Never-Dried Cotton Fibers.   III.   Crystallinity and Crystallite Size

N. MOROSOFF, *Camille Dreyfus Laboratory, Research Triangle Institute, Research Triangle Park, North Carolina 27709*

Synopsis

X-Ray diffraction patterns were obtained for never-dried cotton in water and compared to those for cotton dried once, rewetted, dried a second time, and rewetted again. In addition, cotton reacted with formaldehyde or acrylamide to "fix" the never-dried fibers were examined in the never-dried and rewetted states.   Relative crystallinities were found to be identical for all wet cottons and somewhat lower for dry cotton samples. Half-widths for the equatorial reflections decreased in the order: first-dried, second-dried, never-dried, first-rewetted-second-rewetted for all cottons.   Somewhat broader reflections were observed in the rewetted state for the "fixed" samples than for untreated samples.   The results are consistent with a buildup of stress in the cotton fiber on drying and a relaxation of stress on rewetting with some reorganization of cellulose chains in the first rewetting to form larger or more perfect crystallites than in never-dried cotton.   Such reorganization is inhibited, but not prevented, in the "fixed" samples.

INTRODUCTION

Earlier papers from this laboratory have described the morphology, transport properties, and high strain to break of never-dried cotton,[1] as well as the fixation of the never-dried state[2] resulting in preservation of the never-dried properties after drying and rewetting the fixed fiber.   Never-dried cotton fibers were shown to have high internal mobility resulting in wet extensibilities two or three times that of dried and rewetted fibers. Additionally, it was found that never-dried cotton contains almost 100% more water than can be absorbed during subsequent sorption cycles.   It was found that two drying and rewetting cycles were required to bring the cotton fiber to its final state as the cotton fiber absorbs less water in the initial part of the first rewetting than in any subsequent cycle.

On the premise that the change in mechanical and sorption behavior of cotton fibers after drying is due to the irreversible formation of hydrogen bonds, never-dried cotton was reacted with formaldehyde and with acrylamide in an attempt to block accessible hydroxyl groups and "fix" the never-dried state.[2]   This resulted in cotton fibers in which sorption properties and extensibility to break did not change after drying and rewetting.

An x-ray investigation of the crystallinity of never-dried cotton is clearly of interest both as it relates to the above and because of its relevance to the

mechanism of cellulose biosynthesis in cotton. Up to fairly recently,[3] it was believed that never-dried cotton was largely amorphous, whereas never-dried *Valonia*[4] and never-dried alga *Cladophora*[5] seemed to be crystalline. The first x-ray study of never-dried cotton fibers was carried out by Berkley and Kerr.[6] Using a photographic film method, they concluded that never-dried cotton was amorphous but that crystallinity was induced by stretching the never-dried fibers. Heyn[7] compared diffractometer traces of wet cotton with those of dried cotton, both taken in the reflecting mode, and concluded that the crystallinity of never-dried cotton fibers is low as compared to that of dried fibers. He attributed Berkley and Kerr's result (i.e., no crystallinity observed for never-dried cotton) to the increased background due to scattering from water when nonmonochromatized x-radiation is empolyed. On the other hand, Nelson and Mares[8] concluded that the crystallinity of never-dried cotton is not very different from that of cotton that has been dried on the basis of iodine and dye sorption, deuterium retention, and lateral order distribution studies on cotton of various degrees of maturity studied in both the wet and dry states. Additionally, Ono[9] concluded that the crystallinity of undried cotton is very slightly *higher* than that of dried cotton by using an acid hydrolysis method.

In light of the conflicting data in the literature, it was decided to re-examine the crystallinity of never-dried cotton by an x-ray method that would minimize background due to scattering by water or air (crystal-monochromatized radiation and helium atmosphere) and with the never-dried cotton immersed in water and subjected to a minimum of handling so no question could arise of crystallinity being induced by partial drying or handling. This latter precaution was taken, as Berkley and Kerr[6] stated that never-dried cotton becomes crystalline on stretching and Ingram, et al.[1] demonstrated that the "stretching process itself causes irrecoverable changes in the load–extension properties in contrast with dried and rewetted cotton which only exhibits slight hysteresis on stretching and relaxation." Additionally, the results for never-dried cotton were compared with those for both dried and rewetted cotton, with account being taken of absorption of x-rays in the sample. The x-ray diffraction pattern of fixed cotton was also examined in the never-dried and rewetted states. Preliminary results of this investigation, i.e., the finding that never-dried cotton is not appreciably less crystalline than dried rewetted cotton, were reported in part I of this series.[1]

EXPERIMENTAL

Unopened (green) bolls were obtained from controlled plots and were immediately placed in distilled water to which had been added a drop of chloroform in order to inhibit bacterial growth. They were stored at about 4°C until ready for use. The husks of the boll were gently removed under water to reveal the individual locks of fibers. Small bundles of

fibers would then be cut from the locks using a razor blade. These bundles would then be placed intact into one of two aluminum holders to be described.

The fixed samples studied were one which had been treated for 6 hr with an acidic aqueous solution of formaldehyde yielding a formaldehyde graft content of 0.347% and another containing grafted acrylamide which had received a dose of 8.66 Mrads in the presence of $CuCl_2$ yielding a graft content of 3.7% by weight. Further details may be found in the second paper of this series.[2]

X-Ray diffraction photographs from the samples were obtained using a Nonius Guinier-de Wolff camera and a Rigaku Denki rotating-anode x-ray generator. The camera employs a curved crystal monochromator (set for CuK_α), and the photographs were taken in a helium atmosphere to eliminate air scattering. Plots of intensity of x-ray scattering as a function of the scattering angle 2θ were obtained using a Joyce Loebl Mark III CS microdensitometer.

The Guinier-de Wolff camera[10] is constructed so that separate diffraction patterns may be obtained for up to four distinct samples. The samples are mounted side by side in a planar holder supplied with the camera. The patterns are obtained by transmission, and the incident beam passes through the samples at an angle of 30° with respect to the normal to the sample plane. Two special sample holders were constructed for this work by making two holes in aluminum plates. A cotton sample immersed in water could be mounted in one hole and a sheet of polyethyléne (standard sample) was permanently mounted in the neighboring hole. The diffraction patterns from the two samples could then be obtained simultaneously but separated from one another on the film strip. The intensity of scattering from the polyethylene standard was used as an indicator of the total exposure of the cotton sample to x-rays. The dimensions of the holes in the aluminum plate were smaller than those of the x-ray beam, so that both samples were bathed in x-rays.

One of the sample holders was made from a 0.5-mm-thick plate of aluminum with 0.015-mm-thick aluminum foil windows (holder A). One window was glued to the aluminum sheet with silicone cement. The never-dried cotton sample would then be placed in the sample cavity, a thin coat of silicone vacuum grease would be placed on top of the holder, to be followed by the second aluminum foil. This would be kept in place by a perforated metal plate pressing down on the sample holder. After the exposure, the cotton would be removed from the sample holder and dried overnight in a high-vacuum system. It would be weighed and a portion replaced in the holder so as to obtain a diffraction pattern of the dry sample. The original dried sample would then be immersed in water in a vacuum oven, kept under vacuum for $1/2$ hr, and then kept at atmospheric pressure under water for about 15 min. The thus rewetted cotton would then be replaced in the sample holder and an x-ray photograph of rewetted cotton obtained. The mass ratio of water to cotton in the never-dried and in the rewetted samples was in the range of 2.5–5. Fibers were removed from

some of the never-dried samples in the holder after the x-ray exposure and before drying and were found to deform plastically in the manner typical of never-dried cotton fibers.[1]

The second holder was fashioned from an aluminum sheet 1.25 mm thick, and 0.2-mm-thick beryllium windows were used (holder B). The never-dried sample was loaded as for holder A, and the beryllium windows were secured against the sample holder by means of perforated aluminum plates screwed down against the sample holder. With holder B, the cotton sample was never removed from the sample holder throughout the series of exposures for the never-dried, first-dried, first-rewetted, second-dried, and second-rewetted sample. An 0.5-mm hole had been drilled in the holder parallel to its large surface extending to the sample cavity. This hole was kept sealed by means of a screw during sample exposures. The entire sample holder was placed in the high-vacuum system for drying and immersed in water for rewetting following the procedures outlined for holder A.

In order to check for preferred orientation, holder B could also be mounted in three orientations about the x-ray beam. In addition to the 0° orientation in which both the cotton sample and polyethylene standard yielded diffraction patterns, the holder could be rotated about its normal by 45° and 90° with respect to the 0° orientation. In the latter two orientations, only the cotton sample was irradiated, and the exposure to x-rays was obtained from the height of the water background at $2\theta = 28$-$29°$. The mass ratio of water to cotton for the sample in holder B was 2.3.

The crystallinity of the various samples was obtained from the intensity under the (101), (10$\bar{1}$), (120), and (002) peaks in the diffraction pattern. As this intensity is affected by the separation of crystalline peaks from background, the water background was subtracted from the microdensitometer scans and the result replotted. This operation was facilitated by digitization of the microdensitometer output. A microdensitometer trace was obtained for water in the holder; and this trace, suitably corrected for transmittance of x-rays through the sample, for x-ray exposure, and for mass of water in the x-ray beam, could then be subtracted from the trace obtained for never-dried or rewetted cotton. The results were plotted on a Calcomp plotter Model 565, as were the original traces.

CRYSTALLINITY

A variety of x-ray methods exist for calculation of crystallinity or crystallinity index for dried cotton.[11-14] Most of these involve either a comparison of the areas under the crystalline and amorphous parts of the diffraction pattern or a point-by-point comparison of the diffraction pattern to that of crystalline and amorphous standards. In all cases, great pains are taken to randomize orientation of the cellulose fibers.

We have attempted to subtract the water contribution (see Fig. 2) from a microdensitometer scan obtained for wet cotton fibers (see Fig. 1), thus

yielding the diffraction pattern due to cellulose only (see Fig. 3). This facilitates separation of the crystalline peaks from background. The intensity under the crystalline peaks following this procedure is not very sensitive to the total intensity of the subtracted water curve, i.e., it is fairly insensitive to errors in the estimate of water in the holder. However, the background scattering, i.e., that intensity not included in the crystalline peaks, is obviously directly affected by errors in the estimate of intensity due to water scattering.

For this reason, it was felt that one could obtain more reliable results by comparing the integrated intensity under the crystalline peaks for never-dried cotton with the corresponding quantity from dried and from rewetted cotton. Particularly the latter quantity would be subject to the same random and systematic errors as the data for never-dried cotton. Additionally, it is not necessary that the cotton be randomly oriented for this approach, only that corrections be made for any change in orientation between the different states being examined. As x-ray crystallinities and crystallinity indexes are available for dried cotton, such quantities can be estimated for never-dried cotton from its crystallinity relative to dried cotton.

The method adopted requires that the integrated crystalline intensity be corrected for total exposure to the x-ray beam (i.e., I_t, intensity of incident beam), for absorption of x-rays in the sample (or inversely for T = transmittance of x-rays through the sample), and for the mass of cotton (M) in the x-ray beam. Thus,

$$I_r = \frac{I_0}{I_t \cdot T \cdot M} \qquad (1)$$

where I_0 is the observed integrated crystalline intensity obtained for a sample having a mass M and transmittance T, and exposed to a total incident intensity I_t; I_r is the corrected relative crystalline intensity. As the holders were constructed so that the sample is completely bathed by the x-ray beam, M may be obtained by weighing the dried cotton sample. I_t is obtained from the diffraction pattern obtained for the polyethylene standard which was exposed simultaneously with the cotton sample. As shown in Appendix A-1, T can be obtained from, but is not equal to, the relative transmittance of the sample to the incident beam. The latter was obtained directly for the wet and dry cotton samples.

In order to verify that the degree or kind of orientation does not change drastically on going from cotton in one state to another, two kinds of preferred orientation were considered. The intensities of equatorial reflections on the photographs obtained with holder B at orientations differing by 45° increments about the normal to the holder served to demonstrate that the orientation of cotton fibers was cylindrically symmetric about that normal, for all sample conditions (see results section). Additionally, one might expect that the cotton fibers might have a tendency to lie in the plane of the holder. As there is cylindrical symmetry about the normal to that plane, a

measure of the amount of this preferred orientation is the ratio of the intensity of the (040) reflection to that of the (002) reflection. Since in our photographs the (120) reflection is always a shoulder on the (002) reflection, and as the intensity of the (120) reflection is a small fraction of that of (002), we have obtained the ratios of the intensity of the (040) reflection to the intensity of the (002),(120) composite reflection for all samples. In the case of random orientation, this ratio should be 0.07 as calculated from the intensity data of Mann et al.,[15] suitably corrected by use of the Lorentz polarization factor and multiplicity factor. For perfect orientation of fibers perpendicular to the plane of the sample holder, the (040) reflection would disappear. For perfect orientation of cotton fibers in the plane of the holder, one calculates an increase in the (040) intensity to 2.6 times the random orientation value and a decrease of the (002) reflection to 0.71 the random orientation value. In these calculations, a spiral angle of 22° was assumed, and the geometry of the Guinier-de Wolff camera was taken into account.

Crystallite Size

The breadths of x-ray diffraction peaks were observed as an indicator of the size and perfection of crystallites in the sample. The experimentally observed breadth B must be corrected for instrumental broadening to obtain the true breadth of the peak, β. Normally, the instrumental broadening is obtained from the peak breadth b, obtained for a standard with large perfect crystallites, and which is of the same thickness and absorption coefficient as the sample. We used calcium tungstate dispersed in polyethylene films of various thicknesses for this purpose. The equation

$$\beta^2 = B^2 - b^2$$

is normally used to obtain the true breadth β from the experimentally observed breadth B and instrumental broadening b. This equation is valid for Gaussian peaks. The contribution to β of the crystallite size is given by

$$(\delta S)_c = 1/\bar{D}_{hkl}$$

which is the Scherrer equation with the shape factor set equal to unity. Crystal imperfections would cause β to be larger than $(\delta S)_c$, but more than one order of each reflection is required to extract the contributions of each component to β. As we had measured the breadth of only one order, $(\delta S)_c$ was set equal to β. This yields values of D_{hkl} which are actually lower limits to the crystallite size. Additionally, an apparent decrease in crystallite size may actually represent a decrease in crystal perfection. Further details on this type of analysis may be found in Alexander[16] and Buchanan and Miller.[17]

The (101) and (10$\bar{1}$) peaks of cotton generally overlap. In all microdensitometer traces obtained by us, it was clear that the (101) peak was broader than the (10$\bar{1}$) peak. To express this in quantitative terms, it was necessary to resolve the composite profile into its component peaks. We have

used the method of Patil, Dweltz, and Radhakrishnan,[18] in which the only
assumption made is that the component peaks are centrosymmetric.   Addi-
tional considerations concerning crystallite size measurements are contained
in the Appendix.

RESULTS AND DISCUSSION

Microdensitometer traces of never-dried, first-rewetted, and second-
rewetted cotton obtained for samples in holder B, are shown in Figure 1.
It is immediately clear that never-dried cotton is crystalline and that the
equatorial crystalline peaks (101) at $2\theta = 14.8°$, $(10\bar{1})$ at $2\theta = 16.7°$ and
(002) at $2\theta = 22.8°$ sharpen on going from the never-dried to the first re-
wetted state.   These traces and those in the following figures have been
corrected for differences in incident beam intensity and absorption of x-
rays so that the areas under the crystalline peaks may be directly compared.
The rapidly decreasing intensity between $2\theta = 5°$ and $8°$ is due to diffuse
scattering from the monochromator crystal, which is negligible beginning
at $2\theta = 9°-10°$.   The contribution of x-ray scattering from water is shown
in Figure 2.

The scans with the water contribution subtracted are shown in Figure 3
for never-dried cotton in the 0°, 45°, and 90° orientations of the holder and
for the first and second rewetted cotton at 0°.   Note the change in ordinate
scale.   The trace obtained for the second dried sample (0° orientation) is
included in Figure 3 for comparison.

The relative crystalline intensities (total for (101), $(10\bar{1})$, (120), and (002)
reflections) obtained from such plots are given in Table I.   The relatively
small variation between intensities obtained in the 0°, 45°, and 90° settings
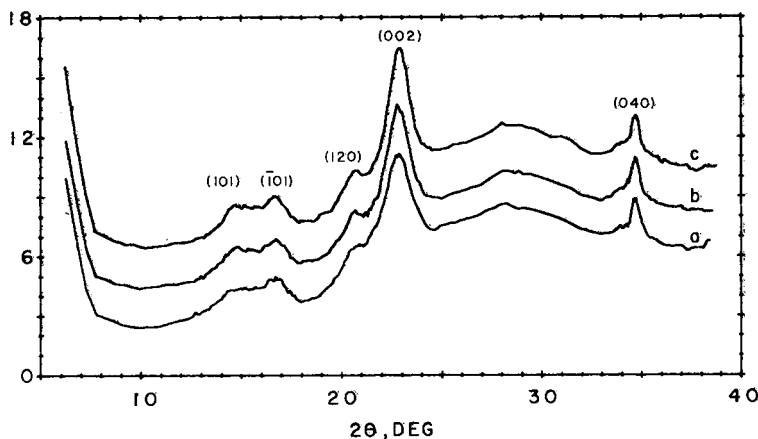for each of the wet states indicates that orientation is cylindrically sym-

Fig. 1. Microdensitometer traces of x-ray scattering patterns obtained for cotton in
water in the (a) never-dried, (b) first-rewetted, and (c) second-rewetted states.   Ordinate
is intensity of scattering in arbitrary units.   The first-rewetted curve is displaced 2
units above its correct position, the second-rewetted curve 4 units, for clarity.

TABLE I
Intensity of Crystalline Peaks (Holder B)

	0°	45°	90°	Mean
Never dried	0.225	0.218	0.205	0.216
First dried	0.186			
First rewetted	0.215	0.195	0.210	0.206
Second dried	0.193			
Second rewetted	0.206	0.217	0.209	0.211

TABLE II
(040) to (002),(120) Intensity Ratios
(Average of Values for the 0°, 45°, and 90° Orientations of Holder B)

Sample condition	Intensity ratio
Never dried	0.060
First rewetted	0.052
Second rewetted	0.054
First dried	0.039
Second dried	0.044

metrical about the normal to the sample holder plane. Additionally, there is no significant difference in relative crystalline intensity between the three wet samples. The dried samples appear to be somewhat less crystalline than the wet.

The data in Table I are not corrected for the effects of preferred orientation. Changes in preferred orientation are indicated in Table II, which lists the ratios of the intensity of the (040) reflection to that of the (002),-(120) reflection for both wet and dry samples in holder B. By reference to Figures 1 and 3, it may be noted that the (040) reflection is superimposed on a broad hump representing the (310),(230) reflections. We have tried to effect a reproducible separation between the sharp (040) reflection and the more diffuse off-meridian reflections. We can limit ourselves to an explanation of the relative values given in Table II.

It would appear that the fibers in the never-dried sample have more of a tendency to lie parallel to the plane of the sample holder than those in the dried and rewetted fibers. This causes a 10–13% drop in the $I_{(040)}/I_{(200),(120)}$ ratio in going from the never-dried to the first- and second-rewetted states. This increase is affected both by an increase in the intensity of the (040) reflection and a decrease in the intensity of the (002) reflection. We have calculated that as one goes from a state of random orientation to one of perfect orientation of fibers parallel to the plane of the holder, the increase in (040) intensity is twice the decrease in (002) intensity. Therefore, one can estimate that there is an approximately 5% increase in intensity from equatorial reflections on going from the never-dried to the rewetted samples due to orientation. This would necessitate a 5% decrease in the intensities given in Table I for the first- and second-rewetted samples if they are to be interpreted as a measure of crystallinity relative to never-dried cotton.
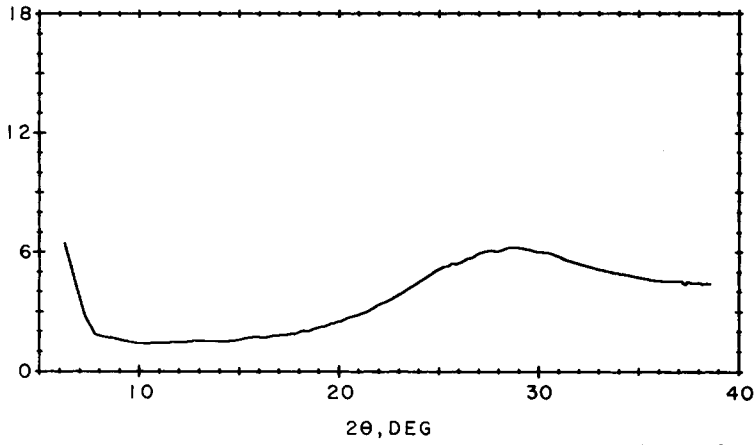
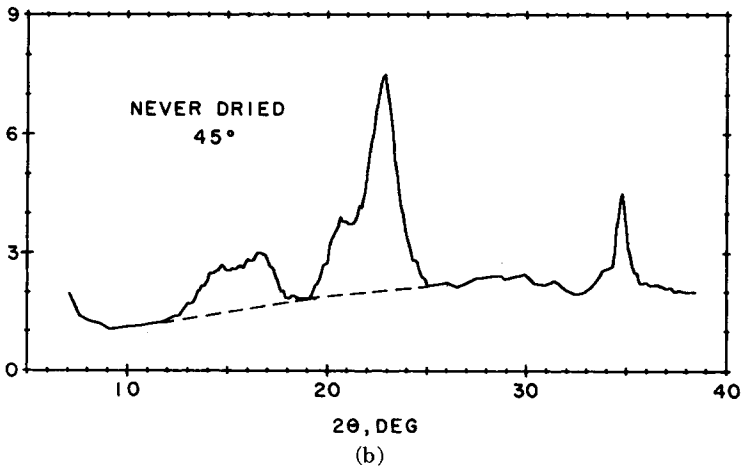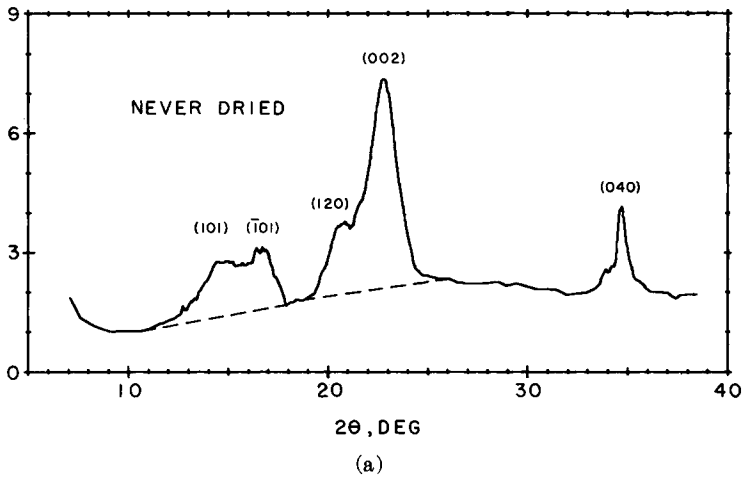g. 2. Microdensitometer trace of the contribution of water scattering to the traces in Fig. 1.

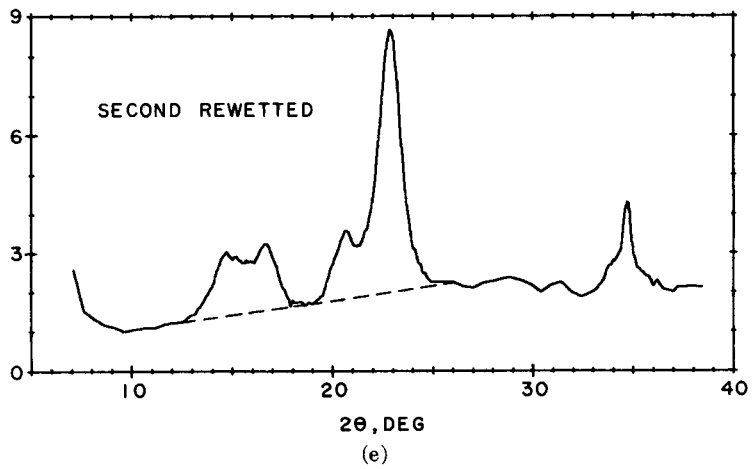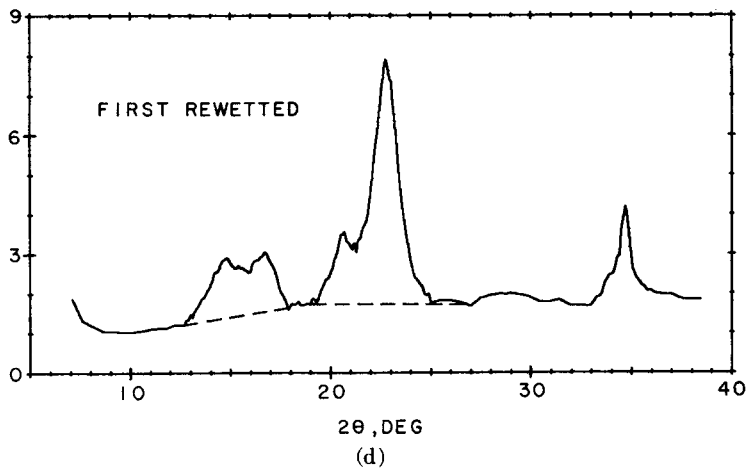(a)

(b)

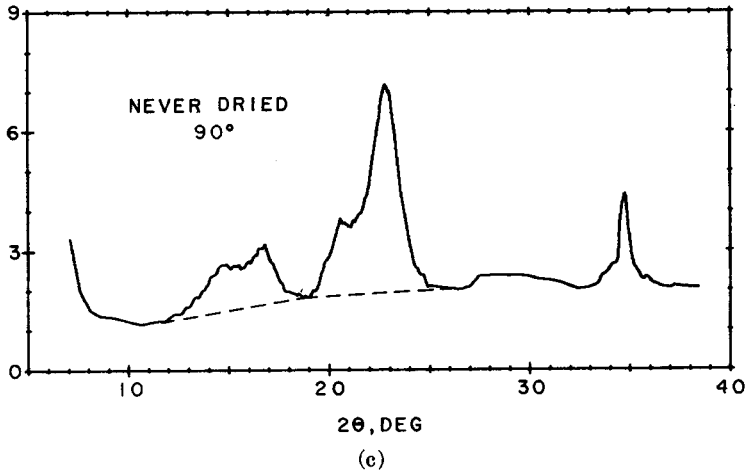Fig. 3 (*continued*)
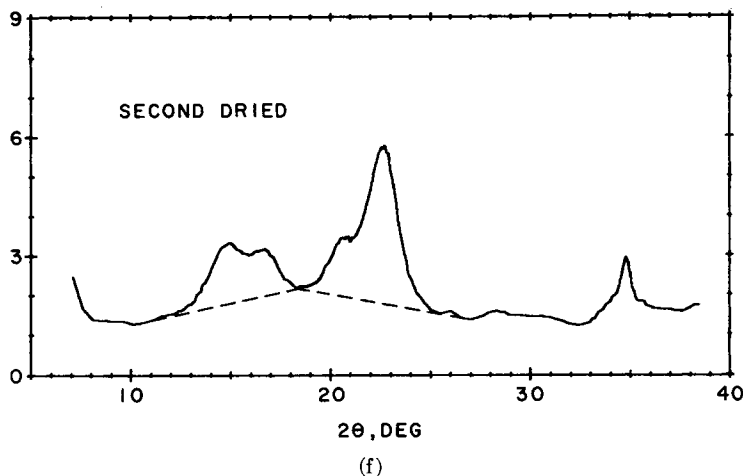
(c)

(d)

(e)

Fig. 3 *(continued)*

Fig. 3. Plots of intensity of x-ray scattering vs. scattering angle 2θ for never-dried and rewetted cotton obtained by subtracting the water contribution (Fig. 2) from traces of the type shown in Fig. 1, as well as for second-dried cotton (f). Dashed lines represent the separation of crystalline scattering from background.

One concludes that never-dried cotton is at least as crystalline as re-wetted cotton and perhaps slightly more so, in agreement with Ono's[9] results. All the wet samples appear to be more crystalline than the dry samples. Consideration of orientation effects, i.e., the results in Table II, would only increase the difference in crystallinity. This result agrees with that of Creely and Tripp,[19] who have obtained x-ray diffractograms for cotton conditioned at 0% and 100% relative humidity, observing less crystallinity and greater (002) half-widths for the former (dry) samples. Seitsonen and Mikkonen[20] have obtained similar results as regards crystallinity for wood pulp samples.

The results discussed above are for one sample. X-Ray diffraction patterns obtained for samples in holder A yielded relative crystalline intensities for four more samples, allowing an estimate of statistical errors, and for four "fixed" samples (two for each treatment). In discussing these results (Table III), it may be noted that the 5% correction for preferred orientation (see above) would be applicable here as well, as data identical to that given in Table II were obtained for the unfixed holder A samples. The numbers in Table III represent the area under the (002),(120) peak of the crystalline diffraction patterns and are in arbitrary units on a scale different from that used in Table I. However it is clear that the crystallinity of cotton is constant within a standard deviation of about 10%, on going from the never-dried to the first- and second-rewetted states. The errors are such that a 5% increase in the relative crystalline intensity of never-dried cotton (correction for orientation) would not be significant. Moreover, it is seen that the fixation treatments do not affect the crystallinity of the cotton either with no further treatment (never-dried state) or on subse-

TABLE III
Intensity of (002),(120) Peak (Holder A)

Cotton	
Never dried	0.116 ± 0.013
First rewetted	0.108 ± 0.011
Second rewetted	0.109 ± 0.010
Formaldehyde-Treated Cotton	
Never dried	0.118 ± 0.004
First rewetted	0.134 ± 0.033
Second rewetted	0.113 ± 0.014
Acrylamide-Treated Cotton	
Never dried	0.115 ± 0.009
First rewetted	0.116 ± 0.021
Second rewetted	0.106 *[a]

[a] Only one sample.

quent dryings and rewettings. The crystallinity of fixed cotton is equal, within experimental error, to that of untreated cotton.

Clearly, the increased extensibility and sorption properties of never-dried and "fixed" cotton cannot be explained on the basis of low crystallinity. Nor is it necessary to postulate a mechanism for the biosynthesis of parallel cotton chains in which crystallization does not occur.[3] Our results indicate that never-dried cotton is similar to crystalline never-dried *Valonia*[4] or never-dried filaments of the alga *Cladophora*.

Our results are obviously in agreement with those of Nelson and Mares[8] and Ono[9] who concluded that the crystallinity of never-dried cotton is not very different from that of rewetted cotton on the basis of iodine and dye sorption, deuterium retention, and lateral order distribution studies in one case[8] and acid hydrolysis in the other.[9] We feel additionally that they are not necessarily in disaccord with the findings of Heyn.[7] Heyn himself states that the relatively low intensities he observed for never-dried cotton can also be observed for "ground, highly crystalline cellulose, when saturated with water....," i.e., for rewetted cellulose. In his Figure 1, one sees diffractometer traces (reflecting mode) for wet cotton compared with those obtained for dried cotton. It is well known that the effective penetration of the x-ray beam into the sample on such a diffractometer is inversely proportional to the absorption coefficient.[21,22] as the x-ray beam is tangential to the sample surface. The effective penetration of the x-ray beam into the sample will increase with decreasing absorption coefficient. It is not surprising, then, to see the intensity of cellulose reflections increasing with decreasing water content in Heyn's Figure 1.[7] Effectively the x-ray beam penetrates deeper into the sample with decreasing water content and "sees" more cotton. To a good approximation, the intensity of scattering from cotton will be proportional to the mass fraction of cotton in the sample consisting of water and cotton. The figure is misleading, then, in that no correction is made for the mass of cotton giving rise to diffracted intensity.

TABLE IV
Apparent Crystallite Dimension, D_{002} (Holder B)

Sample	D_{002}, Å	Sample	D_{002}, Å
Never dried	56	First dried	41
First rewetted	67	Second dried	50
Second rewetted	68		

TABLE V
Apparent Crystallite Dimensions, D_{002} (Holder A)

Sample	D_{002}, Å
Cotton	
Never dried	57 ± 2
First rewetted	67 ± 3
Second rewetted	70 ± 2.5
First dried	44 ± 4
Second dried	53 ± 2
Formaldehyde-Treated Cotton	
Never dried	55 ± 5
First rewetted	61 ± 3
Second rewetted	65 ± 1
First dried	46 ± 1
Second dried	47 ± 2
Acrylamide-Treated Cotton	
Never dried	57 ± 2
First rewetted	58 ± 3
Second rewetted	64
First dried	46 ± 2
Second dried	51

It should be noted that Heyn does not explicitly base his conclusions concerning the crystallinity of never-dried cotton on the results in his Figure 1 but rather on a comparison of the x-ray patterns obtained for indirectly versus directly dried cotton fibers. Our results can be considered a more direct and quantitative comparison of crystallinities.

The breadths of crystalline reflections are a measure of crystallite size and perfection. Accordingly, widths at half-height were measured for the composite (120),(002) reflection. Generally, the (120) reflection is at an angle and of a relative intensity such that it would not contribute to the measured width. The apparent crystallite dimensions obtained for the one sample in holder B are given in Table IV, those for four cotton samples and for four fixed samples in holder A are presented in Table V.

These results complement the water sorption results reported in part I of this series.[1] The latter were interpreted in terms of stresses developed in the fiber during the first drying which are partially relieved during subsequent rewettings. The data in Tables IV and V clearly demonstrate that some reorganization takes place leading to changes in crystallite size and/or

TABLE VI
Crystallite Sizes (Holder A)

Sample	D_{101}	$D_{10\bar{1}}$
Never dried	37 ± 4	57 ± 7
First rewetted	48 ± 5	65 ± 3
Second rewetted	48 ± 1	67 ± 3

perfection on drying and rewetting cotton fibers. The first drying is expected to develop stresses in the fiber because of hydrogen bond formation and the collapse of the fiber to a convoluted state. In fact, Tables IV and V show that the (002) reflection is broader for dried cotton than for never-dried or rewetted cotton. This effect is not an experimental artefact as claimed by Manjunath et al.[21] (see Appendix A-2.) This result would be consistent either with increased microstrain in the crystallites or decreased crystallite size for dried cotton. Stresses are relaxed on the first rewetting, allowing reorganization to take place to the extent that crystallites are larger and/or more perfect in the first rewetted cotton than in the never-dried state.

Comparison of the second drying with the first leads to the expectation that it would involve less stress (convolutions already formed) on a system which starts out with larger and/or more perfect crystallites. In fact, Tables IV and V show that the (002) reflection is broader for the first dried cotton than the second.

The data in Table VI confirm the results obtained from the (002) reflections. All equatorial reflections show an increase in apparent crystallite size on going from the never-dried to rewetted state. In addition, it is seen that the crystallite size perpendicular to the (101) plane is smaller than that perpendicular to the (10$\bar{1}$) and (002) planes. This is the usual result obtained for a variety of celluloses[3] and is a reflection of the fact that hydrogen bonding takes place preferentially along the (101) plane so that this plane is the plane of lamination,[23] as well as the plane of the sheets that are pushed apart on intercrystalline swelling.[24] The nominal 2θ values were 14.75° for the (101) peak and 16.7° for the (10$\bar{1}$) peak (16.65° for second rewetted).

Fixation of the never-dried state does not affect the decrease in crystallite size (see Table V) on the first drying as the stresses associated with formation of convolutions are unchanged. In the second drying, the acrylamide-treated sample attains the same apparent crystallite size as the untreated samples, while that of the formaldehyde-treated sample is unchanged from the first-dried value. This implies that the crystallites in the formaldehyde-treated sample do not grow as much on going through the first-drying–rewetting cycle as in the case of untreated cotton. In fact, the half-widths of both fixed, rewetted samples show that the fixation treatments do inhibit, but not entirely prevent, the reorganization of cellulose chains to form larger and/or more perfect crystals on drying and rewetting. Such an

inhibiting effect would be expected as the fixing agents react with hydroxyl groups on the surface of crystallites in the never-dried cotton to form intramolecular and intrafibrillar crosslinks.

On the other hand, the fact that limited reorganization *does* take place is not surprising if one considers the fact that only a small fraction of the hydroxyl groups accessible to water react with the fixing agent.[25] The mole fraction of hydroxyl groups accessible to water may be obtained by treating the data in the first paper of this series[1] by the method of Brunauer, Emmett, and Teller.[26] This yields 9 mole-% of hydroxyl groups accessible to water.[25] In the case of the formaldehyde-fixed sample, the mole fraction of blocked hydroxyl groups is 1.2%, only 14% of the total accessible to water. Such an analysis is not possible for the acrylamide-treated sample because some polymerization of acrylamide may occur.

CONCLUSIONS

It has been clearly demonstrated that never-dried cotton is just as crystalline to x-rays as cotton that has been dried and rewetted. This has been demonstrated on a sample which was subjected to the bare minimum of handling necessary to place it in a sample holder for the x-ray exposure. The samples were exposed to x-rays in a liquid water environment and were found to deform plastically in the manner typical of never-dried cotton fibers *after* the exposure to x-rays. For these reasons, it seems highly unlikely that crystallinity was induced in the never-dried samples by stretching prior to exposure to x-rays.

Crystallite size and/or perfection is found to decrease on drying in response to the stresses induced on the collapse of the never-dried cylindrical fibers into a convoluted shape. Reorganization takes place on rewetting leading to larger and/or more perfect crystals in the first and second rewetted states than in the never-dried state. The second drying leads to some stress but not to the same extent as the first drying as the convolutions have already been formed. As a result, crystallite size and/or perfection is greater for the second-dried state than for the first-dried, although less than in any wet state.

The fixation treatments previously reported[2] did not affect either the never-dried crystallinity or that found on subsequent rewettings. They did have an inhibiting effect on the reorganization of cellulose chains to form larger and/or more perfect crystals on drying and rewetting.

APPENDIX

A-1. Transmittance of the Cotton Sample

The transmittance T required for eq. (1) can be obtained if one considers that the incident beam enters the sample plane at an angle of 30° with respect to the normal to the plane. If one considers the path of an x-ray beam scattering at an angle of 30° from the backside of the sample plane (that side closest to the x-ray source), we see that it passes through less thickness of sample than a beam that passes through the sample and then scatters at $2\theta = 30°$ from the front of the sample. This is because the scattered x-ray beam from the backside of the sample plane then passes through the sample along the sample normal. The expression for the scattered intensity for the plane sample in the Guinier-de Wolff camera is

$$I\alpha \frac{e^{\left[\frac{-\mu \cdot \rho}{\rho} \frac{l}{\cos 30°}\right]} - e^{\left[\frac{-\mu \cdot \rho}{\rho} \frac{l}{\cos(30° - 2\theta)}\right]}}{\frac{-\mu \cdot \rho}{\rho}\left[\frac{1}{\cos 30°} - \frac{1}{\cos(30° - 2\theta)}\right]} \tag{2}$$

which may be derived from eq. (17) in reference 27. The symbol l represents the thickness of the sample plane, and the transmission for the sample is obtained by dividing eq. (2) by l; μ/ρ is the mass absorption coefficient and ρ is the density of the sample.

The quantity $e^{-\mu \cdot \rho \cdot l/\rho}$ may be obtained by measuring the transmittance of each sample to the incident beam. This quantity is equal to

$$T' = e^{\left[\frac{-\mu \cdot \rho}{\rho} \frac{l}{\cos 30°}\right]}. \tag{3}$$

Clearly, the quantity $\mu \cdot \rho \cdot l/\rho$ can be obtained from eq. (3) and substituted in eq. (2) so as to obtain the quantity T.

The quantity T' was obtained for holder A by direct measurement of the relative transmission of dried and wet cotton samples. This was accomplished by measuring the integrated intensity of the incident beam passing through the cotton sample as compared to that passing through the neighboring polyethylene standard.

In the case of holder B, the relative transmittance of the cotton sample for incident beam (T') was obtained by placing a second sample (PE2) behind the cotton sample and comparing the diffraction patterns from PE2 with those from the neighboring polyethylene standard.

A-2. Crystallite Size Measurements

We have used the same standard to obtain b, the instrumental broadening, for both dry and wet cotton samples. Both samples take up the same volume but the dry sample has a much lower effective absorption coefficient because the volume is largely filled with air. According to Manjunath et al.,[21] it is solely the fact that the instrumental broadening is actually different for wet and dry cotton samples that is responsible for the sharper reflections commonly observed for wet cotton. In our case of transmission, he would claim that because of high absorption of the incident beam in the sample, the bulk of the reflecting intensity would originate at the face of the sample closest to the x-ray beam, as this reflected beam must pass through less sample than the incident beam that passes through to the other face. We have calculated the transmittance for the reflected intensity from the back face (that closest to the x-ray source) as compared to that from the front face of the sample and found that for a wet sample the latter is 75% of the former. We therefore believe that it is permissible to use the same instrumental broadening for both dry and wet samples and that the effects we see are real and not due to errors in estimating instrumental broadening.

In support of this is the experimental measurement of the half-width of the (040) reflection. In all samples, this half-width is close to the instrumental broadening as the coherence length along the chain axis is considerably greater than that perpendicular to the chain axis. There is a small difference between the (040) half-width observed for wet and dry samples, but it is only 20% of the maximum effect one would expect if the assertion of Manjunath et al.[21] were correct and only 10% of the difference in half-width observed for the (002) reflection between the rewetted and dried samples. There is, therefore, no question that the differences in half-widths observed by us for the equatorial (002) reflection of dry and rewetted cotton are not due *solely* to differences in instrumental broadening for the wet and dry samples.

Note added in proof: Since submission of this manuscript another paper dealing with the crystallinity of never-dried cotton [A. K. Kulshreshtha, K. F. Patel, A. R. Patel, M. M. Patel, and N. T. Baddi, *Cellulose Chem Technol.*, **7**, 343 (1973)] has come to our attention. In the work cited, equatorial x-ray diffractograms are obtained from parallel fibers of cotton from bolls picked from 35 days to 65 days after flowering (at 5 day intervals) in both the never-dried and dried states. It is found that the crystallinity increases with maturity and that the crystallinity index of never-dried cotton is always less than that of dried cotton (74.7% vs. 80.8% for the 65 day old sample). However, these results are suspect for two reasons. Firstly, no attempt is made to correct for the effect of possible differences in orientation between samples. Secondly, crystallinity is obtained from the intensity of scattered x rays at $2\theta = 19°$ and 22.8°, including the water contribution in the case of never-dried samples. Using their method, we would obtain a "crystallinity" of 39% for the water curve shown in our Figure 2. Thus the increases in "crystallinity," observed by Kulshreshtha et al., both with increasing maturity of the never-dried cotton sample and on drying, are caused, wholly or partly, by a decrease in the relative contribution of the water curve to the scattering pattern. The data they present cannot be considered a refutation of our observation that never-dried cotton is somewhat more crystalline than dried cotton.

Additionally, Kulshreshtha et al. do not observe the increase in half-width of the (002) reflection, reported here, on going from the never-dried to first dried state. We find that the same half-width of the (002) reflection is observed for first-dried cotton, oriented so that only equatorial reflections are observed, as reported here for diffraction spectra in which the (120) reflection is a shoulder on the (002) reflection. We suggest that the difference in our observation and that of Kulshreshtha et al. may be due to the fact that their samples were dried in the atmosphere, ours under a vacuum of 10^{-6} mm of Hg.

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