Fibrillar Size in Native Cellulose

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Synopsis

Fibrillar size of native cellulose was studied in detail by electron microscopic and x-ray techniques. Samples included natural fibers of cotton and ramie as well as algal and bacterial celluloses. Results indicated smaller sizes for cotton and ramie than was previously reported in the literature and a possible difference in fibrillar sizes among celluloses.

INTRODUCTION

Much ambiguity exists concerning the diameter or size of the cellulose fibril.[1–12] Much of this ambiguity may stem from the techniques and methods used to determine this size.[13] These methods include x-ray small-angle scattering, wide-angle line broadening, and electron microscopic techniques. All of the above methods have been considered suspect. The shape and perfection of the crystallites affect x-ray data. Metal shadow casting and heavy metal staining as used in electron microscopic studies of cellulose have long been a source of controversy. Indeed, all sample preparation methods for electron microscopy are rigorous. As Ohad states, real differences in microfibrillar size do exist both within and between species of cellulose.[14]

Because fibrillar size is an important factor in the chemical modification of cellulose, a detailed study of cellulose fibrillar size was undertaken, and the results are outlined in this article.

EXPERIMENTAL

The celluloses studied were cotton, ramie, an algal cellulose (*Valonia ventricosa*), and a bacterial cellulose (*Acetobacter xylinum*). The cotton fibers were purified according to the ethanol extraction method of Conrad,[15] followed by boiling in 1% NaOH under nitrogen. The ramie was extracted with ethanol, refluxed with monoethanolamine for 2 hr, and boiled in 1% NaOH for 6 hr. The *Valonia* was boiled in the 1% caustic solution for 6 hr and acid rinsed in 0.05*N* HCl. The bacterial cellulose was prepared according to the method of Colvin.[2] After purification, all specimens were washed with water and air dried.

Samples of each specimen were beaten in a Sorval Omni-Mixer homogenizer** in distilled water. The suspensions were dropped onto carbon-coated 200-mesh stainless-steel grids and allowed to dry. Grids were negatively stained with a

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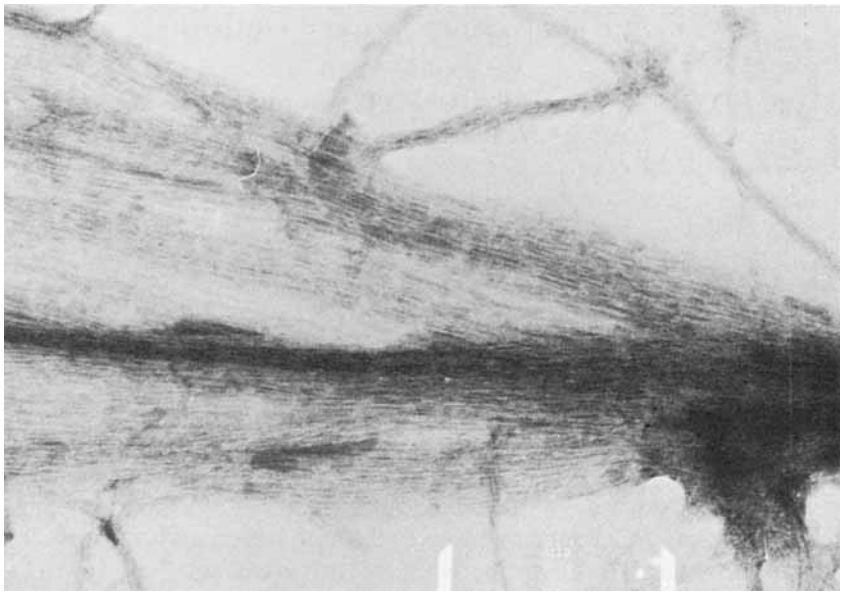

Fig. 1. Cotton fragment negatively stained with phosphotungstic acid; distance between marks represents 0.1 μm.

2% phosphotungstic acid solution, pH 7. The samples were studied in a Philips electron microscope (model EM-300).

For x-ray analysis 150-mg samples of each specimen were ground in a Wiley mill to pass a 20-mesh screen and pressed into a disk under a pressure of 1.7×10^8 Pa. Wide-angle x-ray diffractograms were obtained with a Philips Norelco x-ray defractometer in the focusing geometry arrangement. Allowances for instrumental factors were used according to the method of Stokes.[16] X-ray crystallinity was estimated according to Segal.[17]

RESULTS AND DISCUSSION

Typical results of the heavy metal staining are shown in Figure 1. Results of size measurements by the two techniques are given for each cellulose in Table I. Values reported from EM techniques represent the mean of approximately 500–1000 measurements in all samples. There was a wide distribution of size measurements in all samples. Values as small as 15 Å for ramie to over 100 Å for *Valonia* were observed.

TABLE I
Results of Size Measurements by the Two Techniques

	EM neg stain (Å)	X-ray line broadening (Å)	X-ray crystallinity index (%)	Probable error
Cotton	22	40	88	±0.31
Ramie	25	44	90	±0.20
Bacterial Cellulose	53	55	98	±0.36
Valonia ventricosa	107	112	96	±0.66

The average size for ramie and cotton are considerably smaller than previously reported in the literature.[12] This smaller size is more pronounced in the EM data than in the x-ray results. Crystallinity data given in Table I indicate that cotton and ramie are probably more accessible to the electron dense stain than are the other two celluloses. This accessibility may account for these smaller fibrillar values. This effect would not be as noticeable in bacterial cellulose or *Valonia* where the crystallinity values approach 100%.

Cotton and ramie may possess a different crystalline unit cell than *Valonia* and bacterial cellulose. It may be that a packing variation because of this difference is revealing itself in the electron dense stain.

The data in Table I seem to indicate that there is, as Colvin states,[2] a difference in fibrillar size among celluloses. Cotton and ramie seem to have elementary fibrils that are considerably smaller than those found in the other two celluloses. The samples used for EM work were fragmented mechanically and not with ultrasonics. This technique may not break down the fibril bundles to their minimum size, especially in the highly crystalline samples. This possibility may also explain the close agreement between x-ray and EM values for the higher crystalline materials.

This works suggests that there are several minimum fibrillar sizes among celluloses. Cotton and ramie possess identical basic units, but those in bacterial cellulose are approximately twice this size and as much as four times this size in algal cellulose.

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