignin, thus in these places chemical bonds between lignin and polysaccharides (*13*, *26*) exist. Nevertheless, polyoses may also be deposited within the lignin in varying quantities in different cell wall layers (*27*).

Figure 17b shows a longitudinal view of a 120-Å fibril, with additional consideration of the results of hydrolytic and thermal decomposition. In this section plane, the 120-Å unit is built up of four elementary fibrils. The chain-length measurement of cellulose from thermally treated wood leads to the conclusion that there are sensitive regions located about 300 Å from each other. In the model, the sensitive regions are outlined by dashed lines. Data on the structural character of these regions cannot, however, be given.

There have been many models published in recent years showing a rather different arrangement of the cellulose molecules in the sensitive or accessible regions. Some of the models (*28*) are based on the observations o f Keller (*29* and Fischer (*30*) stating that under special conditions macromolecules are able to form chain-folds. Other models show chains running through the accessible regions in an irregular and/or crossing-over arrangement (*31*). However, in most of the models the accessible zones are very conspicuous so that they should be easily visible in the electron microscope. Generally, in the untreated state the elementary fibrils can be observed as smooth stretched strings. Heyn (*25*, *32*) observed a beaded appearance of the elementary fibrils after freeze-drying the samples.

Loosely arranged accessible zones should presumably give the separated fibrils a high flexibility. In reality, the elementary fibrils seem to be very stiff and as Mühlethaler (*33*) demonstrated, they cannot be bent except in a large radius. It is, therefore, assumed that the sensitivity of certain regions of the elementary fibrils is not caused by a disarrangement of the whole system at that point. Deviation of a few molecules from the complete order causing a lattice defect and/or the presence of molecular chain ends is believed to be a sufficiently good reason for a special sensitivity. A very slight reduction of the lattice order in the accessible regions of regenerated cellulose is assumed to occur by Kiessig (*34*) from the results of X-ray low-angle investigations. Also, Ruck (*35*) concluded from results of mercerizing studies on cellulose that the molecular chains in the accessible regions show only slight deviations from the lattice order. The cross-section model of the cellulose fibrils of Ruck (*36*) considers only the distribution of the extractives and makes no reference to the polyoses.

At the sensitive regions, the cellulose fibrils are also attacked during chemical treatments such as partial acid hydrolysis. The sensitive regions are highly accessible to staining materials and these accessible regions evidently grow toward the less accessible regions, depending on reaction conditions. The influence of a chemical attack on the sensitive regions is indicated by dashed arrows in Fig. 17b.

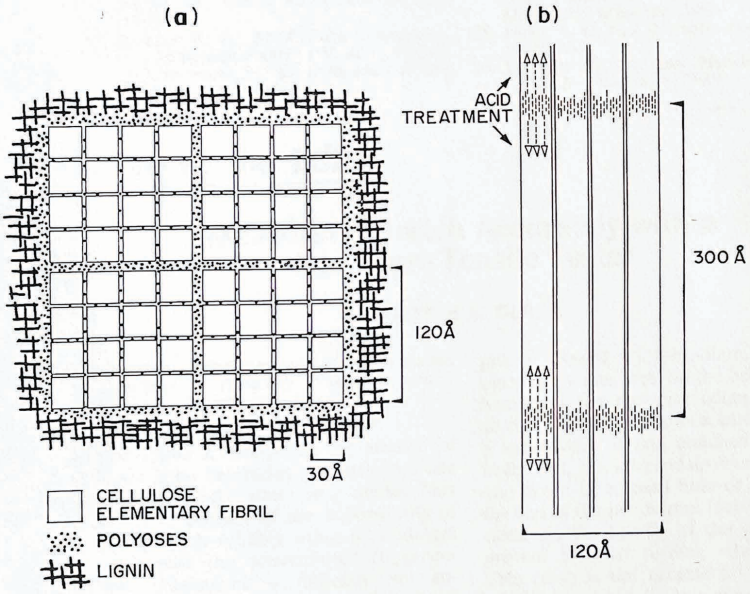

Fig. 17. Model of the ultrastructural organization of a microfibril within the wood cell wall in cross section (a) and model of a 120-Å fibril in longitudinal section (b).

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Table I. Chain Length Fractionation of Cellulose Isolated from Thermally Treated Wood

Temperature, °C	*DP values of kinks in the distribution curves*													
20	...	220	300	400	...	...	...	...	...	930	...	...	...	1770
100	...	230	...	...	450	...	...	...	...	...	1160	...	1560	1780
120	...	...	...	380	...	...	...	730	...	...	1220	1420		
150	...	...	300	...	460	...	630	...	830	...	1190	1380		
180	110	220	...	360	...	560	...	...	...	...	1210	1430		
200	130	220	310	410	...	590								
Multiple × mean factor	2 × 60	4 × 55	5 × 60	6 × 64	7 × 65	9 × 64	10 × 63	12 × 61	13 × 64	15 × 62	19 × 63	23 × 61	25 × 62	28 × 63

CITE THIS PAPER: Fengel, D., *Tappi* 53(3): 497(1970). https://doi.org/10.32964/TJ53.3.497.

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RECEIVED FOR REVIEW July 28, 1969.

ACCEPTED Nov. 2, 1969.

Expanded version of a paper presented at the International Seminar, "Physical and Chemical Properties of Wood Fibers," of the Technical Association of the Pulp and Paper Industry, held in Appleton, Wis., May 12–15, 1969.

The author is very grateful to Dr. N. S. Thompson for kindly presenting this paper at the Seminar.