THE MICROCRYSTALLINE STRUCTURE OF
CELLULOSE IN CELL WALLS OF COTTON,
RAMIE, AND JUTE FIBERS AS REVEALED
BY NEGATIVE STAINING OF SECTIONS

A. N. J. HEYN

From the Department of Biology, Louisiana State University, New Orleans

ABSTRACT

With a new technique of negative staining of sections, it has been possible to observe directly, in ultrathin sections under the electron microscope, the original microcrystalline and microfibrillar structure of cellulose as it occurs in living cells. This method has advantages over the study of isolated fibers used so far by others, in that the original arrangement of microfibrils is better preserved, and their collapse into larger fibrillar units is prevented. With this method, the cell walls of ramie, jute, and cotton fibers have been studied. The size (diameter, 25 to 40 Å) and the longitudinal periodicity observed in the single microfibrils and the orientation and spatial arrangement of the microcrystallite within the microfibrils are found to correspond with the latest models derived by others from data obtained by indirect methods such as X-ray diffraction. The microfibril size of about 35 Å, found by measuring these structures in sections, agrees with the latest conclusions reached by others in recent work with isolated fibrils.

INTRODUCTION

Electron microscope studies of the microcrystalline and microfibrillar structure of cellulose in cell walls of fibers have been limited, until now, almost entirely to the study of air-dried, metal-shadowed fragments and surfaces, shadowed surface replicas, and isolated fibrils. Although valuable information has been obtained on the fibrillar and microfibrillar structure of these materials as a result of these studies (for reviews of the literature, see references 9 and 32), the precise, detailed arrangement and the size and shape of the microcrystallites within this fibrillar structure, could not be studied unambiguously in the ultrathin sections under the electron microscope. The reasons for this are as follows:

The metal-shadowing technique requires the use of fully dried, nonembedded fragments of the material. The removal of water will generally cause a collapse and change of the original microcrystalline structure, as it is present in the living cell, both by the formation of conglomerations of elementary microfibrils into larger composite fibrils and by the transition of amorphous molecules into the crystalline state. When the material is dried, the former type of change is probably more prevalent; X-ray studies of the cell walls of both fresh and dried cotton fibers (18) indicate that the latter change may also occur.

Collapsing must be prevented if the original structure is to be preserved. To achieve this, the study of sections of embedded material appears desirable. Such studies have lagged behind, up to now, because a suitable electron stain for cellulose, necessary for obtaining sufficient contrast in sec-
fibrils to fall over and scatter and conglomerates directly dried material, this procedure causes the fibrils from bacterial cellulose (11) and plant cellulose (24, 27) has been carried out recently, until now this technique (4) has not been used on sections.

Even when used for measurements with isolated single fibrils, the shadowing technique has serious disadvantages, since the relatively heavy metal coating obscures the shape of such small particles, thus preventing reliable measurements. This difficulty has been studied extensively by several authors (25-28) who worked out a method for the adjustment and conversion of measurements obtained from metal-shadowed, isolated fibrils. Colvin (6), however, has criticized some of these authors' conclusions (see Discussion). It should be at least considered that when larger fibrils which consist of conglomerations of microfibrils are studied, the shadowing method would, if the coating is relatively thick, no longer resolve the closely packed constituent microfibrils.

It may be for these reasons that the size of the "ultimate cellulose unit" is still a matter of controversy, and that the measurements obtained by indirect methods, such as X-ray diffractions and X-ray scattering at small angles (16, 17), which allow the study of the undisrupted original structure, do not agree with the direct measurements obtained under the electron microscope.

This paper is a report on the results obtained with negative staining of ultrathin sections. This method can be useful for the direct observation of the microcrystallites and their original arrangement, and for an unambiguous determination of their size and shape. Since the material is embedded and stained without having first been dried, there is reason to believe that the original structure has been more successfully preserved and a collapse of microfibrils into larger composite fibrils more successfully prevented than in material that has been dried in a vacuum and subsequently metal-shadowed. A special feature of this new technique is that the staining is carried out in the uncollapsed, water-swollen state, or in a comparable condition. Since the staining solution penetrates between the microfibrils and is deposited there upon drying and infiltrated by the embedding substance, the collapse and conglomerate of the microfibrils may be largely prevented, and even if some shrinkage should occur, the stain would have already penetrated the structure.

Although negative staining of separate, isolated fibrils from bacterial cellulose (11) and plant cellulose (24, 27) has been carried out recently, until now this technique (4) has not been used on sections.

MATERIALS AND METHODS

The materials studied were the cell walls of ramie, jute, and cotton fibers.

The bast fiber of ramie (Boehmeria nivea), with its large microcrystallites, has been used for a long time as the prototype of a pure cellulose fiber of a high degree of crystallinity. The structure is the most regular one found, in that the cellulose molecules are aligned parallel to the long axis of the fiber. This fiber was therefore selected as the best example for a simple microcrystalline structure.

The bast fiber of jute (Corchorus capsularis L.) has a similar structure and parallel orientation of cellulose molecules, but differs in that it also contains lignin. X-Ray studies have shown that the microcrystallites are smaller than those in ramie fiber.

The cotton fiber is a single cell seed hair (Gossypium hirsutum L.). Its cell wall structure has been studied extensively for practical purposes. The cellulose molecules in the cell wall are arranged in a helix around the central axis of the very long cylindrical cell. Upon drying, the entire cell collapses, twists around its axis, and, at the same time, forms so-called convolutions.

The ramie and jute fibers were available only in the dry state. They were swollen in water for several hours to reestablish, as far as possible, the original swollen structure. The stain was generally added at once to the water in which the fibers were swelling, so that it could penetrate together with the solvent into the interstices between the microcrystallites.

The cotton fiber cell wall was studied both in the nondried fresh fiber and in the reswollen fibers. For the study of the fresh fiber, the material was collected under water from the cotton bolls. Other fibers were dried for several hours or days at room temperature or in a freeze-drying apparatus. The same freeze-drying method was used as in previous X-ray studies (18) which indicated that the original cellulose structure is better preserved in freeze-dried cotton than in air-dried cotton.

An aqueous solution of uranyl acetate was used for staining. The principle of this new method of negative staining of sections of cellulose material is based on the fact that uranyl acetate does not stain...
crystalline cellulose and that the amorphous cellulose can take up only water and aqueous solutions ("intermicellar swelling"). It was to be expected, therefore, that aqueous solutions of the stain would leave the microcrystallites unstained on a stained background of the amorphous phase. Since uranyl acetate is actually observed in the cellulose structure, it can only be in the intermicellar amorphous spaces, since they are the only regions penetrated by the water carrier.

The fibers were embedded in Maraglas 655 (8) or Epon 814 (21), with routine procedures being used. The passings of the fibers through dehydrating alcohols were omitted when the fibers were embedded from the freeze-dried state. The sections were cut on a Porter-Blum microtome with a diamond knife. In most cases, a number of the fiber cross-sections were lost from the entire section, leaving holes where they had been, but with careful preparation a large number remained in place.

The sections were collected on bare grids and examined with a Philips 200 electron microscope at 80 and 100 kv. The microscope was provided with a liquid nitrogen-cooled decontamination device. About 3000 electron micrographs were prepared, but only a few of them are used for illustration. They were magnified 2½, 3½, or 5 times in printing.

The diameters of the microfibrils were measured directly from the original electron micrographs under a traveling microscope at a magnification of 100 or 200 times. The average diameter and the standard deviation were calculated from 50 or 100 measurements.

**Ramie**

Fig. 1 represents a longitudinal section of a ramie fiber at high magnification. Very long microfibrils are seen in the long axis of the fiber, which is vertical in the micrograph. They alternate with darkly stained areas which are presumably the amorphous regions. The lateral width of the fibrils is rather constant and measures 48.0 ± 4.0 X 36.0 ± 4.0 A in electron micrographs. Fig. 2 is the same section showing the "beaded string" appearance. Fig. 3 shows a cross-section of the same fiber. The fibrils appear as nearly circular light areas. This agrees with their longitudinal orientation observed in Figs. 1 and 2. There seems to be a tendency for the microfibrils to bundle up in larger complexes which are seen as larger, lighter areas in the electron micrograph.

**Jute**

Fig. 4 is a longitudinal section of a jute fiber at the same magnification as Fig. 2 of the ramie fiber. The outer edge of the fiber appears as a darker zone.

Fig. 5 is a cross-section of this fiber at the same magnification as Fig. 3 of the ramie fiber. The lateral width of the microfibrils appears to be much less than that of microfibrils of the ramie fiber.

In these micrographs, the lateral width of the microfibrils measures 28.0 ± 3.0 A.

**Cotton**

**Observations of the Unstained Fiber at Low Magnification**

In the cotton fiber cell wall, the following parts can be distinguished.

The primary or outer wall is the original wall and consists of a mixture of cellulose fibrils, waxes, and pectin. The secondary wall is deposited beneath the primary one after the elongation of the cell has stopped. It is generally assumed that this deposition has a periodicity associated with day and night, which results in the formation of so-called growth rings. In the outer primary wall, the cellulose fibrils are arranged almost perpendicularly to the long axis of the fiber, and in the inner part of this wall, they are sometimes parallel to the fiber axis. In the secondary wall, they form a helix around the fiber axis whose direction may change. Between these two wall portions, a third layer of different helical pitch is often recognized, the so-called winding or S layer, which forms part of the secondary wall.

Figs. 6 and 7 show unstained cross-sections of a cotton fiber (this specimen was freshly prepared from the unopened boll). Little detail is seen except at the outer border where a layer of dark dots is observed. These dots originate from the wax in the primary wall which is very strongly electron scattering. In the secondary wall, a faint indication of growth rings is perceived; and towards the center, next to the lumen, some of these rings have been separated, probably as a result of the sectioning. No further details appear in the unstained fiber.

**Observations of the Stained Fiber at High Magnification**

**The Primary Wall**: Fig. 8 shows the primary wall as a dark undulating layer outside the underlying secondary wall. Within the dark layer of wax, some elongated microfibrils are seen faintly. They follow the direction of the primary wall. Fig. 14 shows that the dark color is indeed due to wax; here, a fiber has been treated with 11% caustic soda, which dissolves wax. The primary wall is entirely cleared up, but its fibrils have been so swollen that its structure is no longer seen clearly.

**Inner Cell Wall Border**: Fig. 15 shows the structure of the inner border of the secondary wall neighboring the lumen. The structure apparently does not differ much from that of the rest of this wall. The border line is not entirely smooth, but shows various protuberances toward the lumen.
Figure 1  Ramie fiber. Longitudinal section. Fiber axis vertical. × 312,000.

Figure 2  Ramie fiber. Longitudinal section. Fiber axis horizontal. × 355,000.

Figure 3  Ramie fiber. Cross-section. × 355,000.
Figure 4  Jute fiber. Nearly longitudinal section. Fiber axis horizontal. Fiber edge above. × 355,000.

Figure 5  Jute fiber. Cross-section. × 355,000.
Figure 6  Cotton fiber. Unstained cross-section. × 12,500.

Figure 7  Cotton fiber. Unstained cross-section. Outer edge showing the primary wall. × 31,500.

Figure 8  Cotton fiber. Dark primary wall above secondary wall. Primary wall is folded due to a slight contraction of the fiber. Long microfibrils are seen inside primary wall. × 355,000.
FIGURE 9 Cotton fiber. Cross-section of secondary wall. In right upper corner a small portion of the dark primary wall is seen. × 177,500.

THE SECONDARY WALL: Figs. 9 to 12 are all transverse sections of the stained secondary wall. In Fig. 9, a part of the dark primary wall (upper right) is shown, and the remainder of the section shows numerous tiny microfibrils that are short in appearance. They are cut apparently at slightly varying angles, in transverse and diagonal directions, which accounts for their variation in shape. They all show, however, the same lateral width.

Fig. 11 is a similar section, at higher magnification, in which the microfibrils are cut apparently at a more oblique angle, so that some of them appear in more longitudinal view. Other microfibrils among these, however, show a more circular appearance and are apparently cut in cross-section. Fig. 11 shows a complex of microfibrils at nearly perfect cross-sectional view.

Fig. 12 shows an alternation of layers: in some of the layers, the microfibrils are seen in nearly longitudinal view, and these layers alternate with other layers in which the microfibrils are seen in almost cross-sectional view. This general appearance agrees with what is to be expected if the microfibrils are indeed arranged in helices around the fiber axis, and if they form concentric layers in which the direction of the helix alternates from a left- to a right-handed one.

The average width of the microfibrils, as measured from the electron micrographs, has been found to be 35.0 ± 4.0 A.
The cross-sectional shape of the microfibrils, as studied in various micrographs, appears, in some cases, to be almost round when cut in perfect cross-sections, and more elliptical to very elongated when the plane of sectioning deviates from a 90° angle with respect to the axis of the fibril. (It should be kept in mind that the axis of the microfibril forms, in turn, an angle with the fiber axis). In other cases, however, the cross-sectional shape appears to be almost rectangular (Fig. 11, encircled areas).

Fig. 13 shows that the microfibrils have an appearance somewhat like a string of beads or a twisted cord. Stretches of light and dark shades alternate along the longitudinal axis of the microfibrils.

Fig. 16 shows a complete fiber in nearly longitudinal view. The fiber in question is rather an immature one, i.e., only a small number of cellulose layers have been deposited beneath the outer primary wall so that the lumen has not yet been largely filled as in very mature fibers. The empty lumen is seen in the center of the micrograph. It is bordered on both sides by the cell wall. On the outside of the cell wall is the dark primary wall. Directly under the primary wall, very long microfibrils are seen in almost perfect longitudinal view. Below these a special layer in which the fibrils appear in slightly less longitudinal view is observed; this is apparently the so-called winding or S layer. The secondary wall, which forms about half the thickness of the total wall, is seen below this layer. On the left side of the figure, the microfibrils are seen in nearly diagonal view, but, on the right side, they appear in almost cross-sectional view as light dots.
This is what may be expected if the plane of sectioning is neither exactly parallel to the long axis, nor exactly in the axial plane. In that case, this plane would form a different angle with the fibrillar helix, which has opposite direction in the cell wall at opposite sides of the lumen. If, for instance, the helix has a pitch of 45°, and the plane of sectioning forms the same angle with the fiber axis, this plane would be parallel to the helix at one side of the lumen and perpendicular to the helix at the other side of the lumen.

**Appearance of the Structure After Swelling of the Fiber in Caustic Soda**

Fig. 14 shows the appearance of the fiber cross-section after the fiber has been swollen for 5 min in 11% caustic soda and then washed and stained. The primary wall has been completely cleared up. The winding or S layer is slightly separated from the other layers. The underlying part of the secondary wall has not yet been greatly affected and shows the microfibrils very clearly, possibly owing to a slight intramicellar swelling without the microfibrils themselves being affected yet.

**Observations on Isolated Fibrils**

For comparison with the sections of embedded material, isolated fibrils and microfibrils were also studied.

For the isolation of these structures, the material was prepared in the same way as the sections were except that, instead of being embedded, it was mechanically disintegrated at this point in a Tri-R homogenizer provided with a Teflon pestle. With ultrasonic disintegration, very similar results were obtained. Collodion films and carbon films stripped from glass, according to the new method by Towe (39), were used for support of the material. The appearance of some background structure in the micrographs may be due to absorption of stain by the cellulose nitrate supporting films, but even single microfibrils could still be recognized on this background.

Fig. 17 is an electron micrograph of cotton cellulose microfibrils prepared in this way. Fibrils of various sizes are obtained. They consist of microfibrils similar to those seen in the sections. The finer the fibril, the more evident are the signs of mechanical damage; single microfibrils especially often show such signs. The microfibrils often lie in single layers parallel to the support film so that they can be studied well. The same beaded-string appearance observed in the sections is seen here. The structure is very similar to that of the cellulose models represented in Figs. 18 a, 18 c, and 19, if it is assumed that the amorphous portions which periodically alternate with the crystalline portions take up the stain whereas the crystalline portions remain unstained. In several places, notwithstanding the beaded-string appearance, it is seen that the microfibrils are continuous and uninterrupted.

**Discussion**

The microcrystalline and microfibrillar structure of cellulose in cell walls of cotton, ramie, and jute...
Figure 12 Cotton fiber. Plane of section is at an angle of 45° with fiber axis, and shows layers of microfibrils cut lengthwise alternating with layers of microfibrils cut cross-wise. X 106,500.

Figure 13 Cotton fiber. The microfibrils show the appearance of strings of beads (microcrystalline strings). X 355,000.
fibers as revealed by negative staining of sections is believed to represent the real structure as it occurs in the walls of the living cell. This structure corresponds well with the current model for cellulose derived from analyses with indirect methods such as X-ray diffraction. In this model, microcrystalline regions, in which the molecules have an ordered arrangement, alternate with un-ordered, amorphous regions. The microcrystalline regions have been found to measure about 600 Å in length and 20 to 80 Å in width. The molecules are believed to be longer than the crystalline regions, so that a single molecule may contribute to one or more crystalline and amorphous areas (Figs. 18 a and b).

Since the structural details of fibrils and micro-
fibrils observed in sections are the same as those observed in isolated fibrils and microfibrils, there can be no doubt about the usefulness of the negative staining method employed here for studying the microfibrillar structure in sections.

The main conclusions derived from the present study are that the structure observed under the electron microscope is the original microcrystalline structure as present in the living cell, and that the elementary fibrils, measuring about 35 Å in diameter and observed here for the first time in undisrupted arrangement in sections, are the ultimate structural units, identical with the microfibrils and strings of microcrystallites studied by X-ray methods.

The prevention of the collapse of the microfibrils in the cellulosic cell walls and isolated composite fibrils, which is achieved with the technique used in this study, may be the reason why the results of this technique differ from those obtained with the metal-shadowing technique.

In addition, the lower resolution obtained with the metal-shadowing method may preclude the observation of the constituent elementary com-

Figure 16 Cotton fiber. Nearly longitudinal section, showing lumen with cell walls on both sides. × 97,500.
ponents in composite fibrils, whereas the negative staining method used here singles out the elementary components before any collapse can occur, since the staining takes place when the composite fiber is in a swollen state.

Probably the most accurate method of determining the size of the microcrystallites is the method of X-ray scattering at small angles. With this method (16, 17), the following dimensions for the diameter of the microcrystallites were found, in flax, 27 Å; in jute, 28 Å; in ramie, 43 Å; in fresh cotton, 55 Å. A center-to-center distance of 35 Å was found for microcrystallites of jute in the dry state, and of 53 Å for microcrystallites of jute in the water-swollen state. Sterling (37) confirmed these results, finding slightly higher values which increased from 28 to 118 Å, even for Asparagus fiber, when increased stretch was applied. The diameters obtained by direct measurements on the micrographs in the present study agree very well with the results obtained by X-ray methods, and therefore corroborate the conclusion that the structure observed under the electron microscope is identical with the microcrystalline structure as determined by X-ray methods. The term “microcrystallite” will be used, therefore, in the following

![Figure 17a](image1.png)  
**Figure 17a** Fibril showing component microfibrils. At the left side the microfibrils probably lie in a single layer. The center-to-center distance or maximum diameter of these microfibrils is 42 Å. × 461,000.

![Figure 17b](image2.png)  
**Figure 17b** A few microfibrils at higher magnification. The center-to-center distance or maximum diameter of the two central microfibrils is 37 Å. × 812,000.

![Figure 17c](image3.png)  
**Figure 17c** A very fine fibril consisting of 3 individual microfibrils. × 312,000.
discussion, for components of 35 Å width which are lined up to form the microfibril.

The earlier electron microscope measurements reported by others do not agree with the results of these X-ray studies. It has been the consensus, until recently, that the diameter of the ultimate microfibril should be between 100 and 300 Å (1, 9, 10, 23, 29). These measurements were mostly made, however, with dried, metal-shadowed, isolated fibrils. None of the authors using this method stated whether or not the microfibril is identical with the microcrystallite, except Preston (29, 30) who assumes that the microfibril consists of a core formed by the microcrystallite with a coating of noncrystalline material. This could explain the discrepancy between the results obtained with X-ray methods and those obtained with electron microscopy.

Frey-Wyssling (9), however, stated as early as 1951 that the smallest cellulose fibrils studied under the electron microscope might have a smaller diameter, namely 30 × 100 Å. He termed this smallest unit the "elementary fibril." From 1960 on, an increasing number of authors arrived at still smaller diameters for the ultimate cellulose fibril. Günther (12) made a statistical study of metal-shadowed fibrils from moss spores and, correcting for the metal coating, arrived at an average diameter of 45 Å. This diameter increased to 115 Å 30 days after germination. Ohad, Danon, and Hestrin (23) made a study of metal-shadowed microfibrils from bacterial cellulose, also correcting for the part contributed by deposited metal to obtain the real width. They later published (25) a detailed method for the estimation of the true width of such fibrillar structures from electron micrographs of shadowed materials and arrived at an entirely new value of 35 Å for the microfibril. Mühlethaler (24) and Ohad and Danon (26, 27) recently published similar results for the isolated elementary fibril of bacterial cellulose.

These new results are radically different, and it is no wonder that the discrepancies with the older findings have given rise to controversy. Colvin (6) offered well grounded objections and criticized both the results of Hestrin's group, obtained after correction, with shadowed material, and those of
Mühlethaler (24), obtained with negative staining of isolated fibrils. He pointed out that negative staining might decrease the real diameter of the elementary fibril as much as metal-shadowing would increase it, and that the negative staining method is subject to a bias with relation to the form of the filaments and the variable positive absorption of the stain (phosphotungstic acid, in this case). He believed the new conclusion, "that the true lateral width of native cellulose microfibrils is about 1/3 of the presently accepted value ... is not yet justified by unequivocal direct experimental evidence."

The present results permit the settlement of the above controversy and bring some further experimental evidence. Whereas all previous authors worked with separate single fibrils, the present paper deals with microfibrils in their original arrangement, so that it is possible to make measurements of the distance between the centers of neighboring fibrils. In cotton, this distance is found to be 60 A. It may be concluded, therefore, that the maximum width of a single fibril, if no space is present between fibrils, would also be 60 A. Allowing for some space, a width of 30 to 40 A appears reasonable and is in line with the value obtained here from direct measurements of the microfibril width. This result, however, agrees with the latest measurements on the isolated microfibril as reported by others (24, 26, 27).

The method of negative staining of sections also permits the settlement of the question as to whether the isolated microfibrils studied by others actually exist in the cell wall and fiber, or whether they are artifacts resulting from the breakdown of larger microfibrils during the preparation of the material. Since the diameter of 35 A, found in the present investigation with sections, agrees with the measurements on isolated microfibrils (24, 28), it may be concluded that, in all these cases, one is indeed dealing with the microfibrils as they actually occur in the nondegraded structure.

However, the most significant feature of the present study is that the arrangement and distribution of the fibrils in the composite fiber and cell wall is directly revealed. The most important finding in this respect is that the distribution of microfibrils is completely regular and uniform and that, with the new method used here, no network structure is seen as described by others, e.g., for cotton (23, 38, 39) and viscose. The author believes that such network structures are artifacts, a result of the unequal penetration and internal polymerization of the embedding substance which promotes or causes the collapse of the microfibrils into a denser network, enclosing empty bubbles of the embedding substance. It is found that the average distances between neighboring fibrils, i.e. the regions penetrated by stain, are equal to or less than the diameter of the microfibril under the experimental conditions in the cases studied; but these values will vary, of course, with the degree of swelling.

The question of the cross-sectional shape of the microfibrils will be discussed with some reservation. From Fig. 11, the impression is gained that in cotton fiber the cross-section of the microfibril may be nearly circular to rounded, rectangular, or elongated ellipsoidal (but it should be kept in mind that the appearance will greatly depend on the direction of the plane of sectioning, i.e. whether the latter is precisely perpendicular or more oblique to the axis of the microfibril) and that the microfibrils have variable orientation in this fiber. In the electron micrographs of ramie fiber (Fig. 3), the cross-section appears more circular to elliptical and is more uniform. This uniformity can be explained by the more perfect orientation of the microfibrils in this case. Circular cross-sections have been described by Mühlethaler (24) for isolated microfibrils, but a more elongated cross-section or rectangular shape has been deduced by Kratky and Sembach (20) and Rånby (31) who believe that the ultimate fibril has a lamellar shape. Ohad et al. (25) and Ohad and Mejzler (28) also believe that the cross-section is rectangular.

A last important feature which requires discussion is the beaded appearance of the elementary fibril. In the longitudinal direction, the microfibril shows unstained areas alternating periodically with narrow areas which have taken up the stain (Figs. 13 and 17). It was originally believed that this appearance is caused by a twisting of the fibrils, as had been observed also with fibrils of bacterial cellulose (5, 7). It is more probable, however, that the phenomenon is due to an alternation of stretches of full crystallinity with stretches of less crystallinity, or paracrystalline configurations, the so called "Lockerstellen" observed by Hess, Mahl, and Gütter (15) with iodine-stained isolated cellulose fibrils, and by Hess and Mahl with rayon (14). Such periodic change in crystallinity along and within the microfibrils has also been concluded from small angle X-ray diffraction.
studies of rayon by Kratky and Sekora (19), and of synthetic fibers by Zahn and Kohler (43), Hess and Kiessig (13), Mandelkern, Worthington, and Posner (22), Statton and Godard (36), and others. Fig. 18 c represents Hess and Kiessig's (13) model of microcrystalline structure, and Fig. 18 d, Statton and Godard's (36) model of this structure.

The spacing of the periodicities observed in the present study is about 100 to 150 Å, which corresponds well with the findings of Hess's group. Mühlethalier (24) does not find such periodicities in microfibrils isolated from root tips and stained with phosphotungstic acid. When the present results are compared with the above models (Figs. 18 and 19), a great similarity is obvious. With regard to details, however, different explanations are possible. The broad areas may correspond with the Lockerstellen or the less crystalline areas of the models, if it is assumed that the stain has not penetrated these areas. On the other hand, it is possible that the stain has penetrated the lower density areas, causing the microfibril to look narrower at these places, and that the crystalline portions appear as the broader areas or beads. If the latter assumption is correct, an explanation is still needed as to why, in the present study and in the studies of Hess's group, these areas take up the stain, whereas in the material studied by Mühlethalier this is not observed. One explanation might be that the difference in material may be the cause of the discrepancy; another explanation might be that the different stains used may be the cause. In the studies of Hess's group and in the present study, stains composed of small molecules were used, whereas Mühlethalier used the large molecules of phosphotungstic acid which may not be able to penetrate the Lockerstellen, in contrast with the smaller molecules which do penetrate.

Whatever explanation may apply, it is beyond doubt (see Fig. 17) that some periodicity is present along the length of the microfibrils. It cannot be decided with certainty, however, whether an alternation in width occurs along the microfibril or whether penetration of the stain into the microfibril plays a part. It also cannot be stated with certainty whether any amorphous material occurs between the neighboring microfibrils. If it occurs, however, this material cannot take up more space than half the diameter of the neighboring microfibril. The micrographs of cotton suggest that only very little amorphous material is present between the microfibrils.

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