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CELLULOSE MICELLES IN DEVELOPING FIBERS OF ASPARAGUS

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The voluminous literature on cellulosic structure in walls of mature plant cells reflects the intense research interest in that structure. Nevertheless, relatively few investigations have been concerned with the changes which occur in the size and organization of the cellulose micelles (*i.e.*, crystallites) during ontogeny of the cell. The principal object of developmental studies of micellar structure has been the epidermal hair of the cotton seed (CLARK *et al.*, 1930; HESS *et al.*, 1936; SISSON, 1937, 1938; WERGIN, 1937). HEYN (1933, 1934) has contributed an analysis of the X-ray diagram of the walls of young epidermal cells of the *Avena* coleoptile at a single stage of growth, and polarization-optical studies of the parenchyma cells of the same organ have been made by BONNER (1935), PRESTON (1938), and SÖDING (1934). PRESTON and WARDROP (1949) and WARDROP (1949) have studied micellar size, structure, and arrangement in the cambium of conifers.

Many other polarization-optical accounts have dealt only with micellar orientation in growing cell walls (MAAS GEESTERANUS, 1941; GREEN and CHAPMAN, 1955; MAJUMDAR and PRESTON, 1941; MEEUSE, 1938; RITTER and STILLWELL, 1934; ROELOFSEN, 1951a; ROELOFSEN and HOUWINK, 1953; etc.). Numerous electron microscopic studies also consider the problem of orientation, in this case, of the "visible" microfibrils. However, these investigations indicate little of the changes in size of the micelles, their relationship to other wall constituents, or their relative degree of crystallinity. The assumption, that crystalline micelles are being studied rather than single molecules of cellulose, is implicit in the majority of the works mentioned above.

The present account deals with development of the so-called "pericyclic" (Fischer, 1900) fibers in growing shoots of *Asparagus officinalis* L. The cell walls of the fibers are investigated from the standpoints of cellulose structure, degree of crystallinity of the cellulose, micellar size, and micellar orientation. Essentially this study is a roentgenographic account, which has been supplemented by the findings of polarization microscopy. Asparagus has been chosen

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because of the comparative ease of isolation of its relatively homogeneous fiber region, at the inner border of the cortical parenchyma. The histological development of this fiber tissue plays an important rôle in the assessment of quality of the asparagus shoot as a vegetable.

Materials and Methods

Materials

Emerging "spears" of asparagus shoots were collected in the late spring of 1956 and selected for comparable size and vigor of growth. From each shoot, segments 1 cm. long, 3 mm. wide (tangentially), and 2 mm. thick (radially) were taken at the following distances from the tip: 3–4, 5–6, 8–9, 11–12, and 15–16 cm. Since tissue development proceeds acropetally, these different segments were taken to represent, from apex to base, successively older stages in growth. Elongation was usually just becoming noticeable at the 3–4 cm. level and ending at the level of 11–12 cm. Secondary thickening of the fiber wall, with occasional lignification, was characteristic of the 15–16 cm. level. The segments were preserved in a mixture of formalin, acetic acid, and ethanol according to Johansen (1940).

In order to isolate the fiber region from the cortical parenchyma, on the outer side, and from the "medullary" parenchyma and vascular bundles on the inner side, the segments were first embedded in paraffin by an ethanol-normal butanol-xylol schedule. Cross sections of each segment established the distance of the fiber tissue from the outside (about 180–200 μ) and the radial thickness of the fiber tissue (about 60–75 μ). Preliminary orientation of the segment took place before cutting on a sliding microtome, and a final orientation was made during the first contact cuts. Successive 10 μ tangential sections were made, until reaching just into the fiber zone (about 10 μ into its outer edge). This latter was then isolated as a "fiber segment" slab by a single cut which was 10 μ thinner than the measured thickness of the fiber region.

Because of the cylindrical shape of the shoot, it was possible that curvature of the stem might bring other tissues into the edge of the fiber segment. Therefore, after being cut, the slab was placed in a xylol-filled Petri dish on the stage of a dissecting microscope. Foreign tissue was excised with a spear-pointed needle. Upon several washings in fresh xylol, the segments were ready for use.

X-ray observations

For the different roentgenographic methods it was necessary to vary the conditions of observation somewhat. To obtain X-ray diagrams of the unaltered fibers, tissue segments were superposed, successively, on a specimen holder-tangential faces in contact and longitudinal axes parallel. They were held together by Canada balsam, applied in a dilute solution of xylol. These were then placed in the path of an X-ray beam, Cu-Ka, filtered by a nickel foil, 10 μ thick. The collimator diameter was 0.5 mm., in lead glass, and the

beam was intercepted by Ilford X-ray film in a flat cassette 40 mm. from the specimen.

In order to obtain X-ray diffraction patterns due primarily to the cellulose of the fibers, the xylol-washed segments were brought into water through the agency of ethanol. They were then extracted of non-cellulosic materials in solutions of 0.2 N HCl and 0.2 N NaOH, successively, for 2 hours in each solution at 97° C. in a water bath. Because of the great decrease in rigidity of the segments (Frey-Wyssling, 1952, gives the cellulose content of young cell walls as 2.5 %), it was not possible to mount more than three segments in uniform orientation on the perforated specimen holder. After drying, the segments were exposed to the same X-ray conditions as above, but in a vacuum camera, with the flat film 30 mm. from the specimen. This arrangement served to minimize the amount of X-radiation scattered by air for the very thin samples and also decreased the exposure time.

Crystallinity percentage was determined on other, similarly extracted tissue segments which were packed in random orientation in a small rodlet according to the method of HERMANS and WEIDINGER (1948). However, the sample shape was changed slightly to that of a right cylinder, 1.6 mm. in diameter, after WIJNMAN (1954). The films were photometered with a 5 × translation of the traverse course. The calculated crystalline and amorphous indexes were converted to percent crystallinity by fitting to a crystallinity "nomogram" (Hermans and Weidinger, 1949) [1].

With a smaller sample of the Wijnman type (rod of 0.8 mm. diameter), in which the purified fibers were again packed in random orientation, half-breadth determinations were made for the estimation of micellar size of the cellulose. Exposure of the sample to the X-ray team took place in a cylindrical (Debye-Scherrer type) camera, b7.3 mm. in diameter. The collimator, which must be small relative 5o the size of the sample, had an aperture diameter of 0.25 mm.

Because the collimator was 5 cm. long, the divergence of the beam at the end of the collimator was greatly minimized. Therefore the assumptions and equations of HENGSTENBERG and MARK (1929) were inapplicable. The procedure of calculation was as follows (LONSDALE, 1948): considering that the part of line broadening due to particle size is given by $\beta = \sqrt{B^2 - b^2}$ (where b is the line width, in radians, for particles larger than 1 μ in size and B is the measured line width, in radians, at half the height of the photometered peak above the background), then $t = \frac{C\lambda}{\beta} \sec \theta$. (C is a constant, λ the wavelength of the X-radiation, θ the reflection angle or Bragg angle, and t the diameter of uniform spherical particles with cubic symmetry.) In the present case, b was determined by making a mixture of coarsely-crystalline NaCl and purified cotton fiber in the form of a rodlet, 0.8 mm. in diameter. Thus, b values were established under the same

[1]) The author is greatly indebted to Dr. A. Weidinger for his help with the equipment and calculations in this method.

conditions as B, and b for the desired cellulosic interference was obtained by interpolation.

The least equivocal interference for line breadth determinations in the powder diagram was that of the (002) plane, which gave the micelle diameter perpendicular to that plane. Overlapping of the (021) interference could be corrected easily by establishing the slope of the (002) peak. This was not possible in the overlapping of the (101) and (10$\bar{1}$) interferences. Half-breadth values were corrected for the slope of the background line according to the equation given by Frey-Wyssling (1937). Micelle length is impossible to ascertain in a powder diagram because of the coincidence of 4–10 interferences with the (040) peak [*e.g.*, equatorial (20$\bar{2}$), (301), (30$\bar{1}$), (103) and very likely also (310), (230), (221), (22$\bar{1}$), (130), and (131)].

Polarization observations

For control of observations on micellar orientation, polarized light was employed. For this purpose, 2 μ–5 μ sections were cut transversely and in radial-longitudinal planes from shoot segments embedded in paraffin blocks. After removal of the paraffin, these were either mounted in Canada balsam unstained or stained with Congo red or benzoazurin before mounting. In addition, fiber tissue segments were brought to an aqueous medium (as described above) and then macerated by warming gently in a mixture of 1 % chromic acid–8 % nitric acid, followed by shaking. After washing, the separated fiber cells could be mounted, stained or unstained, in Canada balsam for study. Occasionally the cells were crushed in order that single cell walls might be observed. To enhance sensitivity, a strong white light source was used, and the slow axis of the Red I plate was placed near the plane of the polarizer in the NE-SW quadrants (LAVES and ERNST, 1943).

RESULTS

Crystalline pattern

There is a notable pattern change of crystalline interferences in the untreated fiber tissue as a function of age, as is indicated in Fig. 1–3. These figures show an increase in intensity of the outermost of three rings [at 3.96 – 4.02 Å, perhaps representing the (002) and (021) planes of cellulose], the widening of the middle ring [which has the position of the (10$\bar{1}$) plane of cellulose] to include the spacing which would be the (101) plane of cellulose as a new ring, an intensity increase in the middle ring, and the gradual decrease of intensity of the innermost broad ring, which represents spacings of 9.6 to 15.5 Å. In the original negatives, there is also a gradual decrease of intensity of a weak ring at 2.36 Å, coincident with an increase in intensity of a ring at 2.56–2.63 Å [the latter representing the interferences in the neighborhood of cellulosic (040)].

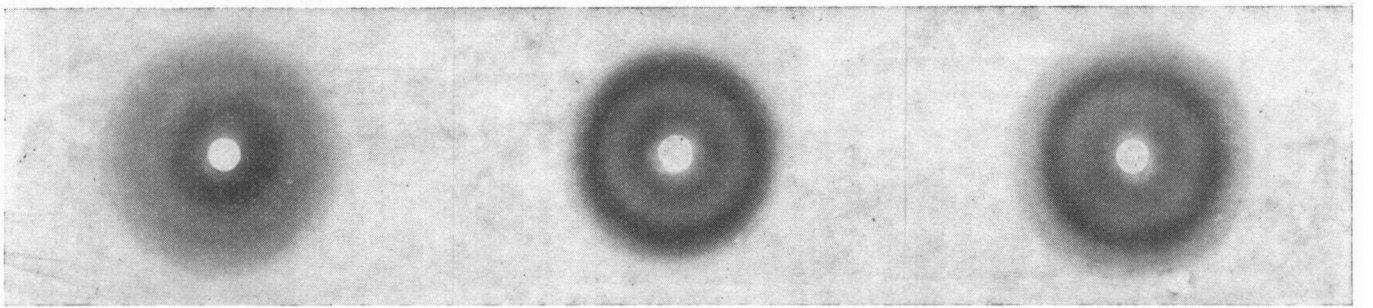

Fig. 1. At 3–4 cm level in shoot (start of elongation)

Fig. 2. At 11–12 cm level in shoot (maximum elongation)

Fig. 3. At 15.16 cm level in shoot (start of secondary wall thickening)

Fig. 1–3. X-ray diffraction patterns of untreated asparagus fiber tissue. Fiber axis vertical. Specimen-to-film distance is 40 mm.

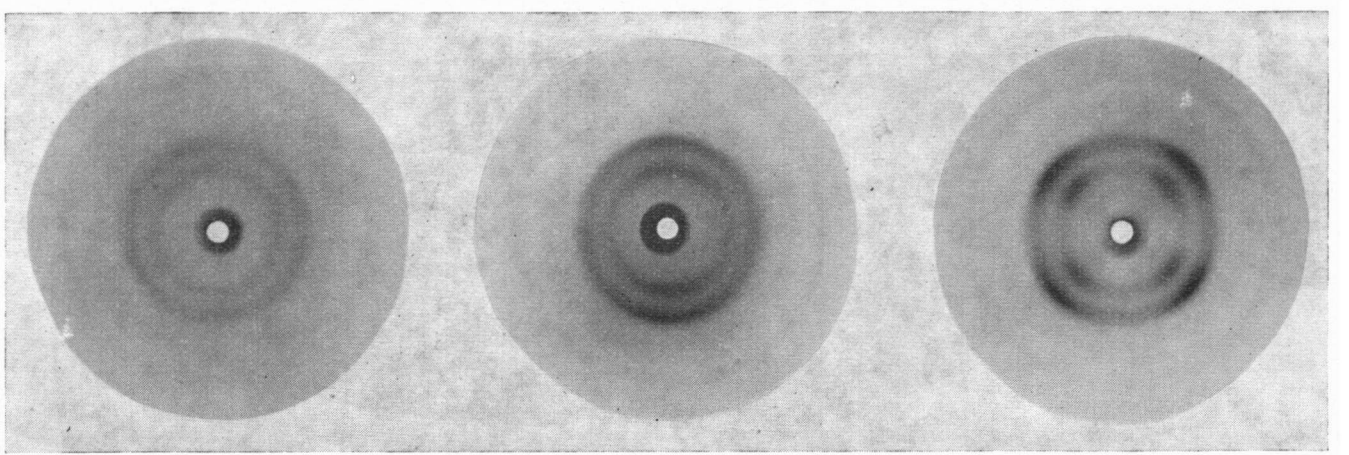

Fig. 4. At 3–4 cm level in shoot.

Fig. 5. At 11–12 cm level in shoot.

Fig. 6. At 15.16 cm level in shoot.

Fig. 4–6. X-ray diffraction patterns of asparagus fiber tissue after extraction of non-cellulosic material. Fiber axis vertical. Specimen-to-film distance is 30 mm.

Organization of cellulose

After the non-cellulosic constituents have been removed from the fiber segments, the cellulosic interferences are visible at all stages of development. Fig. 4–6 indicate that the main crystalline spacings of the cellulose lattice are present at all significant stages: youngest, greatest elongation, and beginning of secondary wall thickening. It may also be seen that the orientation of the cellulose micelles is more or less at random at the 3–4 cm. level (continuous rings), mainly lying in horizontal orientation at 11–12 cm. [the (002) and (101)–(10$\bar{1}$) spacings have a transverse position in Fig. 5], and indicative of a crossed fibrillar arrangement at 15–16 cm. (Fig. 6). In this last figure, the micelles are almost at right angles to each other: the inclination of the micellar long axis to the long axis of the cell is about 48–51°. It is also to be noted that, as in the untreated fibers, there is a progressive differentiation of the diffraction rings. In particular, the (021) spacing becomes quite distinct from that of (002) in the diagram of the 15–16 cm. fibers, both by radial and angular separation.

Degree of crystallinity

A typical comparison photometric recording is given in Fig. 7. The cellulosic diagram is at the right and the comparison substance at the left. The amorphous background is indicated by the dashed line; the air scatter effect by the dotted line. The relative surface area of the curve of cellulosic peaks above the amorphous background is determined for each sample. This value, divided by the peak height of the photometric curve for the comparison substance, gives the

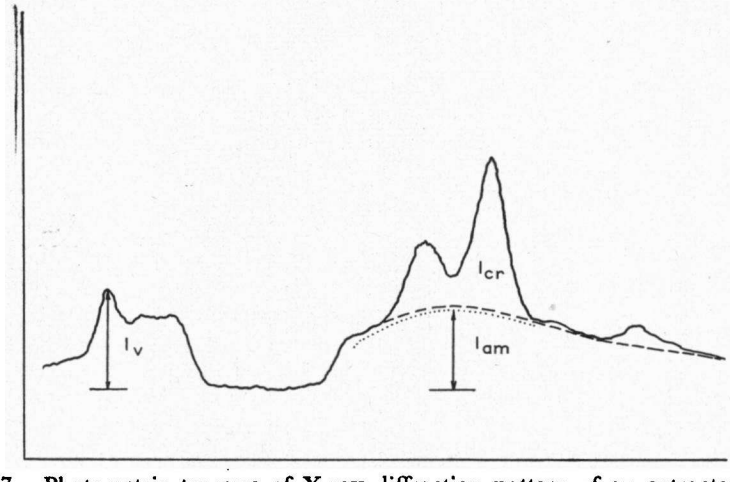

Fig. 7. Photometric traverse of X-ray diffraction pattern of an extracted fiber specimen (at right) and comparison material (at left). This is from the 15–16 cm level. I_{cr} is the crystalline surface area; I_{am} is the maximum height of the amorphous background after correcting for air-scatter; and I_v is the height of the peak of the comparison material.

crystalline index for the sample. The relative height of the amorphous portion of the cellulose curve represents the amorphous index of the sample. Both values establish a point for each sample, and these points have been plotted on the crystallinity nomogram in Fig. 8. The nomogram is determined on the basis of well-defined "crystalli-

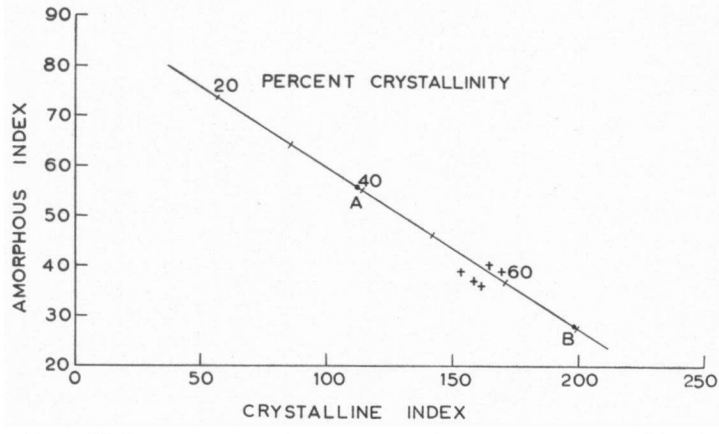

Fig. 8. Crystallinity nomogram. Asparagus specimens are represented by + marks. Points A and B are used to establish the line and the values of percentage of crystallinity. (Further explanation in text.)

nity" values for native plant fibers with highly crystalline cellulose (*e.g.*, ramie, cotton, and flax) at point B and for more amorphous types of regenerated cellulose, such as rayons and cellophane, at point A. (The crystallinity of native fibers is taken as 70 % and that of rayons as 39 %.)

When so plotted, the crystallinity values for the various stages of asparagus development fall within a fairly close range. No principal differences are apparent among the samples with respect to the degree of crystallinity, and no developmental trend is indicated. All the values occur between the points of 55 and 60 % crystallinity.

Micellar size

Table I presents the measured half-breadth values and the steps in calculation of the micellar diameter for the (002) interference in the asparagus samples and in similarly purified cotton and ramie fibers. The value for micelle diameter in ramie was 59 Å. It is of interest to note that HENGSTENBERG and MARK (1929) found the micelle diameter in ramie to be 56 $\pm$ 5 Å, FREY-WYSSLING (1937), 56 Å, and KRATKY *et al.* (1942), 65 Å, with different X-ray techniques and different methods of calculation from that used here. With ultra-sonic disintegration of fibers of ramie, hemp, cotton, etc., WUHRMANN *et al.* (1946) observed that the smallest strands seen in the electron microscope were also 60–70 Å wide.

The micellar diameter for asparagus fiber cellulose increases

slightly during fiber elongation. However, at the end of elongation, the micelle size is not markedly different from that at the initial stages of growth. At the beginning of secondary wall formation, a conspicuous increase in the average micellar diameter is to be seen.

TABLE I

Half-breadth values of (002) interference and calculations for micelle diameter of cellulose in asparagus fibers

Sample Asparagus fibers	Measured half-breadth on film (mm)	B'* (mm)	B'^2	B'^2-b^{2**}	$\frac{\beta}{(\sqrt{B'^2-b^2})}$ (mm)	β (radians)	t*** (Å)
3– 4 cm	1.56	1.50	2.25	2.14	1.46	0.051	28
5– 6 cm	1.46	1.43	2.04	1.93	1.39	0.049	29
8– 9 cm	1.40	1.40	1.96	1.85	1.36	0.047	30
11–12 cm	1.40	1.40	1.96	1.85	1.36	0.047	30
15–16 cm	1.10	1.10	1.21	1.10	1.05	0.037	38
Cotton fibers	0.71	0.71	0.50	0.39	0.63	0.022	64
Ramie fibers	0.77	0.77	0.59	0.48	0.69	0.024	59

* B' is a corrected half-breadth value, obtained by multiplying the measured value on the film by a correction factor which depends upon the relative slope of the background (FREY-WYSSLING, 1937).

** b^2 is 0.11 for the (002) interference under the experimental conditions used here.

*** t is the micelle diameter, from $t = \frac{C\lambda}{\beta} \sec \theta$, where θ is 11.4°, C is 0.9, and λ is 1.54 Å.

The photometric curves of the purified cellulose of the elongating samples (used in the estimation of micellar diameter) are quite similar to one another and may be represented by the curve for the 3–4 cm. sample (Fig. 9). The (002) peak is relatively low, and there is a poor differentiation of such adjoining interferences as (021)–(002) and (101)–(10$\bar{1}$). At the initiation of secondary wall thickening (Fig. 10), the (002) peak is much higher above both the background and the (021) interference, and the (101) and (10$\bar{1}$) peaks are somewhat more distinctly separated. In cotton (Fig. 11), the (002) peak is quite high and narrow, and (021) is clearly set off from it; the tips of (101) and (10$\bar{1}$) are definitely separate and differ conspicuously in height.

Aspects in polarized light

In surface view, the cells at 3–4 cm. are isotropic, but in longitudinal section, the wall edges have a weak positive birefringence with respect to the long axis of the cell, and in transverse section to its periphery. Negative birefringence is first noticeable in the face view of the 8–9 cm. fibers, *i.e.*, in the surface view of the single cell wall as seen in longitudinal section and of the double cell wall, both in section and in macerated whole cells. At this level, the fibers are already 400–500 μ long.

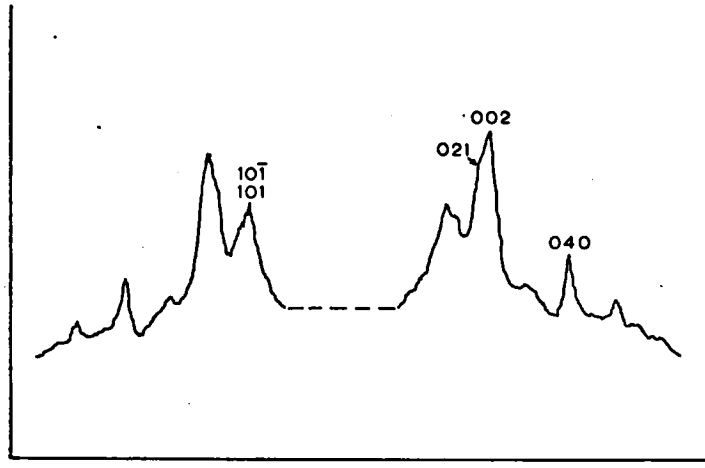

Fig. 9. At 3–4 cm level in asparagus shoot.

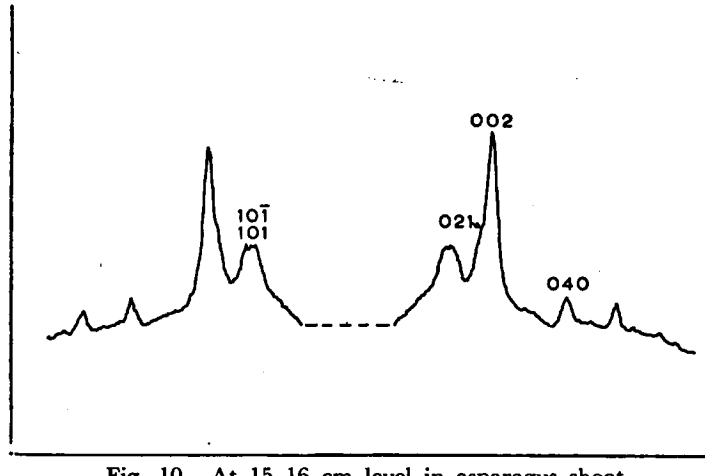

Fig. 10. At 15–16 cm level in asparagus shoot.

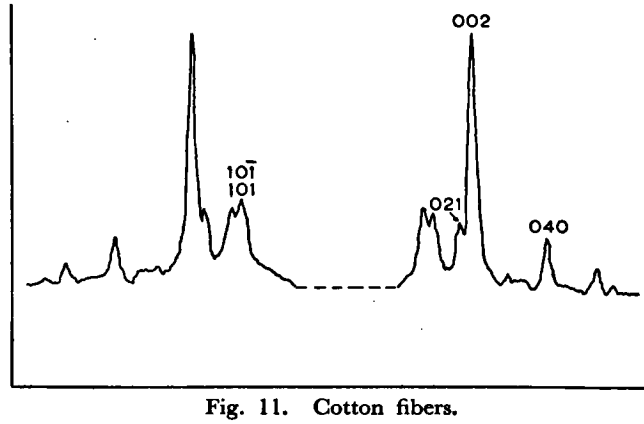

Fig. 11. Cotton fibers.

Fig. 9–11. Photometric traverses of X-ray diffraction pattern of extracted fiber specimens used in determinations of micellar diameters.

At the level of 11–12 cm., the fibers are full-sized, varying in length usually between 700 and 900 μ, hence are almost twice as long as at the 8–9 cm. level. The polarization aspect is much as at 8–9 cm.: in whole cells, both the single and double walls are negatively birefringent in surface views. (The same is true of surface views in section, but the wall edges remain positively birefringent with respect to cell length and cell periphery.) In all cases, the birefringence is relatively weak and needs special techniques for its demonstration, including the benzoazurin staining, strong light source, and altered position of the Red I plate.

At the 15–16 cm. level, the cell wall is considerably thicker and is more strongly birefringent. The double wall is still negatively birefringent with respect to cell length, but single walls have a major extinction position (MEP) at about 45° to the long axis of the cell. When a macerated whole cell is partly stretched, so that some wall tearing occurs with an "unrolling" effect along the line of the MEP, it can readily be seen that the opposite side of the cell has its MEP opposed to the direction of that of the near side.

The opposed directions of the MEP of opposite sides of the cell wall at 15–16 cm. may also be noted without the use of a Red I plate if the cells be stained with safranin. In this case, there is also a blue-yellow color effect. A blue color is achieved when the MEP of the safranin-stained wall lies in the NW-SE quadrants, and a yellow color is produced with the MEP in the NE-SW quadrants. In younger cells and in unstained cells at the 15–16 cm. level, the wall is darkened if the MEP lies in the NE-SW quadrants and is bright if the MEP is in the NW-SE quadrants. These effects appear to be those of crossed-plate relationships, which have been discussed by FREY-WYSSLING (1941), ROELOFSEN (1951), and others.

DISCUSSION

Concerning the broad outlines of micellar orientation in the growing cell wall, this study has corroborated the results of most previous authors. In the youngest stage, the evidence of X-rays and polarized light indicates that the micelles are arranged at random in face view but do tend to have a foliar (uniplanar) texture (Frey-Wyssling, 1930), *i.e.*, at any given depth in the wall, the micelles are arranged in the plane of the wall, perpendicular to a radius from the center of the cell.

During elongation, proportionately more micelles tend to be oriented as well in the transverse direction, to give the so-called "tubular texture" (FREY-WYSSLING, 1930). Apparently, the longer the period of growth goes on, the more predominant becomes the transverse orientation of the micelles. With secondary thickening, the next micelles are laid down in close array in a helix at an angle slightly more than 45° (*ca.* 48–51°) to the vertical axis of the cell. Hence the view through the double wall shows a negative birefringence with respect to cell length.

Various workers have revealed the presence of ambiguous (for cellulose) spacings in the X-ray diffraction diagram of cell walls. These spacings frequently disappear after treatment of the walls for the removal of non-cellulosic components. SEN and WOODS (1949) found spacings of 9.7 and 14.6 Å in jute, SISSON (1938) noted a 14.5 Å equatorial spacing in young cotton hairs, ROELOFSEN et al. (1953) have indicated a spacing of about 13 Å in *Halicystis* (which became intensified after NaOH treatment), and perhaps a similar one may be seen in Fig. 2 of PRESTON's (1951) study on microfibrillar structure in *Valonia*. The present investigation has shown a broad spacing of 9.6–15.5 Å in elongating asparagus fibers. This spacing diminishes greatly in intensity when secondary walls are being deposited, and it disappears completely on treatment of the cells with hot dilute HCl and hot dilute NaOH.

Elsewhere, the author (STERLING, in press) has found a spacing of 12–15 Å as a strong interference in the X-ray diagrams of certain gels of pectic and alginic acids and their calcium salts. He has suggested that this may be a spacing of a polyuronide lattice structure which does not occur in the stretched fibers which are usually made for the study of unit cell construction. Hence, except for the spacing found by ROELOFSEN *et al.* (1953), it is conceivable that the unexplained spacings noted above are those of polyuronide materials. No evidence was found for the "primary substance" rings of HESS *et al.* (1936), which were later considered as those of waxes. However, their ring at 4.2 Å and HEYN's (1933, 1934) of the same size could well correspond to the strong meridional spacing, at 4.2 Å, of pectic materials.

Contrary to the results of CLARK *et al.* (1930) on cotton, the cellulosic interplanar distances of the asparagus fibers do not decrease during growth but remain constant. According to HEYN (1934), the cellulose of the young cell wall is not completely crystalline. Supposedly a regular structure is present at first only in the direction of a spacing d = 4.18 Å [(002) + (021) and/or pectin?]. It is of interest that HERMANS (1949) also conceives of differences of degree of "lateral order" (*i.e.*, crystallinity) in the crystallites of the modified cellulose of rayon, so that a 2-dimensional order in its lattice may be envisioned.

Possibly the delayed appearance of the (101) plane in the X-ray diffraction pattern of untreated asparagus indicates the previous existence of an imperfectly crystalline state. (Of course, orientation of the (101) plane parallel to the tangential plane of the segment is possible, but this has not yet been definitely shown for the cell walls of higher plants. Since the segments of untreated fiber tissue scarcely shrank at all during drying in the xylol-balsam solution and were dried down over an opening in the specimen holder, there was no orienting stress due to drying.) PRESTON and WARDROP (1949) also found that the diffraction spacing for the (101) plane was absent in untreated cambial cell walls although present in purified material. If the lattice structure were incomplete, it could contribute to the

amorphous index in the HERMANS and WEIDINGER (1948) method of crystallinity determination.

Thus, it may be thought that the removal of non-cellulosic substances from the wall does not reveal a crystalline state which is already present but rather permits the better orientation of imperfectly oriented molecules to a lattice structure (WARDROP, 1949). On treating cellulose fibers with acid, HERMANS and WEIDINGER (1949a) obtained an increase in crystallinity in rayons and no change in the crystallinity of ramie. The rayon result was explained in terms of the cutting of chains in the amorphous regions, thereby permitting freer rotation of entangled chains. However, FOSTER and WARDROP (1951) believe that this effect is achieved simply by the hydrolytic removal of the regions of the lowest lateral order and by the removal of the smallest micelles.

HESS *et al.* (1939) are also of the opinion that the cellulose of the primary wall is not yet arranged in a good lattice structure, hence gives a much vaguer type of X-ray diagram. However, after working with young cotton fibers, SISSON (1937) concluded that the process of purifying the cell wall of non-cellulosic substances does not regenerate, hydrolyze, crystallize, or orient precellulosic or unorganized substances, but that it unmasks the crystalline cellulose already present. He explains the non-detection of cellulosic interferences as being due to the very small amount of cellulose in the primary wall (see also FREY-WYSSLING, 1952) and possibly the manner of combination of the non-cellulosic materials with the cellulose. Similarly, ASTBURY *et al.* (1935) believe that the removal of the hemicelluloses from the cell wall simply takes away a more amorphous component, whose direction, spacings, and lateral order in the cellulosic interstices closely resemble those of cellulose.

Few data are available on the proportion of crystalline cellulose in the cellulosic substance of young cell walls. FREY-WYSSLING (1954) has reported 34 % crystallinity in cells of the root tip of corn (also a monocotyledon, like asparagus) and 37 % in the cells of its coleoptile. However, the youngest fiber cells of asparagus have a crystallinity of *ca.* 57 % following a relatively mild extractive procedure. Possibly Frey-Wyssling's material was not adequately purified of non-cellulosic components. It is of interest that the percentage crystallinity here changes little from the youngest stage of fiber development to the stage of secondary wall deposition. The cellulose of older tracheids of *Pinus radiata* and that of fibers of bamboo—again a monocot—likewise have a degree of crystallinity of 50–60 % (PRESTON *et al.*, 1950), although it has been suggested that there were impurities in some of these materials.

Recently it has been speculated that the microfibril of cellulose is essentially a micelle string, *i.e.*, that the diameter of the microfibril is equivalent to that of the micelle (RÅNBY, 1952; BALASHOV and PRESTON, 1955, in *Valonia*). In the asparagus, micellar diameter at the earliest stage is computed to be 28 Å. This agrees quite closely with the dimension of 26 Å, reported by WARDROP (1949) for extracted

primary cell walls of *Pinus* cambium and *Avena* coleoptile parenchyma. However, microfibrils of such small size have not been found in young cell walls. The micellar size increases slowly during cell elongation, as also described by CLARK *et al.* (1930) in cotton. The sudden increase in micellar diameter (*i.e.*, sudden narrowing in half-breadth values) with secondary wall deposition indicates a real change in the new micelles being deposited. Whether that change is actually one of increased diameter or more perfect lattice structure cannot be decided on the basis of the evidence at hand.

It must be pointed out here that there is no general agreement on the validity of estimation of a precise micellar diameter from measurement of line-broadening values. The assumptions, which underlie all such estimations, of uniform micellar size, perfect lattice structure, cubic symmetry, theoretical point source of X-ray beam, etc. are not borne out in actuality. The value obtained is a parameter which is related to micellar size and must be interpreted in this relative context.

SUMMARY

An X-ray diffraction and polarization-optical study of the cell wall of developing fibers of the shoot of *Asparagus officinalis* L. has been made. The cellulose micelles are randomly arranged in very young cells but assume a statistically more transverse position during elongation. The micelles of the secondary wall are deposited in a helical angle of about 48–51° to the long axis of the cell.

Although the degree of crystallinity remains constant at 55–60 % during all stages of development, micellar size appears to increase slowly during elongation and markedly during secondary wall formation. The significance of micellar size determinations and crystallinity interpretations is discussed.

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