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THE VISIBLE STRUCTURE OF THE SECONDARY WALL AND ITS SIGNIFICANCE IN PHYSICAL AND CHEMICAL INVESTIGATIONS OF TRACHEARY CELLS AND FIBERS

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With plates 140–149

INTRODUCTION

THE SECONDARY WALL of plant cells has long been known to be a heterogeneous structure. That it is more or less conspicuously striated and laminated was shown by Mirbel, Von Mohl, Valentin, Meyen, Th. Hartig, and other pioneer anatomists who demonstrated, in addition, that it may be resolved by specific chemical and mechanical treatments into lamellae, fibrils, granules, and other visible units of fairly constant form and size. This led, during the second half of the last century, to prolonged discussions concerning the fundamental structure of cell walls in general, and to much speculation regarding the physiological processes involved in their formation.

Although a voluminous literature developed between 1850 and 1900, no consensus of opinion was reached concerning the exact physical and chemical significance of the visible heterogeneity of the secondary wall. Nor is there a general agreement among different groups of investigators at the present time. It is true that the study of anisotropy, of rod double refraction, of various types of dichroism, and of X-ray diagrams has in recent years contributed much toward a clearer understanding of submicroscopic structures, and regarding the orientation of such structures in the grosser layers of the secondary wall, but it has not afforded as yet an adequate explanation of the finer types of visible heterogeneity.

[1]Parts of these investigations were made by the junior author as a National Research Fellow in Botany.

In view of such facts as these, it seemed desirable to the writers to undertake a detailed investigation of the secondary wall in an endeavor (1) to verify and, if possible, to amplify the observations of previous workers; (2) to correlate results obtained by different techniques and by the study of divergent cell types; and (3) to interpret the visible heterogeneity of the secondary wall in terms of its sub-microscopic structure and of its chemical composition.

In an investigation of this character one is faced, at the outset, by a serious difficulty, upon the solution of which success or failure clearly depends. The range of recorded cases in which the details of wall structure are even vaguely visible — without resorting to the use of softening or hardening processes, of macerating or swelling agents, and of other more or less drastic chemical and mechanical treatments — is very limited. Severe treatments are capable of yielding extremely useful and significant data, but are likely to produce distortions and other artifacts, and therefore must be checked by observations on untreated material. In other words, an adequate system of controls — or means of accurately visualizing the normal structure of the secondary wall — is indispensable.

As indicated in the preceding paper of this series (18), it is possible to section dense woods and other hard tissues without resorting to the use of softening processes which might modify their structure and chemical composition. It seemed advisable, accordingly, to make an extensive survey of a wide range of gymnosperms and angiosperms in search of species that afford clearly defined images of cell wall structure in untreated sections. More than 3000 species, representing 160 families and 40 orders, were examined. It was found that the large-celled woods of various tropical dicotyledons provide unusually favorable material for microscopic investigations. These plants are not bizarre or unusual forms; nor are they confined to any restricted group or genus. They are widely distributed and of not uncommon occurrence in such families as the Theaceae, Monimiaceae, Icacinaceae, Rhizophoraceae, Euphorbiaceae, Flacourtiaceae, etc. When thin (5–10 μ), smoothly-cut sections of the wood are examined in liquids of the right index of refraction, using the best modern optical equipment, the relatively broad expanse of wall in the fiber-tracheids and libriform fibers of certain of these plants reveals finely laminated, striated, and reticulated structures in exquisite detail. By using untreated sections of such plants as controls, it is possible to determine the exact effects upon normal structures of varied chemical and mechanical treatments, and thus to extend the scope of investigation to cover a wide range of less favorable species and tissues.

The following discussion of tracheary cells and fibers is divided into two parts. The grosser and more conspicuous types of layering of the secondary wall are dealt with in Part I; structures which more nearly approach the limits of microscopic visibility, in Part II. As previously stated, considerable is known[1] concerning the physical factors involved in the differentiation of the former structures, which must be clearly visualized and accurately correlated before proceeding to a detailed consideration of the finer types of visible heterogeneity.

TERMINOLOGY

The terms *middle lamella, primary wall, secondary wall,* and *tertiary wall* have been employed in several fundamentally different senses and to designate entirely different structures. This has led to much confusion in the literature and to serious discrepancies, not only in descriptive morphological work, but also in physiological, biophysical, and biochemical investigations. As a result of our detailed study of the cambium and its derivatives and of our preliminary investigations of other meristems and their derivatives, we attempted, in a former paper (18), to clarify the situation by suggesting that (1) the term *middle lamella* be used synonymously with intercellular substance in referring to the truly isotropic material which separates the walls of adjoining cells; (2) the term *primary wall* should no longer be applied to the first-formed layer of secondary thickening, but should be reserved for the original wall of the cell which is formed in the meristematic region and is carried over in more or less modified form into the fully differentiated tissues; and (3) the term *secondary wall* be used in referring to the strongly anisotropic layers of secondary thickening which are formed after a cell has attained its final size and shape. The term *tertiary wall* is so variously used and interpreted and so confusing that its use should be discontinued. We propose to employ our revised terminology in this and succeeding papers.

I. THE PRINCIPAL LAYERS OF THE SECONDARY WALL

A. Layering Due to Physical Factors

The secondary wall of normal tracheids, fiber-tracheids, and libriform fibers commonly consists of three layers of different refractive character; (1) a relatively narrow outer layer, (2) a narrow inner layer, and (3) an intervening layer of variable thickness. When thin, per-

[1]For comprehensive reviews of the literature relating to this subject, the reader is referred to Van Iterson (30, 31) and Frey-Wyssling (13).

fectly transverse sections of such cells are examined in polarized light between crossed nicols, *Fig. 3,* the inner and outer layers exhibit strong double refraction and are brilliant — except in positions of extinction — whereas the central layer is dark or noticeably less birefringent. The conditions tend to be reversed in longitudinal sections, *Fig. 2,* in which the central layer shows intense double refraction, and the inner and outer layers are dark or less conspicuously birefringent. In other words, as shown long ago by Dippel (7) and others, the secondary wall consists of anisotropic layers which are dark or brilliant in polarized light depending upon the plane of sectioning of the cell or upon the angle from which the wall is viewed.

Our extensive survey of gymnosperms and angiosperms has demonstrated that most tracheids, fiber-tracheids, and libriform fibers are provided with a secondary wall of this 3-layered type. The narrow inner and outer layers are of relatively constant thickness, not only in different parts of a given plant but also in plants of different systematic affinities. Variations in thickness of the secondary wall are due, therefore, primarily to fluctuations in the width of the central layer. When the secondary wall is thin, as in the tracheids of the early wood of many conifers, the inner and outer layers are so closely approximated that the tenuous intervening central layer is invisible in polarized light, except in very thin (3–7 μ), perfectly transverse sections of straight-grained tissue. In thicker or obliquely cut sections, the width of the inner and outer layers is much exaggerated by the scattering of light from these intensely birefringent structures. This fogs and conceals the central layer, just as the closely approximated brilliant outer layers of adjacent cells commonly obscure the tenuous primary walls and middle lamella (compare *Figs. 1* and *3*).

Deviations from the normal 3-layered type of secondary wall are of not infrequent occurrence. Thus, many thick-walled libriform fibers and fiber-tracheids have no clearly differentiated inner layer, whereas others have more than three layers of varying width and birefringence, *Fig. 4.* Walls of a multiple-layered, anisotropic type, which are of relatively sporadic occurrence in the fiber-tracheids and libriform fibers of dicotyledons, are characteristic features of the fibers of many monocotyledonous stems. In transverse sections of such fibers, *Fig. 6,* there are narrow brilliant zones in polarized light which alternate regularly with broader and conspicuously less birefringent ones. Variations in the thickness of the secondary wall of these cells are due largely to variations in the number of successively formed layers.

The optical behavior of the anisotropic layers of the secondary wall

of tracheary cells and fibers is closely correlated with the orientation of striations and so-called fibrillar structures, which are visible in cells that have been subjected to various chemical and mechanical treatments. When the striations and fibrils are arranged parallel, or nearly parallel, to the long axis of a tracheary cell or fiber, a layer is dark in sections cut at right angles to this axis, but is brilliant in longitudinal sections and in surface view — except, of course, in the four positions of extinction. The intensity of the birefringence varies in obliquely cut intervening sections, decreasing as the plane of section approaches that of a truly transverse section. On the contrary, where the striations and fibrils are arranged approximately at right angles to the long axis of a cell, a layer is brilliant in cross sections and in surface view, but is dark in thin longitudinal sections, *Fig. 2,* which transect the fibrillar structure. When the striations and fibrils have a helical arrangement and, therefore, are obliquely oriented in relation to the major axis of the cell, a layer is brilliant in surface view and more or less birefringent in both transverse and longitudinal sections. If the helix has a pitch of approximately 45°, an oblique section, which is cut parallel to the striations and fibrils on one side of the cell, will transect these structures on the opposite side of the cell. Thus, in such sections, the layer will exhibit both isotropy and strong double refraction; i.e., it will be dark on one side of the section and brilliant on the opposite side. Changing the fibrillar orientation from a left-handed to a right-handed helix or *vice versa* will not alter the birefringence in transverse or in longitudinal sections so long as the angle of obliquity remains constant.

In the typical 3-layered secondary walls of tracheids, fiber-tracheids, and libriform fibers, the striations and fibrils of the central layer are oriented parallel to the long axis of the cell, or at angles which do not deviate excessively from that axis; whereas those of the inner and outer layers are arranged more nearly at right angles to the major axis of the cell. Thus, the central layer exhibits strong double refraction in longitudinal sections, *Fig. 2,* and isotropy or relatively feeble double refraction in transverse sections, *Figs. 1* and *3;* whereas the conditions are reversed in the case of the inner and outer layers of the secondary wall. In multiple-layered walls of the type illustrated in *Fig. 6,* the orientation alternates regularly from parallelism to the major axis of the cell in the broader layers to marked obliquity in the narrower ones. The former layers exhibit intense double refraction in longitudinal sections; the latter layers, in transverse sections.

In the case of optical anisotropy, the so-called index-ellipsoid has, according to Frey-Wyssling (13), a major axis (N_γ) which is oriented

parallel to the striations and fibrils, and two minor axes ($N\alpha$ and $N\beta$) which are placed at right angles to these structures. On the contrary, in the case of swelling-anisotropy, the ellipsoid of expansion has two major axes which are oriented at right angles to the striations and fibrils, and a minor axis which is parallel to these structures. Therefore, the dark layers of *Figs. 1, 3,* and *6,* which have longitudinal striations, expand laterally, increasing in both width and circumference; whereas the strongly birefringent layers, the striations of which are oriented more nearly at right angles to the long axis of the cell, are unable to do so and expand longitudinally. Where the dark layers are of considerable width, they tend, by their excessive lateral expansion, to disrupt the thin birefringent layers, as indicated in *Fig. 7.*

The strongly anisotropic behavior of the secondary wall suggests that its layers are composed of sub-microscopic units which have definite planes of orientation, and that there is a close correlation between the orientation of these units and of such visible structures as striations and fibrils. It was in fact a consideration of these phenomena which led Nägeli to formulate the Micellar Hypothesis.

More recently, X-ray analyses and other physico-chemical investigations have indicated that native cellulose consists of chains of anhydrous glucose residues which are bound together by secondary valences into a space lattice of definite dimensions. These chains are arranged parallel to each other, and, in the case of the secondary wall of fibers and of *Valonia,* are oriented parallel to the striations and fibrils — as shown by Katz (17) and by Astbury and his co-workers (1). Furthermore, there is much cumulative evidence[1] from detailed investigations of anisotropy, of rod double refraction, of various forms of dichroism, and of X-ray analyses which suggests that the cellulose chains are not uniformly distributed throughout the secondary wall, but are aggregated into more or less vaguely defined anisotropic units the major axis of which is oriented parallel to that of the visible striations and fibrils.

In view of such facts as these, it is evident that layering of the type discussed on preceding pages is not due fundamentally to differences in chemical composition, but rather to changes in the orientation of anisotropic units of cellulose in the successively formed layers of the secondary wall.

B. Layering Due to Chemical Factors

The broad central layers of normal fiber-tracheids and libriform fibers frequently have subsidiary layers of varying width which are much

[1]This evidence has recently been summarized and discussed by Frey-Wyssling (13).

intensified by differential staining, *Fig. 8*. These subsidiary layers, unlike those illustrated in *Fig. 4*, are not closely correlated with variations in the orientation of the anisotropic cellulose, but are due to differences in lignification or to variations in the distribution of non-cellulosic constituents. They may be eliminated by delignification and other standard treatments for the purification of cellulose. It should be emphasized in this connection that the anisotropic layers of normal tracheids, fiber-tracheids, and libriform fibers are coherent even in walls that have been treated to remove their non-cellulosic constituents. There are evident planes of weakness but no actual discontinuities in the cellulosic matrix.

Conspicuous discontinuities are, however, of not infrequent occurrence in the peculiar tracheids of "compression wood," in so-called gelatinous fibers, in certain types of bast fibers, and in sclereids. They are due to narrow layers of *truly isotropic* material which contain little, if any, cellulose. Thus, when sections of unlignified or delignified cells are treated with standard solvents of pectic compounds and hemicelluloses, the layers dissolve and liberate the anisotropic layers of cellulose which may be slipped apart as shown in *Fig. 26*. These truly isotropic layers may be accentuated by differential staining and are clearly visible in ordinary light, *Fig. 21*. They present some difficulties, however, when sections are examined in polarized light between crossed nicols. For example, the entire laminated structure in *Fig. 21*, with the exception of the narrow outer layer, is dark in polarized light, owing to the fact that the orientation of cellulose in the anisotropic layers is parallel to the long axis of the cell. Therefore, the truly isotropic layers are concealed in transverse sections, but they are clearly visible in radial longitudinal sections and appear as dark lines between the birefringent layers of cellulose. There are similar tenuous isotropic films in the fibers of *Pandanus* on the outside of each narrow anisotropic layer, *Fig. 6*. They are masked in both transverse and longitudinal sections, since the broader anisotropic layers of cellulose are dark in cross sections, and the narrower ones are dark in longitudinal sections.

C. Layering in Sclereids and Non-fibrous Sclerenchyma

It should be noted, before passing to a detailed consideration of the finer types of visible structures, that sclereids and other types of non-fibrous sclerenchyma have a fundamentally different type of secondary wall. The anisotropic layers of such cells—at least in tissues of the higher plants that we have examined thus far—show no conspicuous striations or fibrillar structures, either in the untreated or in the swollen

condition of the cell wall. Furthermore, the anisotropic layers are brilliant in polarized light in all planes of section of the secondary wall, but are dark in surface view. The birefringent layers alternate more or less regularly with others which are dark in all planes of view, *Fig. 5*. A detailed discussion of these cells and of other non-fibrous types is reserved for subsequent papers of this series.

II. THE FINER VISIBLE STRUCTURES OF THE SECONDARY WALL

A. Normal 3-Layered Tracheids, Fiber-Tracheids, and Libriform Fibers

As stated in Part I, variations in thickness of the secondary wall of normal tracheids, fiber-tracheids, and libriform fibers are due primarily to fluctuations in the width of the central layer, which may attain a radial breadth of more than 15 μ in the large-celled woods of various tropical dicotyledons. Therefore, the central layer provides more favorable material for sectioning and for study at high magnifications than either the inner or the outer layers which are so tenuous as to present serious optical difficulties.

Figure 10 is a transverse section of the wood of *Siparuna bifida* (P. & E.) A. DC. cut without preliminary softening or other modifying treatments. The broad central layer of the secondary wall is strikingly heterogeneous and exhibits a complex pattern of anastomosing radial striations. The striations are clearly visible in unstained sections mounted in water and in other liquids of varying indexes of refraction; and, in white light, are optically of two types, i.e., light and dark. There are corresponding light and dark striations in tangential longitudinal sections, *Fig. 13*. It is evident, accordingly, that the central layer of the secondary wall in these cells is composed of thin plates or lamellae which have a radio-longitudinal or radio-helical orientation. The lighter lamellae are strongly birefringent in polarized light, *Fig. 13*, except in positions of extinction and in sections cut at right angles to the longitudinal axis of the lamellae; whereas the alternating lamellae are dark, or at least comparatively isotropic, in all planes of view.[1]

The birefringence of the lighter lamellae is not due entirely to rod double refraction, as may be determined by examining sections in a graded series of liquids of varying indexes of refraction. Nor is the

[1]Extremely thin, smoothly cut sections are essential for critical examination in polarized light. If the sections are too thick or are scratched or roughened in cutting, the tenuous dark lamellae will be completely masked by the glare of light from the strongly birefringent lamellae.

apparent isotropy of the intervening lamellae due solely to the masking effects of lignification or to the presence of other non-cellulosic constituents. The walls of immature unlignified cells show identical patterns and a similar differentiation into lamellae of two distinct categories of birefringence, as do delignified cells that are treated for the removal of hemicelluloses and other non-cellulosic constituents.

By subjecting untreated sections to the action of such swelling agents as acids, alkalies, chloro-iodide of zinc or cuprammonium hydroxide, and by carefully controlling the reactions, it is possible to expand the central layer and its constituent lamellae without distorting or seriously modifying the original structural pattern (compare *Figs. 10* and *11*). As the central layer expands and enlarges under the microscope, successively finer details of structure become visible. The lamellae are not discrete homogeneous entities, and are resolved during the expansion of the central layer into aggregations of elongated heterogeneous complexes of varying degrees of fineness which grade down to the limits of microscopic visibility. The darker lamellae are compact sheets of relatively isotropic material which contain a low ratio of birefringent complexes. On the contrary, the lighter lamellae are aggregations containing a high ratio of birefringent complexes and a low ratio of apparently isotropic ones. There are no discontinuities in the structural pattern which is firmly knit together by lateral anastomoses and interlocking complexes.

After treatment for the removal of non-cellulosic constituents, the purified cellulose exhibits a similar structural pattern, which upon swelling, *Fig. 14,* is resolved into a complex and firmly coherent matrix, having elongated, intercommunicating interstices of varying degrees of fineness. The darker and more compact parts of the matrix, which correspond to the lighter lamellae of *Fig. 10,* are strongly birefringent in longitudinal sections and show conspicuous dichroism when carefully stained with congo red or chloro-iodide of zinc; whereas the lighter and more porous parts of the matrix, which correspond to the darker lamellae of *Fig. 10,* are so feebly birefringent that they appear to be comparatively isotropic.

Conversely, when the central layer is freed of cellulose by treatments with 72% sulphuric acid, the details of the swollen pattern are preserved in the so-called "lignin" residue, *Fig. 11,* which also is a complex and firmly coherent structure, having elongated, intercommunicating interstices of varying degrees of fineness. The lighter, finer residues of the originally birefringent lamellae exhibit well defined rod double refraction in longitudinal sections; whereas the darker, denser residues of the originally isotropic lamellae do not.

It is evident from a detailed comparison of *Figs. 11* and *14*, that the denser parts of the "lignin" residue correspond to the more porous parts of the matrix of purified cellulose, and that the "lignin" residue may be interpolated within the interstices of the swollen cellulose. Furthermore, the rod double refraction of the lighter lamellae of the "lignin" residue suggests that the two interpenetrating complexes grade downward in size far below the limits of microscopic visibility. In other words, each of the visible parts of the original structural pattern is heterogeneous and composed of optically different complexes. Removal of either the "lignin" or the cellulose leaves a coherent matrix of varying texture and porosity.

It is possible to reconstruct the structural pattern of the swollen cellulose from the "lignin" residue or *vice versa*, since they are positive and negative images of the same pattern. Although swollen sections of purified cellulose afford excellent preparations for visual examination, they are difficult objects for photographic reproduction. Therefore, a majority of our photomicrographs were made from "lignin" residues.

The structural pattern of the central layer is not a constant; it varies greatly not only in different groups of plants, but also at times in homologous cells of the same plant, and even within the wall of a single cell. For example, in *Siparuna bifida*, the two optically different complexes may be segregated into coarsely radial patterns which are clearly visible in untreated sections, *Fig. 10*, or they may be diffused in finer radio-reticulate patterns, *Fig. 16*, the finest of which are invisible in unswollen sections of the secondary wall. In such cells, conspicuous concentricities usually are due either to abrupt changes in the texture of the structural pattern, *Fig. 11*, or to zones of varying intensities of lignification, *Fig. 9*. The former persist in purified cellulose; the latter are eliminated during delignification.

Structural patterns of a basically concentric type are, however, of common occurrence in the normal tracheids of conifers, *Fig. 18*, and in the fiber-tracheids or libriform fibers of such dicotyledons as *Poraqueiba sericea* Tul., *Fig. 15*. In the central layer of these cells, the optically different complexes are segregated into concentric lamellae of varying widths and spatial groupings. The lamellae are of two types, i.e., strongly birefringent and comparatively isotropic. They are not discrete homogeneous entities, but may be resolved by treatment with swelling agents into complexes of varying degrees of fineness. As in the case of *Siparuna bifida*, the darker lamellae are compact aggregates of relatively isotropic material, *Figs. 15* and *18*, and contain a low ratio of birefringent cellulose; whereas the alternating lighter lamellae are composed largely of birefringent cellulose and contain a low ratio of

isotropic material. The structural pattern persists in delignified sections which are treated with standard solvents of hemicelluloses and of other non-cellulosic constituents. When the purified cellulose is swollen, it appears as a complex and firmly coherent matrix, which exhibits a structural differentiation into compact, strongly birefringent and looser, comparatively isotropic lamellae.

It should be emphasized, in this connection, that the concentric structure of swollen cotton hairs — to which the work of Balls (*2*) has directed so much attention — appears to be due fundamentally to a similar structural pattern. When extremely thin, very smoothly cut sections of raw or purified cotton are treated with diluted Schweizer's reagent and are examined in polarized light between crossed nicols, the lamellae are, during the early stages of swelling, clearly of two optically different types, i.e., strongly birefringent and comparatively isotropic. During subsequent swelling, *Fig. 17,* the central layer is resolved into a complex and firmly coherent, spongy structure, the conspicuously birefringent parts of which are denser and obviously contain a higher ratio of cellulose than the more porous, intervening parts. In other words, the structural patterns of the central layers of cotton hairs, *Fig. 17,* of coniferous tracheids, *Fig. 18,* and of the fiber-tracheids of *Poraqueiba sericea, Fig. 15,* appear to be of a fundamentally similar type. In cotton hairs, as in tracheary cells, the width of the concentric lamellae is not a constant, but varies within relatively wide limits.

The structural pattern of cotton can not be due to a segregation of cellulosic and non-cellulosic constituents, since the central layer of cotton is composed of practically pure cellulose — the low ratio of non-cellulosic constituents in cotton is confined chiefly to the so-called cuticle or primary wall and to the lumen of the cell. Nor can the concentricities be due merely to inequalities in the penetration or modifying effects of the swelling agent, as may be demonstrated by cross-correlating the structural patterns of different hairs from the same boll. For example, in *Fig. 17,* in passing outward from the lumen, there is the following sequence of lamellae: six narrow alternating light and dark zones, an unusually wide light zone, two broad dark zones separated by a narrower light zone, two narrow dark zones and three narrow light zones, and six broad dark zones separated by narrower light zones. The fact that this identical complex of varying concentricities occurs in other hairs from the same boll can not be due to purely fortuitous circumstances, but might be due, either directly or indirectly, to the modifying effects of environmental factors upon the developing hairs.

Nor can the structural patterns of tracheids, fiber-tracheids, and libri-

form fibers be due to inequalities in the penetration and modifying effects of the swelling agents, since the patterns are visible under favorable conditions in untreated sections. Thus, the striking similarities in the finer visible structures of the central layer of unlignified and delignified cells and of "lignin" residues indicate that there are fundamental structural differences in the underlying cellulose to which the pattern of lignification must more or less closely conform.

Combinations of radial and concentric patterns of varying texture and complexity are of common occurrence in the fiber-tracheids and libriform fibers of dicotyledons.[1] In such cells there may be abrupt transitions within the central layer from coarse to fine texture and from radio-reticulate to concentric arrangements and *vice versa*. *Fig. 19* is a transverse section of the wood of *Tetramerista glabra* Miq., cut without preliminary softening or other drastic treatments. It illustrates a type of complex radio-concentric structure which is clearly visible in unstained sections mounted in water and other liquids of varying indexes of refraction. The pattern is complicated, however, as is so often the case in cells of this type, by the presence of zones of varying intensities of lignification. A radio-concentric pattern of much finer texture is illustrated in *Fig. 20*.

In the case of the more heavily lignified zones of such central layers, *Figs. 9* and *20*, both the birefringent and the comparatively isotropic parts of the structural pattern persist in "lignin" residues; whereas, in the less intensely lignified zones, the birefringent parts leave no structural residue. It is of interest, in view of the significance that has been attached to the work of Freudenberg and his co-workers (12), that in longitudinal sections the residues of heavily lignified parts exhibit conspicuous rod double refraction; whereas the residues of the less intensely lignified parts do not.[2] In other words, there appear to be submicroscopic structural differences in the two optically different complexes of the structural pattern which are reflected in their "lignin" residues. Furthermore, as previously noted, when delignified sections are stained with chloro-iodide of zinc or congo red, the strongly birefringent parts of the structural pattern may become markedly dichroic; whereas the more nearly isotropic parts do not.

The observational and experimental data that we have assembled in

[1]Concentric patterns with tenuous radial groupings are of not infrequent occurrence in the tracheids of conifers.

[2]Rod double refraction is visible only in the parts of the lignin residue which are strongly birefringent in the original material. Therefore, it can not be seen in sections which transect the so-called fibrillar structure, since all the cellulose is dark in polarized light in such planes of section.

our extensive survey of a wide range of gymnosperms and angiosperms indicate that the central layer of normal tracheids, fiber-tracheids, and libriform fibers is composed, in all cases, of a complex and firmly coherent matrix of cellulose with elongated, intercommunicating interstices. Within these interstices more or less "lignin" and other non-cellulosic constituents may be deposited. The denser and more porous parts of the cellulosic matrix exhibit striking contrasts in birefringence, which are accentuated by lignification. Where these optically different parts are diffused in various patterns of fine texture — as is usually the case in the tracheids of conifers and in the fiber-tracheids and libriform fibers of many dicotyledons — the structural complexes are invisible in untreated sections of the secondary wall, but may be swollen to microscopically visible dimensions, *Figs. 9, 12, 15, 16, 18,* and *20.* On the contrary, where the two optically different parts are segregated into coarser structural complexities, *Figs. 10, 13,* and *19,* the patterns are clearly visible in unswollen sections.

The cellulosic matrix of the central layer is composed, in all cases, of anastomosing elongated complexes which are oriented parallel to the long axis of the cell or in a helical arrangement. In fact, it is these elongated complexes of two optically different types, *Fig. 13,* which give a longitudinally or helically striated appearance to the central layer and determine its helical or longitudinal planes of cleavage into so-called fibrils. In other words, fibrils are heterogeneous shredded parts of an originally continuous and coherent matrix.

Although there are serious optical difficulties in studying the tenuous inner and outer layers of the secondary wall in sectional view, the striated appearance of these layers in surface view strongly suggests that they have similar structural patterns, the elongated, strongly birefringent complexes of which are oriented more nearly at right angles to the longitudinal axis of the cell.

B. Multiple-layered Fibers

The orientation of the elongated complexes of the structural pattern may be relatively uniform throughout the central layer of tracheids, fiber-tracheids, and libriform fibers, or it may deviate more or less in successively formed parts of this layer. Not infrequently, the changes in orientation are correlated with fluctuations in the texture of the structural pattern. Where the deviations are of considerable magnitude, they may be detected in polarized light, as illustrated in *Fig. 4.* The brilliant internal zones resemble the inner and outer layers of the secondary wall in having their birefringent complexes oriented more

nearly at right angles to the longer axis of the cell, and therefore are bright in transverse sections.

Although there is a superficial similarity between *Fig. 4* and *Fig. 6*, the two cell walls are of a fundamentally different type. In the fibers of *Pandanus, Fig. 6,* as in the libriform fibers of various representatives of the Flacourtiaceae, *Figs. 21* and *26,* and in the bast fibers of ramie and of other dicotyledons, there are, as previously stated, actual discontinuities in the cellulosic matrix produced by narrow isotropic films of a non-cellulosic character. It should be emphasized, in this connection, however, that the individual anisotropic zones of these multiple-layered fibers have complex structural patterns of the general types discussed on preceding pages. For example, *Fig. 21* is a transverse section of the unswollen wall of *Homalium luzoniense* F. Villar. The layers of cellulose have a radio-reticulate pattern, the finer structural details of which are more clearly visible in swollen sections or in "lignin" residues, *Fig. 22*. The elongated birefringent complexes of the structural pattern are oriented parallel to the long axis of the cell. Therefore the entire complex of layers is dark in polarized light in transverse sections. Coarsely radial patterns of the type illustrated in *Fig. 10* are of not uncommon occurrence in the individual anisotropic layers of certain bast fibers; whereas in *Pandanus, Fig. 7,* the two optically different aggregates of cellulose are diffused in a pattern of unusually fine texture. Where the strongly birefringent complexes are oriented closely parallel to the longitudinal axis of the cell, the layer is dark in cross sections, *Fig. 6,* and merges with the truly isotropic film of non-cellulosic material; where they are oriented more nearly at right angles to the major axis of the cell, the layer is brilliant in transverse sections.

Variations in the orientation of cellulose in successively formed parts of the secondary wall have a marked effect upon the swelling of tracheary cells and fibers. Owing to its specific anisotropy, the cellulose expands at right angles to the so-called fibrillar axis, and, during extensive lateral swelling produced by strong chemical reagents, actually contracts in a direction parallel to this axis. In the case of isolated, delignified tracheary cells and fibers having normal 3-layered secondary walls, the laterally expanding central layer frequently splits the tenuous, longitudinally expanding outer layer into a series of constricting rings and helical bands, *Fig. 23,* and bulges outward between these structures. This ring-bead type of swelling occurs in cotton and has received considerable attention in literature dealing with commercial fibers. Although the so-called cuticle or primary wall may aid at times in bead formation, the controlling factor in cotton hairs, as in tracheary cells

and fibers, appears to be differences in orientation of cellulose in the outer and central layers of the secondary wall. There are no transverse plates of non-cellulosic material in the secondary wall which are concerned in ring-bead formation as hypothesized by Lüdtke (21).

In the case of multiple-layered tracheary cells and fibers, it is possible to verify conclusions based upon the study of cells of the 3-layered type. We have shown that the concentric anisotropic layers of various representatives of the Flacourtiaceae, *Figs. 21, 22,* and *26,* are separated by films of non-cellulosic material, and that the orientation of the cellulose is constant except in the outermost layer of the secondary wall, where it is more nearly at right angles to the longitudinal axis of the cell. When such cells are partly or completely delignified and are swollen in cuprammonium hydroxide, the internal complex of anisotropic layers expands laterally and disrupts the tenuous outer layer into constricting rings, *Fig. 27,* or helical bands, *Fig. 24.* The internal layers of cellulose — which may be slipped apart as shown in *Fig. 26* — expand more or less in unison, *Figs. 24* and *27,* and no subsidiary internal constrictions are formed.

On the contrary, in the multiple-layered fibers of *Pandanus* and of other monocotyledons — which have similar isotropic films of non-cellulosic material, but where the orientation of the cellulose changes in the successively formed anisotropic lamellae — each of the narrow anisotropic layers, *Fig. 6,* may be disrupted by the lateral expansion of the broader layers, *Fig. 7,* and in the case of entire, delignified fibers, may give rise to constricting rings and helical bands, *Fig. 25.* In other words, the fiber behaves as if it were composed of several two-layered secondary walls, each of which swells in turn, forming similar ringed and beaded structures, *Fig. 25.* The two outermost layers swell first, the expansion working from the ends towards the center of the cell. The first formed ringlike constrictions commonly determine the position of subsequently formed internal constrictions.

Multiple-layered fibers of the Pandanus type are of common occurrence in the primary tissues of the stems of many monocotyledons. It is evident from Lüdtke's (20, 22) figures and descriptions that the fibers of bamboo are of this structural type, and that they exhibit similar phenomena during their expansion in such swelling agents as cuprammonium hydroxide. It is obvious, in addition, that purely physical phenomena of swelling have been misinterpreted by Lüdtke as evidence for the existence of transverse plates (Querelemente) of non-cellulosic material.

DISCUSSION

A. Concentricities

The secondary walls of tracheary cells and fibers are extremely complex and variable structures. Therefore, it is misleading and fruitless to attempt to homologize all types of fibers in a single structural model. For example, there are five different types of visible concentricities, due to:

1. The segregation of two optically different aggregates of cellulose into concentric patterns.
2. Abrupt changes in the form or texture of the structural pattern.
3. Changes in the orientation of the elongated birefringent complexes of the structural pattern.
4. Varying intensities of lignification or differences in the distribution of non-cellulosic constituents within the structural pattern.
5. Alternation of cellulosic and non-cellulosic layers.

In so far as we are able to judge from a study of a wide range of gymnosperms and angiosperms, most, if not all, tracheary cells and fibers exhibit more or less conspicuous concentricities of the third type, i.e., those due to changes in the orientation of the elongated birefringent complexes of the structural pattern, but the number and magnitude of the deviations in orientation are variable. Inability to detect such concentricities appears to be due to inadequate techniques or to errors of interpretation. In most cases, the third type of layering occurs in association with one or more of the other four types of concentricities. Thus, in the secondary wall of cotton hairs, it occurs with the first type; in the fiber-tracheids of *Siparuna bifida,* with the second and fourth types; in the fiber-tracheids of *Tetramerista glabra,* with the first, second, and fourth types; in the fibers of *Pandanus,* with the first and fifth types, etc.

Variations in the intensity of lignification or in the distribution of other non-cellulosic constituents may at times be closely correlated with changes in the orientation or the texture of the structural pattern. For example, the narrow inner and outer layers of the secondary wall may be more heavily lignified than the central layer or *vice versa.* Similarly, the coarser parts of the structural pattern of the central layer may be more heavily lignified or contain a higher ratio of hemicelluloses than the finer parts or *vice versa.* It is such fortuitous correlations as these which have led, in certain cases, to the unwarrantable conclusion that all types of visible heterogeneities in the secondary wall are due primarily to differences in chemical composition.

There are investigators who believe that all fibers are composed of concentric lamellae of cellulose which are held together by non-cellulosic

material. Thus, Lüdtke (21, 22), who has attempted to homologize all types of fibrous cells in a single structural model, is of the opinion that the lamellae are separated by a "Fremdsubstanz" which differs from both cellulose and lignin in its chemical composition. Ritter (26) argues that it is possible to dissect the secondary wall by chemical means into concentric lamellae which may be slipped apart as shown in *Fig. 26.* Lüdtke's conclusions appear to have been derived largely from a study of bamboo fibers; and Ritter's, from investigations of the libriform fibers of elm. We have shown that the anisotropic lamellae of monocotyledonous fibers frequently are separated by films of non-cellulosic material. The libriform fibers of elm are commonly of the so-called gelatinous type, which also are characterized in many cases by having both cellulosic and non-cellulosic lamellae. In such fibers, where there are actual discontinuities in the structural pattern of cellulose, the anisotropic lamellae may readily be separated by chemical treatments and slipped apart. On the contrary, in cotton hairs and in normal tracheids, fiber-tracheids, and libriform fibers, the entire matrix of cellulose is firmly coherent, and can be dissected only by forcibly tearing or rupturing the structural pattern. In *Siparuna bifida* the more obvious planes of weakness in the cellulosic matrix are radio-longitudinal or radio-helical; whereas in cotton hairs or in *Poraqueiba sericea* they are concentric-longitudinal.

B. "Fibrils" and other "Units" of Cellulose

Since the pioneer days of Von Mohl, Valentin, and Th. Hartig, a succession of investigators have visualized the secondary wall as composed of visible units of cellulose — elementary fibrils, dermatosomes, etc. — that are held together by non-cellulosic material. It is essential to understand the relationship between these units and the visible structural patterns produced by different optical aggregates of cellulose.

We have shown in Part I of this paper that the orientation of the cellulose is correlated with that of the so-called fibrillar structure, as has been demonstrated by analyses of X-ray diagrams, of anisotropy, of dichroism, and of other physical properties of the cell wall. However, these physical correlations are concerned only with the orientation of the fibrillar structure and afford no conclusive evidence that fibrils obtained by chemical or mechanical treatments are discrete entities of constant length or cross sectional area.

Ritter (27) has discussed the length of the so-called fibril and concludes that it is variable. He states that "although fibril segments of only 230 microns in length have been isolated, it seems that some may

be as long or longer than the fiber." Lüdtke (22), on the contrary, claims that the length of fibrils is determined by the presence and spacing of transverse plates of non-cellulosic material. Jancke, working with R. O. Herzog (15), measured the width of fibrils and obtained values of some 0.3–0.5 μ. Balls and Hancock (3), proceeding upon the assumption that lamellae[1] are composed of a single concentric row of fibrils, inferred that the width of both lamellae and fibrils in cotton is 0.4 μ. Frey-Wyssling (13) tabulates the dimensions of fibrils as 0.4 × 0.4 × 100 μ.

Fibrils may be dissected by relatively drastic treatments with oxidizing agents or acids into short segments which are variously designated as dermatosomes, spherical units, ellipsoid bodies, etc. According to Frey-Wyssling (13), dermatosomes have dimensions of 0.4 × 0.4 × 0.5 μ; whereas Farr and Sisson (11) state that ellipsoid bodies prepared from cotton have axes of 1.1 μ and 1.5 μ. Lüdtke (20) believes that dermatosomes are held together by his "Fremdsubstanz"; whereas Farr and Eckerson (9) maintain that the ellipsoid bodies of cotton are jacketed by a pectic cement.

We have demonstrated in Part II that the central layer of tracheary cells and fibers is composed of an extremely complex and firmly coherent matrix of cellulose and that the details of the structural patterns of this matrix grade down to the limits of microscopic visibility. There is no evidence, either in untreated or in carefully swollen fibers, of discrete entities of cellulose, i.e., of fibrils or dermatosomes, which may be liberated simply by dissolving non-cellulosic constituents. The matrix of cellulose is shredded and disrupted during the production of fibrils and dermatosomes, which are heterogeneous fragments of larger size than the finer visible complexes of the structural pattern. In cotton, *Fig. 17,* as in *Pinus, Fig. 18, Poraqueiba, Fig. 15,* and *Siparuna, Figs. 10* and *14,* the lamellae obviously are not composed of a single row of adherent fibrils, but are alternating layers of varying width, porosity, and birefringence. The finer, visible, elongated complexes of the lamellae are 0.1 μ or less in thickness. As indicated at (a) in *Fig. 17,* the cross sectional area of an ellipsoid body of the size postulated by Farr and Sisson covers more than four lamellae and a relatively large number of the finer visible complexes.

The form and size of the fragments which may be dissected from the secondary wall are clearly dependent upon the structural pattern of the matrix of cellulose, and upon the type and severity of the chemical and

[1]Balls did not recognize two distinct categories of lamellae and evidently obtained the value of 0.4 μ by dividing the total width of the wall by the number of denser, strongly birefringent lamellae.

mechanical treatments to which the material is subjected. Splits or cracks develop in the more porous and weaker parts of the matrix, thus liberating the denser parts which contain a higher ratio of birefringent cellulose. In addition, there are submicroscopic, transverse, or oblique planes of cleavage, i.e., "slip planes," to which the work of Von Höhnel (16) and of Schwendener (29) has directed so much attention. It is these slip planes, rather than Lüdtke's hypothetical "Querelemente," which facilitate the dissection of the fiber and of the elongated complexes of its structural pattern into shorter segments.

It is of interest, in this connection, that a fibrillar structure is visible after the action of 72% sulphuric acid upon longitudinal sections of fibers which yield coherent "lignin" residues. By the use of mechanical pressure during the initial stages of the action of the acid, the walls of tracheary cells, *Fig. 12,* and fibers may be resolved into long "lignin" threads, similar to fibrils. These shreds of the originally coherent framework of "lignin" may be dissected by more drastic chemical and mechanical treatments into nearly isodiametric fragments resembling dermatosomes. As previously stated, the amorphous non-cellulosic constituents are deposited within the elongated, intercommunicating interstices of the cellulose matrix, resulting in two continuous, interpenetrating systems. Neither system is composed of discrete entities of visible dimensions, but each may be disrupted into fragments of varying size and form. If there are actual discontinuities in the systems, they must occur in the submicroscopic field, e.g., in the realm of micelles or of molecular chains. It should be emphasized, in addition, that so-called fibrillar structures are not visible in the secondary walls of parenchyma, of sclereids, or of other cells which exhibit statistical isotropy in surface view. The structural pattern of the cellulose matrix in such walls is of a fundamentally different type from that which occurs in fibrous cells.

Dermatosomes, spherical units, and ellipsoidal particles are difficult to homologize, either as regards their size or their form. They are obtained by the action of oxidizing agents or of acids which tend to modify the cellulose. Neale (24) has summarized the modifying effects of oxidation and hydrolysis as follows: "The loss of strength and fall in viscosity which accompany the hydrolysis or oxidation of cellulose are quite irreversible, and the general term degradation is applied to these changes. The degradation of cellulose is accompanied by the appearance of chemical properties foreign to the original material. The hydrolysis of the glucoside-oxygen bridge causes the appearance of reducing sugar properties which may be quantitatively, though arbitrarily, expressed as 'copper number' or 'iodine number.' The reducing

sugar properties also arise as a result of oxidation and may be accompanied by the development of acidic properties, so that oxidized cellulose may retain traces of caustic alkali or absorb basic dyes. This latter property has been put on the quantitative basis so essential in the chemistry of cellulose in the form of the methylene blue absorption test."

Thus, it may be seen that the action of acids, which are supposed to dissolve some cementing substance and to liberate integral units of cellulose, may actually result in partial degradation of the cellulose. We have found that the staining of hydrocellulose and oxycellulose with ruthenium red is similar to the methylene blue absorption values as listed by Dorée (8). Ruthenium red behaves, in some respects, as a basic dye, and the staining of ellipsoidal particles, obtained by treating cotton with relatively strong acid (10), may be interpreted as an indication of the degradation of the cellulose rather than as evidence for believing that the particles are coated with a pectic cement. Ruthenium red is not a specific test for pectic compounds, as botanists have frequently assumed. It is removed from dilute aqueous solutions by coagulated protoplasm and other nitrogenous substances, by gums, mucilages, hemicelluloses, oxycelluloses, hydrocelluloses, and certain lipoids, as well as by pectic compounds.

Any hypothesis concerning the visible structure of the secondary wall must account not only for the varying structural patterns of a wide range of cells, but also for well known facts regarding the physical and chemical properties of cellulose. In the case of the hairs of the cotton plant, the constituents which do not yield glucose upon hydrolysis are small in amount, and are confined chiefly to the so-called cuticle or primary wall and to the lumen of the cell. There obviously is not a sufficient volume of cutinlike substances or of pectic compounds in the secondary wall to serve as a cementing substance of the type postulated by Lüdtke (21) or by Farr and Eckerson (9). Furthermore, when cotton is treated with solvents of such constituents, without degrading the cellulose, the structural pattern is not affected. It persists as a firmly coherent matrix of cellulose.

It is now generally admitted that the cellulose molecule is a long chain of glucose residues bound together by oxygen bridges. Furthermore, there is evidence from X-ray analyses, from anisotropy, dichroism, etc., to indicate that cellulose is built up of submicroscopic, crystal-like aggregates of these chains. The length of the cellulose chain and its arrangement within the crystallite are still subjects of dispute. Thus, it is uncertain whether the chain is shorter or longer than the crystallite or of equivalent length, and whether micelles are discrete and separate entities, or merely parts of a continuous system of overlapping chains.

Estimates of the length of cellulose molecules range from 100–3500 glucose residues. The highest value of 3500 units, i.e., that of Kraemer and Lansing (19), is based upon measurements of viscosity. Such molecules would have a length of approximately 1.8 μ, and would be visible microscopically if they were of sufficient thickness, which they obviously are not. Since the cellulose chains are arranged parallel to the so-called fibrillar orientation, and since there are no visible structures which transect this axis, it is possible to conceive of chains of the length postulated by Kraemer and Lansing arranged in an overlapping manner along the fiber axis.

Our investigations indicate that the cellulosic matrix of the secondary wall is composed of complexes of cellulose of varying birefringence which grade down to the limits of microscopic visibility, and that the fundamental units of cellulose are of submicroscopic dimensions. In the case of cotton, the available chemical and physical data make it appear improbable that the variations in birefringence are due to differences in chemical composition. Correns (5) recognized, more than 40 years ago, that cellulose is heterogeneous and attempted to explain the visible striations and certain types of lamellae as due to differences in water content. This hypothesis originated with Nägeli (23), who postulated water rich and water poor layers as a means of explaining concentricities and still permitting growth by intussusception. Differences in water content apparently do exist, and may be a factor influencing the intensity of birefringence in different lamellae. However, it is difficult to evaluate such differences by a study of dried material. Drying the walls shrinks the cell so that structures just within the limits of microscopic visibility when the preparation is in water, may be contracted to invisible dimensions. Furthermore, differences in water content must be explained in terms of submicroscopic differences in the cellulose which permit varying degrees of hydration. The question whether the variation in birefringence of different complexes of the cellulosic matrix is due to fluctuations in the size, number, or orientation of submicroscopic units of cellulose is one which must be attacked from the physical and chemical, rather than from the botanical, side.

C. Significance of Biological Variables in Physical and Chemical Investigations

Our survey of a wide range of gymnosperms and angiosperms indicates that the secondary wall is a very complex structure, and that the structural pattern of the cellulose matrix varies greatly, not only in different groups of plants but also, at times, in homologous cells of the same plant,

and even in different parts of the same cell. There is a similar variability in the distribution of "lignin" and of other non-cellulosic constituents. Therefore, since all types of secondary walls can not be homologized in a single structural model, there are grave dangers in generalizing from intensive investigations of isolated species, e.g., cotton, spruce, bamboo, or ramie.

Deductions concerning the structure of the cell wall based upon physical or chemical analyses, should be checked by microscopic investigations and by accurate information concerning the numerous biological variables. This is particularly necessary in the interpretation of X-ray diagrams, where the investigator of necessity must deal with complex aggregates of plant material. Van Iterson (31) has shown that certain misconceptions regarding *Valonia* might have been avoided by an acquaintance with the work of Correns (6) and others upon the visible structure of the walls of algae. Preston (25) undoubtedly errs in concluding, from an examination of X-ray diagrams, that there is a single plane of orientation of "fibrils" in the secondary wall of the tracheids of *Sequoia* and of other conifers. Accurate interpretations of X-ray diagrams of growing cells and of differentiating tissues are especially difficult, and such conclusions regarding structural changes as those of Clark and Farr (4) and Ritter and Stillwell (28) must be carefully verified from the histological side.

Although "lignin" residues of thick sections exhibit rod double refraction, as demonstrated by Freudenberg and his co-workers (12), a careful study of the residues of thin sections shows that a considerable proportion of the secondary wall "lignin" is isotropic. Similarly, there are parts of the cellulosic matrix which do not exhibit a clearly defined dichroism when thin sections are stained with chloro-iodide of zinc or congo red. The woods of certain dicotyledons leave no coherent structural residue when subjected to standard treatments with 72% sulphuric acid, as shown by Harlow (14); whereas others leave compact residues such as have been considered to be typical of conifers. In the wood of certain plants, the bulk of the "lignin" is confined to the so-called middle lamella, as Ritter (27) maintains; whereas in others, there is a relatively large proportion in the secondary wall.

It should be emphasized, in conclusion, that most of our own data were obtained from a study of tracheary cells and fibers, and that many additional types of cells must be investigated before it will be possible to visualize the full range of structural variability of the secondary wall. In a succeeding paper, we shall discuss methods that have been perfected for studying the small-celled, lightly lignified woods of dicotyledons of temperate regions.

SUMMARY AND CONCLUSIONS

1. An extensive survey of a wide range of gymnosperms and angiosperms has shown that the structural pattern of the secondary wall is clearly visible in the large fiber-tracheids and libriform fibers of various dicotyledons.

2. By using untreated sections of such cells as controls, it is possible to observe the exact effects of specific chemical and mechanical treatments upon normal structures, and thus to extend the scope of investigation to cover a wide range of less favorable material.

3. The cellulosic matrix of the swollen secondary wall of cotton, as of normal tracheids, fiber-tracheids, and libriform fibers, is an extremely heterogeneous but firmly coherent structure, the finer details of which grade down to the limits of microscopic visibility.

4. There is no reliable evidence to indicate that the matrix is composed of discrete entities of visible size — e.g., elementary fibrils, dermatosomes, ellipsoidal bodies, etc. — that are bound together by non-cellulosic material. On the contrary, our data demonstrate that such putative entities actually are heterogeneous fragments that are shredded or disrupted from an originally continuous and coherent matrix. If there are discontinuities in the structural pattern of the cellulose in normal tracheary cells, they are confined to the submicroscopic field, e.g., to the realm of micelles or molecular chains.

5. The visible structural pattern of the cellulosic matrix varies greatly in form and texture, not only in different plants, but also in homologous cells of the same plant, and even in different parts of the same cell.

6. There are at least two optically different elongated complexes of cellulose which may be segregated into radio-helical, radio-longitudinal, or concentric-longitudinal lamellae, or into various radio-concentric patterns.

7. The orientation of the elongated complexes of the structural pattern fluctuates more or less in successively formed parts of the secondary wall. In the case of normal tracheids, fiber-tracheids, and libriform fibers, there are three layers due to varying orientations: narrow inner and outer layers, in which the orientation is more nearly at right angles to the longitudinal axis of the cell, and a central layer of varying width, in which the orientation is parallel to this axis or does not deviate excessively from it.

8. "Lignin" and other non-cellulosic constituents may be deposited in the elongated, intercommunicating interstices of the cellulosic matrix, thus resulting in two continuous, interpenetrating systems. In heavily

lignified forms, either system may be dissolved without seriously modifying the structural pattern of the remaining system. The purified cellulose and the "lignin" residue reveal positive and negative images of the original structural pattern.

9. Deviations from the typical 3-layered type of secondary wall are of not infrequent occurrence. Thus, many thick-walled libriform fibers and fiber-tracheids have no clearly differentiated inner layer; whereas others have more than three layers of varying "fibrillar" orientation.

10. Conspicuous discontinuities in the structural pattern of the cellulose commonly occur in the multiple-layered walls of so-called gelatinous fibers, in certain types of bast fibers, and in sclereids. They are due to narrow layers of truly isotropic material which contain little, if any, cellulose.

11. There are five different types of visible concentricities which occur in varying combinations, and may be associated at times with radio-helical or radio-longitudinal lamellae. Therefore, it is misleading and fruitless to attempt to homologize all types of fibers in a single structural model.

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DESCRIPTION OF PLATES

Figs. 1–6 and 13 were made from unstained sections and were photographed in polarized light between crossed nicols. All the remaining photomicrographs were made with an arc-light and Zettnow's filter. Figs. 1–6, 8, 10, and 21 were made from sections mounted in diaphane (N = 1.47).

PLATE 140

Fig. 1. *Myodocarpus simplicifolius* Brong. & Gris. Transverse section of the xylem, showing a fiber-tracheid and parts of seven adjoining ones. The thick secondary walls are composed of three distinct layers: a narrow brilliant outer layer, a brilliant narrow inner layer, and a wide intervening dark layer. In a section of this thickness, 15 μ, the isotropic intercellular substance and the feebly anisotropic primary walls are more or less completely fogged or obscured by the brilliant outer layers of the secondary walls (compare *Fig. 3* for a section 5 μ in thickness). × 1750.

Fig. 2. *Urandra corniculata* Foxw. Radial longitudinal section of the xylem, showing the walls of adjacent fiber-tracheids in sectional view. The broad central layers of the secondary walls are brilliant. The intercellular substance, the feebly birefringent primary walls, and the inner and outer layers of the secondary wall are dark. A bordered pit is shown in the center of the photomicrograph. × 1750.

Fig. 3. *Trochodendron aralioides* Sieb. & Zucc. Transverse section of the xylem, showing a tracheid and parts of seven adjoining cells. In a section of this thickness, 5 μ, the outer brilliant layers of the secondary walls of adjacent cells are clearly separated by a narrow intervening dark layer, which actually consists of two feebly birefringent primary walls and a truly isotropic layer of intercellular material. × 1400.

Fig. 4. *Myodocarpus simplicifolius*. Transverse section of the xylem, showing a fiber-tracheid and parts of seven adjoining ones. The thick secondary wall of the central cell consists of a series of alternating brilliant and dark layers. × 1750.

Fig. 5. *Urandra corniculata*. Thick secondary wall of a sclerenchymatous cell in sectional view, showing alternating brilliant and dark layers. × 1750.

PLATE 141

Fig. 6. *Pandanus odoratissimus* L. Transverse section of a group of lignified fibers, showing secondary walls composed of regularly alternating brilliant and dark layers. × 1150.

Fig. 7. *The same*. Transverse section of a fiber after standard treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in balsam, showing residue of secondary wall. The brilliant layers of *Fig. 6* are split and embossed. × 1300.

PLATE 142

Fig. 8. *Siparuna bifida* (P. & E.) A. DC. Transverse section of a fiber-tracheid and of parts of several adjoining cells, stained with Haidenhain's haematoxylin and safranin, showing zones of varying intensities of lignification. × 2000.

Fig. 9. *The same*. Transverse section of a fiber-tracheid after standard treatment with 72% sulphuric acid, staining with Haidenhain's

haematoxylin and mounting in aniline oil, showing finely radio-reticulate pattern and zones due to varying intensities of lignification. Dark zones heavily lignified, light zones less intensely lignified. × 1300.

PLATE 143

Fig. 10. *Siparuna bifida.* Transverse section of a fiber-tracheid and of parts of several adjoining cells, stained with Haidenhain's haematoxylin and safranin. The broad, unswollen central layer of the secondary wall is radially striated. × 2000.

Fig. 11. *The same.* Transverse section of a fiber-tracheid after standard treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in aniline oil, showing radially striated and finely reticulated residue of the central layer of the secondary wall. In the outer part of the central layer, there is a concentricity due to an abrupt transition from coarse to fine texture. The inner concentricity is due to varying intensities of lignification. × 1900.

PLATE 144

Fig. 12. *Tetramerista glabra* Miq. Tangential longitudinal section of the central layer of a fiber-tracheid after treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in aniline oil, showing longitudinal pattern of fine anastomosing threadlike components. The longitudinal orientation has been somewhat distorted during swelling. × 1900.

Fig. 13. *Siparuna bifida.* Tangential longitudinal section through the central layer of a fiber-tracheid mounted in water and photographed with polarized light between crossed nicols, showing alternating birefringent and isotropic striae. × 1900.

Fig. 14. *The same.* Transverse section of a delignified fiber-tracheid, after treatment with diluted cuprammonium hydroxide and staining with congo red. The denser, darker radii of the purified cellulose correspond to the lighter radii of *Figs. 10* and *11*. × 1200.

PLATE 145

Fig. 15. *Poraqueiba sericea* Tul. Transverse section of the secondary wall of a fiber-tracheid after standard treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in aniline oil, showing concentrically lamellated residue of the central layer. × 3200.

Fig. 16. *Siparuna bifida.* Transverse section of the secondary wall of a fiber-tracheid after standard treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin and mounting in aniline oil, showing finely radio-reticulate residue of the central layer. × 3200.

PLATE 146

Fig. 17. *Gossypium hirsutum* L. Transverse section of a cotton hair after swelling with diluted cuprammonium hydroxide and staining with congo red, showing alternating lamellae of varying width and porosity in the inner part of the secondary wall. A particle 1 μ in diameter in the untreated wall would expand to the size of the circle at (a). × 1200. Owing to swelling, the original width of the lamellae has been increased 7500 times in this photomicrograph.

Fig. 18. *Pinus ponderosa* Dougl. Transverse section of the secondary wall of a tracheid after treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in aniline oil, showing concentrically laminated residue of the central layer. × 1900.

PLATE 147

Fig. 19. *Tetramerista glabra.* Transverse section of a fiber-tracheid and of parts of several adjoining cells, mounted in a dilute aqueous solution of iodine potassium iodide and photographed with a Zeiss 70-water-immersion lens. The broad central layer has a coarsely radio-concentric pattern which is complicated by zones of varying intensities of lignification. × 2000.

Fig. 20. *The same.* Transverse section of a fiber-tracheid after treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in balsam, showing finely radio-concentric pattern and broad zones due to varying intensities of lignification. × 1300.

PLATE 148

Fig. 21. *Homalium luzoniense* F. Villar. Transverse section of a libriform fiber stained with Haidenhain's haematoxylin and safranin, showing alternating broad cellulosic and narrow non-cellulosic layers. The radio-reticulate structure of the former layers is vaguely visible. × 3200.

Fig. 22. *The same.* Transverse section of a libriform fiber after standard treatment with 72% sulphuric acid, staining with Haidenhain's haematoxylin, and mounting in aniline, showing residue of both the cellulosic and the non-cellulosic layers. × 1300.

PLATE 149

Fig. 23. *Rhizophora mangle* L. Isolated, delignified, libriform fiber, swollen in diluted Schweizer's reagent, showing beadlike swelling of the central layer of the secondary wall. The outer layer of secondary wall is resolved into a series of constricting rings and helical bands. × 650.

Fig. 24. *Olmediella Betschleriana* (Goepp.) Loes. Isolated, delignified, libriform fiber, swollen in diluted Schweizer's reagent. The outer layer of the secondary wall is resolved into constricting helical bands. × 325.

Fig. 25. *Pandanus odoratissimus.* Isolated delignified fiber, swollen in diluted Schweizer's reagent, showing that each of the internal brilliant layers in *Fig. 6* may be resolved into constricting rings and helical bands. × 650.

Fig. 26. *Olmediella Betschleriana.* Segment of a libriform fiber isolated from a thick transverse section of the xylem after delignification and treatment with 50% sulphuric acid. The concentric cylinders of cellulose are slipping apart. × 650.

Fig. 27. *Olmediella Betschleriana.* Isolated, delignified, libriform fiber, swollen in diluted Schweizer's reagent. The outer layer of the secondary wall is resolved into a series of constricting rings and helical bands. × 400.

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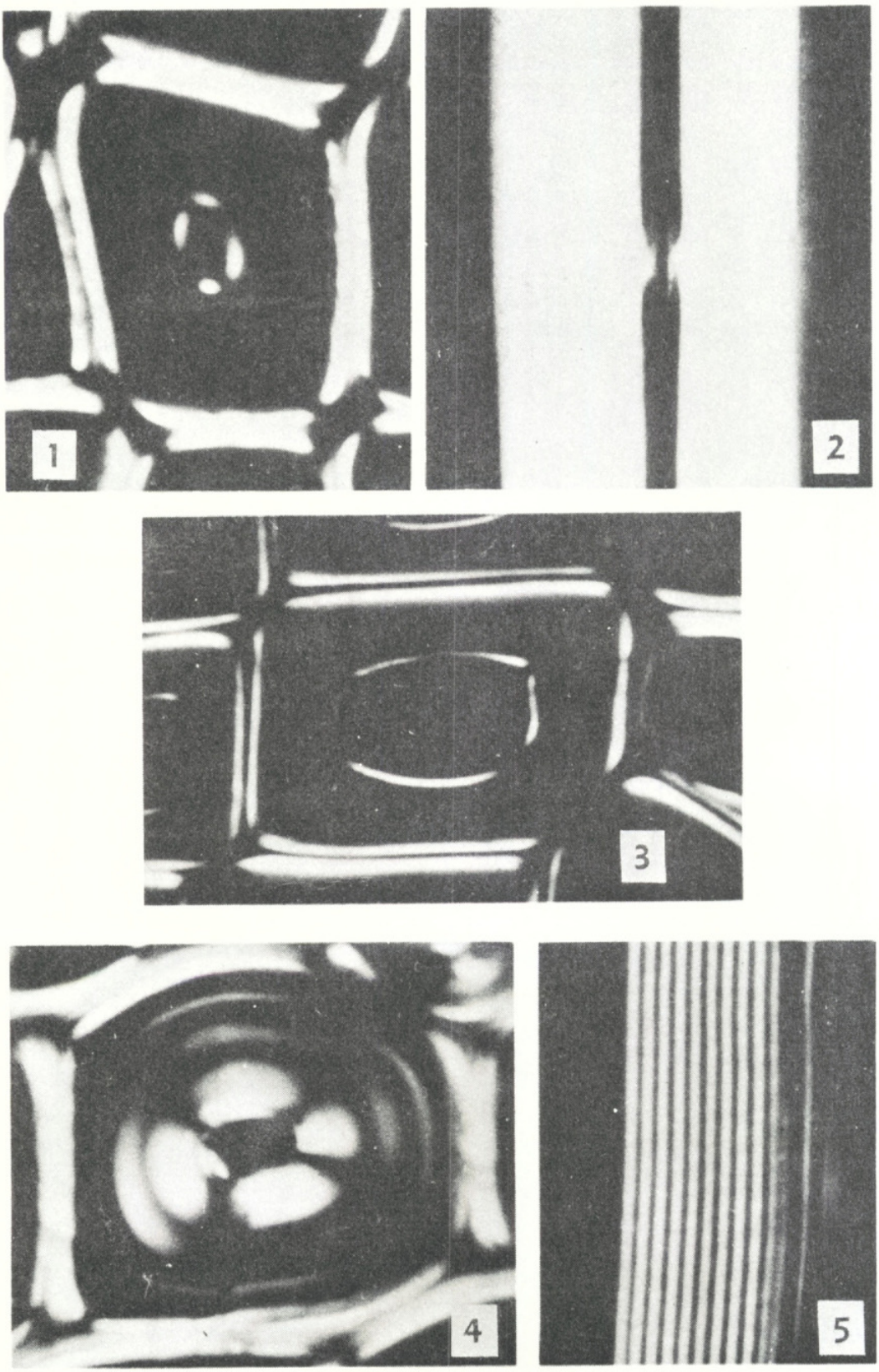
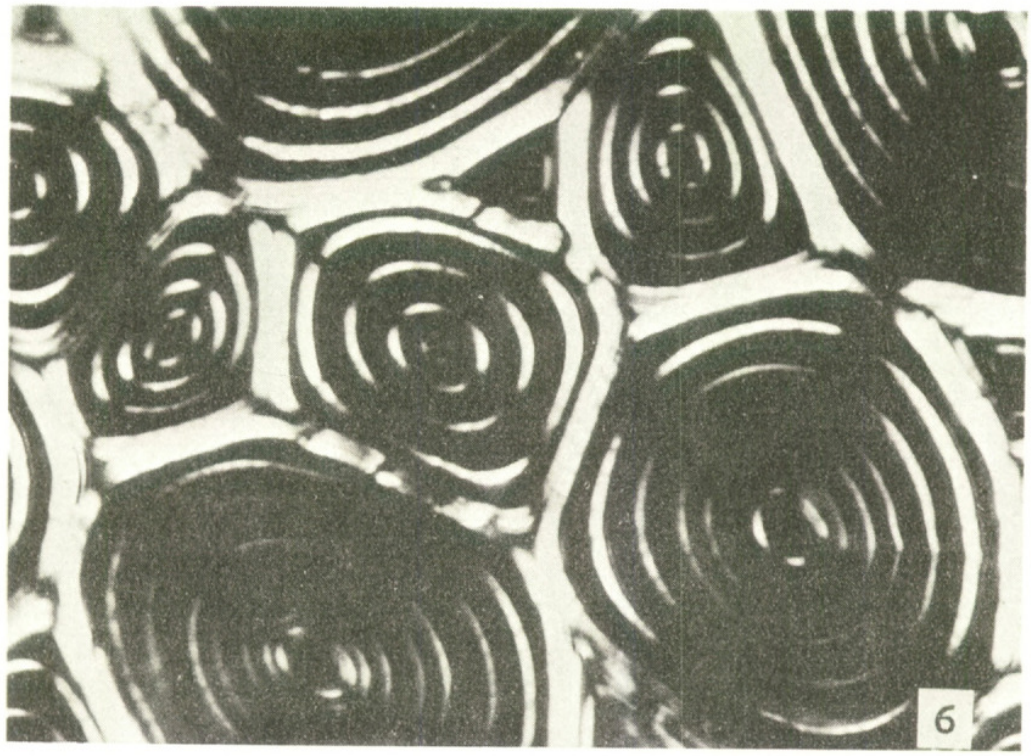
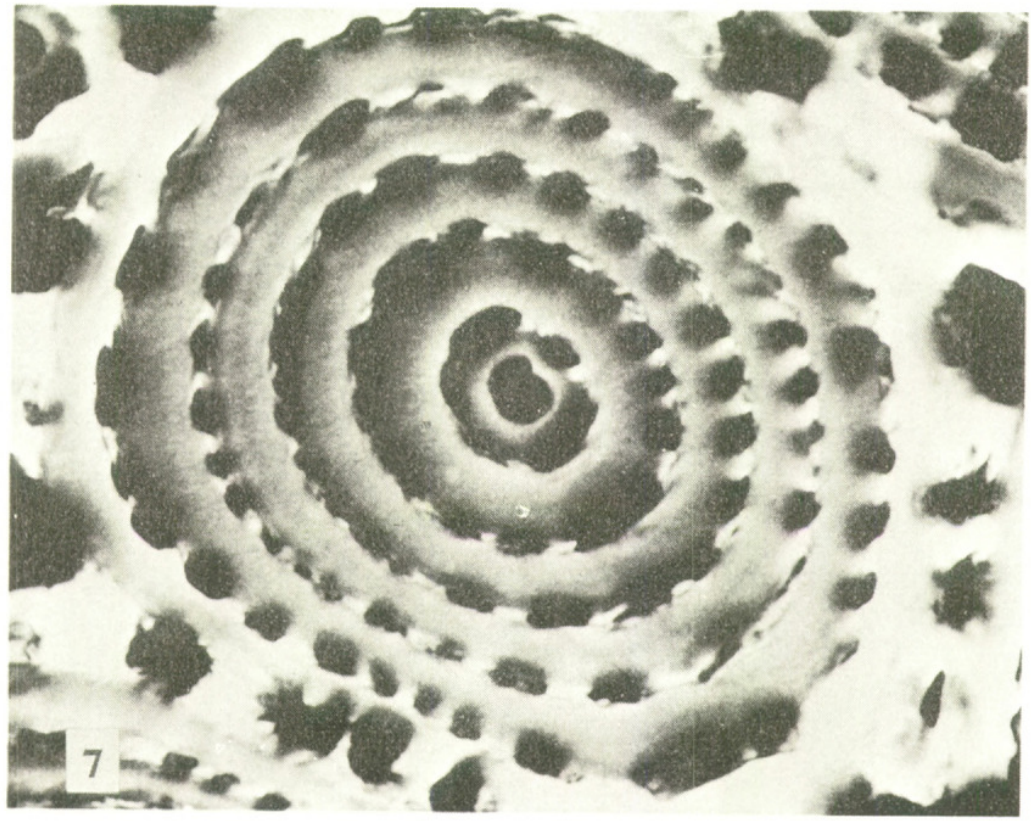


VISIBLE STRUCTURE OF THE SECONDARY WALL





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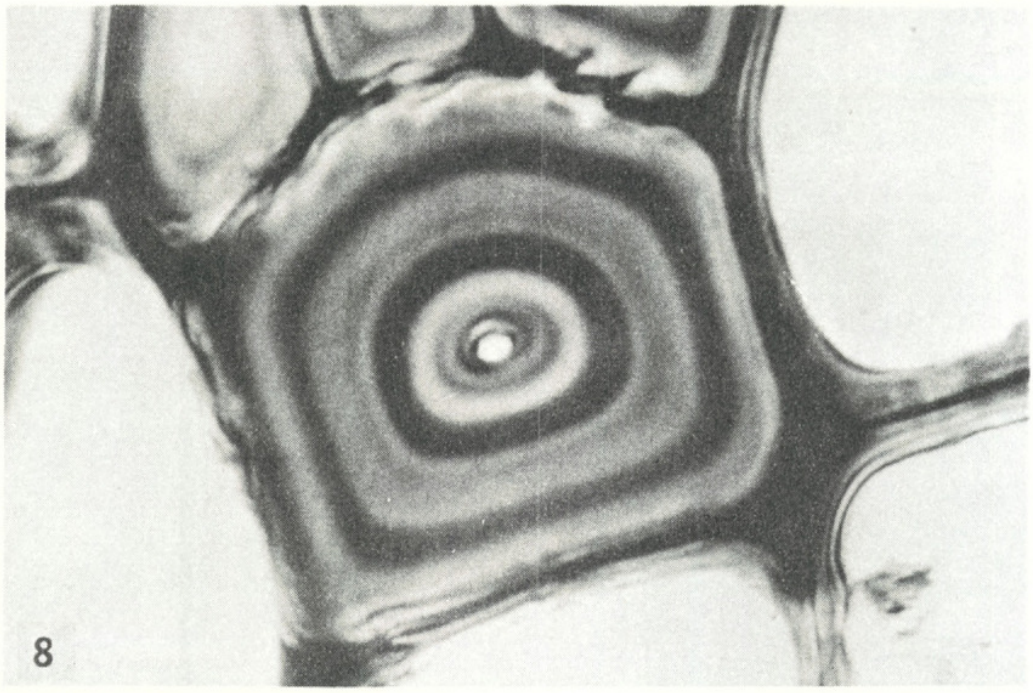

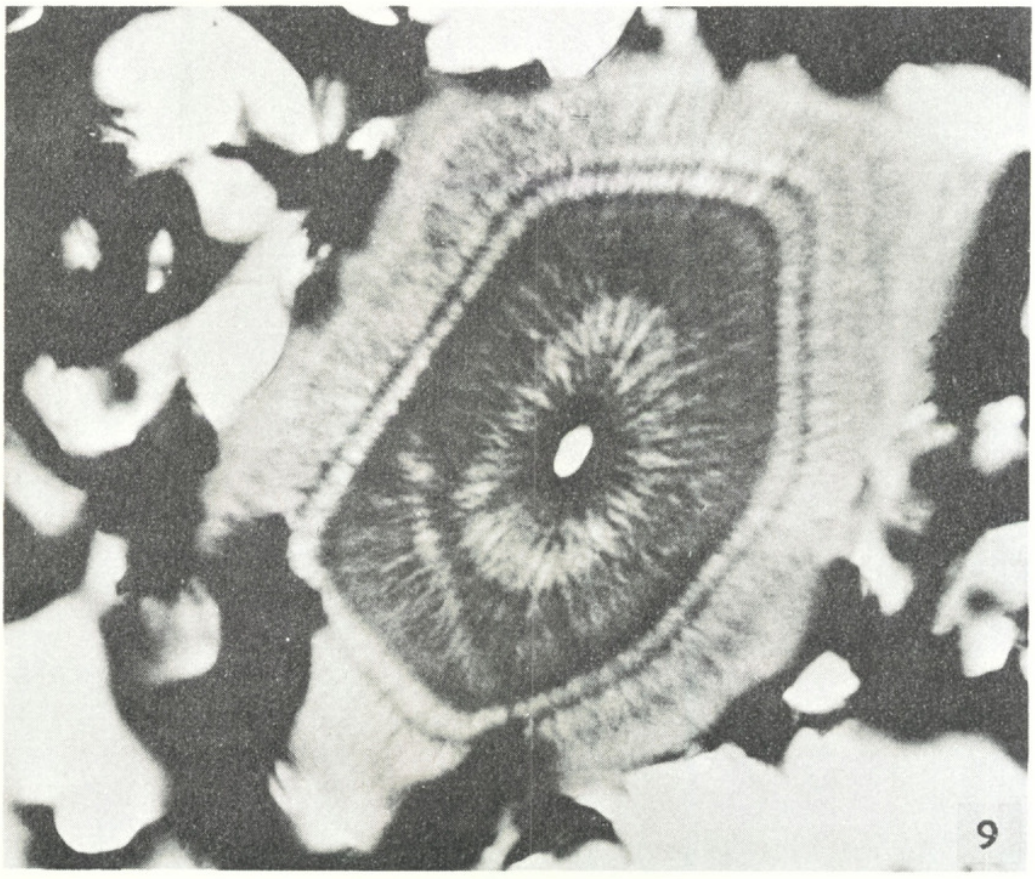

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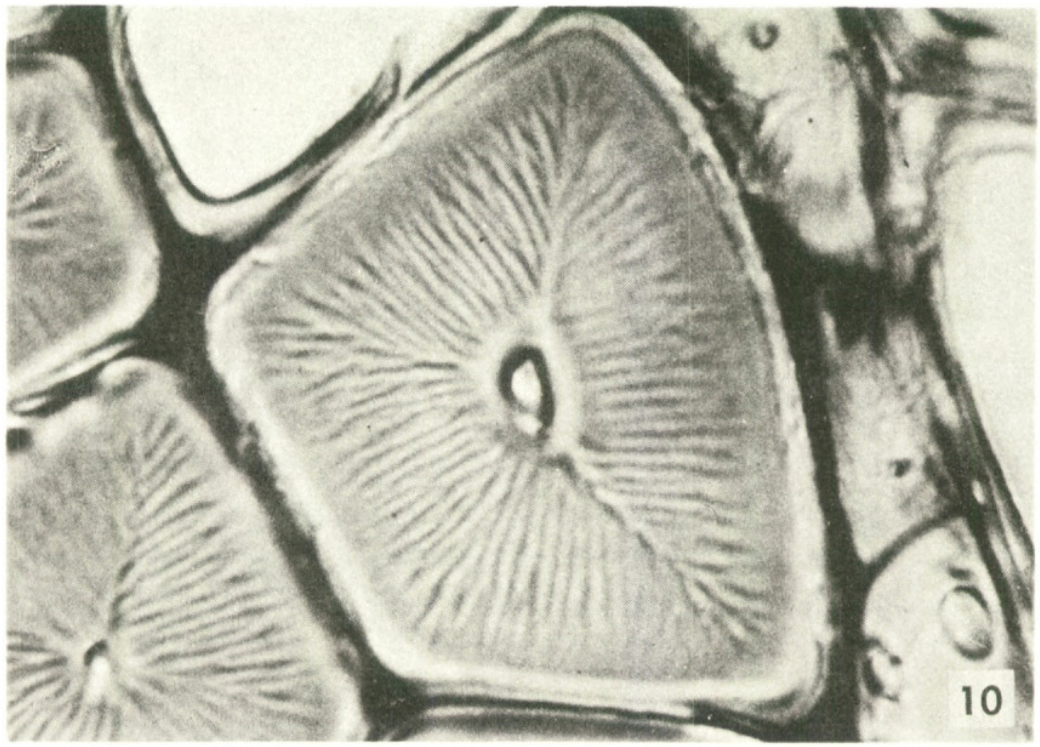

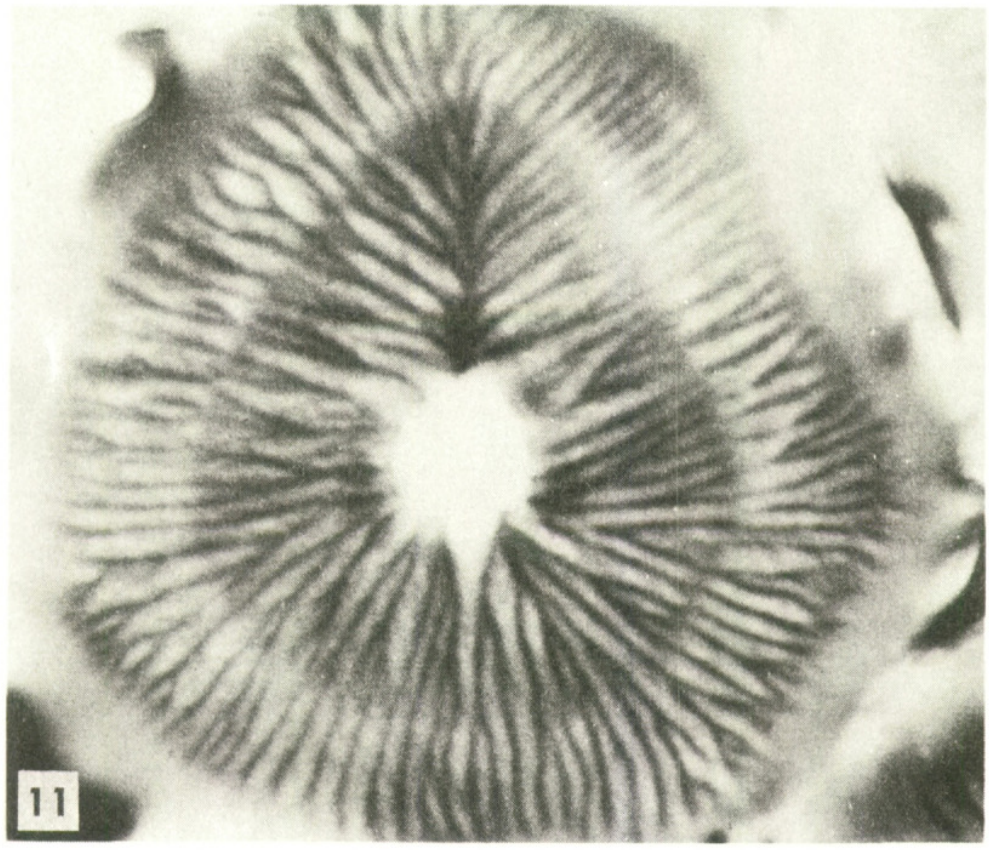

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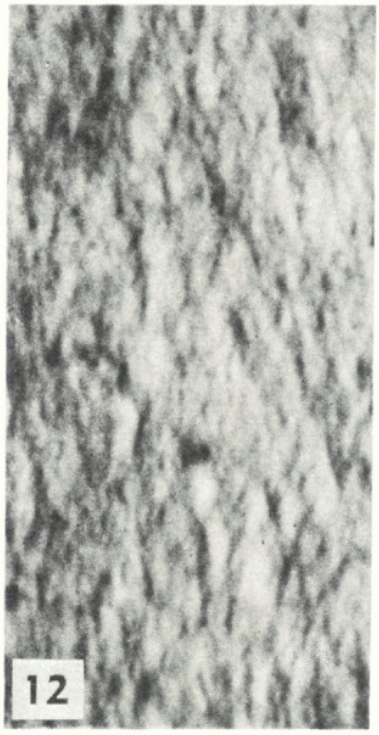

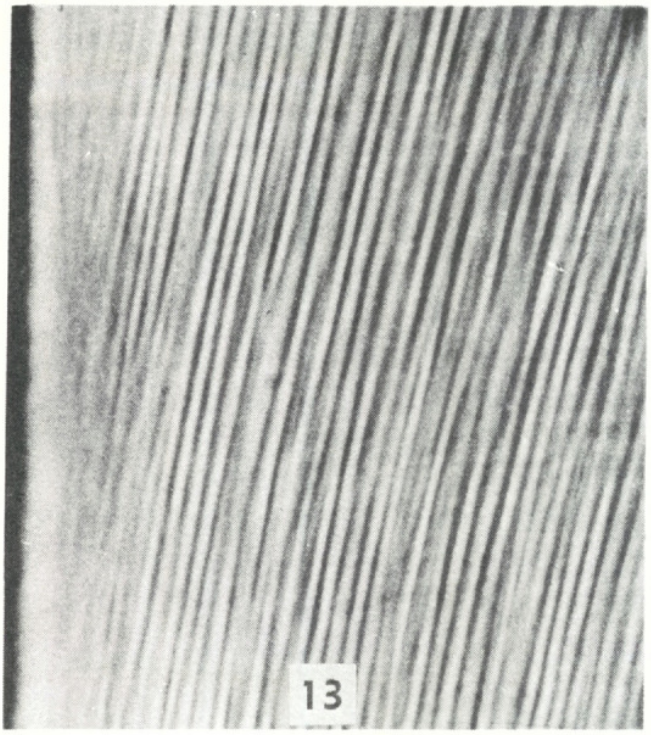

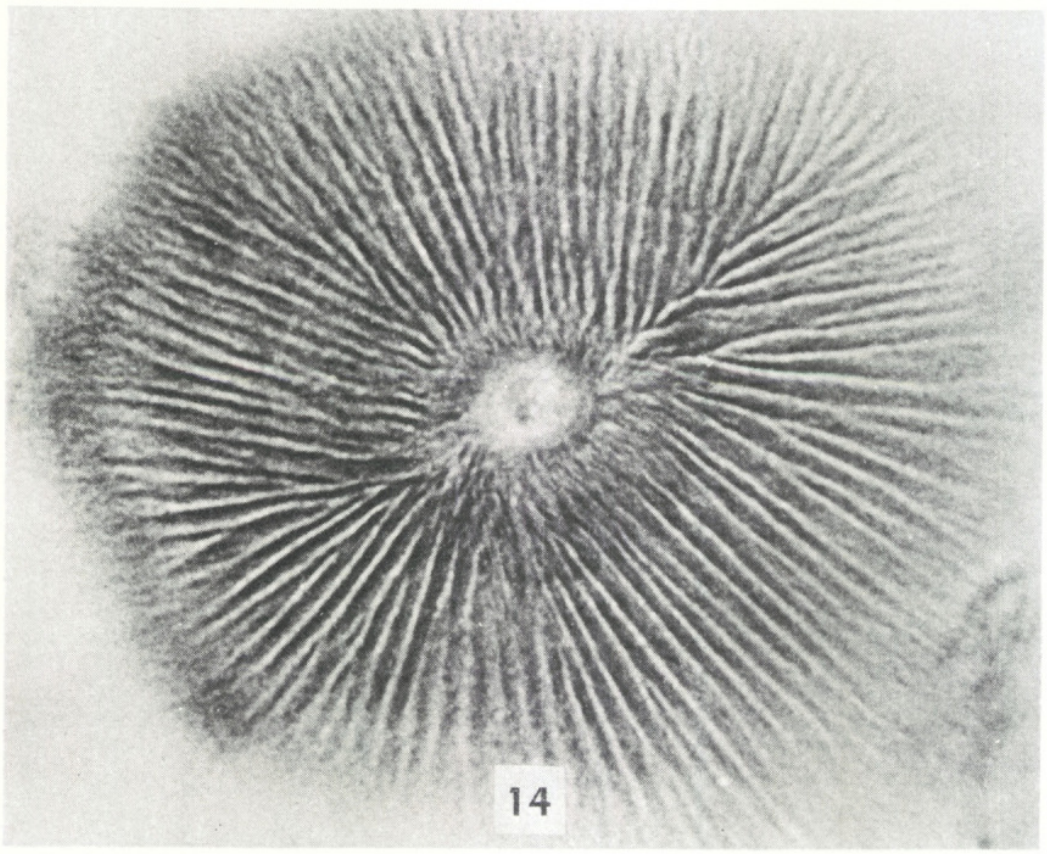

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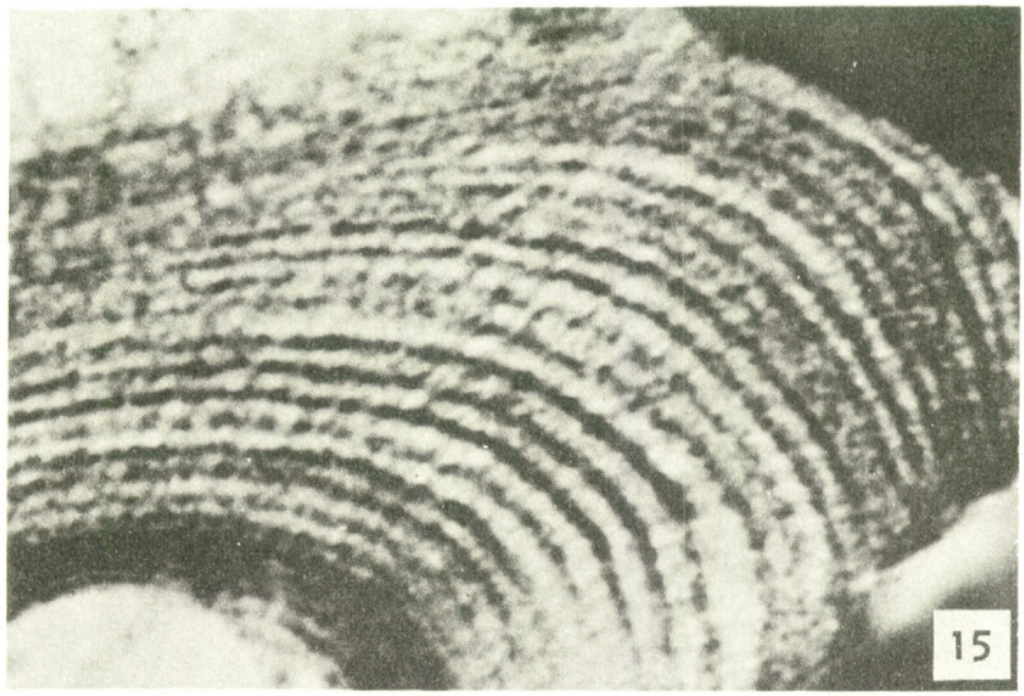
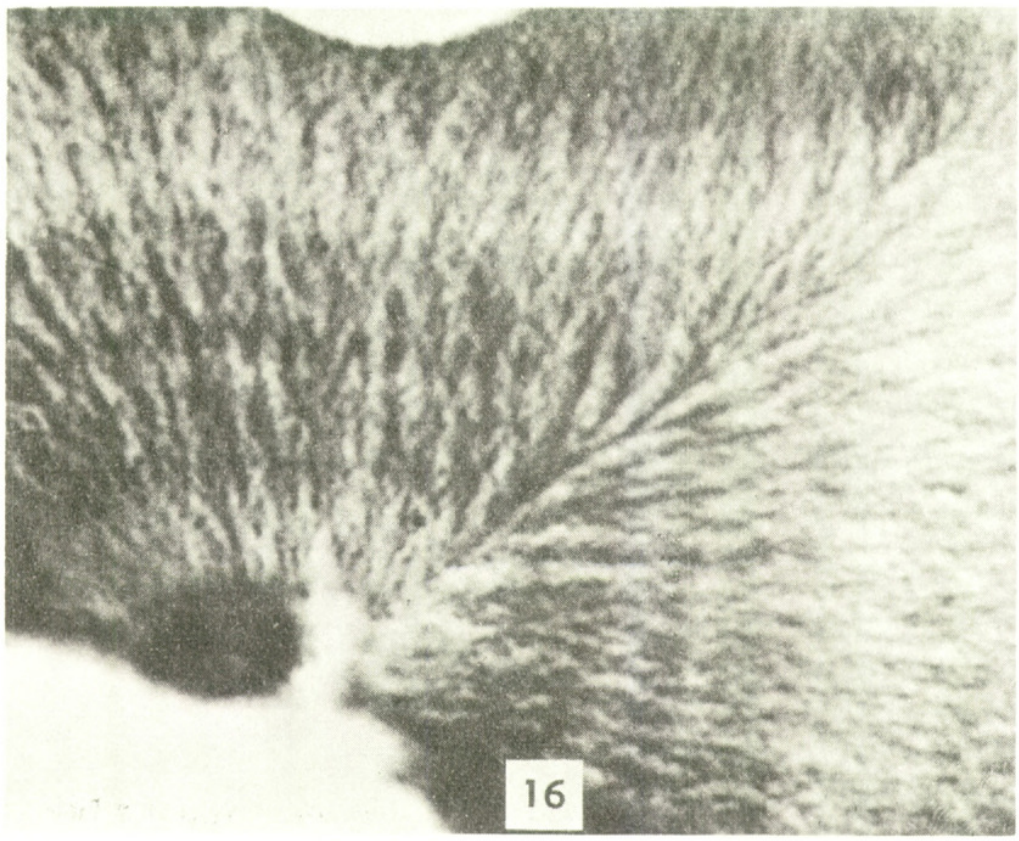
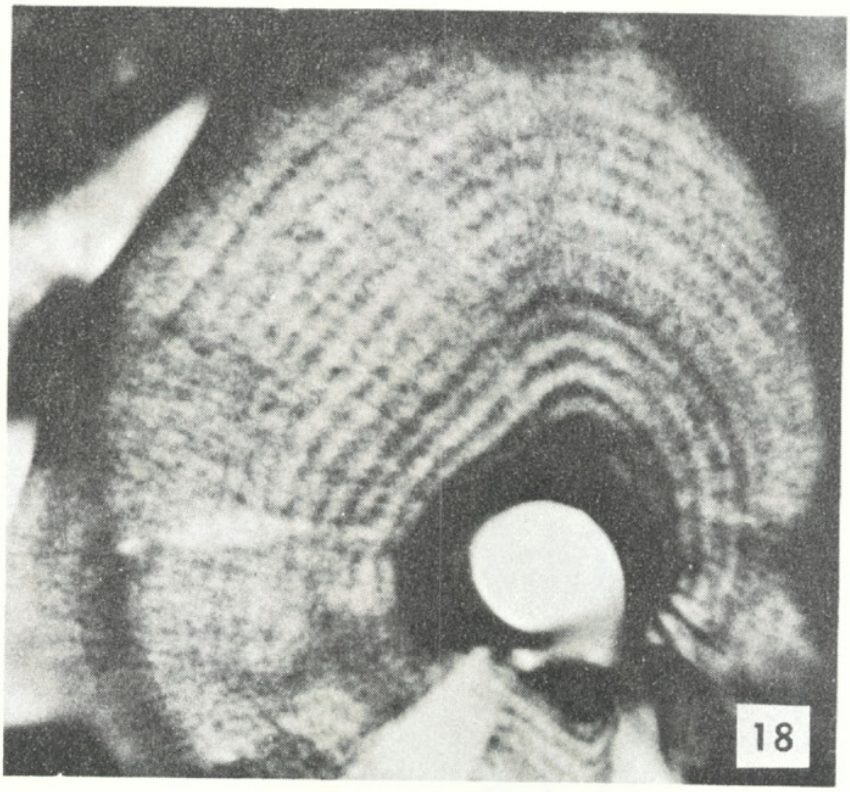




Visible Structure of the Secondary Wall



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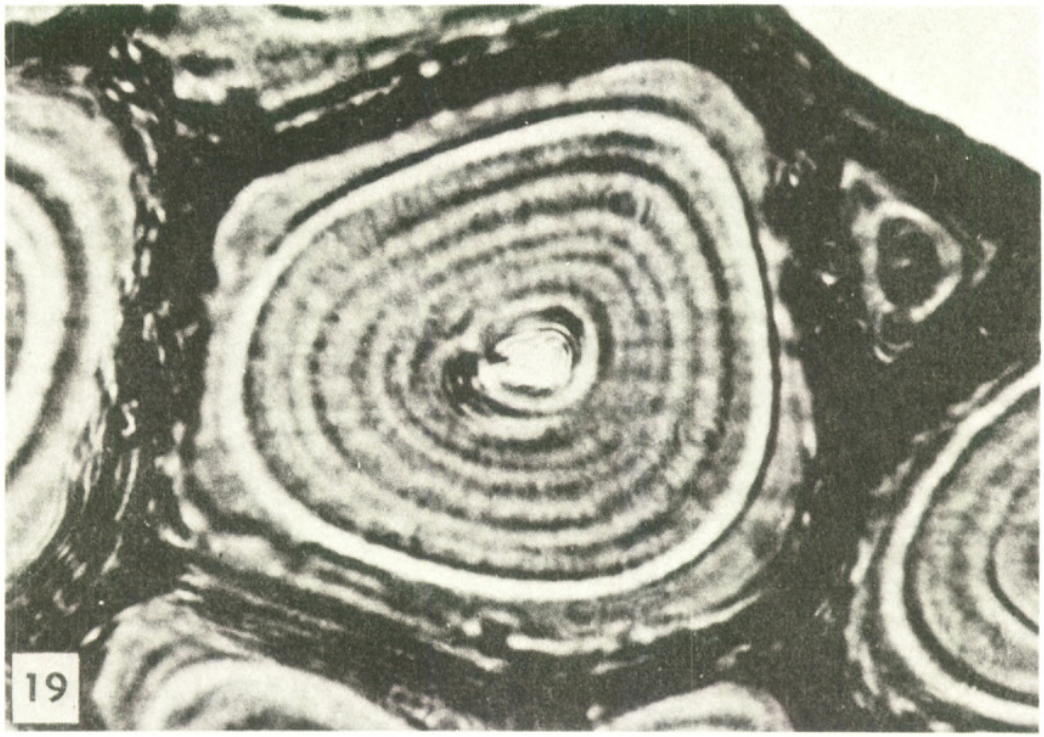

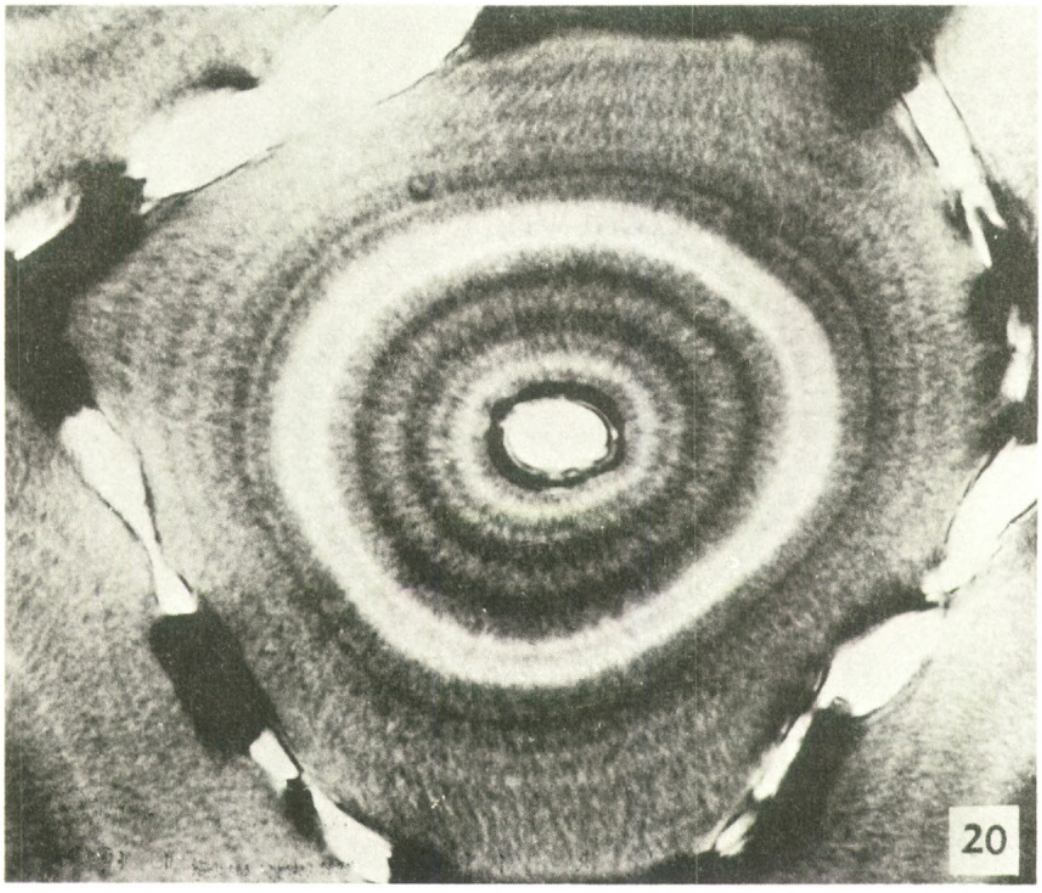

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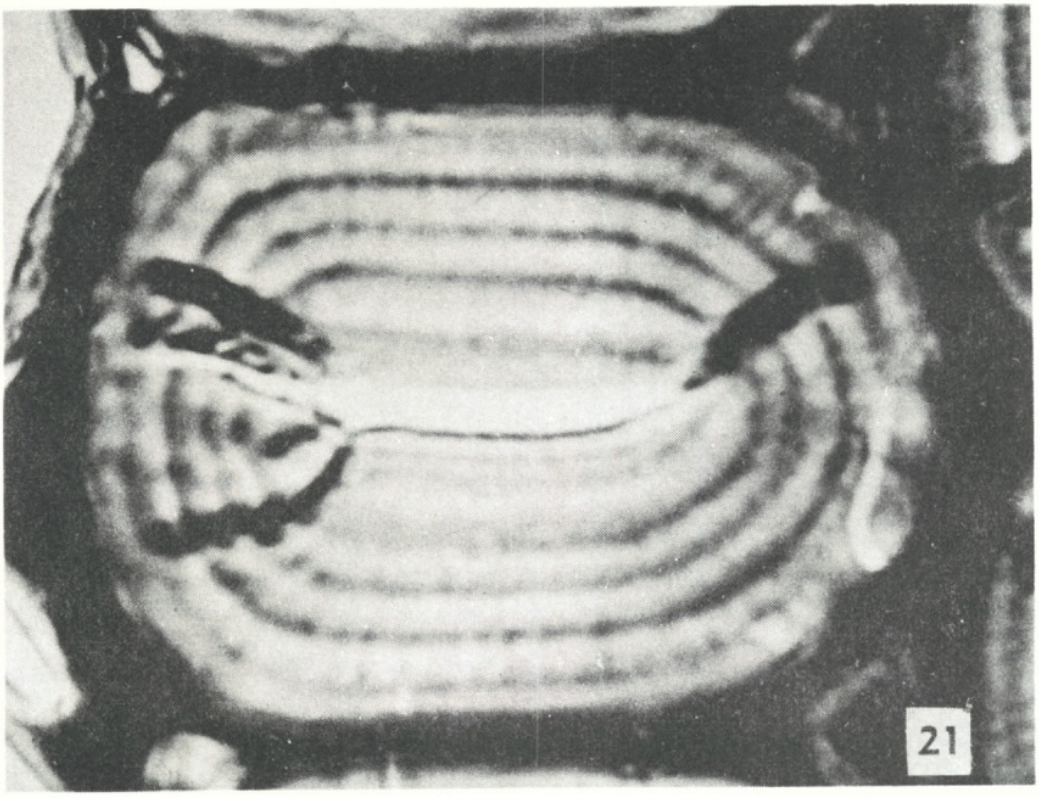

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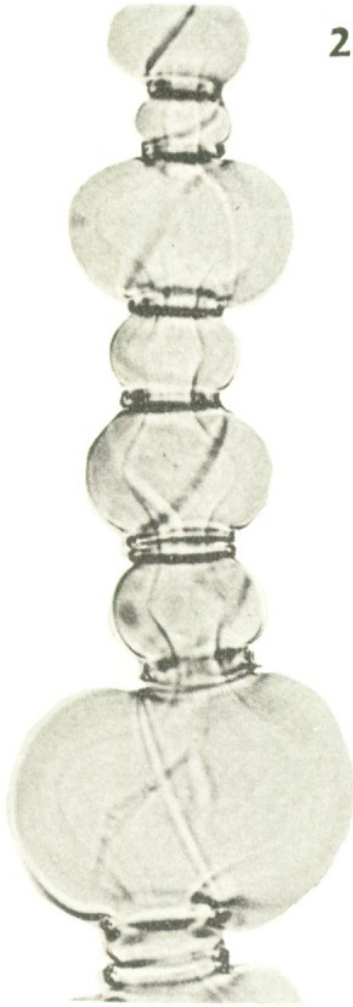

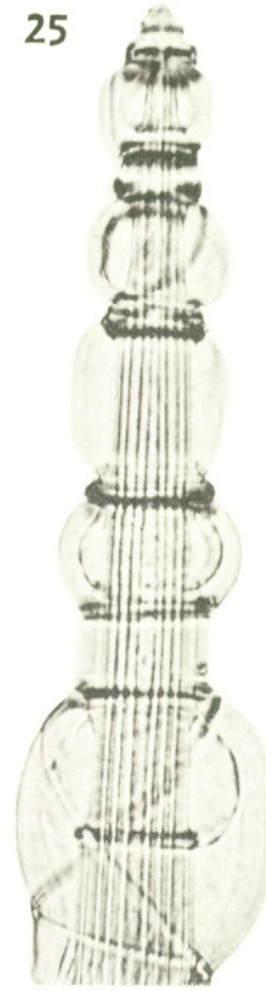

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