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THE FINE STRUCTURE OF CELLULOSE FIBRILS

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Summary

The exceptional stiffness of cellulose chains in solution is discussed in relation to the configuration and the possible conformations of the chains and to postulated intrachain hydrogen bonds. The cellulose chains have a strong tendency to aggregate into partly crystalline fibrils, in native plant celluloses appearing as flat ribbons of about 100 Å width and of indefinite length. Electron microscopy of thin sections of plant cell walls has given good evidence that these fibrils are of native origin — they are embedded in hemicellulose, lignin, etc. — and not artefacts from the preparation of specimens. The crystallinity of the fibrils and their accessibility to, say, swelling or hydrolysis varies with the biological origin of the material and is also affected by the pretreatment. The higher lattice order and the lower accessibility of cotton cellulose fibrils compared with wood cellulose are particularly well studied. When hydrolysed with dilute mineral acid, the native cellulose fibrils ('micelle strings') are attacked at certain points and degraded into rodlike fragments ('micelles'). The fact that extraction of hemicellulose from wood holocellulose and subsequent drying decreases the length of the resulting micelles is discussed in relation to lattice distortion in the fibrils (formation of disordered regions and slip planes) due to the collapse ('crushing') of the cell wall.

Plant fibre celluloses with a low content of hemicellulose (such as cotton hair and ramie) form a group with a higher degree of lattice order than does the wood cellulose group, which also includes straw and cotton stalks. There is experimental evidence that the wood cellulose chains in purified pulps contain a larger number of irregularities like carboxyl or aldehyde groups, than do cotton cellulose chains.

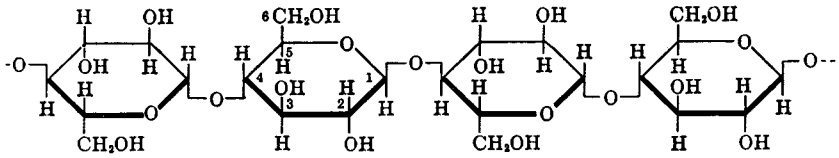
Sulphate pulp cellulose shows a somewhat higher degree of resistance to swelling in caustic soda than does sulphite pulp cellulose. Practically no difference between these pulps is found, however, if the fibres are prehydrolysed with dilute sulphuric acid or if the swelling tests are performed with concentrated phosphoric acid. This indicates that the differences between sulphite and sulphate pulps ('the sulphate effect') is mainly confined to the accessible (non-crystalline) regions of the cellulose fibrils.

Introduction

CELLULOSE is a native polymer of a complex, but largely regular structure. Important properties are its insolubility in all common solvents and its tendency to aggregate to partially crystalline bundles or fibrils of largely parallel chains. The purpose of this paper is to summarise our present knowledge of these fibrils as they occur in native cellulose fibres.

The cellulosic chains

The accepted chemical structure of the cellulose molecule (Fig. 1) is a stereoregular chain, synthesised in nature with a high degree of specificity.



The cellulose chain (the carbon atoms are numbered 1 to 6 in one glucose unit)

Fig. 1

There is good evidence that the chain *configuration* as presented here is basically correct.[1] Possible irregularities in the structure will be discussed later under *Cotton and wood celluloses*. Because of rotation around chemical bonds, however, the cellulose chain could appear in a large number of *conformations* on closely the same energy level.[2] It is concluded from X-ray diffraction work on crystallised cellulose that the cellulose chains form fairly straight and flat

ribbons in the cellulose lattices. A strain-free chain of this shape (Fig. 2) can have its glucose units either in 'chair' or in 'boat' form.[2] Inspection of molecular models indicates that transitions between chair and boat forms are possible in principle, but, because of steric hindrance and potential barriers from strongly polar groups (hydroxyl and ether groups), such transitions appear unlikely under normal conditions. The flexibility of cellulosic chains is, accordingly, thought to be limited mainly to the glycosidic bonds between

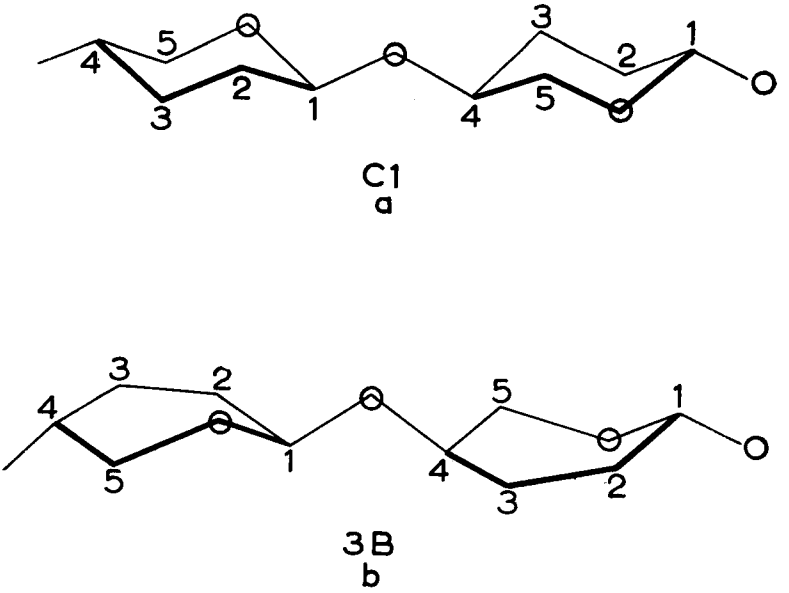


Fig. 2—Cellulose chains in two different conformations—anhydroglucose units in 'chair' (*a*) and 'boat' form (*b*)—group notations C1 and 3B, respectively, from Reeves[2]

the glucose units, where rotation is easier. However, even at these bonds, polarity and crowding of groups would tend to restrict the flexibility. This would in particular be the case for cellulose derivatives with bulky groups of strong polarity—for example, cellulose nitrate.

The limited flexibility of cellulosic chains, as suggested from inspection of molecular models, has been substantially verified from measurements of solution properties. The empirical Staudinger equation modified by Houwink, Kuhn and Mark to—

$$[\eta]=K_m M^a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

is a good approximation of the relationships between intrinsic viscosity $[\eta]$

and molecular weight (M) for cellulose and cellulose derivatives. The parameter K_m is an empirical scale factor while the exponent a can be related to the chain flexibility. According to theory,[3] chain molecules with unrestricted rotation (a random coil) should give an a value of 0.5, while stiff, rod-like molecules should give a values of 1.5—2. For most vinyl and acrylic polymers, a= 0.6—0.8. Cellulosic chains give a values of 0.9—1.0 and they have accordingly low flexibility (*cf.* Table 1), although the chains are far from being rigid rods.

TABLE 1

Parameters in the modified Staudinger equation for cellulose and cellulose derivatives: $[\eta] = K_m \cdot M^a$

Polymer	*Solvent*	$K_m \times 10^5$	a	*Reference*
Cellulose	Cupriethylenediamine..	13.3	0.905	(1)
Cellulose acetate ..	Acetone..	9.0	0.90	(2)
Cellulose trinitrate ..	Ethyl lactate	12.2	0.92	(1)
Cellulose trinitrate ..	Acetone..	17.0	1.00	(3)
Cellulose trinitrate ..	Ethyl acetate	25.0	1.01	(4)

(1) Immergut, E. H., Rånby, B. G. and Mark, H. F., *Ind. Eng. Chem.*, 1953, **45**, 2483
(2) Philipp, H. J. and Bjork, C. F., *J. Polymer. Sci.*, 1951, **6**, 549
(3) Holtzer, A. M., Benoit, H. and Doty, P., *J. Phys. Chem.*, 1954, **58**, 624
(4) Hunt, M. L., Newman, S., Scheraga, H. A. and Flory, P. J., *J. Phys. Chem.*, 1956, **60**, 1278

The degree of flexibility of a polymer chain in solution can be expressed in more specific terms. If the rotation around the chemical bonds is unrestricted, the average end-to-end distance ($\bar{r}^2$) of a polymer chain can be calculated as a 'random walk'[4] giving the relation—

$$(\bar{r}^2)=nL^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where L is the length of the chain element and n the number of bonds in the chain (D.P.=n+1). With restricted rotation, the ($\bar{r}^2$) values can be related to a chain containing the same number of segments (the same D.P.), but with an average *effective bond length* b ($b > L$), which can be calculated if ($\bar{r}^2$) is known —for example, from light scattering measurements. The b values 35Å and 39Å for cellulose trinitrate have been found in two separate investigations.[5,6] Another way of describing chain stiffness is to assume that the chain is substituted with an equivalent chain of the same length when completely extended, but containing a smaller number of *freely rotating but stiff segments*.[7] For cellulose trinitrate, the rod-like segments (valency angle of glycosidic bonds=

110°) would have a length of 20—25 glucose units.[6] Detailed measurements for unsubstituted cellulose chains have not been made, for example, in copper solvents. A corresponding stiff segment length of 10—15 glucose units can be estimated from comparisons of viscosity data. This means that the unsubstituted cellulose has more flexible chains than cellulose derivatives with polar side groups. The stiffness of the cellulosic chains is very pronounced in comparison with vinyl, methacrylic and *iso*butylene chains, which have freely rotating segment lengths of 6—8 Å only.

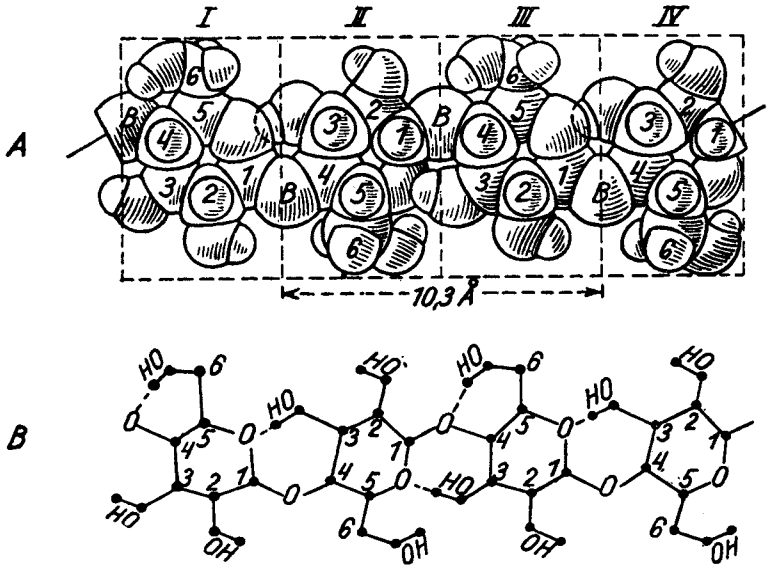


Fig. 3—Cellulose chain with intramolecular hydrogen bonds, from Hermans[8]: *A* as a Stuart's model and *B* as a formula (probable hydrogen bonds indicated by broken lines)

The stiffness of cellulosic chains has been related to intrachain hydrogen bonding. The concept was originally introduced by Hermans,[8] who assumed that the hydroxyl groups on the third and the sixth carbon atoms could form hydrogen bonds with adjacent oxygen atoms (Fig. 3). Experimental evidence to support this assumption has been described by Russell and Moore,[9] working with secondary cellulose acetate. Strongly polar compounds as solvents gave lower intrinsic viscosities than the more inert solvents. This effect was interpreted as an increased chain flexibility due to opening of the postulated intrachain hydrogen bonds (Fig. 4). The assumptions of intrachain hydrogen bonding in cellulose are compatible with recent data on the absorption of polarised infra-red (*see* further *Lattice structure of cellulose fibrils*).

The solubility of cellulose and cellulose derivatives is related to the solvation. This is particularly important with some cellulose ethers such as methyl and hydroxyethyl[10] and ethyl-hydroxyethyl cellulose,[11] which are soluble in water at low temperatures, but precipitate by flocculation when heated to temperatures of 50° — 100°C. The effect has been interpreted as a reversible desolvation causing decreased stability of the solutions to the limit of insolubility. At the same time, the rise in temperature increases the flexibility

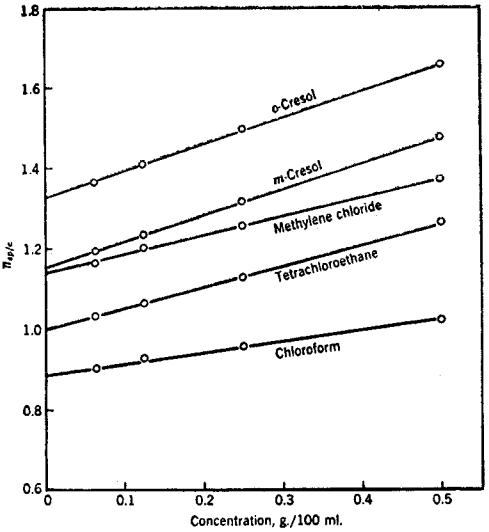


Fig. 4—Viscosity measurements for a cellulose acetate sample in solvents of different polarity, from Moore and Russell[9]

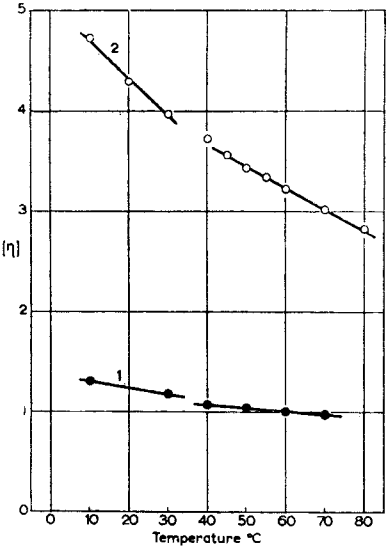


Fig. 5—Intrinsic viscosity as a temperature function for far-hydrolysed cellulose acetates in water solution—(1) with DP_n=190 and (2) with DP_n=380, from Rånby and Griffith (*unpublished*)

of the chains as shown, for example, from the gradual decrease in intrinsic viscosity with increasing temperature (Fig. 5). No flocculation occurs in this case.

Use of the viscosity parameters from the last two lines of Table 1 gives D.P. values about twice as large as those obtained using the earlier parameters. This is largely an effect of the polymolecularity of the cellulose samples. The earlier measurements were based on number average D.P. values (osmotic measurements), while the later two were based, correctly in this case with $a=1$, on weight averages (light scattering). The ratio between weight and number averages is theoretically 2 for a random distribution and values greater than

2 have been obtained from some degraded celluloses. With the new viscosity parameters the DP_w of undegraded native cellulose will reach values as high as 7 000 – 10 000.

Fibrillar structure of native cellulose

Native plant cellulose, as it occurs deposited in the cell walls, shows a fibrillar structure down to the limit of resolution on electron micrographs (20—30 Å). No other structure elements are found. Sub-microscopic fibrils (elementary fibrils or micelle strings) of an average width of about 100 Å were

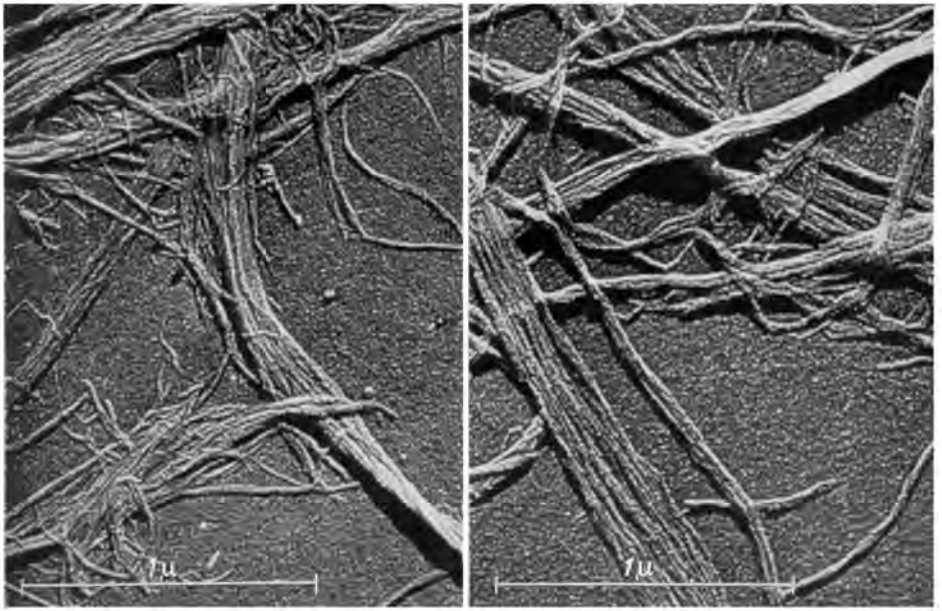


Fig. 6 and **7**—Electron micrographs of cellulose fibrils from purified cotton and chemical grade woodpulps, respectively, dispersed by ultrasonic irradiation (magnification ×39 000, from Rånby[24])

first observed in the 1940s in fragments from mechanically dispersed fibres, treated as a water suspension in a Waring Blendor or with ultrasonic irradiation.[12] With the dispersion methods used, it was still possible that the observed fibrillar fragments could be the result of the mechanical treatment of the fibres rather than native fibrils of definite dimensions. Later investigations, however, carried out with electron microscopy applied to thin sections[13] and to

replicas[14] of native fibres, verified the existence of the same type of fibrils in undisrupted plant cell walls. With these techniques, the resolution has recently been improved, giving average fibril dimensions of about 100 Å in surface replicas (imprint) of spruce wood fibres[15] and dimensions of 50—100 Å in sections of aspen pulp fibres.[16]

Seen in the electron microscope after proper metal shadowing, cellulose fibrils from different plant sources have a similar appearance (Fig. 6 and 7). The aggregation to bundles and lamellae is a very dominant feature with chemical grade pulp fibres, even after intense mechanical treatment. The aggregation has been interpreted largely as a secondary phenomenon, due at least partly to co-crystallisation of cellulose chains of one fibril into an immediate neighbour as shown schematically in Fig. 8. The lamellar structures of fibrils are probably held together by hydrogen bonds, supposed to be well developed in the 101 planes of the lattice, which are parallel to the cell wall plane (*see* further *Lattice structure*). Dispersion into separate fibrils is easily accomplished, if hemicellulose is present.[17] Hemicellulose apparently acts as a protective colloid and prevents solid aggregation (hydrogen bonding and co-crystallisation) of the fibrils (Fig. 9).

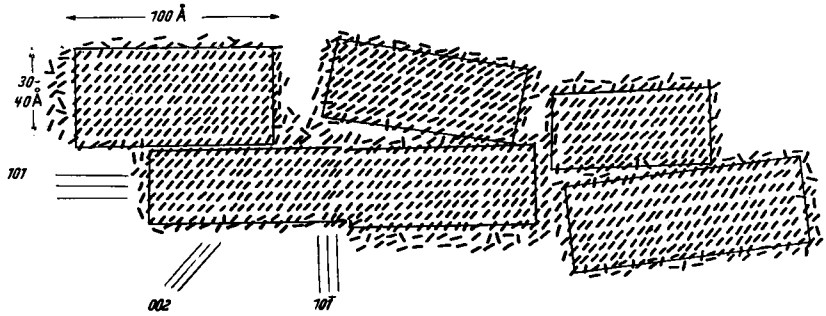


Fig. 8—Schematic cross-section of a bundle of cellulose fibrils in a secondary plant cell wall —101 planes of the lattice are parallel with the plane of the cell wall

There is also electron microscope evidence that the hemicellulose is dispersed between the cellulose fibrils through the whole fibre wall (Fig. 10). The ease of dispersion into fibrils of pulp fibres rich in hemicellulose suggests that the fibrils are largely free entities or units with few, if any, cellulose chains crossing over from one fibril to another. But conclusions in this direction have to be made with great caution, since crossing chains would be much more liable to chemical attack and subsequently more easily broken because of greater accessibility than the aggregated chains in the fibrils. Mechanical

stress on crossing chains could also cause depolymerisation, analogous to the observed degradation of cellulose nitrate in solution by ultrasonic waves.[18]

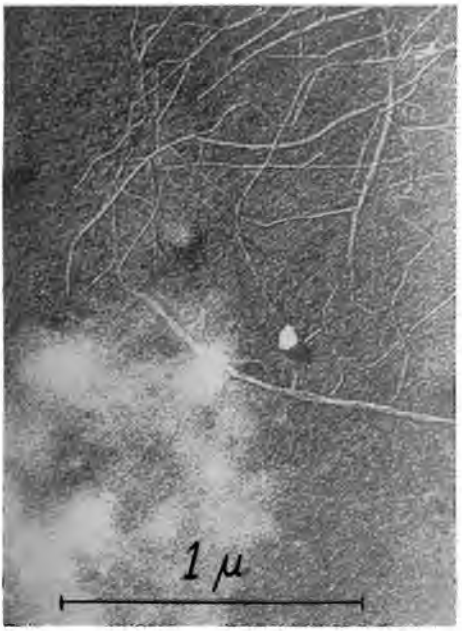


Fig. 9—Electron micrograph of holocellulose from spruce, dispersed by ultrasonic irradiation, showing cellulose fibrils and 'clouds' of hemicellulose, from Rånby[17] (magnification ×40 000)

Fig. 10—A section of an aspen wood fibre (parallel with the fibre axis) stained with thallium ethylate, from Asunmaa[16] (magnification ×52 000)

Dimensions of native cellulose fibrils

It was stated in the preceding section that the native cellulose fibrils show an average width of about 100 Å when observed in the electron microscope. In fact, width data ranging from 50—100 Å to 300 Å have been reported during the last decade.[19] With improved resolution in the electron microscope, there is a tendency towards the lower values. So far, there is no firm evidence that fibrils of 250—300 Å width[20] have significance as structural units in ordinary plant cell walls. These thicker fibrils appear to be aggregates of the type represented in Fig. 8.

Because of the cylindrical orientation of the fibrils in the cell walls, as revealed by X-ray diffraction, it was suggested in the 1930s that the cellulose

fibrils were flat ribbons—that is, had an ellipsoidal or rectangular cross-section.[21] Because of the limited resolution and the flexibility of the supporting membranes, it has been difficult to verify this suggestion by electron microscopy using direct observations and measurements. The first estimates derived

Fig. 11—Micelles from hydrolysed native ramie fibres, deposited on a glass surface, shadowed with palladium at an angle (θ) with $\mathrm{tg}\theta = 0.2$, from Morehead (*unpublished*) (magnification $\times 47\,000$)

from the length of the metal shadow[22] have given values as low as 1 : 5 for the axial ratio of the cross-section. These values are probably influenced by the deformation (imprint or 'sagging') of the supporting membranes. In a recent attempt by Morehead,[23] this difficulty has been overcome by depositing

the cellulose specimen on a polished glass surface. After metal shadow casting, a membrane was deposited and used to strip the specimen from the glass surface for electron microscopy. An average axial ratio of 1 : 3 for the fibril cross-sections of softwood fibres was derived from the length of the shadows in this picture (Fig. 11). This axial ratio would give fibril thicknesses of 30—40 Å in native wood and cotton fibres.

There are data published indicating different dimensions of cellulose fibrils of different biological origin. For cotton, ramie and hemp cellulose, fibril widths of 90—100 Å have been reported, while wood celluloses have given somewhat lower values in the range of 80—100 Å.[24-26] Animal cellulose (tunicin) appears to contain somewhat wider fibrils as does also the unicellular *Valonia* algae. Both have fibril widths in the range of 100—150 Å.[24,27]

Lattice structure of cellulose fibrils

The structure of the cellulose lattices is not resolved in detail. It is generally accepted that the binding forces between the chains are mainly hydrogen bonds, formed by the hydroxyl groups of the glucose units. The native lattice (cellulose I), occurring in the cell walls of all higher plants, was earlier assumed to have the strongest hydrogen bonding in the 002 planes (Fig. 12). It has recently been suggested, however, that the main planes of the lattice with the strongest hydrogen bonding are rather the 101 planes.[28] There are two reasons for this assumption—(*a*) the crystalline fibrils have a larger dimension in the direction of the (101) planes—which are oriented parallel to the surface of the cell walls—indicating that these planes are the main growth plane of the lattice and (*b*) the swelling of the lattice with polar, hydrogen bond breaking reagents gives an expansion mainly in the 101 dimension of the lattice. The 101 plane is probably the main hydrogen bonding plane also in the mercerised cellulose lattice (cellulose II). X-ray diffraction has shown that the formation of practically all addition (or swelling) compounds of cellulose implies an expansion of the cellulose II lattice in the 101 direction only (Fig. 13).[28a] The chains are pushed apart by the entering reagent. The hydrogen bonds between the chains are thereby broken and new bonds are formed with the reagent. The resulting new lattices contain layers (flakes or lamellae) of parallel cellulose chains. There are indications that some of these lamellar structures exist also in concentrated viscose solutions.[29]

It is indicated from infra-red absorption analysis in combination with deuterium exchange[30] that there are four types of hydrogen bonds with different strength in the mercerised lattice (Fig. 14). None of these bonds are properly identified as yet. The dichroism of infra-red absorption in the

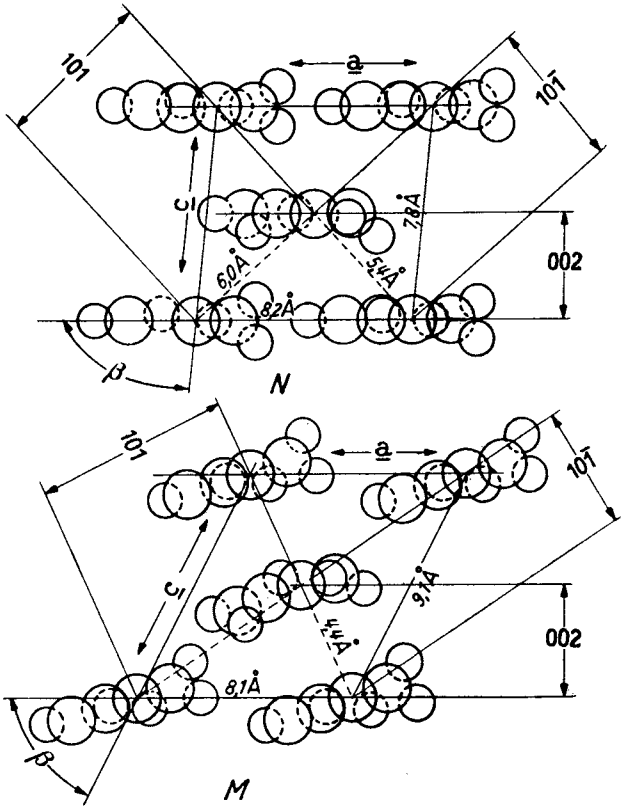


Fig. 12—Schematic cross-sections at rightangles to the chains of the unit cell of native (*N*, cellulose I) and mercerised (*M*, cellulose II)

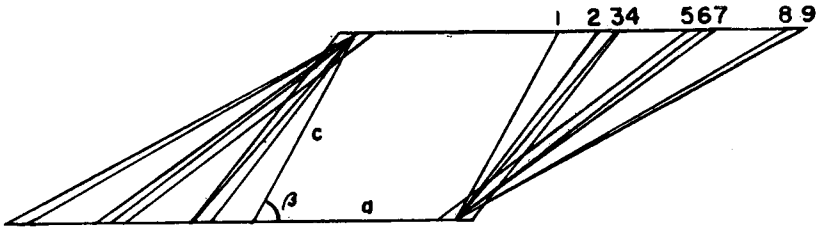


Fig. 13—Schematic drawings of cross-sections of cellulose II lattice[1] (*cf.* Fig. 12), showing lattice swelling with sodium hydroxide, [2,3] ammonia,[3] nitric acid[4] and various quaternary amines,[6–9] from Rånby and Rydholm[28a]

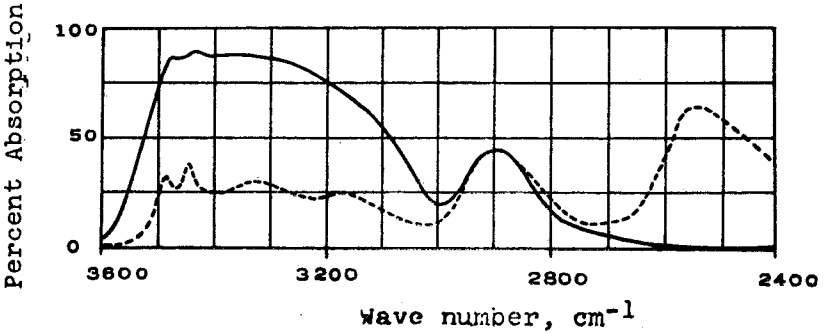


Fig. 14—Infra-red absorption spectra of a regenerated cellulose film (cellulose II) before (—) and after (— — —) exchange with heavy water (deuterium oxide), from Marrinan and Mann[30]

hydrogen bond region (OH stretch vibrations) is parallel with the chains and related to a strong band at 3 050—3 550 cm.$^{-1}$ [31] Native and mercerised cellulose lattices show this type of infra-red dichroism, but the native one to a lower extent.[32] It is possible that the infra-red dichroism of the OH bands in cellulose is related to the postulated intrachain hydrogen bonds (*cf. The cellulose*

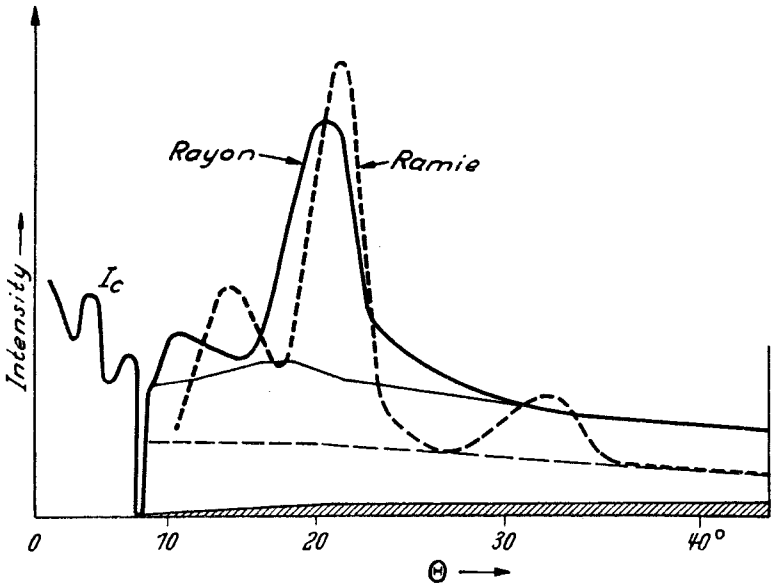


Fig. 15—Sharp reflections and diffuse background scattering in X-ray patterns from native (ramie) and mercerised (rayon) cellulose—I_c shows reflections from a standard sample (NaCl); the shaded area represents the correction for X-ray scattering by air, from Hermans[36]

chains), which accordingly should be stronger in the mercerised lattice than in the native lattice. These results agree well with modifications in the cellulose lattices published recently.[33] It is proposed that the mercerised cellulose chain has its glucose units somewhat rotated out of the planes of the next neighbour units. This position would favour intrachain hydrogen bonding from the hydroxyl on the third carbon atom to the oxygen in the next glucose ring.

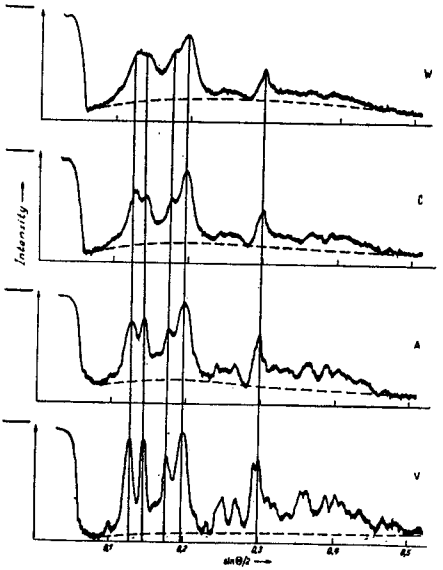


Fig. 16—X-ray diffraction patterns of wood (*W*), cotton (*C*), animal (*A*) and algal (*V*, from *Valonia*) celluloses showing improved lattice order as mentioned, from Rånby[24]

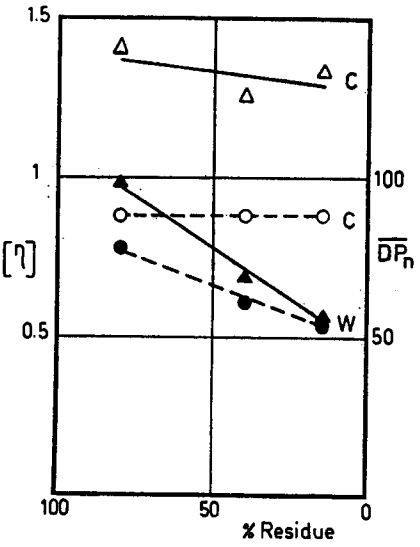


Fig. 18—Effect of prolonged heterogeneous hydrolysis on intrinsic viscosity [η] and osmotic DP_n of native wood (*W*) and cotton (*C*) cellulose—▲ [η] values (DP_w = 144 [η]) and ● DP_n values, from Immergut and Rånby[46]

Crystallinity and chemical accessibility

In any attempt to characterise the crystallinity (the degree to which a native cellulose sample is crystalline), three factors should be considered—(*a*) the cellulose fibrils contain regions of low order, showing high accessibility to chemical reagents, (*b*) the average width of the crystalline regions (the micelles), as well as (*c*) their lattice order varies with the biological origin of the sample.

In the X-ray method,[34] a distinction is made between the discrete reflections (from crystalline cellulose) and the background scattering (from

non-crystalline cellulose). The amounts are derived from the intensities integrated over the scattering angle (Fig. 15). But the sharpness of the reflections —that is, the resolution in the diffraction pattern—varies widely with celluloses of different biological origin (Fig. 16). The algal cellulose from *Valonia* (*V*) shows the narrowest and best resolved reflections. Next in order are animal cellulose (*A*, tunicin) and cotton cellulose (*C*), while the wood cellulose (*W*, a chemical grade pulp from spruce) shows the lowest resolution of the four celluloses. These results indicate a decreasing lattice perfection (lateral order) in the crystalline parts of the samples in the order mentioned. The background scattering, however, is practically the same for the *A*, *C* and *W* samples (Fig. 15), indicating about the same amount of non-crystalline cellulose. The size of the crystalline regions can influence the X-ray pattern in two ways—(*a*) the width of the reflections—according to the diffraction theory—is inversely proportional to the size of the crystalline regions and (*b*) the surface layer of the micelles will to some extent contribute to the X-ray background scattering as non-crystalline material, because the surface represents a discontinuity causing some displacement of the chains. This means, that decreasing size of the micelles will increase the background scattering and also decrease the resolution in the X-ray pattern.

It is concluded from this discussion that a simple figure *per cent. crystallinity* for a certain cellulose sample does not have a well-defined physical meaning. It is a relative figure of value when different methods and different samples are compared. It has been suggested[35] that the crystallinity of cellulose should be described as a *lateral-order distribution function.* Such an attempt seems well justified. The difficulty is, however, that every method used gives its own definition of the distribution function and the relationships between the different functions remain on an empirical basis.

According the reported measurements of crystallinity,[36] Hermans' X-ray method has given very closely the same value for ramie, cotton and wood (chemical grade sulphite pulp) cellulose—31, 30—31 and 30 per cent. non-crystalline cellulose, respectively. Heterogeneous hydrolysis with dilute non-swelling acid[37] has given 6—8 per cent. easily accessible cellulose in cotton and 9—11 per cent. in woodpulp. One reason for the discrepancy between X-ray and hydrolysis data could be a recrystallisation supposed to take place during the hydrolytic depolymerisation of the cellulose chains. Recent measurements, however, have shown that recrystallisation during hydrolysis of native fibres can amount to only 2 or 5 per cent., respectively.[38,39] A more likely interpretation is[40] that the surface layer of the cellulose fibrils (calculated to comprise about 20 per cent. of the fibril volume) gives diffuse X-ray scattering,

although the layer contains hydrogen bonded chains so well ordered that they are not easily accessible to hydrolysis.

Heterogeneous acid hydrolysis disintegrates the cellulose fibrils into shorter fragments, micelles, of the same width as the fibrils.[41] The average length of the micelles is a function of the pretreatment of the fibres; chemical grade cotton linters and woodpulps give average micelle lengths of 500—1 000 Å (corresponds to D.P. values of 100—200), whereas pulps not treated with alkali give much longer micelles upon hydrolysis.[42] It is well established[43] that the average length of the micelles corresponds to the average chain length of the hydrolysed cellulose (length = 5.15 × D.P.).

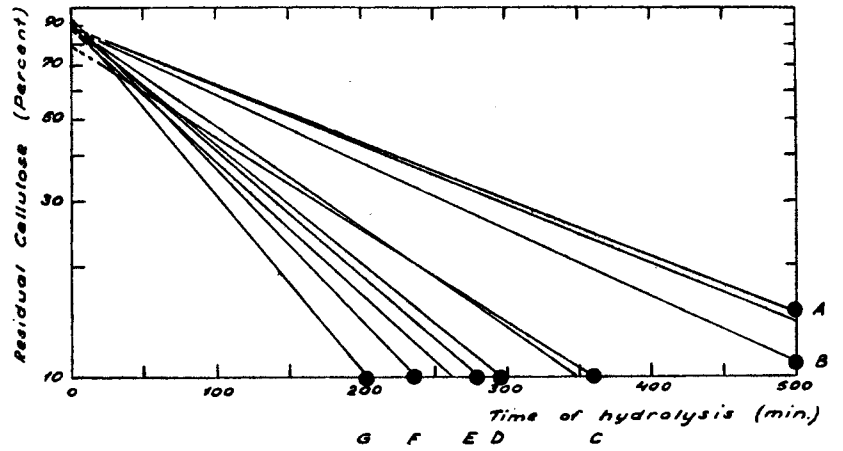


Fig. 17—Hydrolytic degradation of different native celluloses with constant boiling aqueous hydrochloric acid: *A* ramie, *B* cotton, *C* linen, *D* prehydrolysed sulphate and *E*—*G* sulphite pulps, from Millett *et al.*[45]

When native cellulose fibres are hydrolysed in hot dilute mineral acid, two phases of the reaction are observed.[44] The initial fast reaction depolymerises the cellulose to a 'levelling-off D.P.' and removes what is classified as the easily accessible fraction. The following slower reaction is of the first order[45] and with a reaction rate constant that varies with the biological origin of the cellulose (Fig. 17). Of special interest is the difference between cotton and wood cellulose, having a first order rate constant ratio of approximately 1 : 2, respectively. During this heterogeneous, first order reaction, using constant boiling hydrochloric acid, the D.P. of the residual cotton hydrocellulose stayed practically constant down to a residue of only 15 per cent.[46] The wood hydrocellulose, on the other hand, showed a gradual decrease in D.P. during

hydrolysis (Fig. 18). X-ray diffraction and electron microscopy showed further that the dimensions of the cotton cellulose micelles in the hydrocellulose residues remained unchanged in the process, while those of wood cellulose gradually decreased in size. The easiest way to interpret these results would be to assume that the cotton cellulose micelles disappeared by a particle mechanism—that is, to assume that a micelle, once attacked, was rapidly hydrolysed, whereas the wood cellulose micelles were gradually attacked and their cellulose depolymerised.[46] The hydrolysis of cotton micelles has been further investigated by Sharples.[47] He found that the data were compatible with a hydrolytic degradation from the ends of the micelles only, down to a lower limiting length of about 8 glucose units, which is likely to be the solubility limit for cellulose chains. The wood cellulose micelles with their lower lattice order are presumably hydrolysed not only from the ends, but also to some extent on their side surfaces.[46]

As mentioned in a preceding paragraph, the levelling-off D.P. of hydrocellulose from wood (the length of the micelles) is greatly affected by the pretreatment of the fibres.[42] The following experiments were performed to study the effect of drying and alkali treatment.

A holocellulose (*Ho 1*) was prepared from spuce wood chips (*Picea abies*) by treatment four times with aqueous sodium chlorite at pH 4.5 and 60°—70°C to a pulp yield of 67 per cent. of the dry weight of the wood.[48] The wet (never dried) pulp was stored at 4°C. The nitrated pulp had the intrinsic viscosity $[\eta]=24$ in *n*-butyl acetate, indicating a D.P. value of about 3400 (weight average). The wet (*Ho 2*) and the dried (*Ho 3*) pulp (all dryings were made after solvent exchange to absolute alcohol) were hydrolysed with boiling 1.25 M sulphuric acid for 2 hr. (after soaking in the same acid at room temperature for 3 hr.). The never dried *Ho 1* was treated under a nitrogen atmosphere with boiling 1.4 per cent. sodium hydroxide for 1 hr. and then with boiling 3.8 per cent. sodium hydroxide for 15 min., giving a pulp yield of 45 per cent. of the dry wood. After complete washing with distilled water, one part of the pulp (*Ho 4*) was kept wet while the other part was dried (*Ho 5*). These two samples were hydrolysed, using a procedure analogous to that used on *Ho 2* and *Ho 3*. All hydrolysed samples were nitrated in a nitric acid/phosphoric acid/phosphorus pentoxide mixture (48 : 50 : 2) at 0°C for 4 hr. and their intrinsic viscosities $[\eta]$ in *n*-butyl acetate were measured. The nitrated samples *Ho 4* and *Ho 5* were dissolved in acetone, precipitated by pouring the solutions into water under stirring and then recovered by filtration (samples *Ho 6* and *Ho 7*, respectively). Such a reprecipitation removes the main part of the hemicellulose and cellulose of very low D.P.[49] The results

are collected in Table 2, where weight average $\overline{DP}$ values are computed with $K_m = 2.4 \times 10^{-5}$ [5,6] and with the monomer unit molecular weight 290.

The results show that the unextracted holocellulose fibres have a low accessibility to acid hydrolysis (D.P. decreases from 3 400 to 1 200). Drying before hydrolysis has only a minor effect (*cf. Ho 2* and *Ho 3*). Extraction of the main part of the hemicellulose (about 90 per cent.) increases the accessibility (*Ho 4*). Drying the fibres after extraction (*Ho 5*) further increases the accessibility. Reprecipitation removes low molecular weight material giving a net increase of the D.P. of the residue, but the effect of drying the alkali-treated fibres is still clearly shown (*cf. Ho 6* and *Ho 7*).

TABLE 2

$[\eta]$ and $\overline{DP}_w$ values for hydrolysed holocellulose samples

Sample hydrolysed	*Ho 2* w	*Ho 3* d	*Ho 4* w	*Ho 5* d	*Ho 6* w	*Ho 7* d
(η)	8.32	7.94	4.52	2.77	4.84	3.31
$\overline{DP}_w$	1200	1140	650	400	700	480

w = wet (never dried) fibres hydrolysed d = dried fibres hydrolysed

The increased accessibility to hydrolysis of the cellulose fibrils, after removal of hemicellulose and a subsequent drying of the fibres, can be interpreted along two lines. Removal of included (adsorbed) hemicellulose could open more of the cellulose fibril framework to the hydrolytic attack. Furthermore, it is likely that the shrinkage and the partial collapse of the fibre wall could 'crush' the crystalline fibrils. This would distort the lattice at the break-points along the fibrils and make the cellulose chains more accessible to hydrolysis at these points. Increased accessibility from drying of pulp fibres has been previously observed during sulphite cooking and interpreted as a fibre wall opening effect.[50] The occurrence of 'crushing' of the crystalline fibrils as a result of drying is in line with the observation that chemical grade woodpulp fibres give an X-ray diffraction pattern of lower resolution than the original wood.

All holocellulose samples described (Table 2) give considerably higher levelling-off D.P. (that is, longer micelles) than do chemical grade pulps from wood and cotton. In Table 3, a series of D.P. measurements for four commercial pulp samples are presented. The pulps were hydrolysed in boiling 1.25 M sulphuric acid and nitrated in the same way as the *Ho* samples in Table 2.

TABLE 3

[η], $\overline{DP_w}$ and $\overline{DP_n}$ values for chemical grade pulps, hydrolysed with boiling 1.25 M sulphuric acid

Sample	*He 1*	*He 2*	*He 3*	*MB*	*GO*	*CL*
Time of hydrolysis in hours ..	0.5	2	4	2	2	2
(η)	1.61	1.47	1.45	1.45	1.23	1.51
$\overline{DP_w}$	232	212	209	209	177	219
$\overline{DP_n}$	77.8	73.6	72.0	71.8	69.5	77.2
$\overline{DP_w}/\overline{DP_n}$ ..	2.98	2.88	2.91	2.91	2.55	2.84

He=hemlock, *MB*=maple and beech, *GO*=gum and oak, *CL*=cotton linters

The viscometric and osmotic measurements were made in *n*-butyl acetate, giving weight average ($\overline{DP_w}$) and number average ($\overline{DP_n}$) molecular weights, respectively. The *He* and *MB* pulps are chemical grade sulphite pulps from softwood (Western hemlock) and hardwood (maple and beech) having alpha-cellulose contents of 94.9 per cent. and 89.8 per cent., respectively. The *GO*

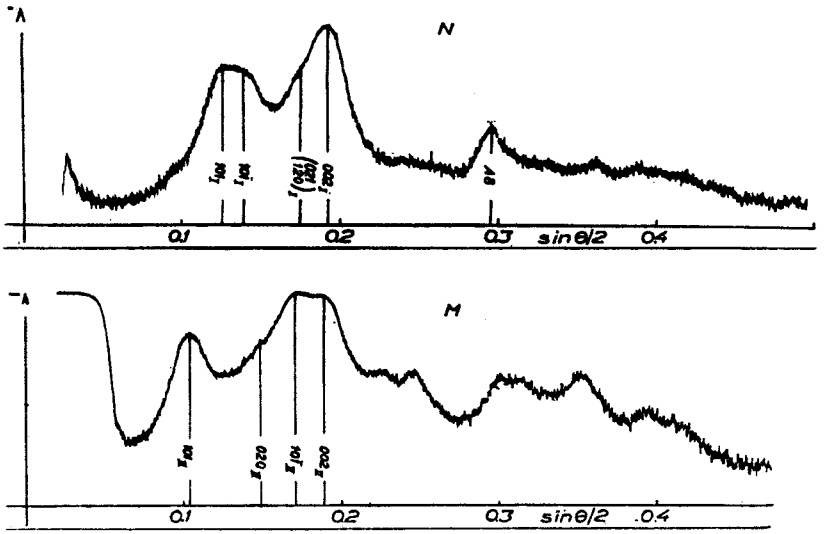


Fig. 19—X-ray diffraction patterns of native (*N*) and mercerised (*M*) celluloses—the intensities of the reflections 101_I and $10\bar{1}_I$ and 101_{II} are used to study the transition to mercerised cellulose

pulp is a prehydrolysed sulphate pulp from hardwood (gum and oak) with 93.5 per cent. alpha-cellulose and *CL* is a kier-boiled cotton linters pulp with 97.6 per cent. alpha-cellulose. All these samples are commercial rayon grade pulps and their levelling-off D.P. values, being of the magnitude 70—80 ($\overline{DP}_n$) or about 200 ($\overline{DP}_w$), are typical for chemical grade pulps.

Lattice order data from swelling experiments

Swelling with aqueous caustic soda is another important method of studying lattice order. Intramicellar swelling at a certain temperature occurs within a well-defined concentration range that is characteristic for the different types of native celluloses.[51] If the caustic soda is washed out with water at a temperature below about 60°C, the original native lattice (cellulose I) is transformed into mercerised cellulose lattice (cellulose II), which is indicated from

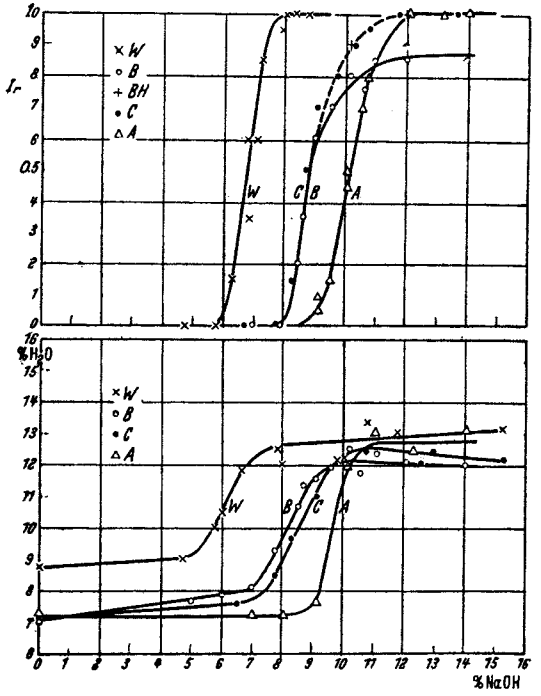


Fig. 20—Transitions to mercerised cellulose of native celluloses of different biological origin by swelling with caustic soda at 0°o—wood (*W*), bacterial (*B*), cotton (*C*) and animal (*A*) cellulose
The transition is indicated by X-ray diffraction (I_r) and by water regain (percentage H_2O) measurements

the X-ray diffraction patterns (Fig. 19). The swelling and the related transition (mercerisation) of the cellulose increases its water regain and the process can therefore be studied by sorption measurements. Transition diagrams for wood (*W*), cotton (*C*), bacterial (*B*) and animal celluloses (*A*), treated with aqueous sodium hydroxide solutions at 0°C are shown in Fig. 20.[52] It is evident that wood cellulose requires the lowest, cotton cellulose an intermediate and animal cellulose the highest caustic concentration range for mercerisation. This is well in line with earlier conclusions from X-ray studies (*cf.* Fig. 16), indicating that wood, cotton and animal celluloses have a more perfect lattice in the order mentioned. The transition studies have been extended to ramie (*RC*) and straw celluloses (*SC*) and the results indicate (Fig. 21) that the former behaves like a cotton cellulose and the latter like a wood cellulose. Ramie and cotton celluloses have nearly the same lattice order, but the crystalline regions in ramie show much better orientation along the fibre axis than those in cotton fibres. Of principal interest is the question whether the whole cotton plant contains the same type of cellulose as the

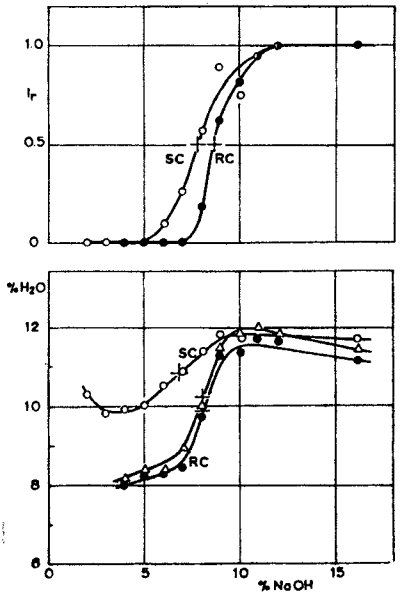


Fig. 21—Transitions to mercerised cellulose of native ramie (*RC*) and straw (*SC*) celluloses by swelling with caustic soda at 0°C

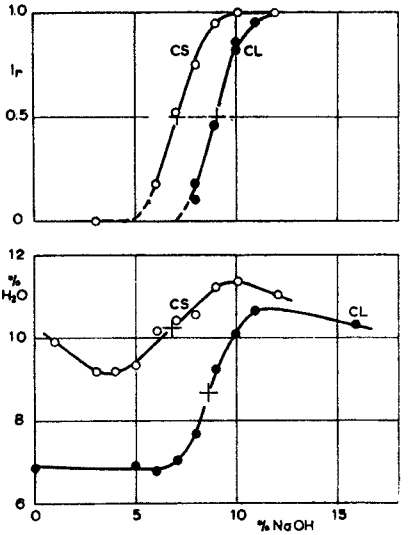


Fig. 22—Transitions to mercerised cellulose of native cellulose in cotton stalks (*CS*) and cotton linters (*CL*) by swelling with caustic soda at 0°C

cotton fibres forming the seed hairs. A sample of holocellulose from cotton stalks was prepared in the following way.

Cotton stalks from mature Empire cotton were supplied by Georgia Experiment Station, Experiment, Ga., U.S.A. Bark, bast and pith were removed and the stalk wood was chopped and cloven into 2—3 mm. thick and 4—5 cm. long rods. The air-dry, alcohol- and ether-extracted material (7.5 per cent. water) was treated with aqueous sodium chlorite at pH 4.6 and 50°—60°c for 4×6 hr. The holocellulose yield was 71.9 per cent. and the intrinsic viscosity $[\eta]$ of the nitrated pulp in *n*-butyl acetate was 21.0. This corresponds to a $\overline{DP}_w$ of 3000, if the pulp is treated as a pure cellulose.

The cotton stalk holocellulose (*CS*) showed closely the same transition region as a wood holocellulose when treated with caustic soda at 0°c (Fig. 22). For comparison, the transition curve for a cotton linters (*CL*) sample is included in the diagram (Fig. 22). Consequently, the cotton stalk cellulose is classified as a wood cellulose according to the swelling experiments.

Swelling experiments have also been performed with phosphoric acid solutions. The cellulose samples (1 g.) were disintegrated air-dry in a Waring Blendor, soaked in 50 ml. precooled (in icebath) phosphoric acid solution under vigorous shaking for 5 min. The closed Erlenmeyer flasks were then transferred to a 20°c thermostat for 60 min. The cellulose was recovered by pouring the reaction mixture into a large excess of icewater (1 litre). The precipitated cellulose was collected on a coarse glass filter and washed with water until a neutral reaction was given. The cellulose was then washed with absolute alcohol and dried in the air. The water regain behaviour of the resulting samples at 65 per cent R.H. (Fig. 23), shows a transition with the 50 per cent. point at 13.4 M phosphoric acid (80.2 per cent.) for the two woodpulps—*MB* is a sulphite pulp from maple and beech and *GO* a prehydrolysed sulphate pulp from gum and oak. The cotton linters pulp (*CL*) shows higher resistance against swelling with phosphoric acid with the 50 per cent. at 13.9 M phosphoric acid (82.3 per cent.). As a comparison, water regain curves for the same pulp samples treated with caustic soda solutions at 0°c are given in Fig. 24. This method also shows a difference between the two woodpulps, the sulphate pulp (*GO*) having a higher resistance towards swelling than has the sulphite pulp (*MB*). Taken together, this data is in line with earlier work [51b] where it was shown that hydrolysis with dilute mineral acid before swelling with caustic soda largely decreased the difference between sulphite and sulphate pulp cellulose. Recent work based on solubility in caustic soda after heterogeneous acid hydrolysis has verified these results.[53] With the assumption that acid hydrolysis of the fibres initially affects mainly

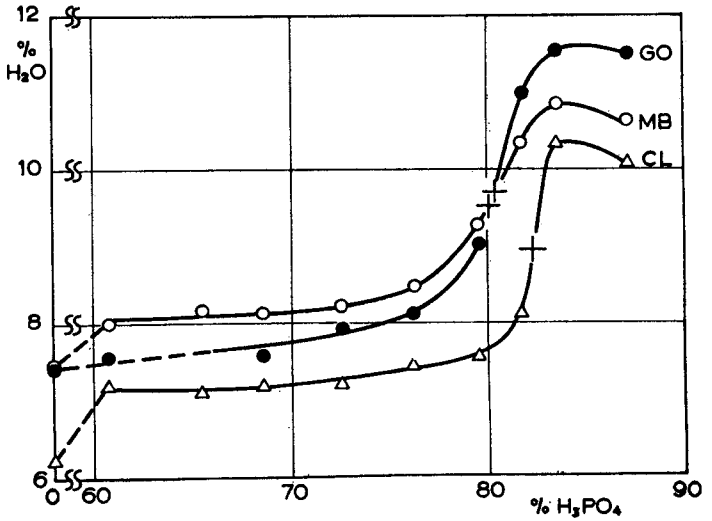


Fig. 23—Intramicellar swelling of two wood (*GO* and *MB*) and one cotton linters (*CL*) cellulose by swelling with aqueous phosphoric acid at 20°C, indicated as a sharp increase in water regain at 65 per cent. R.H.

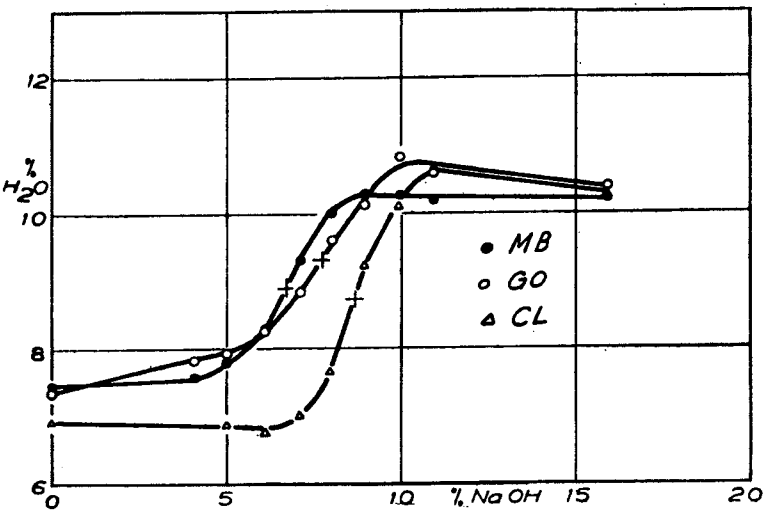


Fig. 24—Transitions to mercerised cellulose of two woodpulps (*MB* is a sulphite and *GO* a pre-hydrolysed sulphate pulp) and one cotton linters pulp (*CL*) by swelling with caustic soda at 0°C

the easily accessible (amorphous) regions, it can be concluded that the difference between sulphite and sulphate pulp celluloses is located mainly in the amorphous regions along with cellulose fibrils. But whether 'the sulphate effect' is a cross-linking, a more intense hydrogen bonding or any other type of closer packing is not known at the present time. It is important to note that the sulphate process has no measurable influence on the lattice order of the micelles after hydrolysis: but the sulphate process gives lower levelling-off D.P. values than the sulphite process (*cf.* Table 3).

Cotton and wood celluloses

The difference between cotton and wood celluloses is of great technical importance. Accordingly, it has been studied extensively. In the preceding two sections, the differences in X-ray diffraction patterns in rate of heterogeneous hydrolysis and in accessibility to lattice swelling have been described. From the following experiments, there is good evidence that the differences found are related to the properties of the fibrils rather than to the gross structure of the fibres.

The native cellulose fibres were hydrolysed with boiling 1.25 M sulphuric acid. After washing out the acid with distilled water, colloidal aqueous sols of cellulose micelles were prepared by peptisation.[55] The micelles were studied in the electron microscope and their transition to cellulose II during mercerisation was followed by electron diffraction.[51c] The transitions occurred within closely the same caustic concentration ranges as those observed before for purified cotton and woodpulp fibres (*cf.* [51b]). This means that the higher resistance against swelling for cotton cellulose compared with wood cellulose is related to the lattice order of the structural elements (the micelles) themselves. The electron diffraction work on micelles has been extended to ramie and wheat straw celluloses (the same samples as those referred to in the preceding section. The ramie micelles showed a transition interval of 8—10 per cent. sodium hydroxide at 0°C and the straw cellulose micelles one of 5—7.5 per cent. sodium hydroxide. These ranges agree fairly well with those earlier found for cotton micelles (8—10.5 per cent.) and wood cellulose micelles (5.5—8 per cent.), respectively.[51c]

A difference between wood and cotton cellulose has been found also in systems where the cellulose chains are in molecular solution—for example, dissolved in concentrated mineral acids, when no influence from fibre structure is to be expected. Cotton cellulose is depolymerised at a rate about half that of wood cellulose (chemical grade sulphite pulp) when dissolved in 80 per cent. phosphoric acid.[56] These results have been verified in later more

extensive investigations using the same solvent at different temperatures.[57] Two interpretations seem possible—(*a*) the greater polymolecularity (wider D.P. distribution) of the wood cellulose gives a larger number of end groups that are known to be hydrolysed at a higher rate[58] and (*b*) the wood cellulose chains have irregularities in their structure that increase the rate of hydrolytic depolymerisation. It does not seem likely that the end group hydrolysis alone can cause the difference, because the rate of depolymerisation is the same both at high and low D.P. levels (from 1 000 to 100). Some type of irregularity in the chain structure is more likely. 'Weak links' with a much higher rate of hydrolysis than the glycosidic bonds according to an earlier hypothesis[59] would give a high initial reaction rate, which has not been found experimentally. It has been observed, however, that the wood cellulose used has a higher content of carboxyl groups (0.39 per cent.—COOH) than has the cotton cellulose (about 0.1 per cent.—COOH). Reduction of the wood cellulose with sodium boron hydride ($NaBH_4$) at pH 9.2 decreased both its carboxyl group content and its rate of hydrolysis in phosphoric acid solution to the same levels as those of cotton cellulose.[57] Oxidation of the wood cellulose with sodium chlorite ($NaClO_2$) at pH 4.5 to a total —COOH content of 0.68 per cent. did not change the rate of hydrolysis, probably because the oxidation mainly affected the end groups. It is therefore conceivable that the presence of a few carboxyl groups along the wood cellulose chains—for example, on the sixth carbon atoms—could make the glycosidic bonds to the adjacent glucose units somewhat more reactive towards hydrolysis. This interpretation agrees well with the fact that reacting cellulose with nitrogen dioxide (NO_2), which preferentially oxidises the sixth carbon atoms, introduces instability in the cellulose chains and makes them depolymerise rapidly even in water solution.[60] Such carboxyl groups could also distort the lattice (decrease the lattice order) and make the wood cellulose micelles more accessible to chemical reagents. The presence of carboxyl groups along the cellulose chains could therefore explain to some extent the behaviour of wood cellulose compared with cotton cellulose, both in solution and in the solid state, but other factors might also be involved. Residual aldehydic or ketonic groups along the wood cellulose chains (not oxidised with sodium chlorite) could also be responsible for the higher rate of hydrolysis and other differences.

It should be observed that the celluloses from the higher plants can be divided into two groups—(*a*) celluloses of high lattice order such as in cotton and ramie, occurring with only minor amounts of other polysaccharides and (*b*) celluloses of low lattice order, such as in wood, stalk and straw and

occurring together with large amounts of other polysaccharides (hemicelluloses).[60a] It has been suggested that the lower lattice order of group *b* (wood celluloses) should be due to the presence of other monosaccharides (D-mannose, D-xylose, etc.) in the cellulose chains. An irregularity of this type would also offer a possible interpretation to the differences between wood and cotton celluloses. The existence of such a copolysaccharide still remains to be proved. The difficulty in removing residues of D-mannose- and D-xylose-yielding residues from pulps using conventional alkali extraction does not prove that these sugars are incorporated in the cellulose chains.[61] A much more complete separation of non-glucose constituents has been obtained by extraction of nitrated fibres with mixtures of ethyl acetate in ethyl alcohol.[62] Furthermore, it has been shown recently that the main part (71 per cent.) of the D-mannose in a sulphite pulp from spruce is incorporated in a glucomannan polysaccharide, which was isolated as a hemicellulose fraction with a mannose/glucose ratio of 3.5 : 1.[63] Small amounts of a similar polysaccharide have been isolated from hemlock pulp.[64] The existence of a glucomannan hemicellulose in wood can explain in a natural way the origin of disaccharides of glucose and mannose in hydrolysed wood cellulose[65] and the origin of the non-cellulosic D-glucose in wood.[66]

Whether the cellulose fibrils form a pure cellulose phase or not in wood fibres has been debated extensively in recent years.[67] The data presented above have shown that the main part of the hemicelluloses can be removed from pulps by extraction. If the small residues left in the fibres should be classified as part of the fibrils or as a separate phase may be a matter of definition. The hemicelluloses are dispersed throughout the whole framework of cellulose fibrils in the fibre wall, probably deposited simultaneously with the cellulose fibrils known to crystallise *in situ* during the growth process. It would not be surprising, if a few hemicellulose chains were trapped in the bundles of cellulose fibrils or even co-crystallised with the cellulose chains to some extent. The last residues of non-cellulosic polysaccharides are indeed tenaciously held by the cellulose framework in the native fibres.

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DISCUSSION

PROF. H. W. GIERTZ: It is obvious that the surface properties of the fibres are of the greatest importance for papermaking and therefore the primary wall (P) and the outer layer of the secondary wall (S1) are of special interest. The primary wall of fibres originating from different types of pulp was studied at the Fibre Chemistry Section of the Swedish Forest Products Research Laboratory during the years 1952-55. The technique used was the same as that used by Dr. Bucher and it is described in my paper for Friday morning (*see* page 397).

At this symposium, when dealing with the surface of the fibre, interest has been concentrated on both the primary wall (Bucher, Giertz) and S1 (Emerton). Before going on to present the results of our investigation, I should like to say why I have drawn the conclusion that the skin-like formations and fragments observed when swelling fibres stained with Victoria Blue in cuprammonium solution originate from the primary wall (and not from S1) —

1. Typical remnants of the middle lamella can be seen on the undissolved skins (Fig. 3 (*b*), page 406), which shows that the skin is the outermost part of the fibre.
2. The pit opening is covered with a membrane on which the *torus* can be identified (Fig. E). If the skin belonged to S1, the pit would be an open hole.
3. If fibres that in unbeaten condition show skins are beaten, the skin is removed, but the fibre in any case swells with ballooning (which demonstrates the presence of S1) and gradually dissolves completely (Fig. 3 (*e*), Page 408).

Using this technique with swelling and partial dissolving in cuprammonium solution, it has been possible to estimate the amount of primary wall that covers the fibre (for details, *see* pages 397, 398).

The quantitative value (as a percentage of the total fibre surface) for the fibre surface area from which the primary wall has been removed is called the *exposed surface*. This exposed surface has been determined for several different kinds of pulp, for instance —

Unbleached pulps	*Exposed surface, %*
Strong sulphite	0
Medium sulphite	14
Rayon grade sulphite	68
Strong sulphate	0
Soft sulphate	0

Bleached pulps

Unbleached sulphite	14
Bleached: Cl — Alkali — Hypo — Alkali — Hypo	72
Unbleached sulphate	0
Bleached: Cl — Alkali — Hypo — Hypo — Hypo	77
Bleached: Cl — Alkali — ClO_2 — Alkali — ClO_2	41

As can be seen from this table, the primary wall is attacked during sulphite cooking. In strong pulps, all fibres are covered with the primary wall; but, in softer pulps and rayon grade pulps, it has been ruptured to a great extent. Unbleached sulphate pulps seem all to be covered. The primary wall is, as could be expected, partly torn off during bleaching, hypochlorite having a more drastic effect than chlorine dioxide.

MR. G. HUNGER: We have clearly seen these parallel features in Mr. Emerton's pictures. It is interesting that, on the inside of the primary wall on top of secondary wall No. 1, we have seen the same features, definitely being microfibrils in our case. It has already been shown by Frey-Wyssling and Muhlethaler that the primary wall goes over at the corners of the cell into microfibrils running parallel to the cell axis. The primary wall of fibres of a spruce pulp, swollen in a swelling agent, are ruptured and fall away from the fibre; only microfibril bundles running parallel to the axis remain. Thus, it seems very likely that these longitudinal features found by us on top of layer S1 may be continued *under* S1, as Mr. Emerton's micrographs have shown.

PROF. B. STEENBERG: All the micrographs have been surface replicas. The technique of Dr. Asunmaa, using transmission electron micrography, gives some further information. In these studies, we have never seen anything we could call a primary wall in accordance with Mr. Emerton's use of the term, but we can see the S1 layer quite distinctly. If you dye it with osmium, S1 differentiates from the S2 layer, the former being more osmium-impregnated. If a flake of the surface comes off, penetration of osmium is possible from both sides, but the other layer is still quite distinctly osmium-coloured, whereas the other is not. So the dyeing of S1 is not just a matter of decreased penetration from the surface inwards. Whether S1 is only two layers or whether it is a multitude of layers, I will not say. From examination of cross-sections, I would say that it is very possible that there are many more than two layers. Of course, looking from the surface as in replicas, you may see whether there is a third and a fourth layer. Cross-sections are valuable here and they indicate the existence of more than two layers.

MR. Ö. ELLEFSEN: I want to make a few comments in connection with the paper read by Dr. Rånby. He referred to work we published a few years ago on cellulose structure.* This investigation was carried out on unoriented specimens of regenerated cellulose from viscose solutions and it is quite correct that we could not find the X-ray results consistent with the well-known chain structure of cellulose suggested by Meier and co-workers.

We tried both models mentioned by Dr. Rånby (the boat form and the chain form) and neither could be interpreted by means of the X-ray results.

In a more recent investigation,† amorphous cellulose, both native and mercerised, has been subjected to the same technique and we have so far not been able to confirm the chain proposed by Meier and Misch. As the distance between the two hydrogen atoms connected with the carbon atoms on both sides of the glucosidic link in this model is only about 1.7Å, we tried to interpret our X-ray findings by means of a certain amount of twisting in the glucose units and, as Dr. Rånby mentioned, we found the best result was obtained when a twist of about 45° was introduced.

I want also to point out that, in recent work carried out by Dr. Norman‡ on oriented specimens of ramie and fortisan, neither model was compatible with the X-ray findings.

I would like also to mention that Dr. Mering and co-workers in France have found in mercerised or regenerated cellulose that the chain structure put forward today by Dr. Rånby and first mentioned by Dr. P. H. Hermans in Holland is the correct one.

Another point is Dr. Rånby's reference to the determination of crystallinity in cellulose specimens by Hermans' method. We also have tried to develop a similar method,† but the results are somewhat different from Dr. Hermans' findings. The general value he found for native cellulose is about 70 per cent. crystalline matter and 30 per cent. non-crystalline matter. With our method, the figures obtained were about 50/50. Of course, as Dr. Rånby pointed out, all these methods are just average methods and what we really need is a method to give a real order/disorder distribution in cellulose specimens. The method we have developed gives automatically a *maximum* value for the amorphous part of the specimens. It is interesting, however, that, if we start with the model of microfibrils 30 Å × 100 Å dis-

* Björnhaug, A., Ellefsen, Ö. and Tönnesen, B. A., 'Interpretation of X-ray Diagrams of Unoriented Organic Chain Polymer Substances — 3. Regenerated Cellulose': *Norsk Skogind.*, 1953, **7** (6), 171

† Ellefsen, Ö., Wang Lund, E., Tönnesen, B. A. and Öien, K., 'Studies on Cellulose Characterisation by Means of X-ray Methods: *Norsk Skogind.*, 1957, **11** (8), 284; (9), 349

‡ Norman, N., Medelelse No. 219, Universitetets Fysiske Institutt (Oslo, 1954)

cussed by Prof. Frey-Wyssling yesterday, we find that, of the 120 cellulose chains that can be present in this structural unit, 48 lie in the surface portion. These 48 chains can easily account for the high amount of non-crystalline material found by means of our method.

Before finishing, may I emphasise that I find it very promising that the problems of cellulose chain structure are being tackled by scientists in different places (Dr. Rånby referred to Japanese work in progress), as I think we really have to know the exact chain structure and arrangement in order to understand why the fundamental microfibrils have just the size we observe in the electron microscope.

DR. J. SIKORSKI: I should like to comment on the following three points—(*1*) the orientation of the crystallographic planes in the microfibrils, (*2*) the morphology of the microfibrils and (*3*) the general significance of the microfibrillar elements in cellulose and other fibres.

The original observations of the preferential orientation of the (101) crystallographic planes in the microfibrils of native cellulose were made[1] on films prepared from dried aqueous suspensions of the colloidal particles obtained from cotton, ramie and jute, using a method of acid hydrolysis similar to that of Rånby.[2] We have not been successful, however, in preparing homogeneous films (containing exclusively individual particles) and it would therefore be of some interest to enquire whether our findings were confirmed or otherwise by other workers. The difference in intensity between the (101) and ($10\bar{1}$) reflections of cellulose (in two diffraction photographs, one with the X-ray beam parallel and the other perpendicular to the surface of the films of particles) could only be interpreted to indicate that the (101) planes are preferentially oriented parallel to the surface of the film and, consequently, to the larger face of the microfibril.[1]

Estimation of the height of cellulose microfibrils involves calibration of the shadowing angle and measurement of the length of the 'shadow' in electron micrographs. With reference to the former, no indication is given of the method used by Morehead for this purpose; in our own work, latex particles were employed throughout (*see* Fig. 6[3]). Rånby suggests, however, that the figures quoted by us for the height of cellulose particles are too low, because of the reduction of the shadow length by the deformation of the supporting membranes. Our observations were not confined to a restricted area of one grid and close examination of the data for the coefficient of

1. Mukherjee, S. M., Sikorski, J. and Woods, H. J., *Nature*, 1951, **167**, 821
2. Rånby, B., *Acta Chem. Scand.*, 1949, **3**, 649
3. Mukherjee, S. M., Sikorski, J. and Woods, H. J., *J. Text. Inst.*, 1952, **43**, T 169

variation obtained in our experiments (*see* Table I[3]) indicates that wide variations in the shadowing angle could not have occurred. The other possibility, that of the formation of 'local imprints' under individual particles, is also very unlikely, as the weights of a single particle and of the equivalent area of supporting film are of the same order of magnitude. Furthermore, some experimental evidence, involving much larger microfibrils (obtained from synthetic polymers) refutes such a suggestion.

The third point, that of the significance of the microfibril, is perhaps of the greatest interest to all of us working in the field of fibrous structures. It is evident that the old concept of a two-phase system (crystalline and amorphous) in polymers requires some revision and one is now forced to accept the well-known idea of Frey-Wyssling advanced before the advent of the electron microscope. It is reasonable to regard the microfibrils as containing a greater proportion of the crystalline material[4] than mainly amorphous interfibrillar regions. Furthermore, it is necessary to accept the views of Fürth[5] who suggested that it is the basic thermodynamic restriction that limits the size of the intrinsic structure elements of all biological objects and crystalline materials in general[4] to an order of magnitude a few hundred times larger than atomic dimensions.

DR. A. MYERS: I wish to draw attention to some of the work we have been doing recently at Leeds University on the structure and composition of the microfibrillar fractions in the cell walls of algae.

In electron micrographs of chemically isolated microfibrils from *Rhodymenia Palmata* (a red alga), the microfibrils appear to be quite normal cellulose fibrils and yet, upon hydrolysis, they yield a mixture of 50 per cent. glucose and 50 per cent. xylose.

Electron micrograph examination of a piece of blended wall of another alga, *Porphyra*, has shown the microfibrils to be surrounded by an amorphous matrix, which can be largely removed by treatment with boiling water. When the wall was further treated by a cold normal solution of alkali, the microfibrils were broken down into particles of pure mannan. The polysaccharides removed during the alkali treatment yielded, on recovery and hydrolysis, a mixture of the sugars galactose, xylose and mannose. In no fraction of the cell wall was glucose detected.

I should like to draw attention to these facts, in view of the assumption often made that all microfibrils are cellulosic.

4. Balashov, V. and Preston, R. D., *Nature*, 1955, **176**, 64
5. Fürth, R., *Exp. Med. Surg.*, 1955, **13**, 17

An electron micrograph of the microfibrils from an unusual wood specimen was examined. It is typical of that obtained from present-day wood, yet the specimen is of oak recovered from the original wall around the city of York and is about 2 000 years old. Analysis shows that only 37 per cent. of the dry weight of this ancient wood is cellulose — a loss of about 15 per cent. compared with modern oak. This 15 per cent. loss has in no noticeable way changed the appearance of the microfibrils. It would be dangerous to deduce anything further from these results, but it is tempting to correlate the loss in weight with the disappearance of amorphous cellulose, which is in the same order of magnitude.

DR. B. G. RÅNBY: I will not comment about the sample of York wood, because it has had plenty of time to crystallise and deteriorate in 2 000 years. Let me say that, of course, it is not only cellulose chains that crystallise into fibrils, because nylon does it and even polyethylene does it, especially when oriented by stretching. We should not be too surprised, therefore, if we find mannans, xylans and other chain molecules forming fibrils. Cellulose has a very strong inclination to form fibrils, because its molecules are stiff, straight and probably unbranched chains.

Let me take the speakers in reverse order.

When I commented on Dr. Sikorski's and Dr. Wood's work on microfibrils some years ago, I may have dealt inadequately with the methods they used.

We did not assume the imprint or sagging of the membranes because of the weight of the objects studied. The mass of cellulose micelles is of the order 10^{-18} g. and the weights involved are negligible in comparison with the surface forces at the contact area between the particles and the membrane. When Dr. Sikorski now presents width/thickness ratios of about 4, I would say this is in fair agreement with our ratio of about 3. We did not agree with your reported fibril thickness 18-16Å, but your present values of about 30Å are very close to ours.

We were very anxious to use the glass surface as membrane support to prevent these imprint or sagging effects, but, if similar results can be obtained without using a glass surface, that is excellent.

I think the evidence Sikorski and Woods brought out about the orientation of the (101) plane in deposited micelle films is very convincing, even if they did not have more than 90 per cent. particles in their specimens. The 10 per cent. aggregates, etc. would cause background scattering, but the evidence is conclusive enough.

On the question of the significance of microfibrils, we have to rely on the evidence from the electron micrographs, which agrees with earlier data from X-ray scattering and surface area measurements.

Now, I wish to comment on Mr. Ellefsen's X-ray data for the amount of cellulose in the micelle surfaces. From earlier work, we obtained an X-ray crystallinity of about 70 per cent., but only some 10 per cent. easily accessible cellulose, for example, in heterogeneous hydrolysis. The difference was explained as a micelle surface effect. With only 50 per cent. crystallinity by the Ellefsen method, we have to assume an even more extensive surface layer. With these data, too, one must accept the fibril surface layer as largely inaccessible to acid hydrolysis. These layers are indeed so well organised and ordered in cotton and ramie cellulose that constant boiling hydrochloric acid does not seem to penetrate or attack the cellulose micelles from the sides, only from the ends according to A. Sharple's interpretation. That is not the case with native wood cellulose, when some hydrolytic attack on side surfaces has to be assumed.

Then there is the question of the carboxyl groups in the wood cellulose, which no one has brought up. Do not quote me as saying that the carboxyl groups are responsible for the high rate of hydrolysis of wood cellulose in phosphoric acid. I have said that we found an increased stability with decreasing carboxyl groups content. We have still to investigate the effect of the aldehyde groups we tried to oxidise. We used sodium chlorite for this purpose and it is quite possible that we oxidised only some of the groups (probably the end groups) and others (of another type) still remained. Maybe, it was just this other type of aldehyde group that we reduced with sodium borohydride ($NaBH_4$) and that was responsible for the stabilisation effect we obtained. Again, we have to say that this is all we know, but the work is in progress. We still have these two possibilities — the carboxyl groups, against which we have evidence and the aldehyde groups, against which we have no firm evidence at the moment, because we lack the analytical data.

It was also said that the carboxyl groups are not reduced by sodium borohydride. At least, we know that the carboxyl group content as we analysed it was decreased when we carried out this reduction. Furthermore, we also tried the reaction with polybutylmethacrylate, which was reduced to about 98 per cent.

MR. H. W. EMERTON: My comments will be largely concerned with dispelling what are, I believe, a few misconceptions. First of all, with one exception, the pictures I showed this morning were light micrographs and not electron micrographs.

Then there is the question of those longitudinal features that I showed. Some people have asked me if there is any possibility that these are the same as the lignin ridges to which Dr. Bucher drew attention yesterday. I do not for one moment think that they are. At the same time, I am not denying the existence of those lignin ridges external to the fibre; both are undoubtedly present.

With regard to the location of the longitudinal features that I showed this morning, I do not say that they would never be on the outside of S1, but quite definitely in certain cases they are on the inside of this wall. In those cases when we can, with complete confidence, say that S1 is folded back so that we observe its inner surface, it can be seen that the longitudinal features are on the inner surface. We have never with certainty identified them on the outside of S1.

Prof. Steenberg mentioned that in some pictures more than two layers might be revealed in S1. I would subscribe to that possibility.

Dr. Sikorski mentioned our figure of $\pm 2°$ in the shadowing angle. These were preparations metal-shadowed for the light microscope, so that, of course, the specimens were dried on a glass slide prior to shadowing. The uncertainty in the shadowing angle is almost entirely due to a difference in the angle subtended by the metal source at the two ends of the slide.

PROF. STEENBERG: Dr. Rånby brought up the question of surface tension, which I wish to comment further upon. I should very much like any of the speakers to go a little further into the details of what effect on the final features the surface tension will have. I am quite sure that this is a very important thing. There may be effects due to surface tension against the surface on which the fibre material is dried. Would you think that it is possible to say how much of these different structural features are influenced by surface tension, especially surface tension against the surface on which the material is evaporated?

DR. RÅNBY: This is very important and I think it should be observed. I have been told by papermakers that you cannot make paper from a pulp suspended in an organic liquid. In this case, we did not put the particles on the glass surface. The collodion or formvar membrane was coated on the glass surface; the cellulose suspension was then dried on the membrane and shadowed. The membrane was then stripped from the glass and studied in the electron microscope. This technique has been developed in our laboratories by F. F. Morehead and we think it is a safe method to avoid

membrane distortion effects. The results show that we have somewhat higher values for the fibril thicknesses than have Sikorski and Woods.

Frankly, we do not know very much about contact angles or the adhesion between cellulose and collodion and even less about the surface tension between cellulose and formvar. There is a lot to be done here and I hope those who are still working with the electron microscope will do it.

PROF. STEENBERG: If you take a wet membrane from a cellulose or some cellulose derivative preparation and dry it on glass, it may stay like a membrane on the glass; if you dry it on teflon foil, you may find no membranes, but a woolly, hairy-looking material. They look like two entirely different materials. Surface tension may change the appearance of very fine particles: that is the point I feel is very important.

MR. EMERTON: If I may comment on this question of surface tension, I have three illustrations to show. Surface tension has very marked effects both upon the fibre itself and upon the membranes of microfibrils that peel away from it. When a wood fibre is dried on to a glass slide, we observe that the lumen usually collapses and the fibre is pulled down flat by surface tension. So far as pine and spruce are concerned only a small proportion of latewood fibres resist this and, as beating proceeds, these too tend to collapse.

Fibrillated sheets of microfibrils are apt to react to surface tension in two ways. They tend to be pulled into close contact with the substrate and, if this is cellulosic (or even glass), they bond firmly to it so that they are prevented from shrinking on themselves. Such membranes are usually taut, because at quite an early stage of drying they bond to the surface with which they are pulled into contact; any fibres to which they are attached continue to lose water and shrink, thereby stretching the membrane. Secondly, the contracting water envelope surrounding a membrane that is suspended between two fibres gathers up the sheet of microfibrils and it finally bonds to itself, as Steenberg said, like a curling-up leaf. These effects are illustrated in Fig. F and G.

Fig. E—The primary wall from a strong sulphite fibre after the secondary wall has been dissolved in cuprammonium solution — note the dark longitudinal formations that originate from the middle lamella and the *torus* fixed on the membrane in the pit opening

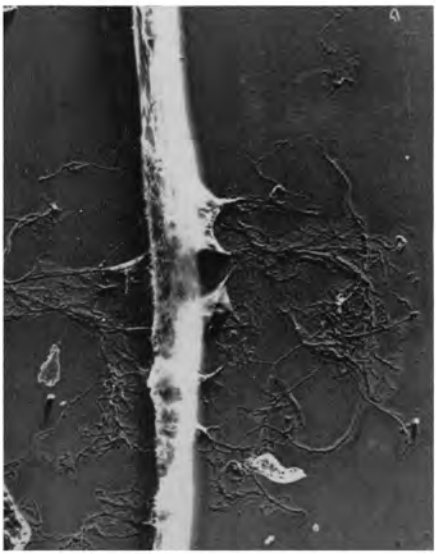

Fig. F — Bonding of fibrillated membrane to substrate and effect of surface tension (light micrograph × 250)

Fig. G — Effect of surface tension on a fibrillated membrane between two fibres (light micrograph × 250)