ON THE DIMENSIONS OF CELLULOSE MICROFIBRILS

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INTRODUCTION

The width of *metal shadowed* cellulose microfibrils from plant (1, 2), bacterial (3–6), and animal (7) sources was found to vary between 70 and 300 A according to different authors. During an electron microscope investigation on the formation of extracellular cellulose fibers by *Acetobacter xylinum* the image width of shadowed microfibrils, including the added metal, was found to be about 12 mμ, their thickness as calculated from the shadow length being about 2 mμ (8). It was demonstrated that the real width of a microfibril could be deduced by accounting for the contribution to the image width by the shadow casting metal (9). The width thus found for the bacterial cellulose microfibril was 3 mμ (8, 9). Moor reported the dimensions of 3 to 4 mμ for the elementary fibrils of plant cellulose, using a platinum-carbon replica method (10). Similar width dimensions were reported by Mühlethaler for plant cellulose (11) and by Frey-Wyssling and Mühlethaler for bacterial cellulose microfibrils (12) using the negative staining technique. However, in a recent paper J. R. Colvin (6) maintained that the diameter of a microfibril is 15 mμ to 20 mμ rather than about 3 mμ.

In Frey-Wyssling and Mühlethaler's papers the 10 to 20 mμ microfibrils are composed of elementary fibers (3.5 mμ). The microfibrils of these authors correspond to what we have called in a previous paper (8) "composite fibers," and their "elementary fibril" corresponds to what we have called a "microfibril." Colvin, in his paper (6), does not distinguish between these two structural units. He denies the existence of "microfibrils" or "elementary fibrils" about 3 mμ in width.

In the present communication results of analysis of plant and bacterial cellulose by negative staining and shadow casting are presented.

EXPERIMENTAL

Cellulose from corn coleoptiles was prepared as described elsewhere (13). Native *A. xylinum* cellulose was prepared and purified as previously described (14). Specimens for electron microscopy were prepared from both corn and bacterial cellulose suspensions containing about 20 μg dry weight cellulose/ml.

Negative staining (15) was carried out with 2 per cent phosphotungstic acid (PTA) (final concentration), adjusted to pH 6.4 with NaOH, containing 0.2 per cent sucrose and traces of an anionic detergent.

RESULTS AND DISCUSSION

Electron micrographs of metal shadowed corn and bacterial cellulose show microfibrils as well as composite fibers (Fig. 1). In corn cellulose preparations networks resembling membrane structure are also observed (Fig. 2). The average image width of corn and bacterial microfibrils perpendicular to the direction of the shadow was 9 and 12 mμ, respectively. The minimal width measured was 4 mμ and the maximal 15 mμ.

The height-to-shadow ratio, as measured with the aid of latex spheres on the preparation, varied from 1 to 4 to 1 to 6. When the measured image widths of several shadowed fibrils were plotted against the sine of the angle (β) between the long axis of the fibril and the direction of the shadow, and extrapolated to $\beta = 0°$ (9), the real width was found to be about 3 mμ (Fig. 3). Similar dimensions were measured on PTA preparations of bacterial (Fig. 4 *a*) and corn cellulose (Fig. 4 *b*). There are two peaks in the graphic representation of the measurements, 3 mμ and 2 mμ. The 3 mμ peak is by far predominant (Fig. 5).

The results obtained from the measurement of shadow-cast material yields 2 mμ for the height (deduced from the shadow length) and 3 mμ for the width (according to the extrapolation method). The data in Fig. 5 show that in the negatively stained material the 3 mμ width is predominant, some (rare) fibers showing up to 4 mμ. In view of these results it follows that the cellulose microfibril (elementary fibril in Frey-Wyssling and Mühlethaler's nomenclature) is of a rectangular cross-section about 2 by 3 mμ. This is in only slight disagreement with the results of Frey-Wyssling and Mühlethaler (11, 12) who consider the elementary

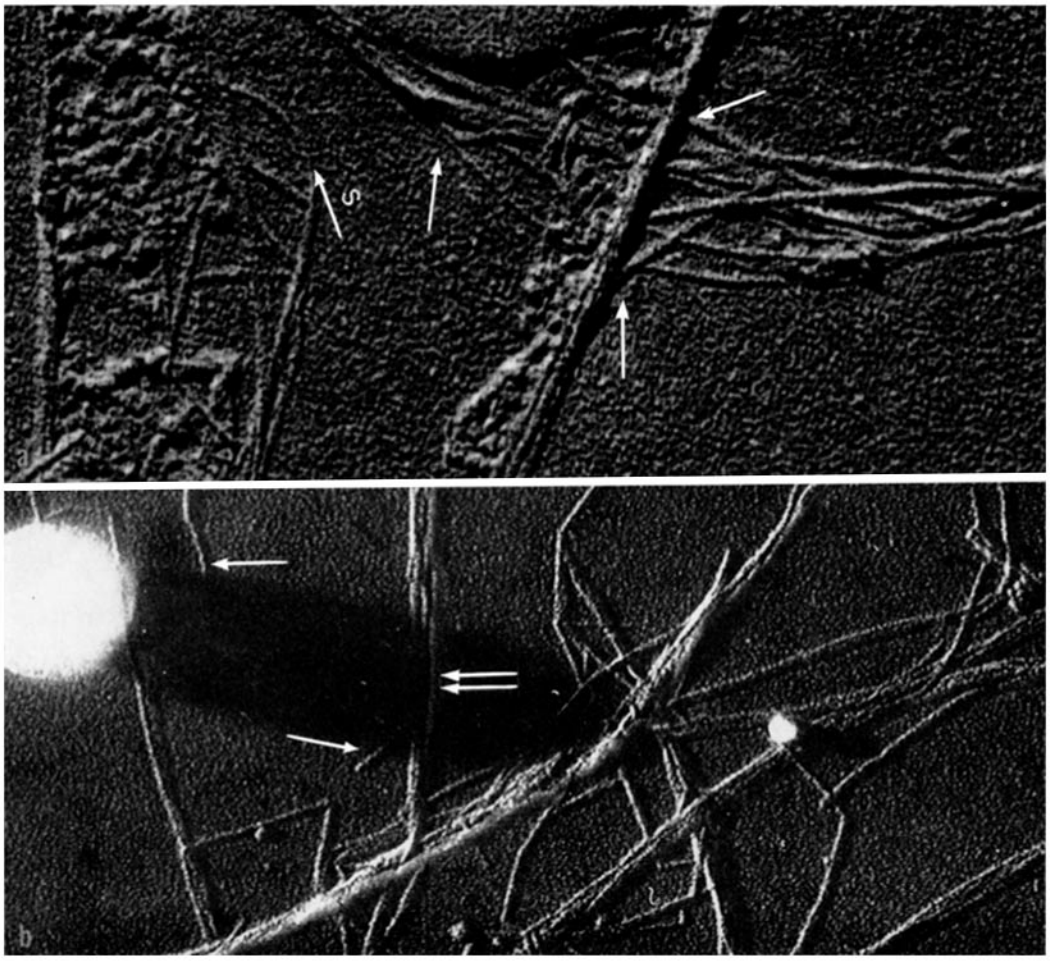

FIGURE 1 Metal shadowed preparations of bacterial and corn cellulose showing microfibril and composite fibers. Note the microfibrils disappearing in the shadow (arrow) and the composite fibers still recognizable in the shadow (double arrows). The image width of the microfibrils diminishes as a function of the angle between the shadow direction and their long axis ($\overset{s}{\longrightarrow}$). The thicker appearance of the microfibrils in the bacterial cellulose preparation is due to heavier shadow.

Fig. 1 *a* is bacterial cellulose; Fig. 1 *b* is corn cellulose. Shadow cast with platinum at a height to shadow ratio of 1 to 5. × 100,000.

fibril's cross-section as a square of 3.5 × 3.5 mμ. However, we find a second group of width measurements in the negatively stained material with a peak at 2 mμ. These are relatively rare and we consider them as images of microfibrils standing on their narrow edge (Fig. 5, see also Fig. 4).

Colvin's criticism of the accuracy of the measurement of negatively stained fibrils (6) is based on the assumption that cellulose microfibrils are circular in cross-section. Since the cross-section of the microfibril was previously found to be rectangular (8, 11, 12, 16) and confirmed in the present work, an error due to an eventual circular cross-section is very unlikely.

The extent to which metal added to the microfibril by shadow casting contributes to the width of the image has been discussed by many authors (10, 17–21). The image width is enlarged by a multiple of the thickness of the deposited film when measured in the direction of the shadow (19). The argument that "metal shadowing as commonly practised need not bring to erroneous conclusions"

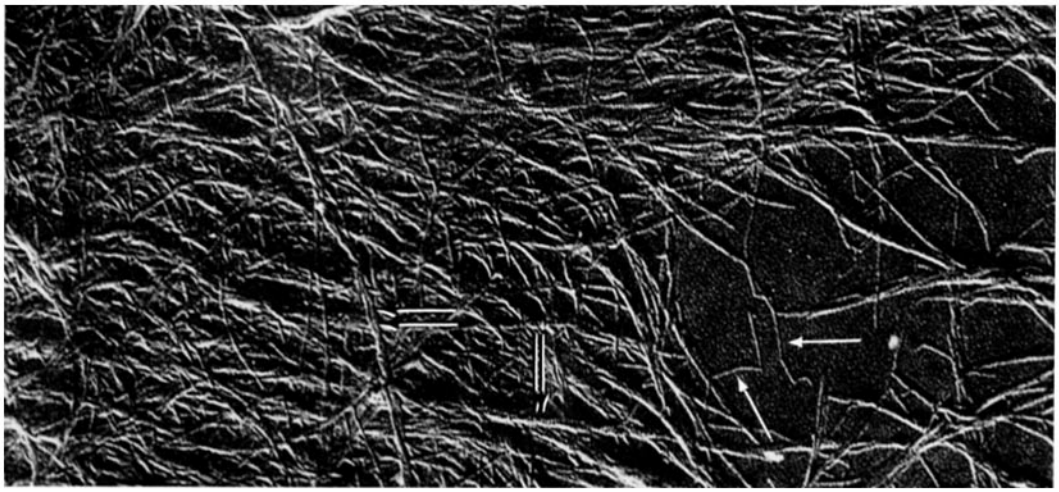

FIGURE 2 Corn coleoptile cellulose showing networks resembling membrane structures. The presence of microfibrils (single arrow) together with composite fibers (double arrows) and membrane-like structures indicate a rather mild sonication during the preparation of the cellulose fibers for electron microscopy. × 50,000.

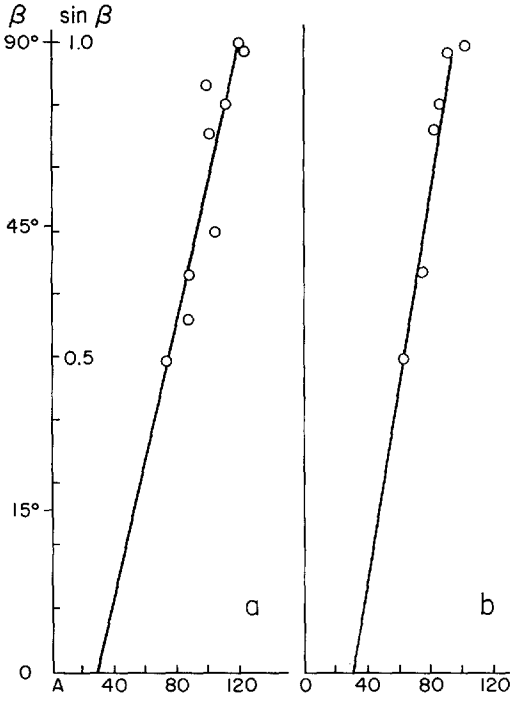

FIGURE 3 Measurement of the width of cellulose microfibrils on micrographs of shadow cast preparations. The image width is plotted against the sine of the angle β between the long axis of the fibril and the direction of the shadow. Extrapolation to $\beta = 0$ (9) yields the real width of about 3 mμ for both (*a*) bacterial microfibrils and (*b*) corn microfibrils.

was ruled out in a previous paper describing the method for estimating the width of elongated particles by the amount of metal added as a function of the angle between the direction of shadow and the long axis of the particle (9). Fig. 3 of the present paper confirms these findings for cellulose microfibrils.

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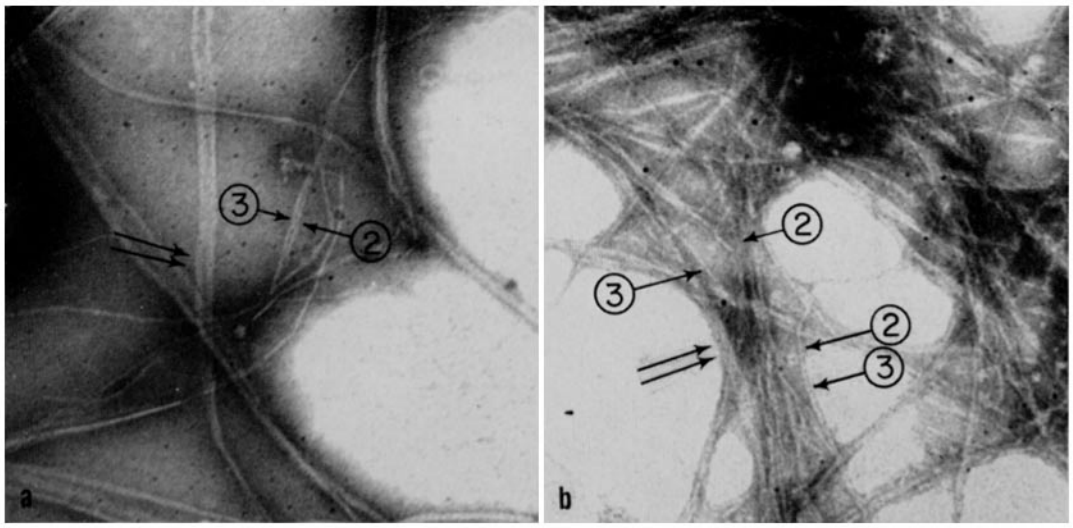

FIGURE 4 Electron micrographs of negatively stained cellulose fibrils. ③→ measured width about 3 mμ; ②→ measured width about 2 mμ; (⇉) composite fibers.

Fig. 4 *a* is bacterial cellulose; Fig. 4 *b* is corn cellulose. ×100,000.

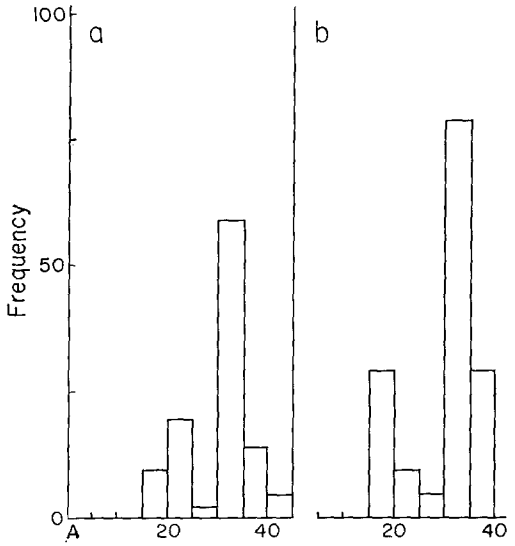

FIGURE 5 Distribution of width of microfibrils measured in electron micrographs of negatively stained preparations, showing two peaks—at 3 mμ and 2 mμ; the 3 mμ peak is predominant.

Fig. 5 *a* is bacterial cellulose, Fig. 5 *b* is corn cellulose.

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