THE SUBMICROSCOPIC ORGANIZATION OF THE WALLS OF CONIFER CAMBIUM

by

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INTRODUCTION

The physiological significance of the cambium as the major lateral meristem of arborescent plants has long been recognized and has been the subject of intensive investigation, notably by BAILEY (1923) and by PRIESTLEY (1930). Information regarding the submicroscopic organization of this tissue is, however, meagre and investigation from this point of view in conifers has hitherto centred more on the secondary wall of the xylem tracheids which arise from the cambium by its regular division. The structure of the secondary walls is now known in some detail (BAILEY AND VESTAL, 1937; PRESTON, 1946; WARDROP AND PRESTON, 1947) and it is therefore important to examine the cambium from this point of view.

Differences in structure between the primary walls of the cambial initials and the secondary layers of tracheids are clearly to be expected, since the cambium remains alive throughout the life of the tree and its walls continue to undergo dimension changes, during each growing season, over a period of 30–50 years or more, while the tracheids reach their final size soon after differentiation. Such differences as these may well have an important bearing on the processes of growth.

In attempting to elucidate the structure of the wall in such a growing tissue, it is clearly essential to observe material under conditions as nearly as possible like those obtaining in the living tissue. This is always the major difficulty in attempting observations of a biophysical nature and we cannot in the present instance claim to have overcome it completely. It is possible that certain of the more confusing aspects of our present knowledge of wall structure in growing cells is to some extent attributable to various degrees of success achieved by different workers in preserving the tissues in their natural state.

In problems of submicroscopic structure of growing cells generally it is to be remembered that there are many aspects of molecular organization to be assessed, of which perhaps the most important are:

1. The presence or absence of cellulose, and the nature of other substances present and of their inter-relations.

2. Whether the cellulose possesses micellar organization and, if so, the size of the micelles.

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3. The relative amount of cellulose which is organized into micelles — the so-called crystalline/non-crystalline ratio.

4. The preferred orientation of the micelles, if any.

5. The angular dispersion about the direction of preferred orientation.

On very few of these points has general agreement been reached. Thus BERKELEY AND KERR (1946) claim that in cotton hairs in the fresh condition the molecular chains of cellulose in the wall, while lying more or less parallel to each other as shown by the birefringence, are not organized into micelles since no reflection of X-rays occurs such as one expects from material with crystalline organization. On stretching or drying the cells, however, the cellulose apparently "crystallizes" and gives a cellulose diffraction pattern of the normal type. From these observations BERKELEY AND KERR concluded that in the fresh condition the chains of cellulose are separated by water films probably two molecules thick .On the other hand the same authors could not demonstrate these phenomena in young stems of flax where the X-ray diagram typical of micellar cellulose was obtained under all conditions, and attribute this to the early operation of growth stresses. It is not clear, however, that other factors are not involved and these will be discussed later in the paper. The frequent difficulty found in staining young growing walls with I_2 and H_2SO_4 might also be explicable along these lines, although here again many factors are concerned since difficulty of staining is encountered with other cells in the walls of which micellar cellulose is definitely present (ASTBURY AND PRESTON, 1940). The percentage cellulose in growing walls is known to be low (ALLSOPP AND MISRA, 1940; BONNER, 1935), but beyond the general picture presented by BERKELEY AND KERR nothing is known concerning the crystalline/non-crystalline ratio.

As regards the orientation of the micelles it is agreed that, in a wide variety of elongating cell types, the micelles tend to be in a comparatively flat spiral (VAN ITERSON, 1935; PRESTON, 1947; MAAS GEESTERANUS, 1941; FREY-WYSSLING, 1948) though in some of these cases it is not clear that the observations made are of a type which allows of unambiguous interpretation and in some cases (KUNDU AND PRESTON, 1940; PRESTON, 1941) it is suggested that at least in some stages of elongation the micellar spiral is steep. The most interesting feature of growing cells with a demonstrably flat spiral organization, is that the pitch of the spiral remains unaltered during elongation.

It is against this general background that the present structural determination was attempted on the primary walls of cambial initials in the conifer. Previous investigations have shown that the birefringence of cambial initials is negative (VAN ITERSON, 1935) indicating an angle between the general micelle direction and cell length greater than 45° and that in the primary layer which still surrounds the mature tracheid the corresponding angle is in fact about 80° on the average (PRESTON, 1947). It was thought desirable to establish the condition in the cambium itself by an X-ray method and the present paper deals largely with the result of the consequent investigation.

X-RAY AND OPTICAL INVESTIGATION OF CONIFER CAMBIUM

The bulk of the X-ray investigations was made on air dried material and this will be considered first leaving, the work on wet material to be briefly discussed later in this paper. The major difficulty in the X-ray examination of the cambium is to obtain specimens of sufficient thickness to give a good diffraction photograph. This was achieved by peeling the cambium from young specimens of *Pinus sylvestris* early in the growing

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season in the manner long used in this laboratory and as described by PRIESTLEY AND MALINS (1933), the material being collected when the cells were in active division and before any secondary wall deposition had occurred. The long delicate strips of tissue, some 3–4 cells thick, were kept in dilute preservative and subsequently prepared for examination by washing in distilled water. A block some 3 mm wide and 10 mm long was built up by laying successive strips over each other in parallel orientation, which were then dried flat on a glass slide. During drying, the cambial cells collapsed and became flattened in the plane of the strips so that in effect the block was a series of superposed parallel walls. The diffraction photographs from specimens thus prepared, however, showed considerable diffuse scattering tending to mask the diffraction arcs so that, after preliminary experiments, the strips of cambium were first extracted for 90 minutes with 0.1 N HCl and then built into a block as described above. The only visible effect of this treatment on the X-ray diagram was the clearing up of the background, with no effect on the position of the diffraction arcs themselves.

In determining the micellar orientation, photographs were taken with the X-ray beam directed in turn along three mutually perpendicular axes of the block.

1. Normal to the broad face of the block (*i.e.*, perpendicular to the cell surface and to the longitudinal cell axis, Fig. 1A).

2. Parallel to the broad surface of the block with the beam in the plane of the wall and perpendicular to the longitudinal cell axis, Fig. 1B.

3. Parallel to the broad surface of the block with the beam in the plane of the wall and parallel to the longitudinal cell axis, Fig. 1C.

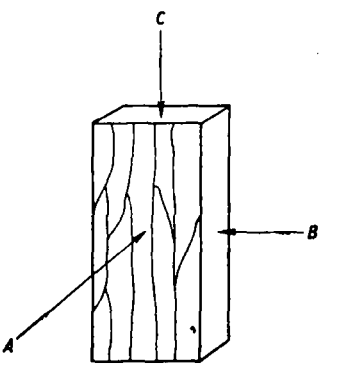

Fig. 1. For explanation, see text

The corresponding X-ray diagrams are reproduced in Plate I, Figs 1A, 1B, and 1C, respectively. In all cases there can be no doubt that cellulose is present in the dried walls and under these conditions is aggregated into micelles though the radial breadth of the arcs suggests that the micelles here are smaller than usual, a point which has been made elsewhere (PRESTON, 1947), and is in agreement with a similar observation by FREY-WYSSLING (1936) in the elongated parenchyma of oat coleoptiles. These considerations apply, it is to be noted, to dried specimens. In view of the work of BERKELEY AND KERR (1946) already mentioned, the condition of wet specimens was briefly examined. When fresh cambium, preserved in dilute aqueous preservatives, was photographed soaking wet then the water haloes were so intense as to mask any cellulose diagram. The presence of water haloes only is no guarantee that the cellulose diagram is absent. When, on the other hand, the material was put into equilibrium with an atmosphere of 98% R.H. and maintained in that condition during X-ray examination, with no further drying at any time, then the intensity of the water haloes was reduced and the normal cellulose diagram was clearly present. The cellulose is therefore organized into micelles under these conditions.

Returning, therefore, to the diagrams of dried specimens the breadth of the arcs makes it difficult to estimate the exact interplanar spacings but Table I gives the average of many determinations made visually and with a photometer. The length of the unit cell along the *b* axis (parallel to the chains) is undoubtedly 10.3 Å and there is no doubt that the spacing of the 002 planes is approximately 3.9 Å. The $10\bar{1}$ planes are represented

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by an arc corresponding to 5.5 Å, but the diffraction arc from 101 planes is apparently absent. Possible reasons for this will be discussed in a later paper, but it may be noted now that in purified cambial cellulose the arcs corresponding to both these planes are clearly present (Plate I, Fig. 2).

TABLE I

SPACINGS OF MOLECULAR PLANES IN CAMBIUM

Direction of beam in specimen (see Fig. 1)	Spacings (Å)		
A	3.97	5.54	2.55
B	3.91	5.55	2.55
C	3.97	5.54	2.55

In addition to the arcs already mentioned, others corresponding to spacings of 8.3–8.7 Å were sometimes observed. These can just be detected, for example in Plate I, Figs 1B and 1C. It is difficult at the moment to explain the presence of these anomalous arcs in terms of the accepted structure of cellulose, although preliminary investigations indicate that they are strongly reduced in intensity, or indeed completely absent, in diagrams of cambial cells from which pectins and lipophillic substances have been removed. These arcs are also absent in the diagram of purified cambial cellulose.

As regards the micellar orientation in cambial cells the diffraction photograph corresponding to position 1 above and shown in Plate I, Fig. 1A consists in the main of meridional arcs drawn out into almost complete circles. This diagram, even assuming the cells have not collapsed into flat plates, would indicate the presence of micellar aggregates lying approximately transversely to the length of the cell. This is made clear by consideration of the nature of the spiral diagram. Thus it is well known that the diagram expected from a spiral molecular configuration should consist of arcs in sets of four, symmetrically disposed about the centre of the diagram, and it has recently been shown (PRESTON, 1946) that these fuse into two meridional arcs if the angle of the spiral to the longitudinal cell axis exceeds a certain figure, which depends on the interplanar spacings concerned and the angular dispersion about the direction of micellar orientation existing in the specimen under examination. The figures for undispersed cellulose can be calculated as 73.5°, 81.8° and 82.8° for planes 3.9, 5.4 and 6.1 Å apart respectively. If considerable dispersion exists in the specimen then fusion of the arcs takes place with steeper spiral configurations. Now the outer arcs in Plate I, Fig. 1A correspond to planes of spacing 3.9 Å and the inner arcs roughly to 5.5 Å. The photograph therefore makes it certain that the cellulose micelles in the walls of cambial initials lie more or less transversely to cell length without, however, allowing any precise figure to be estimated. An average value of 80° for the primary walls of conifer tracheids, as found earlier (PRESTON, 1947), would be in harmony with these findings. There can, however, be little doubt that the cells are actually collapsed so that the photograph corresponds to that of a series of crossed crystal plates and the tilt of the micellar direction in either plate (wall) cannot be much less than 80° to the longitudinal cell axis.

The diagrams presented in Plate I, Figs 1B and 1C add some further information.

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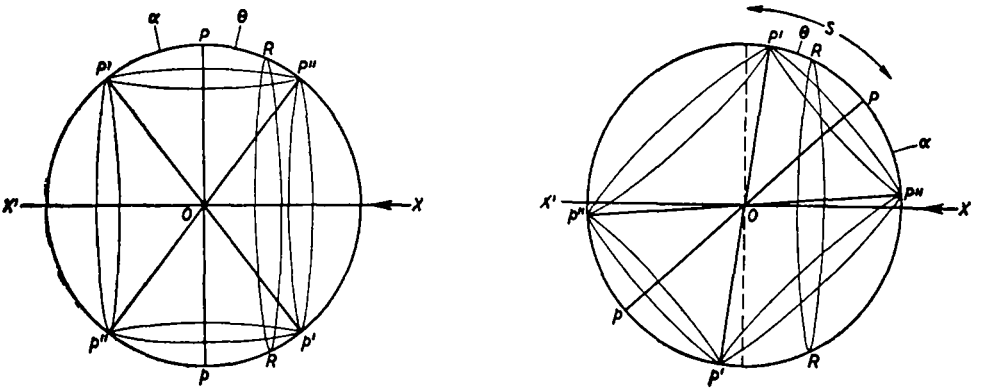
If the micelles were, in fact, not materially dispersed about their preferred, almost transverse orientation, then Fig. 1B, Plate I, might have been expected to show a series of circles since the X-ray beam would have been parallel to the micelle length. In fact, however, the intensity is much less along the meridian than along the equator. At first sight this could be taken to imply a condition similar to that found in the walls of algae (PRESTON AND ASTBURY, 1937; ASTBURY AND PRESTON, 1940; NICOLAI AND FREY-WYSSLING, 1938; PRESTON AND NICOLAI, 1949), *i.e.*, a preferred orientation of certain molecular planes parallel to the wall surface. In view of the fact, however, that the arc corresponding to planes of spacing 5.5 Å is present in all photographs (Plate I) this condition cannot occur in cambium. The correct interpretation of the photographs in Plate I, Figs 1B and 1C will be clear from the pole figures given, for two possible configurations, in Figs 2(a) and 2(b). Fig. 2(a) shows the condition in which the molecular chains of cellulose are truly transverse to the cell length. The specimen is considered to be located at O in the path of a beam of X-rays XOX'. A sphere P'P"p'p", the projection sphere, is constructed round O as centre and, concentrating on the planes of 3.9 Å



(a) Fig. 2. For explanation, see text (b)

spacing, parallel to the chain length, the normal OP to these planes in any one transversely oriented micelle is erected from O intersecting the sphere at P, OP being perpendicular to XOX'. Since in the real specimen there is, in fact, some angular dispersion about the preferred direction of orientation, as is clear from the presence of an arc of 2.56 Å spacing in Plate I, Fig. 1B, and from other observations described below, the line POp can be considered to "wobble", defining the solid cone P'OP" and p'Op" of half angle α. If the micelles are completely dispersed around the axis of common orientation this can be taken into account by revolving the cones around the axis XOX' so that the pole of all the possible positions of the normal becomes a broad band limited by two small circles P'p" and P"p'. If the dispersion α is sufficiently great this band will intersect the reflection circle RR in a circle, so that the X-ray diagram would be a complete and uniform circle. If, however, the cellulose chains are not truly transverse, but make an angle S with the transverse direction, then this can be taken into account by tilting the pole figure through an angle S as shown in Fig. 2(b). It is to be noted that since the configuration is spiral, opposite walls in a cell will be tilted in opposite directions so that two pole figures are required. Only one of these is drawn in Fig. 2(b) since the presence of the other, tilted in an anticlockwise direction through an angle S, would make the diagram unduly complicated. Under these conditions the intensity in the

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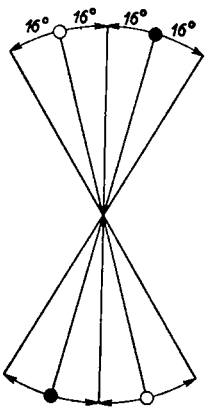
region of the point R is reduced. In fact, if $S > \Theta + \alpha$ the intensity near R would be zero. In the diagram Plate I, Fig. 1B the intensity along the meridian is, in fact, strongly reduced, but not to zero. It may therefore be said that the cellulose chains are tilted to the transverse plane by an angle which is greater than zero but less than $\Theta + \alpha$ or, approximately for the planes of 3.9 Å spacing, $11.50 + \alpha$. The spread of the arcs in Plate I, Fig. 1A shows that the dispersion is not greater then $\pm 32°$ so that these considerations show that the inclination of the chains to the transverse is less than 43°.



Since, further, a dispersion about the chain direction of front and back walls of $\pm$ 16° would give a total spread of the arcs of $\pm$ 32° (Fig. 3) it is apparent from further examination of Plate I, Fig. 1A that the angle of inclination of the micelles to the transverse is not greater than 16°. The three photographs of Plate I, Fig. 1 are therefore, mutually consistent with this figure.

Fig. 3. Diagrammatic interpretation of the X-ray photograph of cambium with the beam normal to wall surface (Plate 1, Fig. 1A). The solid circles represent the mean positions of the 3.9 Å arcs corresponding to upper walls of the cells and open circles the corresponding position in lower walls. An angular dispersion of $\pm$ 16° can then give a meridional arc of total extent $\pm$ 32°, as observed on the photograph. If the angular distance of the circles from the meridian is greater than the 16° shown here, then the arc would be resolved into two.

TABLE II

THE BIREFRINGENCE IN TRANSVERSE SECTION OF CAMBIUM AND THE PRIMARY WALLS OF DIFFERENTIATING TRACHEIDS FROM *Pinus sylvestris*

Specimen	Phase difference* (degrees)	Section thickness (μ)	Birefringence
Cambium			
Radial Walls	8.3	8.0	0.001_7
Tangential Walls	7.8	8.0	0.001_6
Primary Walls of Differentiating Tracheids			
Radial Walls	8.3	8.0	0.001_7
Tangential Walls	8.3	8.0	0.001_7

* Averages of 20 measurements

These conclusions are supported by optical evidence such as that of KERR AND BAILEY (1934) which shows that the cambium is birefringent in both longitudinal and transverse section, an observation which has been repeated during the present investigation and which suggests that considerable angular dispersion of the micelles occurs about their preferred orientation. This is supported also by the magnitude itself of the birefringence of the cambium. From Table II it is clear that the birefringence is of the order of 0.001 to 0.002 in transverse section. If the cellulose content of the wall is taken as 25% (ALLSOPP AND MISRA, 1940) then the observed value is still considerably lower than might be expected. For, assuming the usual mixture formula (see, *e.g.*, HERMANS, 1946) to hold and that the remaining 75% of the wall has a refractive index of n_r then, ignoring small density corrections,

$$n_\gamma' = 0.25n_\gamma - 0.75n_r$$

$$n_\alpha' = 0.25n_\alpha - 0.75n_r$$

and

$$n_\gamma' - n_\alpha' = 0.25\,(n_\gamma - n_\alpha)$$

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PLATE I

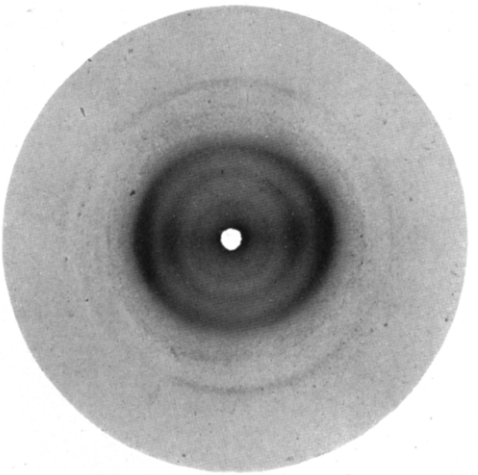

Fig. 1A. Beam normal to cell wall surface, length of initials parallel to length of page, (see Text Fig. 1A). Specimen-film distance = 2.86 cm

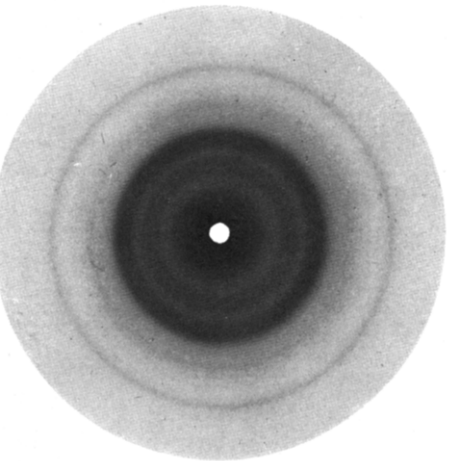

Fig. 1B. Beam parallel to wall surface and perpendicular to longitudinal axis of cells. Cell length parallel to longer edge of page, (see Test Fig. 1B). Specimen-film distance = 2.54 cm

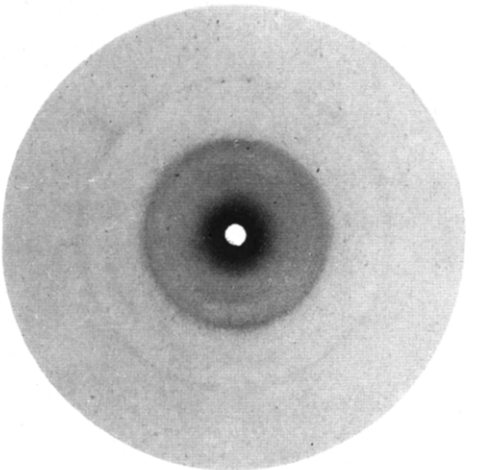

Fig. 1C. Beam parallel to wall surface and to longitudinal cell axis. Wall surface parallel to longer edge of page, (see Text Fig. 1C). Specimen-film distance = 2.86 cm

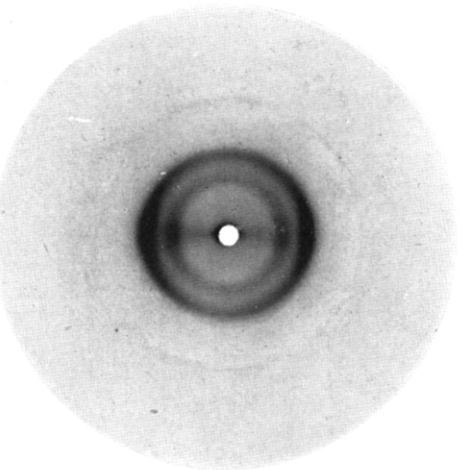

Fig. 2

Fig. 1A, 1B, 1C. X-ray diagrams of dried cambial tissue. CuK_α radiation, flat film.

Fig. 2. X-ray diagram of a pellett of purified cambial cellulose. Note the presence here of diffraction rings corresponding both to planes of 6.1Å and 5.4 Å spacing (inner two circles) whereas only the 5.5Å ring is present in Fig. 1

where $n_\gamma' - n_\alpha'$ is the birefringence to be expected in a body containing 25% cellulose whose intrinsic birefringence is $(n_\gamma - n_\alpha)$. Taking the angle of the chains to the transverse to be 16° which is the greatest possible, then $(n_\gamma - n_\alpha)$ should be of the order 0.047, assuming the micelles are all parallel and hence

$$n_\gamma' - n_\alpha' = 0.012$$

approximately. This gives the minimum possible figure for undispersed cellulose. Since the real figure is 0.001 to 0.002 there must be either a large angular dispersion or a low crystalline to non-crystalline ratio, or both. The X-ray diagrams show clearly that the former at least is involved.

The above X-ray and optical evidence may be interpreted diagrammatically as in Fig. 4 representing a type of micellar organization essentially similar to that described by FREY-WYSSLING (1930) on the basis of optical evidence and termed by him "Tube Structure". The present investigation provides the only complete X-ray evidence demonstrating such a structure though the writers have applied the same technique in demonstrating this structure in the parenchyma of oat coleoptiles (WARDROP AND PRESTON, 1949).

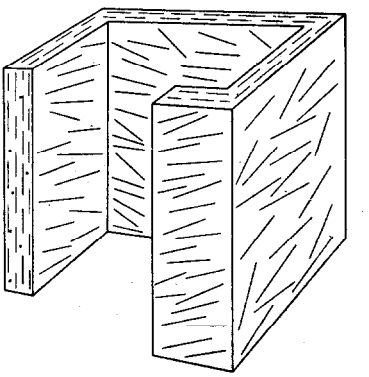

Fig. 4. Diagrammatic representation of a portion of a cambial initial with parts of two walls removed. The lines represent the orientation of the micelles in the walls.

THE INTERMICELLAR SYSTEM IN CAMBIUM

The organization of the crystalline portion in cambium cell walls may be taken therefore as fairly well understood. Since, however, some 75% of the weight of the tissue is non-cellulosic it would seem profitable to examine briefly the main features of this non-crystalline material. The chemical analysis of the cambium carried out in this laboratory by ALLSOPP AND MISRA (1940) and that of oat coleoptiles by BONNER (1935) are presented in Table III.

TABLE III

THE MAJOR CONSTITUENTS OF CAMBIUM AND OF *Avena* COLEOPTILES

Specimen	Cellulose %	Pectin %	Hemicellulose %	Protein %
Conifer Cambium . . . . . (ALLSOPP AND MISRA, 1940)	25.1	16.6	*ca.* 6	20.8
Avena coleoptile . . . . . (THIMANN AND BONNER, 1933)	42	8	38	12

In cambium the cellulose constitutes only one quater of the dry weight of the wall substance while in *Avena* coleoptiles it is present to a considerably greater extent. Greater interest, however, centres on the relative volume of the cellulose in fresh material. The fact that, on drying, the wall of each type of cell shrinks to about $^1/_3$ of its thickness when wet suggests that this volume is rather low, and FREY-WYSSLING (1936) estimates that in the parenchyma of oat coleoptiles the cellulose occupies approxi-

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mately 14% of the wall volume. Similar estimates for wet cambium would suggest a figure of approximately 8%. From this figure it is possible to estimate the order of magnitude of the distance separating the micelles. If, for instance, it is assumed that all the cellulose occurs in micelles of d Å (circular) diameter separated uniformly by a distance of m Å, then in a 1 cm^2 area perpendicular to micelle length there will be $(1/m)^2 \cdot 10^{16}$ micelles, each $\pi\ (d/2)^2 \cdot 10^{-16}$ cm^2 in cross-sectional area. In a cube 1 cm deep, therefore, the total micelle volume will be $(\pi\, d^2/4\, m^2)$, assuming the micelles to be at least 1 cm long. This is the relative volume of the cellulose and hence

$$(\pi\, d^2/4\, m^2) = 0.08$$

and therefore

$$m = 3.3\, d$$

It will be shown in a later paper (WARDROP AND PRESTON, 1949) that d is of the order of 20–30 Å and so the intermicellar distance would be of the order of 70–100 Å. If, however, it is assumed that only 60% of the cellulose is crystalline then this distance will be increased by $\sqrt{(5/3)}$ and would then be of the order 90–120 Å. While this latter condition is most likely to attain, it is probable that the actual distances are very variable.

It is perhaps also interesting to note that if the wall could be considered as consisting of individual cellulose chains arranged strictly parallel to, and equidistant from, each other then the distance between them would be of the order of 13 Å. This is of the order of three molecular diameters and compares favourably with the bimolecular separating films suggested by BERKELEY AND KERR (1946). If the cellulose chains are indeed spun in the surface of the cytoplasm in regular distribution this might be expected as the distance separating them.

DISCUSSION

From the foregoing it will be seen that in cambial cell walls the micellar organization approximates to the tube type of FREY-WYSSLING (1930) and is characterized further by the relatively small volume occupied by the skeletal micellar system. It is also of interest that in the absence of lignin there is a close association between the various cell wall constituents although as yet there is no definite evidence of linkages of a chemical kind existing between them. Further the cell wall is intimately related to the cytoplasm as shown by the staining reactions of cambial tissue*. There is already a good deal of evidence in the literature suggestive of such a relation in the walls of growing cells, notably in the demonstration of the difficulty of plasmolysis of meristematic cells and in the fact that in the growing terminal cells of algae and fungi, although the cytoplasm does move away from the wall in the regions of the filament removed from the apex, in the actual region of growth the cytoplasm adheres firmly to the wall. The physiological significance of the observations most probably lies in the fact that cells which are capable of extension growth usually possess tube structure. In this discussion it is useful therefore to explore what correlations may exist between the structural and physiological aspects of extension growth.

In the first place it would appear unwise to envisage any rigid separation between the wall of the cambial cell and its cytoplasm, at least in the sense used in cells capable

* These staining reactions will be discussed in a later paper on primary walls generally.

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of ready plasmolysis. In this regard, then, it is possible that the wall itself represents the boundary of the cytoplasm which ramifies and interpenetrates the cellulose micelles of which its structural skeleton is composed.

Further support for this view may be found in the organization of the cellulose skeleton itself and of its relation to cell dimensions. The micellar orientation of the wall in growing cells is usually maintained approximately transverse to the longitudinal cell axis irrespective of the cell length. This is suggested by the results of MAAS GEESTERANUS (1941) on the stellate pith cells of *Juncus* and of others, (see FREY-WYSSLING, 1948) and is in sharp contrast to the condition attained in secondary walls. Thus PRESTON (1934, 1948) has shown in the case of conifer tracheids that the micellar orientation is such that as the cells become longer the micellar spiral in the secondary wall becomes steeper and the same has now been found true with some fibres (PRESTON AND MIDDLEBROOK, 1949, PRESTON AND SINGH, 1949). Hence it seems legitimate to infer a relationship between wall and cytoplasm different in primary from that obtaining in secondary walls.

As regards the suggestion of CASTLE (1937) and of VAN ITERSON (1946) and others, that membrane tension in cylindrical cells is the factor governing micellar orientation it is to be pointed out that such orientation could arise in isolated cells, as for example, in algal and fungal filaments and the stellate pith cells of *Juncus*, only from strains operating in the growing cell which accompany dimensional changes during growth. However, in the bulk of the cells investigated the actual dimension changes during growth are in a direction perpendicular to the directon of micellar orientation, that is the cells extend in length rather than in diameter, whereas cambium is one of the few cases where during differentiation the strain operates in the direction of micellar orientation. In the differentiating cambium there is then a case where conceivably strains arising during growth could govern micellar orientation. Even in this case there are serious objections. In the first place, as the above investigations show, orientation exists in the cambium before dimensional changes accompanying differentiation occur. In particular the longitudinal tangential wall of the fusiform initials do not extend laterally, at any stage of differentiation. Nevertheless these walls are largely responsible for the diagrams presented above so that here, too, the micellar orientation is almost transverse. This is a clear demonstration that some factor other than simple strains in the cell wall must govern orientation. Secondly, the cells of the cambium are not isolated but are closely packed, so that the osmotic forces of the cell will not be wholly resisted by the cell wall and will be counteracted by similar stresses operating in adjacent cells.

All the above considerations point to the underlying significance of the cytoplasm as the factor governing orientation and extension of the cell wall. Nevertheless extension of the cells must involve molecular displacement under conditions such that the net micellar orientation is not materially affected. It would therefore be of considerable interest to enquire how far the cell wall structure envisaged in the present discussion will allow displacements of this kind.

In view of the fact that the bulk, though not all, of the work described in this paper has been carried out on dried material, it becomes pertinent to look into the evidence presented by BERKELEY AND KERR (1946) that the chain molecules of cellulose, even in the secondary walls of cotton hairs, while oriented with respect to one another are not aggregated into micelles. It was further suggested that under these conditions the cellulose molecules were separated by bimolecular water films so that no X-ray diffrac-

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tion pattern was obtained such as characterizes crystalline substances. In our present investigation the X-ray diffraction pattern of fresh cambium was examined at different relative humidities and in all cases up to 98% R.H. the cellulose was clearly present in micellar aggregates. A preliminary note on this point, including similar observations on algal cells, has already been given (PRESTON, WARDROP, AND NICOLAI, 1948).

The possibility that in growing cells the cellulose skeleton is not organized into micelles is, in fact, not new for it was suggested by HEYN (1940) some years ago. Unfortunately it is difficult to assess the merits of the idea as proposed by BERKELEY AND KERR (1946) in terms of the X-ray diagrams they present. Although the same fibre bundle was used in the observations presented in their paper (Fig. 2, p. 305) it is not explicitly stated that the specimen was exposed to X-rays for the same period under the different conditions. This leaves open the possibility that in the fresh material the water halo was of sufficient intensity to mask completely the X-ray diffraction pattern of cellulose, and in this regard it is to be noted that quite generally fresh cellulose material has a higher water content than it has after drying and rewetting. This effect may well be exaggerated in the present case since in the fresh material, at least up to the time of cessation of secondary thickening the lumina of the cells are water filled whereas after drying and rewetting it is possible that the (collapsed) lumina would not refill with water. This is, in fact clear from the diagram in Fig. 2 of their paper since the dried, rewetted bundle shows no water halo at all. The complete absence of a water halo here is indeed puzzling. There is again the further point that in fresh material the micelles may be, and probably are, more dispersed around their common direction of orientation than after drying. The X-ray diagram would then be more diffuse and more easily masked by the water halo. This may also explain the appearance of a cellulose diagram in wet cotton after stretching, for elongation would again reduce the dispersion. Thus it seems that any conclusions based upon these diagrams are at least open to question. In any case the complete absence of diffraction arcs does not necessarily imply complete separation of the molecular chains of cellulose, for according to FANKUCHEN AND MARK (1946) diffraction arcs are not to be expected from micelles less than a certain diameter (*ca* 20 Å).

If, as our evidence suggests, the cellulose is always aggregated into micelles in the cambium then the micellar structure must be sufficiently flexible to allow considerable dimensional changes to occur. This point will, however, be discussed in a later paper.

SUMMARY

An X-ray and optical examination has been made of the walls of cambial initials in conifers, and it has been concluded that the structure of these growing cells approximates to the tube structure of FREY-WYSSLING. This is the first demonstration of this structure made by X-ray methods. The molecular chains of cellulose are inclined to the transverse at an angle which is less than 16° but greater than 0°, and the "micelles" are narrower than in secondary walls and have considerable angular dispersion about their common direction of preferred orientation. X-ray examination of fresh cambium at high relative humidities indicate that the same structure obtains in the fresh tissue.

RÉSUMÉ

L'examen optique et l'étude par les rayons X des parois des cellules terminales du cambium chez les conifères a montré que la structure de ces cellules en voie de croissance se rapproche de la structure des tubes de FREY-WYSSLING. C'est la première démonstration de cette structure faite par les rayons X. La chaîne moléculaire de cellulose forme avec la perpendiculaire à l'axe de la

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cellule un angle compris entre 0° et 16°; les "micelles" sontplus étroites que dans la formation secondaire et présentent une dispersion angulaire considérable autour de l'orientation générale. L'étude par les rayons X du cambium frais à forte teneur en eau montre que la même structure existe dans les tissus frais.

ZUSAMMENFASSUNG

Die Wände der Endzellen des Kambiums der Koniferen wurden optisch und mit Röntgenstrahlen untersucht und es wurde gefunden, dass die Struktur dieser wachsenden Zellen der Röhrenstruktur von FREY-WYSSLING ähnelt. Dies ist der erste Nachweis einer solchen Struktur mit Röntgenstrahlen. Die Zellulosemolekülkette bildet mit der Senkrechten zur Achse der Zelle einen Winkel von 0° bis 16° Spannweite; die "Mizellen" sind schmäler als in den sekundären Wänden und zeigen starke Abweichungen rund um die allgemein bevorzugte Richtung. Eine Röntgenstrahlenuntersuchung des frischen Kambiums mit starkem Feuchtigkeitsgehalt zeigt, dass frische Gewebe die gleiche Struktur besitzen.

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