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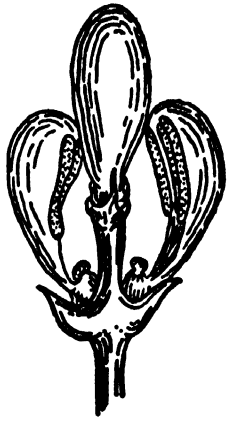

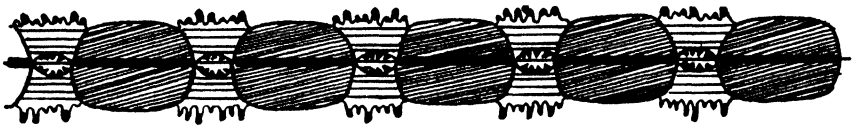

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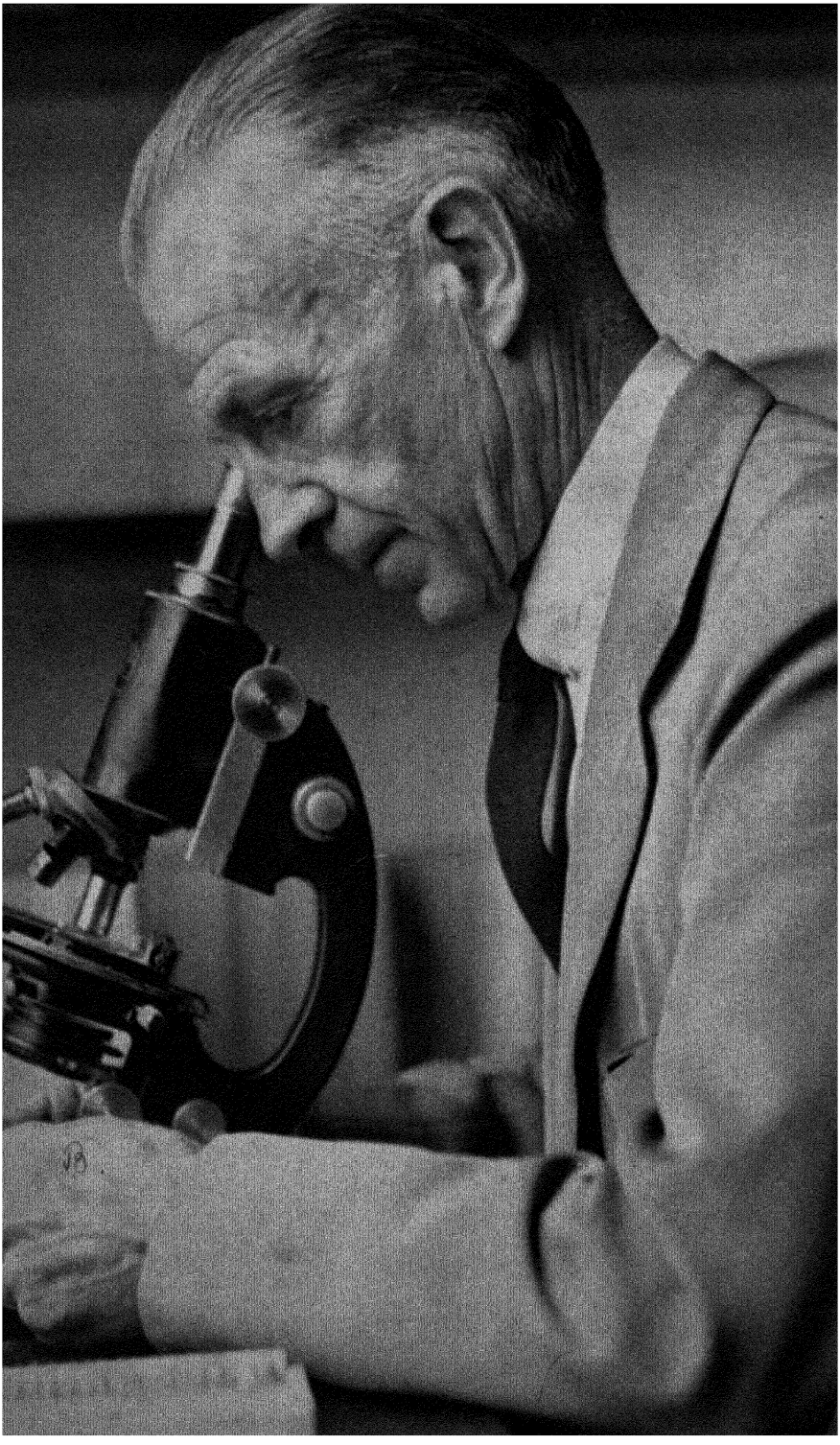

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by IRVING W. BAILEY, Sc.D.

Professor of Plant Anatomy and Chairman of the Institute for Research in General Plant Morphology, Harvard University

1954

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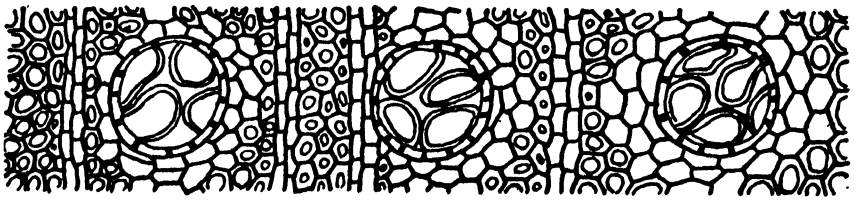


Irving Widmer Bailey

A Biographical Sketch

In the history of botanical science in the United States few individuals have made so many contributions to so many diverse areas of botany as has Irving W. Bailey. Attracted first, professionally, by the more practical applications of plants to human affairs, especially in forestry and silviculture, his interests and basic contributions have ranged widely from the pragmatic to the abstract; from the observational to the analytical. A continuing intellectual absorbtion with major questions involved in the origin, organization and evolution of plant life, coupled with a keen alertness to the fortuitous and unforeseen factors revealed in the solution of biological problems have made Irving Bailey's contributions a lasting addition to botanical knowledge. It is the purpose of this volume to draw together and to make more readily accessible certain selections of Professor Bailey's work which are representative of the breadth and depth of his scientific interests, and also to make better known among students of plant life the many facets of his personality as a botanist and as a scientist.

Irving Widmer Bailey was born in Tilton, New Hampshire, August 15, 1884, son of Solon Irving and Ruth Poulter Bailey. Several years after his birth his father was appointed to the staff of the Harvard College Observatory and later became Acting Director of the Observatory and Philips Professor of Astronomy in Harvard University. One of his father's early responsibilities was the management of the Observatory's southern hemisphere station near Arequipa, Peru. These duties necessitated many years residence in the high Andes. Numerous botanical interests manifested in later years doubtless stem from Bailey's boyhood spent in the coastal lowlands, the eastern jungles, and the high mountains of Peru. As attested by the writings of Darwin, Humboldt, Spruce and many other naturalists, there are few parts of the world which provide more diverse climate, more varied geologic features and greater range in types of vegetation than this relatively small geographic area of western South America. As a boy, keenly interested in the natural phenomena of his environment, Irving Bailey learned early what many botanists learn only in their later years, *viz.*, the extraordinary and bizarre development of plant life in tropical regions. He also, as a result of his years in South America, achieved a lasting interest in archeological and ethnological problems of early man in the Americas,

through exploration with his father of the then imperfectly known remains of ancient Peruvian cultures. These boyhood impressions, frequently renewed by return to the temperate environment of New England, have extended their influence both on BAILEY's scientific interests and on his personal, avocational interests throughout his life.

IRVING BAILEY entered Harvard College in 1903, not yet certain of his field of special interest, though he soon chose botanical science as an area of specialization. A latent interest in organic chemistry shows in many of his later scientific papers, but fortunately he chose to specialize in botany and forestry and entered the Graduate School of Harvard University in the Fall of 1907. He received the degree of Master of Forestry in 1909. It is significant that his first paper, "The Structure of the Wood in the *Pineae*" was published in this second year of graduate study. In the autumn of 1909, BAILEY became Instructor in Forestry in the Graduate School of Applied Science and began his long career in the many and varied activities into which Harvard's botanical development has led him. He was appointed Professor of Plant Anatomy in 1927.

During a period of World War I, BAILEY was in charge of the Wood Section, Materials Engineering Division of the Bureau of Aircraft Production stationed at Wright Field near Dayton, Ohio. His assignment here was in connection with the then critical problem of selection of wood for the manufacture of airplanes, a practical application of his extensive knowledge and experience with the structure and physical properties of wood.

At the close of World War I, BAILEY returned to the staff of the Bussey Institution and began his intensive studies on the histology and cytology of the cambium, a field of botanical interest which held his attention for many years and which lead him eventually into his pioneer researches on the structural organization of the plant cell wall.

During this period of his career BAILEY also began collaborative studies with his senior colleague at the Bussey, the distinguished entomologist and Dean of the Bussey Institution, Professor WILLIAM MORTON WHEELER. In 1920 they worked together in British Guiana, Trinidad and the West Indies, investigating the curious relationships which exist between ants and certain tropical plants. Resulting from these field studies is a unique series of papers dealing with the minute structural and anatomical features of myrmecophytes, or the so-called "ant plants" of the American tropics.

Resulting from his many years of productive research and teaching in forestry Professor BAILEY was appointed in 1926 as a member of the Committee on Forestry Research of the National Academy of Sciences. This committee, formed by the National Academy at the request of Colonel W. B. GREELEY, then chief of the United States Forest Service, was established to study the national resources and future needs in production of timber and wood products. In connection with this work, in which he took a leading part for several years Professor BAILEY travelled extensively in the United States, Canada, and Europe, surveying laboratories, tree plantations, and forest experiment stations with a view to formulating a long range program for research in American forestry. The result of this study was published

jointly with Dr. HERMAN A. SPOEHR of the Carnegie Institution of Washington in 1929, in a book entitled *The Rôle of Research in the Development of Forestry in North America,* portions of which are reprinted in this volume.

Although his contributions to botany are featured by a wide diversity of interests, a central theme may be discerned in many of BAILEY's investigations. This theme, which occupied his attention from early professional years became focused on the problem and enigma of the origin and evolution of the Angiosperms. A deeply embedded interest in phylogeny is certainly the most important factor in giving coherence to his numerous descriptive studies on the comparative anatomy of vascular plants. The interpretative, analytical, and essentially phylogenetic viewpoint strongly expressed in BAILEY's anatomical and cytological contributions has resulted in a unique synthesis of knowledge regarding the evolution of vascular tissues in land plants. Of especial interest and importance is elucidation of the progressive sequences in complexity which have characterized the evolutionary specialization of cells and tissues in the wood of arboreal flowering plants. These studies firmly established the significance of the structure of the secondary xylem as an essential tool in solving problems of phylogeny in the angiosperms, and demonstrated that one of the most outstanding evolutionary documents to be found in the plant kingdom lies in the progressive modification of the secondary body. Interest in the evolution of structure logically led into questions concerning the anatomy and morphology of plants of the past, and among his papers are a number of highly individual and significant contributions to paleobotany.

Long experience, accruing from a quarter century of study of the anatomy of woody plants prepared BAILEY for his investigations of the microstructure, histochemical, and physical organization of the plant cell wall. These studies, which were begun in 1933-34 in collaboration with THOMAS KERR, extended over a period of 10 years and resulted in the clarification of controversial interpretations concerning the organization and physical relationships of cellulose, lignin, and other constituents of the plant cell wall. It is a significant tribute to Professor BAILEY's acuteness as an investigator that newer techniques developed in very recent years for the study of organic microstructures, especially improvement in X-ray photomicrography and the development of electron microscopy have confirmed, and little altered, his interpretation of cell wall structure in woody plants based on optical and histochemical studies.

To students of taxonomy and comparative morphology of the Angiosperms it is probable that BAILEY's later contributions will have the greater appeal and interest. These investigations are based on the utilization of the totality of anatomical evidence available in all organs and tissues of the plant to solve problems of phylogenetic relationship within families and orders of the Dicotyledons. With the collaboration of graduate students and other co-workers, intensive studies have been made of many families of the Ranalean complex, with special emphasis on delineating the structural organization of the primitive carpel, stamen, pollen and leaf in this group of the least specialized of living Angiosperms. Investigations of the floral morphology of certain monotypic and problematic families in the Ranalean

complex have revealed much that is new concerning the structure and morphology of the primitive carpel and the primitive pollen bearing organs among Angiosperms. Establishment of these morphological criteria represents a major advance in the solution of that ultimate and persistent botanical riddle: the origin of the Angiosperms from their geologically ancestral stock.

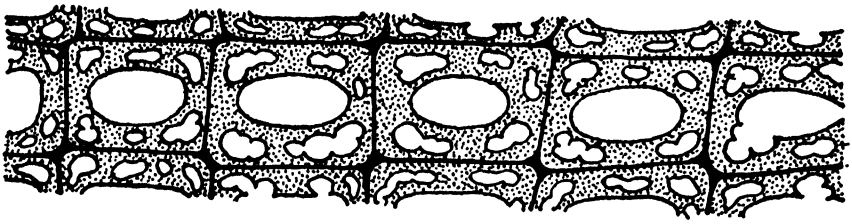
During World War II Professor BAILEY accepted the responsibility, delegated to him by the Dean of the Faculty of Arts and Sciences of Harvard University, to draft a plan for the re-organization and closer integration of Harvard's botanical resources both in the morphological-taxonomic and in the experimental areas. The result of this effort, in which he was generously and enthusiastically assisted by many of his colleagues, was a report entitled *Botany and its Applications at Harvard,* submitted in June, 1945. This report, subsequently approved by the Harvard Corporation, contained a recommendation for the erection of a botanical building, centrally located in Cambridge, and designed to house the herbaria, associated libraries and other botanical collections. The new botanical building, recommended in his report, was erected in 1953-54. As a result of the reorganization plan of 1946, two botanical budgetary and administrative areas were created, the *Institute for Research in Experimental and Applied Botany* and the *Institute for Research in General Plant Morphology,* Professor BAILEY was appointed chairman of the latter by the Corporation of Harvard University in 1946, a position which he has held since.

Many honors have been conferred upon IRVING BAILEY in recognition of his contributions to botanical science. Among these honors are election to the National Academy of Sciences, the honorary degree of Doctor of Science conferred by the University of Wisconsin in 1931, election to Foreign Member of the Swedish Royal Academy of Sciences, President of the Botanical Society of America in 1945 and Vice President of the Seventh and Eighth International Botanical Congresses.

It is perhaps appropriate to close this brief record of Professor BAILEY's career with a few words regarding his approach toward scientific problems. Much of his success in the clarification of both practical and theoretical questions and in discerning their essential and significant factors has been due to an awareness of the variables which characterize biological phenomena and to an ever present alertness to the significance of the unexpected and seemingly irrelevant. His intellectual qualities, scientific curiosity and capacity for sustained effort have always been manifest throughout his career. It is hoped that these excerpts from long and fruitful years of work will serve to make better known to botanists and other biologists IRVING BAILEY'S diverse and lasting contributions to botanical science.

ELSO S. BARGHOORN

July 1954



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108. The conduplicate carpel of dicotyledons and its initial trends of specialization (Am. Jour. Bot. 38: 373-379) (*with* B. G. L. SWAMY).

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AUTHOR's PREFACE

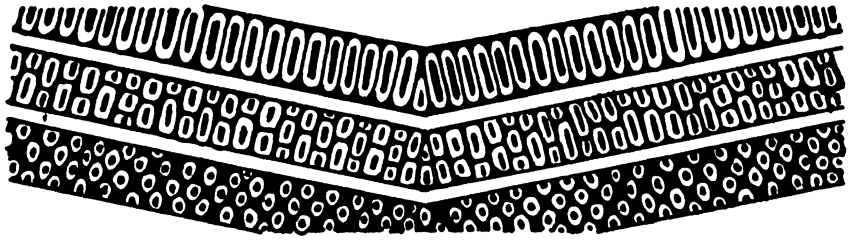
When approached by Doctors BARGHOORN *and* VERDOORN *with the request that I make a selection of my papers for publication in book form, it soon became evident that some central theme was essential to bind such a selection together. Historically, the first half of the 20th Century — the period during which I received my scientific training and have conducted my investigations — has witnessed a gradual and significant change in the fundamental concepts and methodologies of Plant Anatomy. At the beginning of the century, the older botanical disciplines had become rigidly compartmentalized and concerned with the application of stereotyped techniques. At present, the fixed boundaries between scientific disciplines are rapidly breaking down and diversified new techniques are being commonly applied. Thus, the papers in this book have been selected and arranged to illustrate successive attempts between 1909-1953 to bridge the gaps between Plant Anatomy and other fields of scientific endeavor. Obviously, little could have been accomplished in these directions without the willing and active cooperation, not only of numerous botanists, but also of entomologists, biochemists, biophysicists and others, to whose helpful assistance I owe so much.*

As indicated in the Table of Contents, the selected papers are grouped in eight parts, each of the first seven of which is concerned with the relation of Plant Aanatomy to some other discipline or disciplines. At the end of each of these parts, references are listed to additional papers of the author dealing with this particular aspect of his work. Throughout the book, figures in italics between parentheses refer to the numbered list of the author's publications on pages xiii-xviii. In general, the selected papers have been reprinted with only minor changes in the text and text-figures. However, it was essential to reduce the plates to approximately one half of the original number. This necessitated the elimination of some and the combination and rearrangement of others, with concomitant changes in magnification and numbering.

I am greatly indebted to Dr. B. G. L. SWAMY *for the preparation of the sectional title pages, headpieces and vignettes* (cf. p. 259), *to Dr.* ROBERT C. FOSTER *for his laborious help in reading the page-proof, and to Mrs.* VERDOORN *for the compilation of the index of plant and animal names, the author index, and miscellaneous proofreading.*

Autumn 1954

I. W. Bailey



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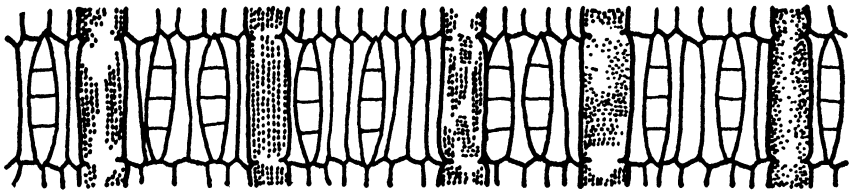

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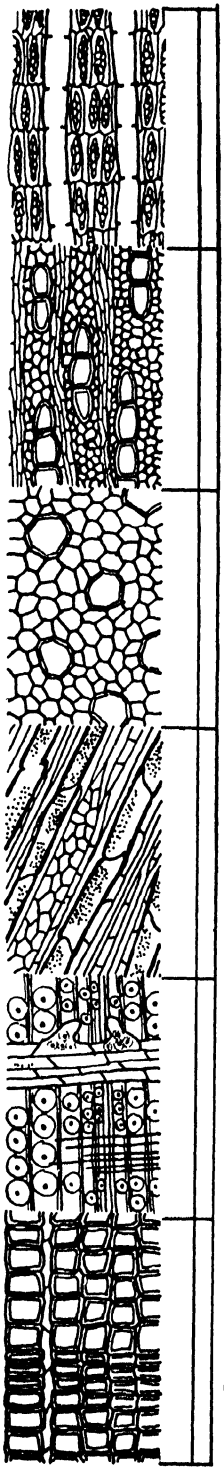
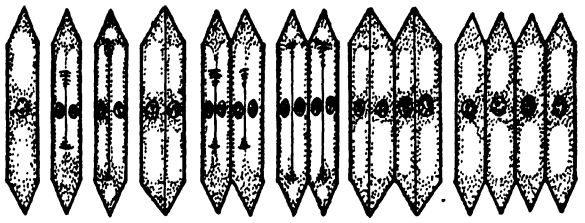
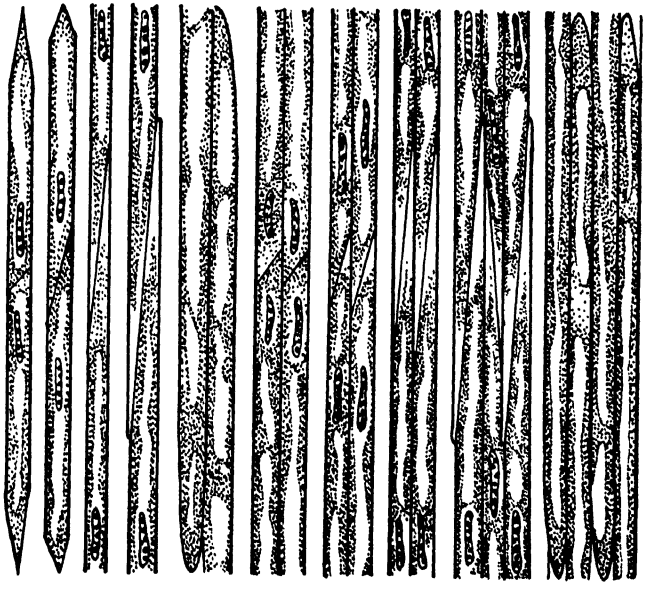




Part I

CYTOLOGY and ONTOGENY



Chapter 1

PHENOMENA OF CELL DIVISION IN THE CAMBIUM OF ARBORESCENT GYMNOSPERMS AND THEIR CYTOLOGICAL SIGNIFICANCE *

IN CONNECTION with a series of investigations upon the variations in size and structure of tracheary cells in vascular plants, the writer had occasion to search for information concerning the processes of growth and cell division in the cambium of arborescent plants. He was unable to find satisfactory descriptions of these phenomena in the botanical literature, and was assured by several cytologists that comparatively little was known about them; in all probability, owing to the difficulty of sectioning the lateral meristem (cambium) of woody plants. It seemed advisable, accordingly, to initiate a special histological and cytological investigation of this important tissue.

During 1917 and 1918, specimens of the cambium of *Pinus Strobus* L. were collected—from trees of different ages and varying environments—at frequent intervals during the growing season, April to September. Methods were perfected for fixing, sectioning and staining this material, which was compared with that secured from 14 other genera of Coniferae. The results of this investigation and of a similar study of arborescent dicotyledons will be published *in extenso* at a later date, but certain of the cytological phenomena encountered are so significant as to merit a brief preliminary description in these pages.

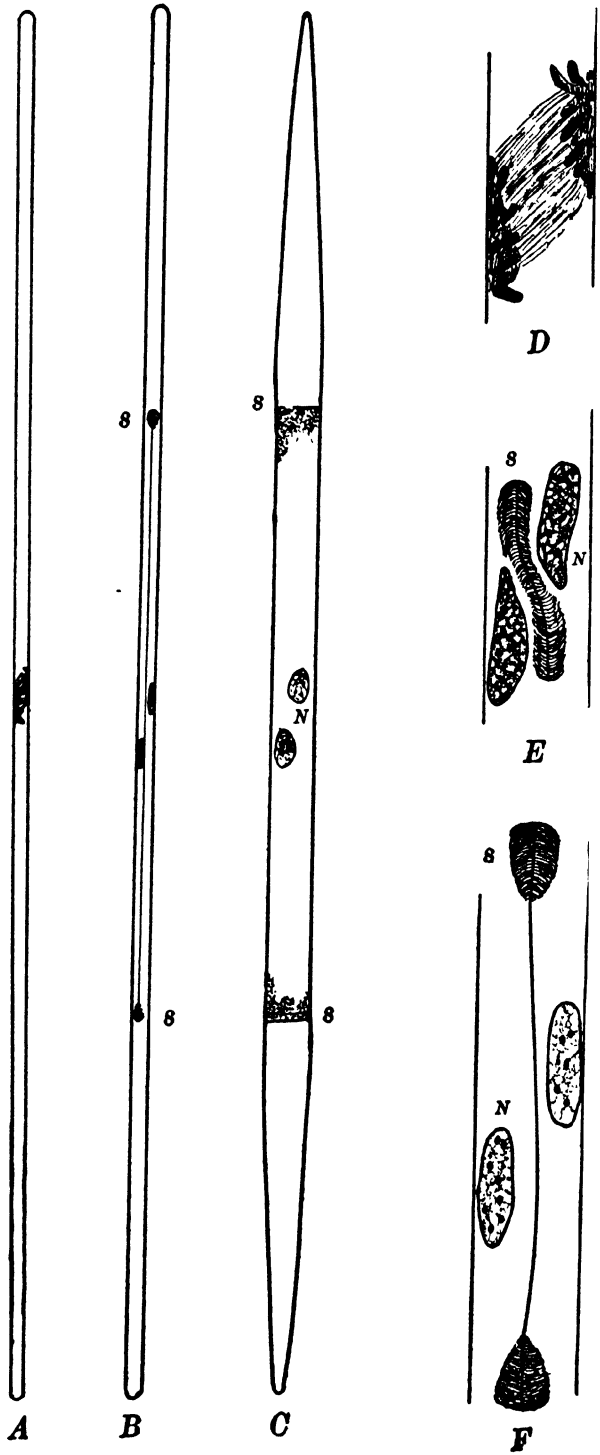
The cambium, although essentially an undifferentiated or 'embryonic' type of tissue, is composed of cells which are considerably elongated. In *Pinus Strobus* L., as in other arborescent gymnosperms, the cambial initials are commonly from one hundred to several hundred times as long as they are wide (radial diameter). From the point of view of SACHS' and STRASBURGER'S theories of the 'working sphere of the nucleus' and the observations of TREUB, KALLEN, BUSCALIONI, PIROTTA and BUSCALIONI and others upon the occurrence of many nuclei in elongated protoplasts of the higher plants, it might have been expected *a priori* that these long meristematic cells would contain more than one nucleus each.

This did not prove to be the case, however, in any of the material examined by the writer. Each cell contains a single nucleus, which is centrally located and placed so that its longest axis is approximately parallel to the long axis of the cell.

During mitosis, on the other hand, the polar axis of the division figure—late prophase, metaphase, anaphase, early telophase—*does not stand,* in most cases at least, *at right angles to the long axis of the cell.*

As is shown in TEXT FIGURE 1 (*A* and *D*) the mitotic figure is placed diagonally across the cell, at an angle of from 20 to 40 degrees. That this

* Reprinted from the Proceedings of the National Academy of Sciences 5: 283-285, 1919.


A
B
C
D
E
F
S
N

phenomenon is not an artifact, *i.e.*, due to the displacement of an ordinary spindle, is indicated by the fact that the whole mitotic figure is asymmetrically developed, in conformity with its diagonal position (*D* and *E*).

Of course it is well known that the longitudinally dividing cells of the cambium form an exception to SACHS' law of 'rectangular intersection of successive division-planes' and HERTWIG's modification of SACHS' hypothesis, but it has been assumed by GIESENHAGEN and others that, in the cambium, the karyokinetic figures lie with their polar axes perpendicular and their equatorial planes parallel to the long axes of the cells.

The formation of a cell-plate starting from one of these obliquely placed spindles is a very interesting phenomenon. The spindle becomes greatly extended laterally by the addition of peripheral fibers and gradually assumes the curved form shown in (*E*). As more peripheral fibers are successively added the remains of the central fibers disappear from about the cell-plate, leaving two separate aggregations of fibers which are connected by the first formed portion of the cell-plate (*F*). These aggregations of kinoplasmic fibers, which may be called *kinoplasmasomes*, have a very characteristic form and structure. They extend across the cell—at right angles to its longitudinal axis—from one radial wall to the other (*C*), and are located in the centre of the protoplast midway between its tangential surfaces (*F*). In sectional view (*F*), they have a somewhat wedge-shaped outline, bluntly convex in front and tapering to a point at the rear along the cell-plate. The *kinoplasmasomes* move in opposite directions towards the ends of the cell (*B* and *C*). As they move forward, the cell-plate is extended until it eventually reaches the two ends of the cell, thus dividing the protoplast into two similar portions each of which contains one of the daughter nuclei. The latter remain close together near the centre of the cell during the process of cell-plate formation. The writer has been unable to demonstrate any visible connection between the daughter nuclei and the kinoplasmasomes or their constituent fibers. Except at the beginning of cell-plate formation the daughter nuclei are in a semi-'resting condition' and are provided with a clearly defined nuclear membrane and numerous nucleoli. Not infrequently the distance traversed by the kinoplasmasomes, in passing from the vicinity of the daughter nuclei to the ends of the protoplast, may be from one to several millimeters.

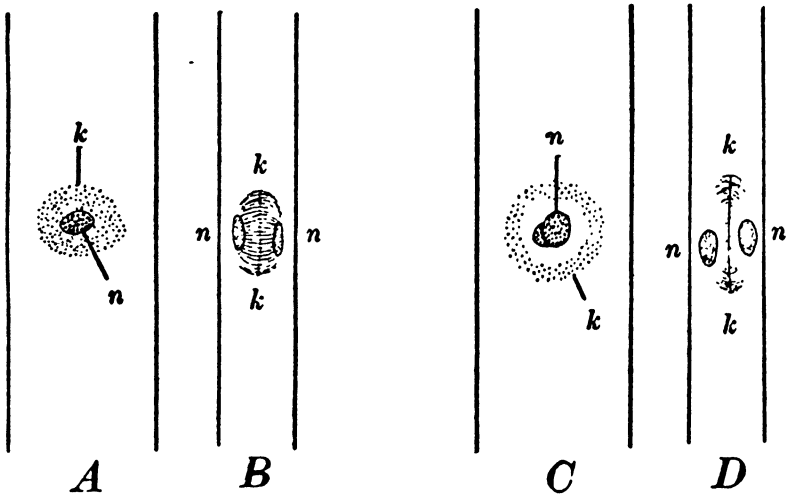
This type of cell division, in which the process of cell-plate formation is so greatly extended—both as regards space and time—and so clearly dissociated from the usual phenomena of karyokinesis, promises, upon further analysis, to be of some significance in the discussion of the dynamics of cell division.

TEXT FIGURE 1 (*opposite*).— *Pinus Strobus*. *A*, cambial initial, figured in radial longitudinal extension, showing oblique position of karyokinetic figure; *B*, the same, showing daughter nuclei and process of cell-plate formation; *C*, cell B, figured in tangential longitudinal extension; *D*, highly magnified view of karyokinetic figure shown in *A*; *E*, central portion of cambial initial, figured in radial extension, showing the beginning of cell-plate formation; *F*, the same, showing later stage of cell-plate formation; *n*, daughter nuclei; *s*, aggregations of kinoplasmic fibers, kinoplasmasomes, which are concerned in the formation of the division membrane.

Chapter 2

THE FORMATION OF THE CELL PLATE IN THE CAMBIUM OF THE HIGHER PLANTS *

In a previous note (34) the writer called attention to a remarkable type of cytokinesis that occurs in the cambium of Coniferae. The process of cell plate formation is greatly extended, both as regards space and time and is clearly dissociated, except in its initial stages, from the usual phenomena of karyokinesis. Since this type of cell division promises to be of considerable significance in the study of various cytological and physiological problems it is desirable to determine whether it is an isolated phenomenon, *i.e.*, confined to the Coniferae, or one that is characteristic of the cambia of all of the higher plants, angiosperms as well as gymnosperms.



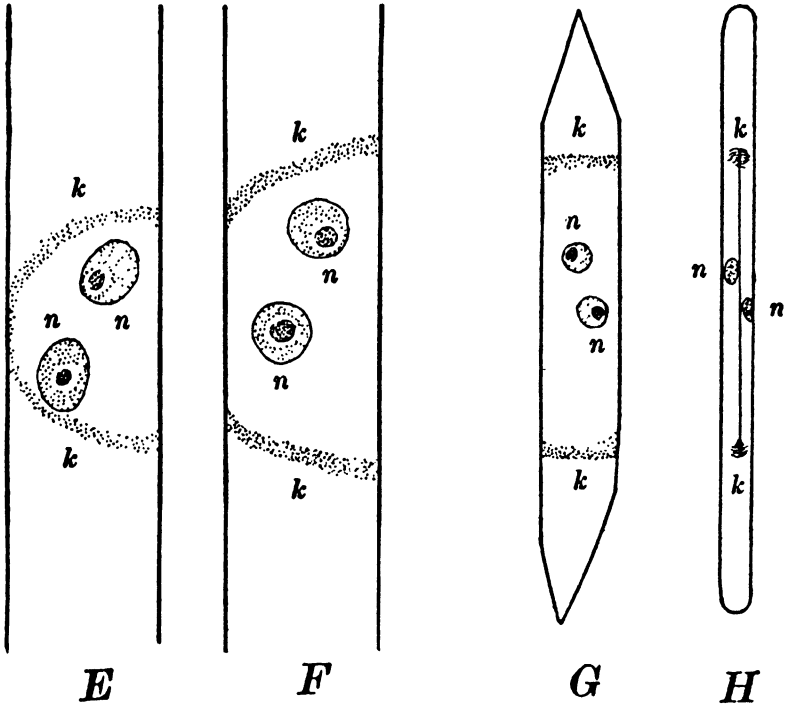
Text figure 1.— *Robinia Pseudo-Acacia.* *A*, central portion of cambial initial, in tangential longitudinal extension, showing early "disk" stage in the formation of the cell plate; *B*, the same, radial longitudinal extension; *C*, central portion of cambial initial, in tangential longitudinal extension, showing "ring" or "halo" stage in the formation of the cell plate; *D*, the same, radial longitudinal extension.

During the last growing season, I secured specimens of the cambium from an extensive series of angiosperms. Selections were made so as to include representatives of all of the larger and more important orders of the dicotyledons and of certain arborescent monocotyledons which have "secondary" growth in thickness. Specimens were obtained from both tropical and temperate environments.

Although the cambial initials in mature stems of angiosperms are on an

* Reprinted from the Proceedings of the National Academy of Sciences 6: 197-200, 1920.

average considerably smaller than homologous elements of gymnosperms[1] the salient features of cytokinesis are the same in both subphyla. In the former, as in the latter group, each initial contains a single nucleus which is centrally located and divides mitotically. The spindle becomes extended laterally by the addition of peripheral "fibers" [2] and gradually assumes the



TEXT FIGURE 2.— *Robinia Pseudo-Acacia.* *E*, central portion of cambial initial, in tangential longitudinal extension, showing disappearance of two arcs of the kinoplasmic ring; *F*, central portion of cambial initial, in tangential longitudinal extension, showing early kinoplasmasome stage in the formation of the cell plate; *G*, cambial initial, in tangential longitudinal extension, showing later kinoplasmasome stage in the formation of the cell plate; *H*, the same, radial longitudinal extension; *n*, daughter nucleus; *k*, kinoplasma.

form of a disk (TEXT FIG. 1, *A* and *B*). As more fibers are successively added the original "connecting fibers" disappear from about the cell plate, leaving a ring-shaped aggregation of kinoplasmic fibers, which in tangential longitudinal sections of the cambium appears as a halo about the daughter nuclei (TEXT FIG. 1, *C*). This ring increases in circumference until it intersects the radial walls, when it becomes more or less flattened on two

[1] The average length of the cambial initials in 152 stems of gymnosperms was computed as approximately 3400 micra; in 275 stems of dicotyledons as approximately 600 micra.

[2] The word fibers is used without reference to whether the fibrillae are true threads or lines of protoplasmic streaming in the living cell.

sides. As soon as the cell plate intersects the radial walls of the cell, the fibers in these two sides disappear leaving two separate aggregations of kinoplasmic fibers which are connected by the cell plate (TEXT FIG. 2, *E*, *F*, *G*, *H*). These aggregations of fibers, kinoplasmasomes, have the same characteristic shape and structure as those which occur in the Coniferae. They extend across the cell—more or less at right angles to its longitudinal axis—from one radial wall to the other (TEXT FIG. 2, *G*) and are located in the center of the protoplast, midway between its tangential surfaces (TEXT FIG. 2, *H*). They move in opposite directions—*usually at equal rates*—towards the ends of the cell. As they move forward, the cell plate is extended until it eventually reaches the two ends of the protoplast, thus dividing it into halves, each of which contains one of the daughter nuclei. The latter usually remain close together near the center of the cell during cytokinesis, as in the gymnosperms; and I have been unable to find any visible connection between them and the kinoplasmasomes.

Having determined that this type of cytokinesis is characteristic of the cambia of both gymnosperms and angiosperms, it is of interest to inquire whether it ever occurs in other somatic tissues, and what relation it bears to those types of cell plate formation which previously have been described by TREUB, STRASBURGER and SCHÜRHOFF. The writer has accumulated considerable evidence which indicates that the phenomenon in question is not confined to the cambium, but occurs in other somatic tissues, in elongated or much flattened cells whose planes of division have one long and one short dimension. Furthermore, a comparative study of cytokinesis in different somatic tissue and in cells of different shapes and sizes suggests that the various types of cell plate formation, described by TREUB, STRASBURGER, SCHÜRHOFF and the writer, are but different phases or stages of a single general or fundamental type of cytokinesis. The particular expressions of the phenomenon which may occur in any given cell are dependent upon the dimensions of the latter, its plane of division and the size and location of the nucleus. Thus, in very small, isodiametric cells, having a large centrally located nucleus, the cell plate quickly intersects the walls of the cell, without any extensive lateral growth—by means of additional peripheral fibers—of the spindle. In somewhat larger elements there is sufficient room for the process of cytokinesis to reach the "halo" stage before the cell plate intersects the sides of the cell. Only in elongated or much flattened elements, *i.e.*, cells which are not isodiametric, is it possible for the phenomenon of cell plate formation to pass through the "spindle," "disk," "halo" and "frame" stages and finally to form two entirely separate aggregations of kinoplasmic fibrillae, the kinoplasmasomes.

It is evident, accordingly, that the remarkable type of cell plate formation which was described in a former note, is not an isolated or unusual phenomenon, but is of frequent occurrence in the somatic tissue of the higher plants, gymnosperms and angiosperms. It promises to be significant in any general discussion concerning the dynamics of cytokinesis and karyokinesis.

Chapter 3

THE CAMBIUM AND ITS DERIVATIVE TISSUES

III. A RECONNAISSANCE OF CYTOLOGICAL PHENOMENA IN THE CAMBIUM *

IN THE second paper of this series, the writer (38) called attention to the fact that the dimensions and volume of the undifferentiated, actively dividing and growing cells of the lateral meristem or cambium vary greatly, not only in plants of different systematic affinities, but in different parts of a given individual and in stems grown under different environmental conditions. Therefore, the cambium provides an unusually favorable medium for the study of a number of fundamental cytological problems, particularly those of the "working sphere of the nucleus," the much discussed "nucleocytoplasmic relation," and phenomena of karyokinesis and cytokinesis in cells of unusual shapes and sizes.

SACHS (1893) held that, although plants vary enormously in their linear dimensions, their constituent cells are minute and of relatively uniform size. His student AMELUNG (1893) endeavored to prove, by means of an extensive series of measurements, that variations in the size of an organ or plant are due to differences in cell number rather than to fluctuations in cell size; a view which subsequently was championed on the zoological side by CONKLIN, RABL, DRIESCH, SCHULTZ, and others. SACHS (1892, 1893, 1895) and STRASBURGER (1893) concluded that the size of uninucleated cells, particularly of the undifferentiated cells of embryos and meristems, is determined by the "energizing" or "working sphere" of the nucleus, which they considered to be very restricted. Both investigators noted that unusually large or much elongated protoplasts tend to be multinucleate, and SACHS emphasized the fact that large, uninucleated cells either contain much passive (non-cytoplasmic) material or are relatively inactive until energized by the formation of numerous nuclei.

Are Cambial Initials Multinucleate?:—The cambium is composed of elements of two distinct shapes and sizes. The ray initials commonly are roughly isodiametric and of the same general order of magnitude as the cells of embryos and terminal meristems. The initials which divide to form the elongated elements of the xylem and phloem, on the contrary, have one long and two short dimensions and are relatively large. In gymnosperms and less highly differentiated dicotyledons, adjacent fusiform initials vary greatly in length and volume. The questions suggest themselves, accordingly: are the ray initials uninucleate and the elongated cells multinucleate, and are the striking variations in the length and volume of the fusiform initials closely correlated with fluctuations in the number of nuclei that are

* Reprinted from the American Journal of Botany 7: 417-434, 1920.

contained within them? SCHACHT (1856) and RUSSOW (1882) were of the opinion that the elongated cells of the cambium contain more than one nucleus each, but STRASBURGER (1891) questioned the accuracy of their conclusions.

During the last few years, I have secured material of the cambium from a wide series of gymnosperms and angiosperms of both temperate and tropical regions. The specimens were removed from various parts of the stems, roots, and branches and from trees of different sizes and ages, and, in the case of certain species, were collected at frequent intervals throughout the growing and resting seasons. The tissues were transferred as rapidly as possible to various killing fluids, of which the chrom-acetic-urea solution proved to be the most effective. In none of this material have I found cambial initials which contained more than one nucleus each. The elongated initials, in tangential, longitudinal sections of the cambium, frequently appear to be multinucleate, but this is due to the fact that several radially flattened cells are exactly superimposed so that their nuclei lie close to the same focal plane. It is evident, accordingly, that the fusiform cells of the lateral meristem do not resemble other types of large or much elongated protoplasts, such as have been shown by SCHMITZ, TREUB, JOHOW, KALLEN, HABERLANDT, PIROTTA and BUSCALIONI, SMOLÁK, NĚMEC, LUNDEGÅRDH, and others, to be multinucleate. Each initial contains a single nucleus which is centrally located and retains this position during growth and karyokinesis. In other words, not only is there a much greater variability in the size of the meristematic cells than hypothesized by SACHS or STRASBURGER, but in the lateral meristem the nucleus may extend its "energizing" influence to a distance of several thousand microns. STRASBURGER found that the average diameters of the more or less isodiametric cells of terminal meristems varied between 5 and 24 microns. In certain gymnosperms, the cambial initials may attain a length of more than 9,000 microns and a volume of approximately 10,000,000 cubic microns.

Nucleo-cytoplasmic Relation:—STRASBURGER'S measurements led him to believe that there is a close correlation between cell size and nuclear size, a conclusion which was strongly supported by the experimental investigations of GERASSIMOW (1902), and which has led to considerable controversy among zoologists and botanists as to whether the so-called nucleo-cytoplasmic relation is a constant and self-regulating ratio. The painstaking investigations of a number of zoologists indicate that, although in general large cells tend to have larger nuclei than small ones, the nucleo-cytoplasmic ratio fluctuates within rather wide limits, not only in different organisms, but also during different stages in ontogeny and under different environmental conditions. Highly specialized tissue cells tend to have very different ratios from those characteristic of undifferentiated or embryonic elements.

The cambial initials of arborescent dicotyledons contain smaller nuclei than do homologous cells of gymnosperms. Whether this difference is due entirely to the fact that the cambial initials are smaller in the former than in the latter group of plants is a question which must be reserved for discussion in a subsequent paper. In Coniferae, as illustrated by *Pinus Strobus*

L., the dimensions and volume of the nuclei vary considerably in cambial initials of different shapes and sizes. The large, fusiform initials have larger and more elongated nuclei than the small, roughly isodiametric, ray initials (TABLE I). Furthermore, the large cambial initials of stout, mature stems tend to contain larger nuclei than do the small, meristematic cells of young shoots (TABLE I). However, as shown in TABLE II, the nuclei of

TABLE I: *Pinus Strobus* L.:—

	Nucleus				Cell				Ratio Between Volume of Nucleus and Volume of Cell
	Dimensions			Approximate Volume	Dimensions			Approximate Volume	
	VD	RD	TD		VD	RD	TD		
	Cambium from 1-year-old stem								
	microns	*microns*	*microns*	*cu. mic.*	*microns*	*microns*	*microns*	*cu. mic.*	
Ray initials	10 8	8.7	6.5	350	22.9	17 8	13.8	5,000	1:14
Large initials	63	3.2	5.8	1,000	870	4.3	16.	60,000	1:60
	Cambium from 60-year-old stem								
Ray initials	12.4	12 5	9.9	850	24.8	26 6	17.0	10,000	1.12
Large initials	82	5 9	8 9	3,500	4,000	6.2	42.4	1,000,000	1:286

Basis. dimensions of cells and nuclei are averages of 50 measurements.

fusiform initials are subject to variations in their longitudinal dimensions (VD) which are not closely correlated with fluctuations in the length of the cells. The most striking of these variations are seasonal. In the stem of the white pine, the "resting" nuclei of the fusiform initials tend to be much longer and narrower during the fall and winter than during the spring and summer (TABLE II). This change, if it occurs, is much less conspicuous in the root. It should be noted, in addition, that the variations in the shape of the nuclei are not paralleled necessarily by similar fluctuations in their volume. This is due to the fact that the changes in length may be more or less completely neutralized by concomitant changes in cross-sectional area.

Although the values in TABLE I indicate that the ratio between cell size and nuclear size may remain relatively constant in ray initials of different sizes, they show very clearly that this ratio varies greatly in fusiform initials of different dimensions and volumes. The nuclei do not elongate to any considerable extent as the fusiform initials increase in length. In other words, these meristematic cells do not contain very abnormally elongated nuclei, such as have been described and figured by MOLISCH (1889) for highly specialized tissue cells of certain monocotyledons. As previously stated, the relatively small, centrally located nucleus must, in certain cases, extend its energizing influence for several thousand microns in order to

control processes of growth and longitudinal division in the cambial initials of various gymnosperms.

Boveri (1902, 1905) found that the size of the nuclei in echinoderm larvae is dependent upon the number of chromosomes which enter into the nuclei, and concluded that "die Grösse der Larvenzellen ist eine Funktion der in ihnen enthaltenen Chromatinmenge, und zwar ist das Zellvolumen der Chromosomenzahl direkt proportional." A few years later Gates (1909) showed that *Oenothera gigas,* a tetraploid mutation of *Oe. Lamarckiana,* is composed of larger cells than the species from which it

Table II· *Pinus Strobus* L.—

Date	Source of Cambium			Nuclei		Fusiform Initials	
	Age of Tree, Years	Portion of Plant	Number of Rings Under Cambium	Dimensions (Microns)		Dimensions (Microns)	
				VD	TD	VD	TD
3/25/18	36	Stem	1	64	4 5	910	18 6
3/21/18	36	Stem	8	68	8 1	2090	23 7
3/9/18	36	Stem	27	67	8 9	2780	29 5
2/25/18	36	Branch	1	60	4 6	830	20.1
2/25/18	36	Branch	12	60	9 1	1950	27.7
4/22/18	67	Stem	1	56	4 3	800	16 3
3/24/18	67	Stem	48	82	8 9	3890	42.2
4/28/18	67	Stem	48	*41*	*17.3*	4000	38.5
3/24/18	67	Stem	61	84	7.9	3000	32.0
3/23/18	67	Large root	32	42	17 2	2760	40.5
3/2/18	25	Stem	1	56	6 1	890	16.3
3/2/18	25	Stem	15	57	7.9	2650	39.1
4/22/18*	25	Stem	19	71	6 3	1700	23 5
4/22/18*	25	Stem	19	*34*	*13 6*	1700	23.5
5/19/18	25	Stem	15	*36*	*15 3*	2400	35 3
6/17/18	25	Stem	17	*39*	*12 8*	3260	35.2
7/15/18	25	Stem	17	32	*16.3*	2970	36 8
8/19/18	25	Stem	17	*39*	*13.2*	2580	34 4
10/13/18	25	Stem	19	59	8 3	1710	29.8

Basis Dimensions of cells and nuclei are averages of 50 measurements.

Italicized figures indicate that the nuclei are from cambial initials which have been dividing for some time previously.

* Both types of "resting" nuclei were present in the same sections, due to the fact that certain initials had divided, whereas others were still in the winter condition.

originated. Similar phenomena have been observed by Gregory (1912), Winkler (1916), and others. The work of Gregory (1909) and Keeble (1912) indicates, however, that in certain giant forms of *Primula sinensis,* which have larger cells than the typical form, there is an increase in the size, but not in the number of chromosomes. Winkler's paper is particularly significant, in this connection, owing to his general discussion of the relation between cell size and chromosomal number in the higher plants. He reaches the conclusion that there is a very close correlation between cell size and chromosomal mass, both in meristematic and non-meristematic, somatic

tissue. He states that in embryonic types of tissue (lateral and terminal meristems) the cells are roughly isodiametric, of nearly uniform size, and always contain the diploid number of chromosomes. In non-meristematic tissue, multinucleate protoplasts, nuclear fusions, and changes from the diploid to the tetraploid or the polyploid condition are of common occurrence, and many cells depart widely from the inherited, specific cell size of the plant. He infers that such cells tend to be hyperchromatic, much elongated elements containing more than one nucleus each and other types of large cells an abnormal number of chromosomes.

It is evident that the cambium provides a favorable medium for testing WINKLER'S generalizations. The fusiform initials, *which vary greatly in size,* obviously are not multinucleate. Are they hyperchromatic? Before attempting to answer this question it is essential to devote some attention to a discussion of karyokinesis in the lateral meristem.

Karyokinesis in Cambial Initials:—As far as I have been able to determine, the nuclei of cambial initials always divide mitotically. STRASBURGER (1891) observed fragmentation in young sieve tubes of larch. My own preparations indicate that amitosis may occur in slightly differentiated cells of the cambial layer—which later are to develop into tracheary elements—just before the beginning of the growing season. Vacuolated nuclei also tend to be present at this time. However, I have never seen any indication of either of these phenomena in undoubted *initials* of the lateral meristem of either gymnosperms or dicotyledons.

The cambium varies so greatly in its activities during different seasons, in different plants, and in different parts of given individuals, that the only rules which I have been able to formulate for obtaining division figures are, to collect specimens at frequent intervals, from as many individuals as possible, and from all parts of each plant. Initials may be dividing actively in one portion of a stem when those in adjacent portions are inactive. Similarly, numerous mitotic figures may be present in a given individual when a neighboring plant appears to be entirely devoid of them.

The karyokinetic figures in ray initials (PLATE 1, FIGS. 30-32) generally resemble those that occur in parenchyma and terminal meristems. The figures vary considerably in fusiform initials, depending upon the shape and size of the nuclei and cells, and upon the plane in which the cells are dividing. Of course, the principal plane of division in elongated initials is periclinal or parallel to tangents to the circumference of the stem or root. In other words, the fusiform initials divide in a tangential, longitudinal plane which is a division plane of maximal area. Although the tangential diameter of the cambial initials increases to a certain extent during the earlier stages of enlargement of stems, roots, and branches (TABLE 1), it falls far short of being sufficient to compensate for the rapid increase in the periphery of the cambium. NÄGELI (1864*a*) inferred from this fact that the fusiform initials must divide periodically in a radial, longitudinal plane. These hypothetical radial, longitudinal divisions are described and figured in many botanical textbooks, but I have been unable to find them in any of the gymnosperms and less highly specialized dicotyledons that I have studied.

As shown by ROBERT HARTIG (1895) and KLINKEN (1914)—for *Pinus sylvestris* L. and *Taxus baccata* L.—the fusiform initials elongate, sliding by one another, until they have attained a certain length. They then divide, by means of a more or less oblique, transverse partition into two short cells, which in turn elongate and divide. Thus, the increase in the periphery of the cambium is due primarily, not to radial, longitudinal divisions of the fusiform initials, accompanied by lateral enlargements of the products of such divisions, but to the sliding growth of periodically elongated and dividing cells. During the process of elongation, between successive pseudo-transverse divisions, the initials continue to divide in a tangential, longitudinal plane.

In dicotyledons (*e.g., Robinia*) having short initials and small nuclei, the polar axis of the karyokinetic figure in a longitudinally dividing cell is placed at right angles to the main axis of the cell (FIG. 25). In *Pinus Strobus* and other gymnosperms, on the contrary, it tends to assume a diagonal position during the late prophase, metaphase, anaphase, and early telophase (FIGS. 17-19). That this is not an artifact, due to the displacement of an ordinary spindle during fixation or sectioning, is indicated by the fact that the whole-mitotic figure is asymmetrically developed in conformity with its oblique position (FIGS. 17-19). Furthermore, it should be noted that the radial diameter of the initials is so short that there is not sufficient room to permit the elongated nucleus to shift into, or the karyokinetic figure to develop in, a transverse position. Of course, the position of the mitotic figure in pseudo-transversely dividing fusiform initials (FIGS. 28, 29) cannot be due to such factors as these, but is closely correlated with the orientation of the division membrane. When the partition is exactly transverse, the polar axis of the karyokinetic figure is parallel to the long axis of the cell (FIGS. 28, 29), but when it is oblique the division figure tends to assume a diagonal position. In ray initials and in longitudinally dividing fusiform initials of *Pinus Strobus,* the chromosomes tend to be twisted and crowded together in the nuclear plate, but in wide,[1] pseudo-transversely dividing fusiform initials, the nuclear plate may be more extensive and the chromosomes so arranged that they are nearly all visible in a single focal plane. Furthermore, the chromosomes in adjoining cells may vary considerably in shape, in certain figures resembling hooks, and others V's or U's of varying widths. The shape of the karyokinetic figures, particularly during the prophase, is profoundly affected by the shape of the "resting" nuclei. Thus, the spiremes formed in the stem at the beginning of the growing season, when the nuclei are much elongated, may be entirely unlike those which are formed subsequently from shorter and wider nuclei.

Nucleo-cytoplasmic Relation (*continued*):—In order to determine whether large meristematic cells are hyperchromatic, I have devoted considerable attention to a study of the number and size of the chromosomes in the cambium of *Pinus Strobus* L. Sections were secured showing adjacent meristematic cells of various dimensions and volumes in equivalent stages

[1] Tangential dimension.

of karyokinesis. The large, fusiform initials do not contain the tetraploid or polyploid number of chromosomes. All cells regardless of their size have approximately the diploid number (24).[2] It is evident, accordingly, that the variations in the size of the nuclei and cambial initials of *P. Strobus* are not dependent upon variations in the numbers of chromosomes. Are they correlated with fluctuations in the size of chromosomes?

ERDMANN (1908) concluded from her investigations upon sea urchins that chromosomal mass, rather than chromosomal number, is the size-determining factor of cells. CONKLIN (1912) considered that in comparable cells of *Crepidula* large protoplasts have larger nuclei and chromosomes than small protoplasts. HEGNER (1920) reaches similar conclusions in a recent paper upon *Arcella.* If there is a close correlation between chromosomal mass and volume of cytoplasm, the adjacent cells of the cambium vary so greatly in volume that there should be a striking contrast in the size of their chromosomes. This does not appear to be the case, however, in any of the material that I have studied. The chromosomes in a small ray initial having a capacity of 3,000-10,000 cubic microns may be fully as long and thick as those which occur in an adjoining fusiform initial with a volume of 1,000,000-5,000,000 cubic microns. FIGURES 17, 18, 28, and 29 are from fusiform initials having capacities of 800,000-1,200,000 cubic microns, and FIGURES 30, and 31 from ray initials with volumes of 5,000-10,000 cubic microns. Of course, as admitted by CONKLIN, it is difficult to determine the volume of individual chromosomes with any considerable degree of accuracy, but comparisons indicate very clearly that the large, fusiform initials of the lateral meristem are not hyperchromatic. The striking variations in the size of the nuclei are due to differences in the volume of their achromatic portions. Thus, the volume of nucleolar matter is much greater in the large nuclei. Furthermore, the staining reactions of the "resting" nuclei suggest that the chromatin is more concentrated in the smaller than in the larger nuclei. In chromatin stains, the small nuclei of ray initials are very heavily overstained long before the large nuclei of adjoining fusiform initials become clearly differentiated.

It is evident, accordingly, that, although in certain cases variations in the volumes of cells are closely associated with fluctuations in the number of chromosomes (BOVERI, GATES, WINKLER) and in others with fluctuations in the size of chromosomes (ERDMANN, GREGORY, KEEBLE, CONKLIN, HEGNER), the undifferentiated, actively dividing cells of the lateral meristem may vary greatly in size without corresponding variations in chromosomal size or number.

Cytokinesis:—Certain of the fusiform initials in Coniferae are several hundred times as long as they are wide (radially), yet they divide longitudinally. What then is the nature of cytokinesis in cells of such extraordinary dimensions? During the telophase (FIG. 17) the central spindle expands laterally by the addition of peripheral fibers and gradually assumes the form of a more or less warped disk (FIG. 18). The connecting fibers,

[2] Miss FERGUSON (1904) has shown that the haploid number is 12.

and later the accessory fibers, thicken to produce a cell plate in the usual manner, and then the fibers disappear except for a circular rim of kinoplasm. In tangential, longitudinal sections of the cambium, this ring-shaped aggregation of kinoplasmic fibrillae forms a halo about the daughter nuclei. The ring increases in circumference by the addition of new peripheral fibers and extends the cell plate as it does so. When it intersects the radial walls of the cell, it becomes more or less four-sided. As soon as the cell plate comes in contact with the radial facets of the protoplast, the kinoplasm disappears from two sides of the frame-like figure, leaving two entirely separate aggregations of kinoplasmic fibrillae, which are parallel and which cross the cell from one radial wall to the other. These rod-like masses of kinoplasm (kinoplasmasomes) move in opposite directions, thereby extending the cell plate towards the ends of the cell. In radial, longitudinal sections of the cambium, the kinoplasmasomes are seen in section (FIGS. 20, 21). They are located midway between the tangential facets of the cell and usually are equidistant from the daughter nuclei or approximate center of the protoplast. This indicates, of course, that they move forward at equal rates. As shown in FIGURE 22, they have a wedge-shaped outline, bluntly convex in front and tapering to a point at the rear along the cell plate. They are composed of fibrillae which resemble those that occur in the spindle. The threads or "lines of flow" do not extend across the cell from one tangential membrane to the other, but lie free in the cytoplasm. Nor are they connected with the daughter nuclei, which remain in their original position near the center of the protoplast. As new peripheral fibers are successively added in front, those at the rear disappear from about the recently formed portion of the cell plate (FIG. 22). The latter is gradually extended in this singular fashion, often for a distance of several thousand microns, until it eventually reaches the ends of the protoplast; thus dividing the latter into halves, each of which contains one of the daughter nuclei. The nuclei become enclosed in a nuclear membrane and reform their nucleoli long before the kinoplasmasomes reach the ends of the cell.

Similar phenomena occur in dicotyledons during longitudinal division of the fusiform initials (FIGS. 23-25), but, owing to the smaller size of the cells, the kinoplasmasomes and cell plates are smaller than in the Coniferae. The oblique partitions of fusiform initials, and the longitudinal and oblique divisions of their derivative cells, are also formed by the intervention of these extraordinary cell plates. Furthermore, the writer has accumulated considerable evidence which indicates that the phenomena in question are not confined to the cambial layer, but that they occur in other somatic tissues of the higher plants, in elongated or much flattened cells whose planes of division have one long and one short dimension.

Types of Cell Plate Formation in the Higher Plants:—It is of interest to inquire what relation this type of cell plate formation—which is so greatly extended, both as regards space and time, and so clearly dissociated, except in its initial stages, from the usual phenomena of karyokinesis—bears to those types which previously have been described by TREUB (1878), STRASBURGER (1880), and SCHÜRHOFF (1906). In TREUB's first type, the

nucleus is centrally located and the central spindle merely expands symmetrically until it touches the four walls of the isodiametric cell. In his second type, the nucleus lies near one wall and the daughter nuclei and "fibrillar-complex" migrate to the opposite side during cytokinesis. STRASBURGER described a third type, in which the daughter nuclei remain on one side of the cell and the "fibrillar-complex" crosses it. SCHÜRHOFF found a fourth type, in large cells having centrally located nuclei and small spindles. It differs from the preceding types in that "mit der Neubildung der peripheren Cytoplasmafäden geht die Auflösung der zentralen Strahlungen Hand in Hand." In other words, a ring or halo of kinoplasm is formed such as occurs in cambial initials during incipient stages of cytokinesis.

A comparative study of cell plate formation in different somatic tissues, and in cells of different shapes and sizes, suggests that the various types of phenomena, described by TREUB, STRASBURGER, SCHÜRHOFF, and the writer, are but different phases or stages of a single general or fundamental type of cytokinesis. The particular expressions of the phenomenon which may occur in any given cell are dependent upon its dimensions, its plane of division, and the size and location of the nucleus. Thus, in very small, isodiametric cells having a large, centrally located nucleus, the cell plate quickly intersects the walls of the cell without any extensive lateral enlargement of the central spindle. In larger cells with small nuclei, there is sufficient room for the process of cytokinesis to reach the halo or frame stage before the cell plate intersects the sides of the cell. However, only in elongated or much flattened elements is it possible for the phenomenon of cell plate formation to pass through the spindle, disk, halo, and frame stages, and finally to form two entirely separate aggregations of kinoplasmic fibrillae, kinoplasmasomes.

All five types of cytokinesis may be found in the cambium. TREUB's first type occurs in ray initials (FIG. 31) and in transversely dividing fusiform initials (FIG. 29). SCHÜRHOFF's type also is present in certain ray initials (FIG. 32) and transversely dividing fusiform initials. Although the nuclei are centrally located under normal conditions, I have found them in a lateral position in *Sequoia*, in fusiform initials which were dividing to form callus. During cytokinesis the daughter nuclei remained on one side of the cell, as in STRASBURGER's preparations, or one or both of them migrated across the cell, as in TREUB's second type of cytokinesis.

Significance of Cytokinesis in the Cambium:—The formation of the cell plate in cambial initials promises to be significant in the discussion of a number of fundamental cytological and physiological problems.

STRASBURGER's (1875, 1880, 1882) suggestion that spindle fibers are of cytoplasmic origin and his conclusion that the cell plate originates in swellings of the connecting spindle fibers have been questioned by various investigators. TREUB (1878) held that the cell plate arises from free cytoplasmic granules which migrate into the equatorial region of the spindle, and ZACHARIAS (1888) contended that the spindle is of nuclear, and the cell plate of cytoplasmic, rather than of kinoplasmic, origin. MOTTIER (1900) found that in certain of the higher plants the connecting fibers do not

thicken appreciably in the equatorial region, and do not lie sufficiently close together to enable the slightly thickened middle parts to meet and fuse. He concluded from this "that the cell plate is formed by a homogeneous plasma which is conveyed to the cell plate region and deposited there by the connecting fibers." STRASBURGER's generalization concerning the kinoplasmic origin of the cell plate was ably defended by TIMBERLAKE (1900), who concluded, however, that the nucleus "is the center of metabolic processes concerned in the production of the kinoplasm."

STRASBURGER (1880) and WENT (1887*a*) noted that the connecting spindle fibers appear to increase in number prior to the formation of the cell plate, and the former investigator subsequently suggested that this increase might be due to a splitting of the original fibrillae. TIMBERLAKE endeavored to prove that the lateral expansion of the spindle is not due to the formation of new fibers, but to the separation and enlargement of the original connecting fibers, coupled in certain cases with the addition of peripheral threads, formed from fibers which radiate from the daughter nuclei.

HOF (1898) and NĚMEC (1899) showed that after the cell plate is complete the connecting fibers appear to be "drawn in" toward the cell plate and their place taken by granular cytoplasm. TIMBERLAKE stated that "concurrent with the growth of the cell plate in extent and the disappearance of the fibers from a central portion of the spindle the nuclei come to lie nearer the cell plate." He concluded that "all of the fibers which form cell plate elements are completely used up in the growth of the cell plate," and that "during the period of growth the cell plate may so shift its position as to lie in a plane different from that in which it was first formed."

The formation of the cell plate in cambial initials is significant in these connections. There is an enormous increase in the number of "peripheral" fibers during cytokinesis. As in the case of the radial fibrillar systems, which HARPER and DODGE (1914) have shown to function in the formation of the capillitium of certain Myxomycetes, the kinoplasmic fibers have no visible connection with the nuclei. They are so far removed from the nuclei (FIGS. 20, 21) during the later stages of cytokinesis that one is inclined to question TIMBERLAKE's generalization concerning the nuclear origin of kinoplasm. The phenomena appear rather to justify STRASBURGER's contention that the accessory fibers are of cytoplasmic origin. As the original connecting fibers "draw in" or disappear, the nuclei tend to move towards the cell plate (FIGS. 19 & 32), but subsequently may move away from it (FIGS. 21 & 27). That the connecting fibers may thicken considerably in the equatorial region of the spindle is shown in FIGURE 30; but a discussion of the finer details of cell plate formation is reserved for a subsequent paper. The width of the kinoplasmasomes closely approximates that of the central spindle. In other words, broad kinoplasmasomes tend to occur in cells having large karyokinetic figures (FIGS. 27 & 29) and narrower ones in elements having smaller spindles (FIGS. 17-20, FIGS. 25, 24, 23, and FIGS. 31, 32).

The phragmoplasts develop along straight lines or curves depending upon the orientation of the karyokinetic figure and the plane in which the

initial is dividing. In longitudinally dividing fusiform initials, in which the polar axis of the karyokinetic figure is transverse (FIG. 25), the kinoplasmasomes move toward the ends of the protoplast along its longitudinal axis (FIGS. 23, 24). When the mitotic figure occupies a diagonal position (FIG. 17), the phragmoplast, during its earlier stages, curves toward the center of the protoplast avoiding the tangential facets of the cell (FIGS. 18, 19), and subsequently straightens out as shown in FIGURES 20 and 21. In obliquely dividing cells having diagonally oriented division figures, either the phragmoplast develops in a single plane, or the kinoplasmasomes meander more or less, forming undulating or curved partitions. In the formation of new ray initials, which are carved out of fusiform initials, both kinoplasmasomes may, in certain cases, curve toward and intersect the same radial surface of the cell.

THOMPSON (1917) is of the opinion that ERRERA's (PLATEAU's) law of minimal area is not invalidated by the occurrence of oblique or curved partitions provided these membranes are sigmoid and intersect the older walls at right angles. DE WILDEMAN (1893) found that in certain cells the polar axis of the division figure shifts to a diagonal position previous to the formation of the cell plate, which curves during its development so as to intersect the sides of the cell at right angles. That all diagonal partitions do not develop in this manner is indicated very clearly by the phenomena in the cambium. The writer (36) has shown that the kinoplasmasomes, cell plates, and young membranes intersect the sides of the cell at varying degrees of acuteness, as well as at right angles. This suggests, of course, that in dealing with cytokinesis we are not concerned with protoplasm in liquid or semi-liquid phases. In other words, cellular membranes, at the moment of their formation, frequently do not assume the forms which would be assumed, under similar conditions, by liquid films destitute of weight (PLATEAU).

Nucleoli:—The cambium of Coniferae appears to be an unusually favorable medium for the study of the structure and function of nucleoli, which are unusually large and conspicuous in most representatives of the family. In *Pinus Strobus* they vary greatly in size, shape, and number in different initials. They are clearly visible during the early prophases and late telophases. Many of my preparations suggest that the large nucleoli are composed of aggregations of smaller nucleolar masses. During the process of aggregation hollow spheres may be formed which resemble "vacuolated" nucleoli. The larger nucleoli also appear to fuse in many cases, particularly in cells which are differentiating into tracheary elements or sieve tubes.

If nucleoli actually are concerned in the growth or enlargement of chromosomes, as is maintained by various botanists, one might expect the nuclei of fusiform initials, which contain a relatively large volume of nucleolar matter, to form much larger chromosomes than the small nuclei of ray initials. This is not the case, however, in *Pinus Strobus*, as I have shown on preceding pages.

There is a striking contrast between the cambial nucleoli of gymno-

sperms and those of dicotyledons. The latter tend to be surrounded by a conspicuous halo, which is absent in the Coniferae. Of course, this halo is supposed to be an artifact (NÄGELI), yet the fact that it occurs so constantly in one group and not in the other suggests that it may be significant from the morphological or the physico-chemical point of view.

SCHWARZ (1884) and ZACHARIAS (1895) noted that during the differentiation of tissue elements in the growing points of plants, the volume of the nucleoli, as of the nuclei, first increases and then decreases. Similar phenomena occur in the cambial zone of *Pinus Strobus*. During the early stages of the differentiation of tracheids and sieve tubes, the nuclei become coarsely granular. The nucleolar mass subsequently decreases, and the nucleus becomes profoundly modified in shape and loses its staining capacity. As suggested by STRUMPF (1898), the crumpling of the nucleus in young sieve tubes may be mistaken for fragmentation.

In conclusion, it is to be emphasized that this paper merely presents the results of a reconnaissance of cytological phenomena in the cambium. Its primary object is to outline salient features of the more striking phenomena encountered, and to pave the way for subsequent and more detailed investigations, on the part both of the writer and of others who may become interested in the cambium as an unusually favorable medium for the study of certain cytological and physiological problems.

SUMMARY AND CONCLUSIONS

1) The initials of the lateral meristem or cambium, which may attain a length of more than 9,000 microns and a capacity exceeding 10,000,000 cubic microns, are uninucleate. The working sphere of their nuclei must extend in certain cases for a distance of several thousand microns.

2) The nucleo-cytoplasmic ratio may be relatively constant in ray initials, but varies enormously in fusiform initials.

3) All of the cambial initials of *Pinus Strobus*, regardless of variations in size, contain the diploid number of chromosomes.

4) Small ray initials having a capacity of 5,000-10,000 cubic microns may contain as large chromosomes as adjacent fusiform initials with a volume of 1,000,000-5,000,000 cubic microns.

5) Fusiform initials, which frequently are several hundred times as long as they are wide, divide longitudinally by an extraordinary extension of the cell plate.

6) The various types of cell plate formation that have been described by TREUB, STRASBURGER, SCHÜRHOFF, and the writer appear to be merely different phases or stages of a single general type of cytokinesis.

7) The significance of the cambium in the investigation of various cytological problems, particularly of those relating to the cell plate and the dynamics of cytokinesis, is briefly discussed.

Chapter 4

THE CAMBIUM AND ITS DERIVATIVE TISSUES

IV. THE INCREASE IN GIRTH OF THE CAMBIUM *

SINCE the publication of NÄGELI's (1864) "Dickenwachsthum des Stengels," most botanists, if we may judge from statements in standard textbooks, have assumed that the increase in girth of the lateral meristem, during successive stages in the enlargement of a stem or root, is due to "radial," anticlinal divisions of the cambial initials. Not all investigators, however, have accepted NÄGELI's generalization. ROBERT HARTIG (1895) inferred from the structure of the secondary xylem of *Pinus sylvestris* L. that the increase in the periphery of the cambium in conifers is due primarily to the elongation of transversely dividing fusiform initials. KLINKEN (1914) reached a similar inference from the study of serial sections of the phloem of *Taxus baccata* L. He concluded that there are two fundamental types of meristematic activity, one characteristic of the conifers and the other of the dicotyledons. NEEFF (1920) subsequently found evidences of HARTIG's and KLINKEN's type of cambial activity in the xylem and phloem of *Tilia tomentosa* which led him to believe that there is no such fundamental distinction between the lateral meristems of gymnosperms and dicotyledons.

There is, of course, a considerable element of uncertainty in ascribing a particular type of meristematic activity to large groups of the vascular plants, either upon the basis of *a priori* deductions or upon that of indirect evidence obtained from the study of the xylem or phloem of one or two supposedly representative species.

In 1917, the writer perfected methods for sectioning the cambium and for investigating its histological and cytological details. As stated in the third paper (*40*) of this series, he did not succeed in finding evidences of the hypothetical radial, anticlinal divisions which are described and even figured in botanical textbooks. The normal, anticlinal divisions of the fusiform initials, in all of the gymnosperms and less highly specialized dicotyledons examined, were transverse or more or less oblique. That radial longitudinal divisions do occur, at least in certain cases, is suggested however by KLEINMANN (1923), upon the basis of the orientation of karyokinetic figures in the cambium of *Raphanus*. In view of these facts, it is evident, on the one hand, that the increase in girth of the lateral meristem is not the simple phenomenon that NÄGELI hypothesized, and, on the other hand, that there may be more than one fundamental type of cambial activity in the vascular plants.

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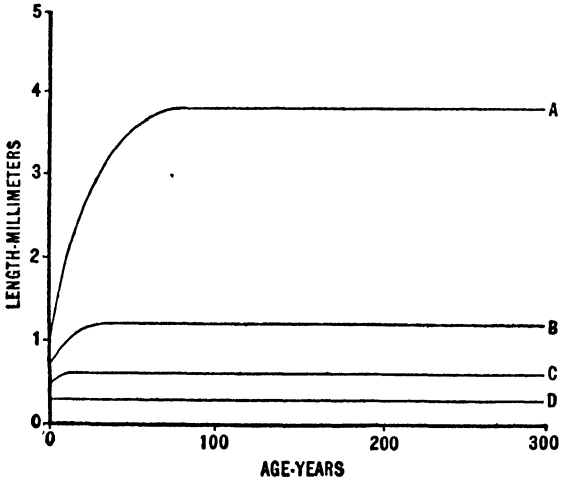
Analysis of the Problem:—In NÄGELI's formulae for computing the frequency of radial divisions, during a given increase in the diameter of a stem or root, the size of the cambial initials is treated as a constant. It is well known, however, that the cambium or lateral meristem is composed of initials of two distinct shapes and sizes: (1) *fusiform initials*, relatively large, elongated elements whose derivatives become differentiated into tracheids, fibers, vessels, sieve tubes, etc.; and (2) *ray initials*, scattered aggregations of small, more or less isodiametric cells which divide to form the horizontal sheets of radially disposed parenchyma, the so-called medullary rays (PLATE 2). Therefore, upon the basis of *a priori* considerations, the increase in girth of the lateral meristem might be due to one or more of the following factors:

1) An increase in the tangential diameter of the fusiform initials.
2) An increase in the length of these cells.
3) An increase in the number of these cells.
4) An increase in the diameter of the ray initials.
5) An increase in the number of these cells.

In many of the vascular plants, as the writer has shown in previous papers of this series (*36*, *38*, *40*), the initials tend to be larger in old stems than in young shoots, but they do not continue to increase in size through-



TEXT FIGURE 1.— Normal curves, showing average lengths of cambial initials at successive stages in the enlargement of a stem. *A*, Conifer or vesselless dicotyledon; *B*, less specialized type of dicotyledon; *C*, highly specialized type of dicotyledon; *D*, highly specialized type of dicotyledon, having a stratified cambium.

out the entire life of an individual. Thus, in the conifers and less specialized dicotyledons, where the fluctuations in cell size are considerable, the normal curve of average length of the fusiform initials at successive ages is of the general type illustrated in TEXT FIGURE 1. There is a rapid increase in length for a period of years until a certain size is attained which then remains more or less constant during succeeding growth of the plant.

Are these variations in cell size significant factors in the increase in girth of the lateral meristem during the earlier stages in the enlargement of a stem? Some typical measurements taken from *Pinus Strobus* L. are of interest in this connection.

One-year-old stem

Radius of woody cylinder	2,000 microns
Circumference of cambium	12,566 microns
Average length of fusiform initials	870 microns
Average tangential diameter of fusiform initials	16 microns
Average tangential diameter of ray initials	14 microns

60-year-old stem

Radius of woody cylinder	200,000 microns
Circumference of cambium	1,256,640 microns
Average length of fusiform initials	4,000 microns
Average tangential diameter of fusiform initials	42 microns
Average tangential diameter of ray initials	17 microns

The increase in width of the original 724 fusiform initials would produce, during the 59-year interval, an arc of 30,408 microns, and the increase in the diameter of the 70 ray initials, during the same interval, an arc of 1,190 microns; or a total circumference of 31,598 microns as compared with the actual circumference of 1,256,640 microns. In other words, the enlargement of the cambial ring, during the 59-year interval, is due primarily to an increase in the number of initials; from 724 to 23,100 fusiform initials and from 70 to 8,796 ray initials.

It is to be emphasized in this connection that such a multiplication of cambial initials—*as seen in any given transverse plane or cross section of a stem*—is not due necessarily to cell division. For, if the initials elongate and slide by one another, the number which intersect a given transverse plane will be continually augmented. However, if the increase in the number of fusiform initials in *Pinus Strobus* were due entirely to longitudinal sliding growth, the original initials, during the 59-year interval, would have to attain an average length of approximately 26,800 microns instead of 4,000 microns. The ray initials do not elongate to any considerable extent.

In exceptional cases, *e.g., Sequoia,* the fusiform initials may attain a maximum variability in length of 9,000 microns and in width of 60 microns, but the rate of increase in size during a given period of years is not much in excess of the values recorded for *Pinus Strobus.* Furthermore, the ray initials in certain dicotyledons may have a maximum enlargement in diameter of from 30 to 40 microns, but the variability in the size of their fusiform initials is much less than that which occurs in most conifers.

It is evident, accordingly, that, although the increase in size of the cambial initials, during the earlier stages of the enlargement of certain plants, is by no means a negligible factor, the rapid increase in girth of the lateral meristem must in general be due largely to a progressive increase in the *number,* rather than in the *size,* of its constituent cells.

What then is the relative significance of the increase in the number of the two types of cambial initials? In the young shoot of *Pinus Strobus,* the combined diameters of the 70 ray initials form an arc of 980 microns,

whereas those of the 8,796 ray initials in the 60-year-old stem constitute an arc of 149,532 microns, or approximately one eighth of the total circumference of the cambium. Many of the dicotyledons have a much higher percentage of ray initials. Indeed, in extreme cases more than one half of the circumference of the lateral meristem may be occupied by ray initials. Therefore, in discussing the *modus operandi* of the increase in girth of the cambium, it is essential to distinguish between the effects of (1) those anticlinal divisions, which are concerned in the origin and multiplication of ray initials, and (2) those which produce an increase in the number of fusiform initials.

The reader should bear in mind in this connection that NÄGELI's generalization is based upon the assumption that the divisions in both types of initials are radial, whereas the conclusions of HARTIG, KLINKEN, and NEEFF refer to the activity of the fusiform initials. The writer will likewise confine his attention in the following pages to the fusiform initials, reserving further discussion of the ray initials for a subsequent paper of this series.

Significance of the Arrangement of Fusiform Initials:—In the gymnosperms and less specialized dicotyledons, the fusiform initials are not arranged in regular rows, whereas in certain of the more highly differentiated dicotyledons they are symmetrically grouped in parallel, horizontal series. The question suggests itself, accordingly, is this striking dissimilarity in the architecture of the lateral meristem indicative of fundamental differences in the growth and division of the fusiform initials, and, if so, what factors are concerned in the transitions from a non-stratified to a stratified arrangement?

As shown in FIGURES 4-6, PLATE 2, the "fusiform" initials in stratified meristems are roughly hexangular with long parallel sides and abruptly tapering ends, and the elements of adjacent horizontal series do not overlap to any considerable extent. In other words, the form and the arrangement of the initials indicate very clearly that the increase in girth of the cambium cannot be due to the *elongation* of transversely dividing cells; for, if it were, the superimposed initials must necessarily crowd by one another and ultimately break up the stratified arrangement. Conversely, if the anticlinal divisions are radio-longitudinal, the products of successive divisions should be grouped in horizontal rows, unless this arrangement is modified by differences in the elongation of adjacent elements. In non-stratified cambia (FIGS. 1-3) the adjacent, overlapping fusiform initials vary considerably in length, but, as the writer has previously stated, the *average* length of these elements does not increase appreciably during the later stages of the enlargement of a stem or root. Thus, the increase in the number of fusiform initials in non-stratified meristems cannot be due solely to radio-longitudinal divisions; for, if it were, there would have to be a general increase in the length of the initials during all stages of the enlargement of the plant.

Such facts as these suggest that there are two fundamentally different types of meristematic activity—so far as the fusiform initials are concerned—in the vascular plants; *i.e.*, that the increase in girth of non-stratified

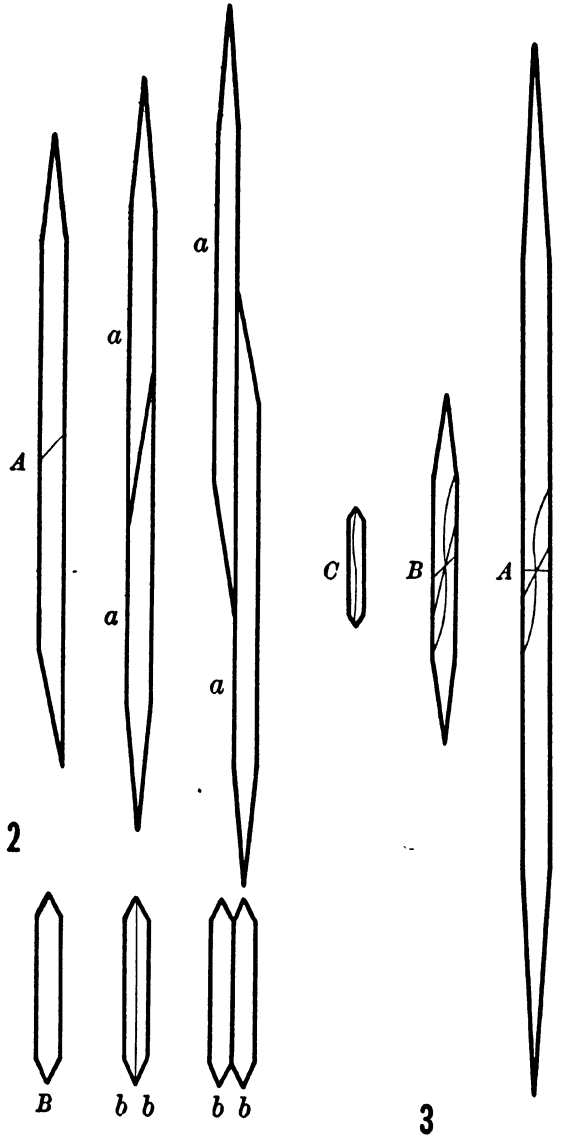
cambia is due to the elongation of transversely dividing initials and that of stratified cambia to radio-longitudinal divisions of these elements. Detailed investigations of the cambium and of the histology of its derivative tissues, in numerous representatives of the gymnosperms and dicotyledons, strongly support such an assumption.

The writer has already referred to the fact that in all of the lateral meristems of conifers and less specialized dicotyledons investigated by him —which are, of course, of the non-stratified type—the anticlinal divisions are transverse or oblique (FIGS. 8, 9). The orientation of the cell plates and recently formed anticlinal partitions in the stratified meristems of *Robinia Pseudo-Acacia* L. and of *Diospyros virginiana* L., on the contrary, is radio-longitudinal (FIGS. 6, 7).

Furthermore, since the fusiform initials leave a record of their activities in the xylem and phloem, it is possible to trace successive stages of their growth and division in serial, tangential sections of these tissues.[1] Although this indirect method of studying cambial activity must be applied with a considerable degree of caution, particularly in the case of dicotyledons, it enables one to investigate a large number of plants of which suitable material of the lateral meristem is not readily available. In all of the various genera (62) of gymnosperms and dicotyledons that the writer has examined in this way, the evidence indicates very clearly that the anticlinal divisions in non-stratified meristems are pseudo-transverse, whereas those in stratified cambia are radio-longitudinal. In meristems of the former type, the fusiform initials elongate, sliding by one another, until they attain a certain size. Then they divide by means of a more or less oblique partition into two short halves which in turn elongate and divide (TEXT FIG. 2, *A*). Owing to the fact that the cells do not divide and elongate in unison, there is a very considerable variability in the length of adjacent initials. In addition, owing to the fact that the frequency of the anticlinal divisions and the elongation of the fusiform elements are not constant, the size of the initials tends to fluctuate more or less in different parts of a given individual; *e.g.*, they tend to be considerably shorter in slender shoots than in robust stems (TEXT FIG. 1). Thus, the non-stratified arrangement and the variability in size of the fusiform cells are both due primarily to elongation following pseudo-transverse, anticlinal divisions. In meristems of the latter type, on the contrary, the initials divide radio-longitudinally and the products of such divisions expand laterally, but they do not elongate to any considerable extent (TEXT FIG. 2, *B*). The structure of the secondary tissues in the Calamariales, Sphenophyllales, Lepidophytineae, and Cycadofilices indicates that the cambia in these primitive groups of vascular plants were of the non-stratified type.

In view of these facts, we appear to be justified in concluding that there are at least two distinct, fundamental types of cambial activity in the vascular plants. In the vascular cryptogams, gymnosperms, and less specialized (structurally) dicotyledons, the anticlinal divisions are more or less transverse and the products of these divisions elongate and crowd by one another,

[1] For detailed descriptions of this method, the reader is referred to KLINKEN's and NEEFF's papers.



TEXT FIGURES 2-3.— FIG. 2: Diagrams illustrating *modus operandi* of the increase in girth of cambium in non-stratified and stratified lateral meristems. *A*, fusiform initial from non-stratified cambium, dividing pseudo-transversely; *a*, *a*, products of this division which elongate and slide by one another; *B*, fusiform initial from stratified cambium; *b*, *b*, products of the radio-longitudinal division of this initial, which expand laterally but not longitudinally. — FIG. 3: Types of anticlinal divisions in fusiform initials. — *A*, Typical fusiform initial of a conifer; *B*, fusiform initial of a dicotyledon, having non-stratified cambium; *C*, fusiform initial of a dicotyledon, having a stratified cambium.

producing thereby an increase in the girth of the cambium and a non-stratified arrangement of its cells. In certain of the more highly differentiated dicotyledons, on the other hand, the anticlinal divisions are radio-longitudinal and the products of these divisions expand laterally, thereby increasing the circumference of the cambium, but they do not elongate to any considerable extent, and thus become symmetrically grouped in parallel, horizontal series.

The Transition from the Non-stratified to the Stratified Arrangement:—In previous papers of this series, the writer (29, 38) has called attention to the fact that in the dicotyledons there is a progressive reduction in the length of the fusiform initials and of their derivatives, which closely parallels successive stages in the differentiation of highly specialized types of vascular tissues. As indicated in TABLE 1, the vesselless dicotyledons (*Tetracentron, Trochodendron,* and *Drimys*), whose secondary xylem closely resembles that which occurs in the vascular cryptogams and gymnosperms, have large fusiform initials; fully as large as those of most gymnosperms, for example. Dicotyledons with vessels, on the contrary, are characterized by having much smaller meristematic cells which become shorter and shorter as the tracheary elements become more and more highly specialized. Furthermore, stratified meristems tend, in general, to be composed of smaller fusiform initials than non-stratified cambia. It should be

TABLE I: *Length (in microns) of adjacent fusiform initials in random samples of the lateral meristem of old stems:—*

NON-STRATIFIED CAMBIA

Gymnosperms

Species	Max.	Ave.	Min.
Ginkgo biloba L.	3,000	2,200	1,400
Pinus Strobus L.	4,000	3,200	2,300
Picea Abies (L.) Karst.	4,200	3,300	2,400
Juniperus virginiana L.	3,000	2,200	1,000
Larix decidua Mill.	5,000	4,000	2,500
Sequoia sempervirens Endl.	8,700	6,600	4,200
Agathis robusta F. M. Bailey	7,700	6,800	4,100
Tsuga canadensis (L.) Carr.	4,400	3,200	2,200
Podocarpus Nagcia R. Br.	5,000	3,800	2,300
Cedrus Libani Barrel	4,100	2,900	2,100
Average	4,910	3,820	2,450

Dicotyledons

A. Vesselless

Trochodendron aralioides Sieb. et Zucc.	6,200	4,400	2,800
Drimys Winteri Forst.	4,500	3,300	2,400
Average	5,350	3,850	2,600

B. Vascular tissues not highly differentiated

Betula populifolia Marsh.	1,160	940	700
Myristica philippensis Lam.	1,620	1,310	990
Altingia excelsa Noronha	2,300	1,900	1,200
Liriodendron tulipifera L.	1,500	1,100	700
Urandra luzoniensis Merr.	1,700	1,400	1,100
Dillenia philippinensis Rolfe	2,300	1,600	1,000
Gordonia Lasianthus L.	1,700	1,300	1,000
Cornus florida L.	1,400	1,100	800
Symplocos tinctoria L'Hér.	1,400	1,100	600
Halesia diptera L.	1,100	900	800
Average	1,620	1,260	890

C. Vascular tissues highly specialized

Carya ovata (Mill.) C. Koch	600	520	420
Litsea glutinosa C. R. Rob.	700	550	390
Prunus serotina Ehrh.	590	460	320
Excoecaria Agallocha L.	870	630	410
Mangifera monandra Merr.	830	570	390
Acer rubrum L.	610	490	320
Garcenia dulcis Kurz.	1,020	740	520
Vatica Mangachapoi Blanco	810	610	410
Barringtonia racemosa (L.) Roxb.	900	720	500
Psychotria luzoniensis F. Vill.	1,080	700	450
Average.	800	600	410

STRATIFIED CAMBIA

Dicotyledons

D. Vascular tissues highly specialized

Species	Max.	Ave	Min
Grewia multiflora Juss.	370	250	160
Thespesia populnea (L.) Soland. ex Corr.	280	250	210
Bombycidendron Vidalianum Merr. et Rolfe	430	360	320
Heritiera littoralis Dryand.	360	300	270
Klainhovia hospita L.	480	360	270
Pterospermum niveum Vid.	430	370	320
Tarrietia sylvatica Merr.	340	280	210
Sterculia foetida L.	450	370	320
Robinia Pseudo-Acacia L.	210	170	140
Diospyros virginiana L.	520	410	320
Average	390	310	250

Basis: 50 measurements of fusiform initials.
Probable errors of individual averages 10–15 percent.

noted, in addition, that, during this sequence of changes, the size-on-age curves (TEXT FIG. 1) are depressed and ultimately become approximately horizontal; *i.e.*, the length of the fusiform initials is stabilized in plants having stratified meristems.

These facts are of considerable interest in discussing the *modus operandi* of the transitions from one fundamental type of meristematic activity to the other. In most of the non-stratified meristems that the writer has studied, the orientation of the anticlinal partitions fluctuates between a transverse position and varying degrees of obliquity (TEXT FIG. 3). As the fusiform initials become shorter, the ends of the more oblique partitions tend to approach the extremities of the cells, or, in other words, to become more and more nearly radio-longitudinal. Thus, certain of the more highly differentiated dicotyledons have transitional types of meristems, which show incipient stages of stratification. Furthermore, the variability in the size of adjacent fusiform initials in stratified meristems (TABLE 1) is due, at least in part, to the fact that many of the anticlinal divisions are somewhat oblique.[2]

How much significance should be attached to the close parallelism in the sequences of changes in the cambium and vascular tissues? Is the progressive reduction in cell size in the lateral meristem due to the increasing specialization of the vascular tissues, or *vice versa*? Many morphologists interpret such correlations as due to cause and effect. There is, however, a very considerable element of uncertainty in so doing, where the basis of comparison is time, as KARL PEARSON has so clearly shown. Thus, in the absence of reliable collateral evidence it is not possible to determine whether the changes in the cambium are due to those which occur in the vascular tissues or *vice versa*, or whether the parallel sequences are both due to some third factor or group of factors.

SUMMARY AND CONCLUSIONS

1) There are two fundamental types of cambial activity in the vascular plants: one characteristic of the Calamariales, Sphenophyllales, Lepidophytineae, Cycadofilices, Gymnospermae, and less differentiated (structurally) Dicotyledoneae, and the other of certain highly specialized dicotyledons.

2) In the former type the fusiform initials are not arranged in regular rows. The anticlinal divisions are pseudo-transverse and the products of these divisions elongate and crowd by one another, thereby producing an increase in the girth of the cambium.

3) In the latter type, in which the fusiform initials are relatively short, of nearly uniform length, and more or less symmetrically grouped in parallel, horizontal series, the bulk of the anticlinal divisions is radio-longitudinal, and the increase in the periphery of the cambium is due primarily to the lateral expansion of the products of these divisions.

4) The transition from the non-stratified to the stratified arrangement closely parallels successive stages in the specialization of the vascular tissues, *e.g.*, the differentiation of vessels, libriform fibers, etc., and appears to be due to a progressive reduction of cell size and of longitudinal sliding growth in the cambium.

[2] The size and the arrangement of the fusiform initials is also modified by the ray initials, new aggregations of which are periodically carved out of the elongated elements.

*

Chapter 5

THE CAMBIUM AND ITS DERIVATIVE TISSUES

V. A RECONNAISSANCE OF THE VACUOME IN LIVING CELLS *

Introduction:—In previous papers of this series, the writer has called attention to the significance of the cambium in the discussion of various histological, cytological, and physiological problems, particularly such as are concerned with cell-size and the nucleocytoplasmic ratio, the phragmoplast and the mechanism of cytokinesis.

An extension of the scope of these earlier investigations to cover a more detailed and accurate study of the protoplast and of its various constituents has necessitated the development of special techniques: (1) methods for examining living cells of the cambium, and (2) essential modifications of the usual methods of fixing and of embedding plant tissue. The writer has concerned himself with the former, and Doctor CONWAY ZIRKLE with the latter, of these tasks. Thus, by a concatenated effort, it has been possible, not only to compare the results secured by two entirely different techniques, but also to observe the progressive effects of specific fixatives and reagents upon the visible structures of the living cell.

As will be shown in the following pages, the cambium is particularly significant in any general discussion of plant vacuoles and of various theories that have been formulated regarding their origin, function, and distribution. In fact, the results secured in the study of the cambium have necessitated a reinvestigation of terminal meristems, an account of which is presented by ZIRKLE (1932) in a separate paper.

Present Status of Theories regarding the Vacuome:—The view that meristematic or embryonic cells—which are concerned primarily with cell division and the synthesis of protoplasm—are non-vacuolate, and that vacuoles arise as concomitants of increasing hydration, cell enlargement, and tissue differentiation has dominated botanical literature from the days of VON MOHL to the present. The persistence of this conception in modern textbooks is undoubtedly due, in part to the prestige of its earlier advocates, *e.g.*, HOFMEISTER, NÄGELI, SACHS, DE BARY, PFEFFER, VAN TIEGHEM, STRASBURGER, and in part to the efforts of numerous investigators who periodically claim to have observed, or to have induced experimentally, the development of vacuoles *de novo*.

Between 1886 and 1890 WENT (1887*b*, 1888) presented extensive data in support of his contention that all plant cells are vacuolated, and that all vacuoles of the higher plants are derived from divisions of the vacuoles of the egg or embryo-sac. He emphasized the necessity of distinguishing

* Reprinted from Zeitschrift für Zellforschung und microskopische Anatomie 10: 651-682, 1930.

between normal vacuoles, such as occur in unmodified living cells, and abnormal vacuoles, such as may be induced during protoplasmic injury, degeneration and death; the former may fuse or divide but never originate *de novo*. Unfortunately, WENT's work served as one of the main supports for the Tonoplast Theory of DE VRIES (1885) and has been cited in most cases merely in connection with critiques of that hypothesis. It should be emphasized, however, in fairness to WENT, that his observational data related to vacuoles as such. The demonstration of vacuolar membranes was more or less incidental. Nor did WENT concern himself with those hypothetical units—"inactive tonoplasts" resembling microsomes—which were assumed by DE VRIES to form vacuoles much as plastids produce starch or chlorophyll.

The most extensive and detailed work upon the vacuoles of plants is that of the DANGEARDS and of GUILLIERMOND and his students. Since 1916, these investigators have published more than 90 papers covering a wide range of tissues, organs, and groups of plants. In view of the fact that the investigations have periodically been summarized and re-summarized by GUILLIERMOND, it is not essential to discuss the successive individual papers, but merely to outline the salient generalizations that have been drawn from the work as a whole.

The DANGEARDS (1923) and GUILLIERMOND (1929) support WENT's conclusion that all plant cells are vacuolated, but, in so doing, they modify the classical conception of the plant vacuole, and define the vacuome as including certain structures which previously have not been classified as vacuoles.

In the undifferentiated or meristematic portions of plants,—*e.g.*, growing tips of hyphae, terminal meristems of the higher plants, the vacuome is represented by minute bodies—granules, rods, filaments, or nets—which closely resemble various forms of mitochondria. These *metachromes* (P. A. DANGEARD) or *primordia of vacuoles* (GUILLIERMOND) are composed of viscous colloidal material which stains uniformly and intensely with various basic intra-vitam dyes, particularly Brilliant Cresyl Blue and Neutral Red. In the differentiating portions of plants, the pseudomitochondrial forms of vacuome swell by hydration and gradually coalesce to form typical, conspicuous,. liquid vacuoles. With increasing hydration, the vacuoles as a whole stain faintly, if at all, in basic vital dyes, but their colloidal constituent appears as a deeply colored precipitate. Similar forms of precipitates are produced by many reagents used in fixation.

P. A. DANGEARD holds that all plant vacuoles are characterized by the presence in varying concentration of a specific substance (or specific category of similar substances), the so-called *metachromatin*. He contends that metachromatin resembles the mitochondrial substance of animals, and, therefore, that the pseudomitochondrial forms of the vacuome and the animal chondriome are homologous cell constituents. Furthermore, the DANGEARDS support WENT's conclusion that vacuoles are self-perpetuating and never arise *de novo*. At critical periods in the life cycle of plants, the original, viscid, pseudomitochondrial forms of the vacuome may be restored by the fragmentation and dehydration of large liquid vacuoles.

GUILLIERMOND, on the contrary, maintains in his later papers that vacuoles do arise through neoformation. He visualizes the protoplasm as constantly building diverse materials or by-products,—colloids which are not miscible with the cytoplasmic colloids. These diverse colloidal substances, —*e.g.*, alcohol soluble proteins, aromatic compounds, metachromatin, etc., being of the same semi-fluid consistency as mitochondria, are physically made to assume the same patterns as the latter. They possess a strong power of imbibition and hydrate readily into liquid vacuoles. They behave similarly toward basic intra-vitam dyes. Indeed, Neutral Red may be considered virtually a specific stain for the vacuome. In addition, GUILLIERMOND contends that the vacuome is entirely distinct from, and not under any circumstances to be confused with, the chondriome either of plants or of animals. In plants, the homologues of the GOLGI apparatus (HOLMGREN canals) of animals are to be found in the vacuome, a conclusion that has been championed upon the zoölogical side by PARAT and his coworkers.

Methods and Materials:—In the case of the more complex and highly organized plants, it is not possible in most instances to observe the normal structure of living tissue cells *in situ*. Therefore, the investigator is forced to perfect methods for isolating or "explanting" groups of living cells. The technique used by the writer in his investigations of the cambium and of its derivative tissues involves the following procedures: (1) Carefully prepared blocks of living tissue are clamped in a microtome and sectioned. (2) The sections are rapidly mounted in suitable liquid media and are examined at once. (3) Selected groups of living cells are then kept under continuous or intermittent observation to determine what structural changes occur at successive intervals. Although the technique appears at first sight to be relatively simple, there are numerous difficulties to be overcome in its practical application. The most serious of these is the task of securing sections of such quality that mechanical injuries are reduced to a minimum, and of such thickness that the finer details of cytological structure are clearly visible under high magnifications. Unfortunately, there is no simple formula for solving this difficulty, since the details of the technique must constantly be varied in dealing with material from different sources. In other words, skill in mechanical manipulation and an accurate visualization of the numerous variables in the material to be sectioned are prerequisites for obtaining favorable results.

Having solved the primary difficulty of securing sections of suitable quality and thickness, the investigator must face the task of finding media in which the tissue cells will remain alive for considerable periods of time. Here, again, there is no simple formula for solving the problem. The media must be varied more or less in dealing with different plants, with different tissues of a single individual, and with specimens collected at different seasons of the year. In the case of the cambium and its derivative tissues, the writer has, in general, secured better results with sugar solutions of varying concentration than with physiological salt solutions or the more complex types of culture media. Under favorable conditions, the cambium initials have been kept alive and actively streaming for 500 hours in solu-

tions of lactose. Similar results have been secured with sucrose and with medicinal liquid petrolatum ("White Russian Oil" [1]).

An additional difficulty, and one that has been overlooked or ignored by most students of the cambium, is the problem of accurately distinguishing the cambial initials from their incompletely differentiated daughter cells, and of distinguishing the various categories of the latter cells from one another. This difficulty is more serious than might be expected *a priori,* and the problem can be solved in most cases only by an intensive cytological study of each species with which the investigator is concerned. During the successive stages in the differentiation of the derivatives of the cambium, more or less characteristic changes occur in the nuclei, nucleoli, mitochondria, plastids, cell wall, vacuoles, protoplasmic streaming, etc. These changes must be studied in detail and used as diagnostic criteria. The mere occurrence of mitotic figures, phragmoplasts, and other evidences of cell division is by no means a dependable criterion in identifying the cambial initials, since certain of the differentiating daughter cells divide. Variations in the size, form, and orientation of the division figures must be taken into consideration before the phenomena of karyokinesis and of cytokinesis can be relied upon in this connection.

The student of explanted groups of living tissue cells eventually must face the problem of determining to what extent the structure and activities of the cells have been modified during the process of removing the tissue from its normal environment to an abnormal one. Scant attention has been devoted thus far to this fundamentally important problem. Most cytologists have concerned themselves primarily with the task of developing techniques for demonstrating specific structures[2] in living, as contrasted with killed and fixed, cells rather than with the problem of determining to what extent the cells may have been modified by the treatment to which they have been subjected. Not infrequently, a considerable interval of time elapses before the explants are examined microscopically. Thus, in much of the recent work upon animal tissue cultures, detailed cytological observations can be made upon those cells only which grow out of, or migrate away from, the explants. Although these cells may live and even divide for considerable periods of time—thereby providing favorable material for certain specific lines of cytological and physiological research—they obviously are much modified tissue elements. There are no direct means of studying the changes that have occurred during the interval between the removal of the explants and the examination of the migrant cells.

It is evident that, if the normal structure of living tissue cells is to be observed, the cells must be examined before they have had *time* to become modified. In other words, the interval between the removal of the explants and the microscopical study of the living cells must be reduced to a minimum. Of course, the objection may be raised that the changes induced

[1] This oil was used originally as a control upon sudden osmotic changes that might be induced in vacuoles by aqueous media. The fact that explants remain alive in this medium, and that spores of various fungi germinate and grow luxuriantly in it, raises a number of interesting physiological questions.

[2] E.g., mitochondria, chromosomes, vacuoles, GOLGI apparatus, etc.

by traumatic and other stimuli are so rapid that the time interval cannot be abbreviated sufficiently to enable the investigator to observe the unmodified or normal structure. It is essential, therefore, to determine whether this objection is valid in the case of the cambium and of its derivative tissues.

A priori, the massive stems and roots of arborescent Gymnosperms and Dicotyledons should provide particularly favorable material for investigation. The tropistic responses of these organs are, in general, relatively slow. There are no "trigger" mechanisms or special adaptations for the rapid transmission of traumatic stimuli. Furthermore, blocks of tissue may be removed from the trees and kept alive for considerable periods of time[3] merely by coating them with paraffin to prevent desiccation. By examining these blocks, as well as the trees from which they were removed, at successive intervals, it is possible to demonstrate that the *visible* effects of the *original* injuries are restricted for some time to the cells in the immediate vicinity of the abraded surfaces. Hours and even days or weeks may elapse before the cells in the interior of the blocks become conspicuously modified. Thus, in the writer's technique for the investigation of the living tissue, the critical time factor is the interval between the cutting of the sections and the microscopical examination of the uncut cells. The time consumed in removing the masses of tissue from the tree and in preparing the blocks for sectioning is not a significant factor,—at least in the case of the species which have served as a basis for the writer's investigations.

Sections of the cambium and its derivatives may be cut, mounted in liquid media, and placed under microscopic observation in less than 60 seconds. To what extent do the cells become modified structurally during such an interval of time? In the more seriously injured cells at the margins and upon the upper and lower surfaces of the sections, changes are initiated very rapidly, as shown by the fact that certain of them are already in progress when the sections are first examined. Similar changes occur in the less seriously injured cells, but they are initiated and progress more slowly. Cells in the interior of the sections, which have not been cut, torn, or excessively compressed, remain visibly unmodified for a varying length of time after they are placed under observation. This suggests that, in the case of these cells, the interval between stimulus and visible structural response is longer than the period of time required in preparing the sections for microscopic examination,—a deduction that has been corroborated by the study of rapidly killed and fixed blocks of tissue.[4]

Properly prepared sections of the cambium provide an unusually favorable medium for the cytological investigation of living tissue elements. Owing to the size, form, and orientation of the cells, such structures as the nuclei, nucleoli, phragmoplasts, mitochondria, vacuoles, etc. are more clearly visible than in most other internal tissues of the higher plants. The vacuoles, and in many cases the mitochondria, may be studied without resorting to the use of so-called vital dyes,—*e.g.*, Neutral Red and Janus

[3] Specimens cut during the late fall and winter have been kept alive for several months.

[4] A detailed account of improved techniques for studying fixed material of the cambium and its derivative tissues will be presented in a subsequent paper.

Green B. Thus the writer has used Neutral Red primarily as a hydrogen ion indicator, and as an aid in photomicrography rather than as an indispensable reagent for demonstrating the vacuome in cambial initials.

P. A. DANGEARD, GUILLIERMOND, and PARAT, in their investigations of the vacuome, have placed much emphasis upon the use of basic vital dyes,—*e.g.*, Neutral Red, Brilliant Cresyl Blue, Methylene Blue, Nile Blue, Toluidine Blue, etc. Neutral Red is the most generally useful of these dyes and, as previously stated, is now regarded by GUILLIERMOND, PARAT, and others as being practically a specific stain for the vacuome,—just as Janus Green B is considered virtually specific for the chondriome. In addition, Neutral Red is one of SØRENSEN's standard hydrogen ion indicators and has been employed, particularly by PIERRE DANGEARD, as a means of determining whether the contents of vacuoles are alkaline or acid. No concerted effort has been made, as yet, to develop a standardized technique for the vital staining of the vacuome. Each investigator tends to follow a procedure of his own, and the dye used is referred to merely as Neutral Red without stating the particular brand or make. The uniformity of the results secured by different groups of investigators suggests, however, that the vacuome or its colloidal constituent stains readily under a relatively wide range of experimental conditions.

Different samples of Neutral Red, as shown by WILSON (1927), vary in their physical characters, in chemical constitution, in color and in color intensities at the same concentration, in percentages of impurities, and in their physiological effects,—*e.g.*, toxicity. In view of these facts, and in order to eliminate as many variables as possible, it seemed advisable to the writer to use a single sample of Neutral Red in all of his work. After a series of preliminary tests with different brands of Neutral Red, the product (Lot No. 3486) of the National Aniline and Chemical Company was selected for the purpose. This Neutral Red has a total dye content of 91 per cent and is certified by the Commission on Standardization of Biological Stains as NXI. It is employed by the writer in a ratio of one part of a saturated aqueous solution to 1000 parts of liquid media. Other dilutions are used frequently as controls upon this stereotyped procedure.

There is considerable confusion in biological literature concerning the color changes of Neutral Red. For example, in acid solutions, it is variously referred to as red, rose violet, magenta, etc. Therefore, if Neutral Red is to be employed as a hydrogen ion indicator, it is essential to have a set of permanent color standards, specially prepared for the particular dye and ratio of dilution used in vital staining.[5] From p_H 8.0 to p_H 6.8, the usual working range of Neutral Red, the color of NXI varies from orange to magenta. It is orange-red or reddish-orange between p_H 7.0 and p_H 7.8. The dye is blue in extremely acid solutions, and, between p_H 5.6 and p_H 0.0, the color varies from a conspicuously bluish-magenta to violet. Of course, in dealing with the vacuome, there are no means at present of determining the magnitude of "salt" and "protein" errors or of allowing for fluctuations

[5] Since Neutral Red is rather unstable, the colors in solutions of known p_H must be matched with stable dyes. This was done for the writer by the La Motte Chemical Products Company of Baltimore, Md.

in shades of color due to varying concentrations of dye in the vacuoles. Thus, it is not possible to measure the exact p_H of specific vacuoles or to distinguish minor variations of acidity or of alkalinity. The investigator must confine his attention to conspicuous changes of color rather than to slight differences of intensity of the same color. Unfortunately, most of the standard indicators,—*e.g.*, the sulfonphthaleins, kill the cells or do not visibly stain the vacuoles, at least in the case of the technique employed by the writer. Methyl Red, with a working range of p_H 4.4 to p_H 6.0, is, however, of considerable value in verifying the acidity of vacuoles which have been classified by means of Neutral Red as more acid than p_H 5.6.

The data that are to be recorded in the following pages are based upon the study of material secured from large stems of the following species of arborescent Gymnosperms and Angiosperms:

Gymnospermae, Coniferae	Angiospermae, Dicotyledoneae
Pinus Strobus L.	*Populus grandidentata* MICHX.
Pinus resinosa AIT.	*Juglans cinerea* L.
Pinus rigida MILL.	*Carya ovata* (MILL.) K. KOCH
Pinus sylvestris L.	*Ulmus campestris* L.
Larix laricina (DU ROI) KOCH	*Robinia Pseudo-Acacia* L.
Picea rubra (DU ROI) DIETR.	*Cladrastis lutea* (MICHX. f.) KOCH
Abies balsamea (L.) MILL.	*Acer rubrum* L.
Tsuga canadensis (L.) CARR.	*Tilia americana* L.
Thuja occidentalis L.	*Nyssa sylvatica* MARSH.
Juniperus virginiana L.	*Fraxinus americana* L.

The Vacuome: Cambial Initials:—The cambium is composed of initials of two distinct shapes and sizes: (1) *fusiform initials*,—large, much elongated elements, and (2) *ray initials*,—small, more nearly isodiametric elements of the same order of magnitude as the cells of the terminal meristems. The derivatives of the fusiform initials differentiate into tracheids, fibers, segments of vessels and of sieve tubes, etc. The ray initials divide to form the horizontal sheets of radially disposed parenchyma, the so-called medullary rays.

The fusiform initials are much compressed radially, and, therefore, are best studied in tangential longitudinal sections of the stem. As shown in the accompanying text figures and photomicrographs, these elongated meristematic elements are highly vacuolated, but the form of the vacuome varies considerably at different seasons of the year and in different groups of plants. During the active growing season, the fusiform initials of the Coniferae are characterized by having a large elongated vacuole (TEXT FIG. 1). The parietal protoplasm, with its included oil globules and mitochondria, rotates rapidly about this vacuole in a single ascending and descending stream. It is the most spectacular case of protoplasmic streaming in the internal tissues of the higher plants. The large nucleus is centrally located and serves as an obstruction around which the protoplasm flows. The tonoplast, or visible surface layer between the vacuole and the protoplasm, is tenuous and moves with the streaming protoplasm. In longitudinally

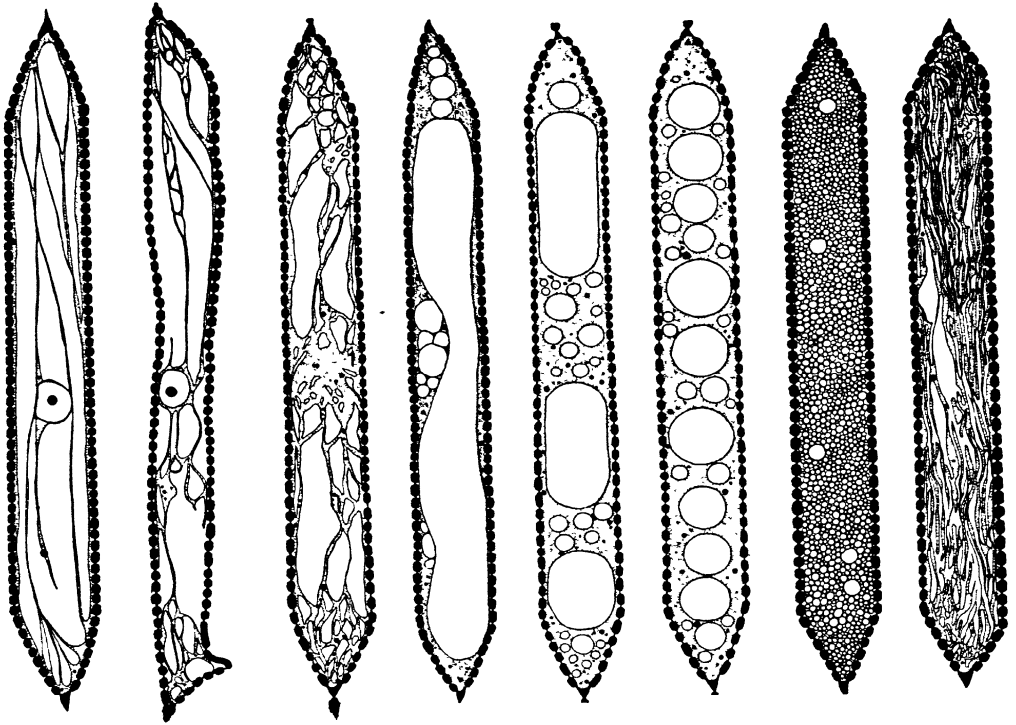
dividing initials, the expanding phragmoplast moves through the vacuole and splits it into elongated[6] halves; the protoplasmic streaming is not arrested during the process.

The fusiform initials of the Dicotyledons also contain a huge, elongated vacuole during the active growing season (TEXT FIGS. 5, 6, and PLATE 3,

TEXT FIGURES 1-4 (*from left to right*).— *Pinus Strobus*. Diagrammatic illustration of various forms of vacuome in fusiform initials of the cambium. — FIG. 1: Growing season. — FIG. 2: Transitional "myelin" form of vacuome. — FIGS. 3 and 4: Resting season. — Protoplasm stippled, vacuome not stippled. Actual dimensions of initials: length 4000 μ, diameter 42 μ.

FIGS. 10, 11). The protoplasm circulates around and through this vacuole in a system of anastomosing threads. Thus, these meristematic elements resemble the plant hairs that are figured in botanical textbooks as illustrations of extreme specialization of the protoplast in fully differentiated cells.

[6] For detailed descriptions of the remarkable phenomena of cytokinesis in cambial initials, *see* BAILEY (*34, 35, 40*).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The protoplasmic streaming of the fusiform initials, at least in the case of the Coniferae, appears to be continuous during the warmer months and to be arrested, during the colder months, only when the temperature drops close to or below zero degrees Centigrade. In *Fraxinus americana* the form of the vacuome remains more or less unmodified throughout the year, regardless of whether the protoplasm is streaming actively or not. On the contrary, in the remaining species investigated by the writer, the form of the vacuome fluctuates considerably during the late fall, winter, and early spring. When the temperature is low, the vacuome of the inactive (non-streaming) fusiform initials tends to be divided into a varying number of independent vacuoles. The size and the number of these vacuoles vary, not only in different plants, but also in material collected on different dates from the same tree, and even in different cells of the same section (TEXT FIGS. 3, 4 & 8-11). During transitions from the inactive to the streaming condition and *vice versa*, the relations between the protoplasm and the vacuome frequently become more or less unstable. While the protoplast is in this condition, the vacuoles may coalesce and divide and may be modified into a variety of unusual and constantly changing forms and patterns. At times, a portion of them tend to assume elongated, threadlike or "myelin" forms resembling those illustrated in TEXT FIGURES 2, 7 and 12.

That the physico-chemical constitution of the protoplast is altered during the transition from the actively growing to the so-called resting season is well known. At the height of the former period, the "hydrated" fusiform initials are extremely sensitive to abnormal environmental factors such as low temperatures, intense illumination, hypertonic solutions, etc. Unless these factors are carefully controlled, explanted cells die within a relatively short time. In other words, from an experimental point of view it is difficult to modify the normal form of the vacuome without killing the cells. On the contrary, during the "resting" season, the "dehydrated" initials are more resistant or adaptable, and it is possible to induce many reversible changes in the vacuome merely by varying the temperature and the osmotic pressure of the saccharine solutions. *This can be done without plasmolyzing the cells.* Not only is it possible to duplicate those varied forms of the vacuome which occur under normal conditions, but to observe successive stages of the transitions from one form to another (PLATE 3, FIGS. 7-9 & 13). It should be emphasized, in this connection, that the smallest vacuoles and the most attenuated of the "myelin" forms of the

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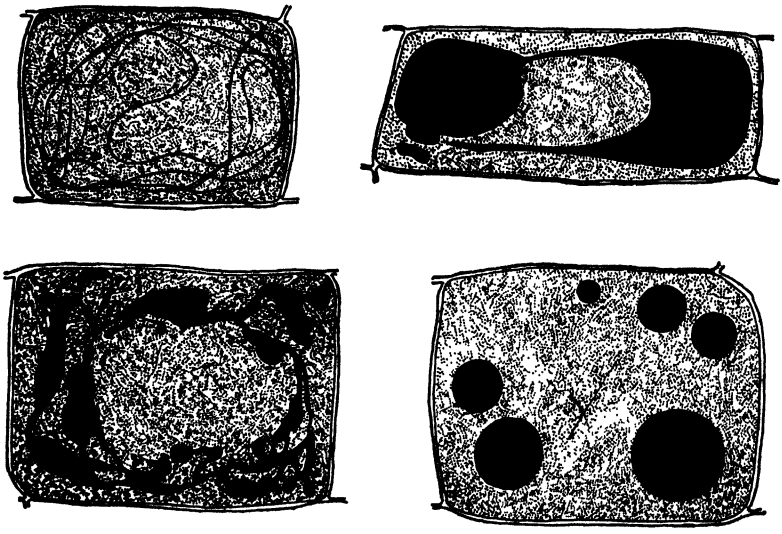
TEXT FIGURES 5-12 (*opposite*).— *Robinia Pseudo-Acacia.* FIG. 5: Diagrammatic illustration of highly vacuolated fusiform initial of the cambium. Growing season. Protoplasm stippled, vacuome and nucleus not stippled. — FIG. 6: *Fraxinus americana.* Diagrammatic illustration of highly vacuolated fusiform initial characteristic of the cambium. Protoplasm stippled, nucleus and vacuome not stippled. Actual dimensions of the initial: length 290 μ, diameter 28 μ. — FIG. 7: Transitional form of the vacuome. — FIGS. 8, 9 and 10: Three forms of the vacuome in adjacent cells of material collected in February 1929. — FIG. 11: Form of the vacuome in material collected in February 1928. — FIG. 12: Transitional "myelin" form of the vacuome. — Protoplasm stippled, vacuome not stippled. Actual dimensions of initials shown in FIGS. 5 and 7-12: length 180 μ, diameter 20 μ.

vacuome never have been observed to arise *de novo*. They originate by division or modification of the larger vacuoles.

The ray initials of the cambium also are conspicuously vacuolated, but, in these cells, the ratio of volume of vacuome to total volume of the protoplast is, in general, considerably smaller than in the fusiform initials. This is due, in part, to the fact that the ratio of volume of nucleus to total volume of the protoplast is much greater in the former than in the latter initials. As shown in TEXT FIGURES 13-16, the form of the vacuome is not constant in ray initials. It varies more or less, not only from species to species and from season to season, but also from cell to cell of the same section. Threadlike (TEXT FIG. 13), reticulate, branching or convoluted (FIG. 14) forms are of common occurrence, as are isolated vacuoles (FIG. 15) of varying size and number. In the more highly vacuolated condition of the cells (FIG. 16), the vacuoles frequently coalesce about the

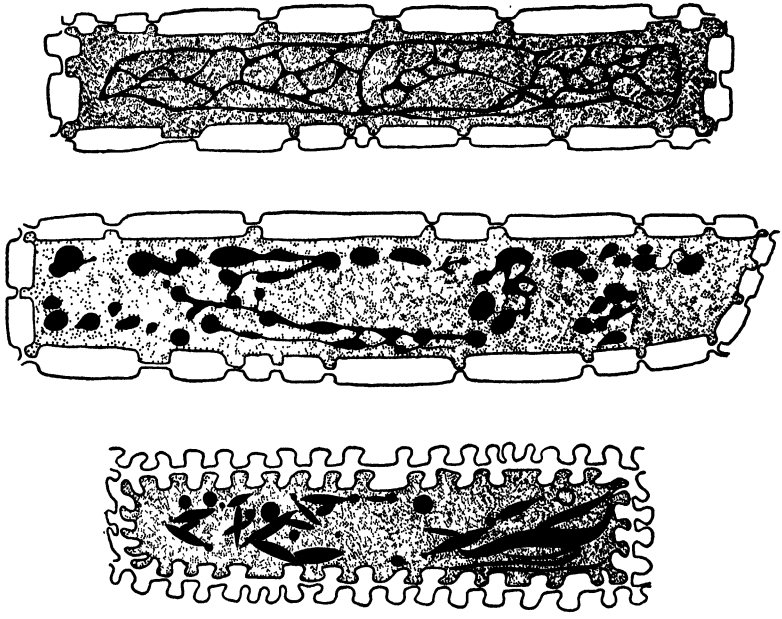


TEXT FIGURES 13-16.— Diagrammatic illustration of various forms of vacuome in ray initials of the cambium of gymnosperms. — FIG. 13 (*left, top*): *Abies balsamea*. — FIGS. 14 and 15 (*bottom*): *Pinus sylvestris*. — FIG. 16 (*right, top*): *Tsuga canadensis*. — Protoplasm stippled, vacuome in black.

centrally located nucleus. During the growing season, the form of the vacuome appears to be less stable than in the actively streaming fusiform initials. Such movements of the protoplasm as have been observed in ray initials are relatively sluggish. They are most conspicuous when associated with the so-called "myelin" forms of the vacuome. In explanted ray initials, the form of the vacuome changes not infrequently in a comparatively short interval of time. The specific changes that occur are dependent upon the condition of the initials at the time when they are removed from the tree and upon the particular combination of environmental influences to which they are transferred subsequently. The normally occurring threadlike or reticulate forms of the vacuome readily give rise to isolated spherical vacuoles and *vice versa*.

The Vacuome: Derivatives of the Cambium:—In radio-longitudinal sections of the stem, it is possible to compare the cambial initials with their serially arranged centripetal (xylem) and centrifugal (phloem) derivatives. Thus, during the growing season, the investigator may observe, within the limits of a single section, successive stages of the enlargement and differentiation of the elements of the xylem and phloem.

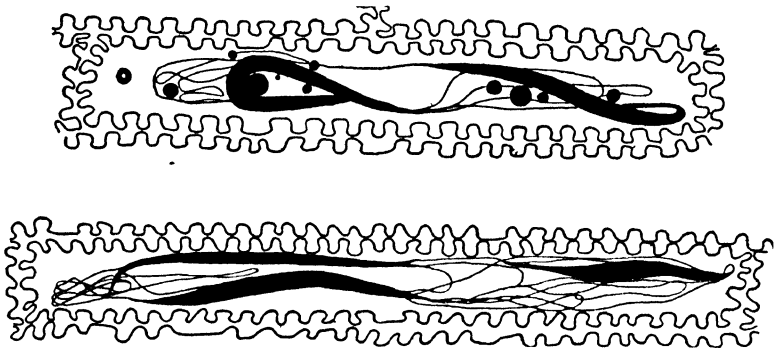
As previously stated, the actively streaming and dividing fusiform initials are characterized by having a large, elongated vacuole which is split longitudinally by the phragmoplast. This form of vacuome may be retained by the differentiating or fully differentiated derivatives of the fusiform initials or it may be modified in various ways. In those cells of the Coniferae



TEXT FIGURES 17-19.— Diagrammatic illustration of various forms of vacuome in fully differentiated cells of the ray parenchyma. — FIGS. 17 and 18 (*top and middle*): *Picea rubra.* — FIG. 19 (*bottom*): *Abies balsamea.* — Protoplasm stippled, vacuome in black.

which complete their development into tracheids and sieve tube segments during the current growing season, the vacuoles merely expand as the cells enlarge. On the contrary, in those which do not complete their development until the following spring, the vacuoles become modified during the fall and winter much as do the vacuoles of the fusiform initials themselves. Not only may the form of the vacuome fluctuate more or less during the differentiation of parenchyma, but also in the fully differentiated parenchymatous cells. The original large elongated vacuoles may be divided into spherical vacuoles (PLATE 3, FIG. 12) of varying size and number, or, at times, into irregular threadlike or vermiculate forms, which subsequently coalesce to reform large vacuoles.

The most varied and spectacular changes in the form of the vacuome, encountered by the writer, are those that occur in the centripetal derivatives of the ray initials of the Coniferae. In the radially elongating and differentiating ray cells, the protoplasm rotates more or less rapidly about the relatively large, centrally located nucleus. The vacuoles, as well as the mitochondria, plastids, and oil globules, commonly tend to circulate in the streaming protoplasm. As they do so, the vacuoles, or a portion of them, may coalesce and divide and become modified into a great variety of forms and patterns. Furthermore, as shown in TEXT FIGURES 17-21, the form of the vacuome is extraordinarily variable even in fully differentiated ray parenchyma of the sapwood. The number, size, and form of the vacuoles fluctuate, not only from species to species and from season to season, but



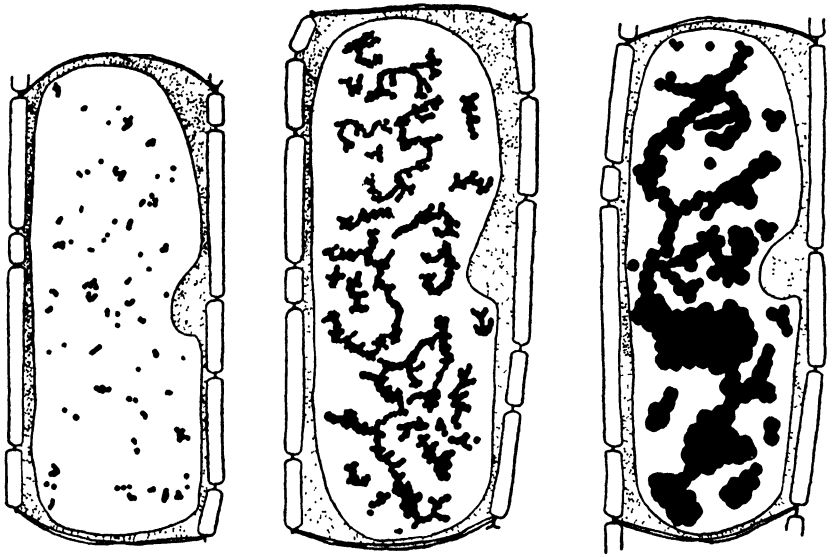
TEXT FIGURES 20-21.— Diagrammatic illustration of skein-like forms of vacuome in fully differentiated xylem ray parenchyma of *Abies balsamea*. Protoplasm not stippled, vacuome in black.

also in adjacent cells. The various changes in the form of the vacuome are reversible and may be observed in explanted tissue merely by controlling such environmental factors as temperature, osmotic pressure, etc. During the differentiation of ray tracheids and phloem ray parenchyma, the ratio of volume of vacuome to total volume of the protoplast becomes conspicuously greater, whereas, in the differentiating xylem ray parenchyma, it may remain more or less constant or even decrease. In other words, the expansion of the latter cells is due largely to an increase in the volume of the nucleus and of the cytoplasm, rather than of the vacuome.

The Vacuome: Vital Staining:—Considerable confusion has been introduced into biological literature by applying the term "vital" to those types of staining which immediately precede, or are concomitants of, the degeneration and death of the protoplast. The only structures that the writer has succeeded in staining thus far, *in cells which remain alive for considerable periods of time,* are the cell wall and the vacuome. The vacuoles of the cambial initials may be stained and destained without killing or seriously injuring the cells, as shown by the fact that fusiform initials have been kept alive and actively streaming for 500 hours after an intense staining of the vacuome with Neutral Red. The protoplasm, nucleus,

nucleoli, chromosomes, and phragmoplasts do not stain in normal cells. Therefore, in explanted tissue, the staining of these cell constituents may be used as a valuable aid in differentiating the injured and dying protoplasts.

During the so-called resting season, the vacuome of the cambial initials of the Coniferae stains reddish-orange with Neutral Red. It does so in liquid media of varying composition, temperature, and osmotic pressure, and in different dilutions of the dye, *provided the media are not too acid.* Vacuoles that stain rapidly and intensely in neutral tap water and in alkaline or nearly neutral solutions, frequently do not do so in ordinary distilled water and in other acid media. It is significant, furthermore, that there is no close correlation between the size and form of the vacuoles and the intensity of the coloration. The largest vacuoles commonly stain as deeply



TEXT FIGURES 22-24.— *Pinus resinosa.* Diagrammatic illustration of three successive stages in the formation of products of condensation or precipitation in the vacuoles of the phloem parenchyma. Protoplasm stippled, precipitate in black.

and uniformly as do the smaller ones. There is no evidence to indicate that the larger vacuoles arise by the hydration and dilution of the contents of the smallest ones. Nor do the larger vacuoles form deeply colored precipitates. The reddish-orange color in Neutral Red indicates that the vacuoles are relatively alkaline.

The vacuoles of the fully differentiated parenchyma of the xylem and phloem are of two conspicuously different colors: (1) reddish-orange and (2) bluish-magenta. The former, as in the case of the cambial initials, are relatively alkaline and stain homogeneously without forming precipitates. The latter stain homogeneously at first in both acid and alkaline media, but subsequently tend to form a more or less copious dark magenta precipitate (TEXT FIGS. 22-24). That these vacuoles are more acid than p_H 5.6 is suggested by their bluish-magenta color in Neutral Red and by their

magenta ("red") color in Methyl Red. As the precipitate increases in amount (TEXT FIGS. 22-24), the remaining portion of the vacuolar substance may lose its color entirely.

The abundance and distribution of the two categories of vacuoles vary considerably in the different types of parenchyma and in different representatives of the Coniferae. For example, in *Pinus Strobus* the vacuoles of the phloem parenchyma are acid, whereas those of the xylem ray parenchyma and of the epithelium of the resin canals are prevailingly, if not entirely, alkaline. In the phloem parenchyma of *Thuja occidentalis* and of *Juniperus virginiana,* both types are of common occurrence. In *Tsuga canadensis,* acid vacuoles are particularly abundant even in the xylem. Vacuoles of markedly different p_H may occur not only in adjacent cells, but *even within a single protoplast.* The latter phenomenon is of not infrequent occurrence in certain cells of the phloem parenchyma, particularly of *Thuja* and of *Juniperus.* The large, central vacuoles are bluish-magenta, whereas the smaller peripheral ones are reddish-orange.

At the height of the growing season, the vacuoles of the actively streaming fusiform initials commonly do not stain under those experimental conditions which produce an intense coloration during the resting season. The large, elongated vacuoles of the developing sieve tubes become increasingly alkaline (orange), but those of the fully differentiated sieve tubes give no color reaction in Neutral Red. The vacuome of the differentiating tracheids stains, if at all, a dilute magenta. In the differentiating parenchyma, the more conspicuous color changes are from reddish-orange to magenta,—*i.e.,* from slight alkalinity to acidity.

In most of the Dicotyledons, as in the Coniferae, the vacuome of the cambial initials stains readily and intensely during the resting season, regardless of the size and form of the individual vacuoles, but the color reactions in Neutral Red vary considerably from species to species. For example, the vacuoles of the fusiform initials are bluish-magenta in *Fraxinus americana,* reddish-orange in *Robinia Pseudo-Acacia,* and orange in *Acer rubrum.* In other words, the apparent p_H of the vacuome fluctuates more than it does in the cambial initials of the Coniferae. The vacuoles of certain of the Dicotyledons,—*e.g., Cladrastis lutea,* do not stain uniformly. A colored precipitate is rapidly formed and the rest of the vacuolar material stains faintly, if at all.

The vacuoles of the fully differentiated tissues commonly are of two conspicuously different types: (1) relatively alkaline (reddish-orange) vacuoles and (2) markedly acid (bluish-magenta) vacuoles. The abundance and distribution of these categories of vacuoles vary from tissue to tissue and from species to species. *Both types may occur within a single protoplast.* The correlation between the color of the vacuome and the formation of precipitates is not so close as it is in the case of the Coniferae. Although the bluish-magenta vacuoles tend to form precipitates, and the reddish-orange vacuoles commonly do not, there are numerous exceptions. The large bluish-magenta vacuoles of *Fraxinus americana* do not form precipitates readily, whereas certain of the reddish-orange vacuoles of *Populus grandidentata,* and of other species may do so.

The phenomena of precipitation in Neutral Red vary considerably from tissue to tissue, from species to species, from season to season, and apparently also under different experimental procedures. The vacuoles may stain uniformly and intensely before precipitation occurs, or precipitates may appear during the earlier stages of the staining of the vacuoles. In the case of the bluish-magenta vacuoles, the precipitation may progress internally, as illustrated in TEXT FIGURES 22-24, or it may result in the segregation of deeply stained masses that are external to the remaining portion or portions of the vacuole. As the dark magenta material increases in volume, the color fades from the rest of the vacuole. In the less intensely staining reddish-orange vacuoles, one or more deeply colored spheres may be formed internally. Not infrequently, the precipitate adheres to the surface of the tonoplast, forming crescents or curved rims. In exceptional cases, LIESEGANG rings and sphaerocrystals may be formed in the more intensely stained alkaline vacuoles.

At the height of the growing season, the fusiform initials of the Dicotyledons stain faintly, if at all, under those experimental conditions which produce an intense, uniform coloration during the resting season. Changes in p_H, in intensity of staining and in the formation of precipitates, may be observed in the differentiating derivatives of the cambium. These changes may or *may not* be correlated with variations in the size and form of the vacuome.

Vacuome vs. Chondriome:—Even in unstained living cells of the cambium, the vacuoles may be distinguished readily from the other constituents of the protoplast. They are much less refractive than the oil globules, and rarely resemble mitochondria either in size or in form. The mitochondria, when visible, appear in white light as shadowy, uniformly bluish green dots, slender rods, or fine threads. The vacuoles have a more or less conspicuous bluish green surface layer or tonoplast of varying thickness and a lighter colored interior.

The oil globules and mitochondria of the species investigated do not stain in Neutral Red. Furthermore, although the normal bluish-green color of the mitochondria, as of certain of the oil globules, may be intensified slightly in dilute solutions of Janus Green B,[7] the writer has not obtained a truly vital staining of the chondriome in unmodified living cells. In fact, the work of CHAMBERS and others suggests that the so-called vital staining of the chondriome is actually a concomitant of fixation or of other irreversible changes in the mitochondria and cytoplasm.

Discussion: — The generalizations of WENT, of the DANGEARDS, and of GUILLIERMOND concerning the vacuolated condition of terminal meristems of the higher plants are open to various criticisms. It may be objected that the tenuous "myelin" or pseudomitochondrial forms of the vacuome are not present in the normal promeristem, but are induced by the dyes employed in vital staining and by the reagents used in fixation. Similarly, it may be argued that the vacuoles observed by WENT and others in unstained

[7] Janus Green B of HÖCHST.

preparations were produced by the treatment to which the material was subjected. That vacuolation of the protoplasm may be induced by mechanical injuries, by water and hypotonic solutions, by changes of temperature and illumination, and by alkalis, acids, fat solvents, and other reagents is maintained by many physiologists. The possible effects of such factors as these are commonly overlooked or ignored by cytologists, both in the study of living and of fixed material.

In the investigation of the living cells of the terminal meristems, the growing points are cut, crushed, or dissected. The liquid media in which the living cells are examined commonly are referred to merely as solutions of Neutral Red, Brilliant Cresyl Blue, etc. PIERRE DANGEARD (1923) evidently used dyes in tap water, whereas WENT (1888) employed 5 per cent solutions of sugar without vital stains. The media obviously are hypotonic in certain cases and may have been in all. Furthermore, no reliable data are presented concerning the interval of time that elapses during the preparation of tissues for microscopic observation. There are no means of determining what structural changes may have occurred during this interval. According to the observations of NĚMEC (1901), the time factor of traumatically induced vacuolation is relatively brief in terminal meristems, particularly in the case of cells in close proximity to cut or abraded surfaces. Although the effects of mechanical injuries may be avoided by sectioning blocks of rapidly killed and fixed tissue, such a procedure introduces a new set of factors, the exact effects of which can be determined only by comparisons with living tissue. Vacuoles may be made to appear and disappear, and different reagents produce entirely different fixation images of the vacuome. Thus, the only reliable control upon "good" fixation is the normal structure of the living cell.

As previously stated, the student of the lateral meristem or cambium may simplify the problem by eliminating the use of vital dyes. Owing to the size, form, and arrangement of the cambial initials and to the character of their contents, the vacuoles are clearly visible in unstained cells. Furthermore, in the writer's technique, such environmental factors as temperature, illumination, and the p_H, osmotic pressure and composition of the liquid media are controlled. The uncontrollable factors are the traumatic ones, but it is significant, in this connection, that the critical time factor—the interval between the cutting of the sections and the microscopical examination of the uncut cells—may be reduced to less than 60 seconds. To argue that large vacuoles and an elaborate system of protoplasmic streaming are induced, and the transformation entirely completed, in less than one minute leads to a *reductio ad absurdum*. Even the so-called phenomenon of aggregation in insectivorous plants and in *Mimosa* involves no such extraordinary modification of the volume of the protoplasm.

NĚMEC and others have shown that in injured terminal meristems the visible structural changes progress gradually from the more seriously to the less seriously affected cells, and that the tropistic responses are retarded or restricted at low temperatures. Such progressive structural changes may be observed in explanted sections of the cambium. In the case of material collected during the warmer periods or seasons of the year, erratic and

irregular protoplasmic movements, followed by cessation of streaming, appear first in the more seriously injured cells upon the margins and upper and lower surfaces of the sections, and subsequently and more gradually in the less seriously affected cells. A smooth, even, uninterrupted flow of the protoplasm along characteristic and regular channels continues for some time in the cells of the interior of the sections only. The equilibrium of these cells may be upset, however, and the protoplasmic streaming more or less profoundly modified, or inhibited, by intense or prolonged illumination, by hypotonic and hypertonic solutions, by abnormal temperatures, etc.

In the case of specimens collected during cold weather and maintained at a temperature close to zero degrees Centigrade, the protoplasm is inactive, but the cells are highly vacuolated. *The tropistic responses of the chilled tissue are extremely sluggish.* If the temperature is raised and other environmental factors are controlled, protoplasmic streaming is induced, and the vacuome is modified more or less in form but not conspicuously in volume. The transitions from the inactive to the actively streaming condition are gradual. The first movements of the protoplasm are sporadic and irregular, and considerable time elapses before the smooth even lines of flow become characteristically established. Furthermore, during the warmer months of the year, protoplasmic streaming may be induced at times in normally inactive cells of the xylem and phloem by specific combinations of environmental factors. However, the transitions from the inactive to the streaming condition are gradual, not instantaneous. Similarly, the varied changes that may be induced in the form and volume of the vacuome by fluctuations of temperature, of osmotic pressure, and of other factors do not occur abruptly, at least in cells which remain alive for any considerable interval of time.

Such facts as these, corroborated as they have been by the study of fixed material, justify the writer's conclusion that the cells of the lateral meristem or cambium are vacuolated. Not only are the normal cells conspicuously vacuolated, but in many of them the ratio of volume of vacuome to volume of protoplasm is fully as high as it is in plant hairs and other cells which are commonly cited as illustrations of extreme specialization of the protoplast in fully differentiated cells. It is evident, therefore, that the classical conception of non-vacuolated meristems, and the various physiological generalizations that have been deduced therefrom, must be modified, at least as regards the cambium.

Nor are certain generalizations of P. A. DANGEARD and of GUILLIERMOND applicable to the cambium. According to these investigators, the vacuome is represented in meristematic tissue by minute, viscous, deeply and uniformly staining bodies resembling mitochondria. These metachromes swell by hydration and coalesce to form the typical, conspicuous liquid vacuoles of fully differentiated tissues. With increasing hydration, the vacuoles stain faintly, if at all, but their colloidal constituent appears as a deeply colored precipitate.

It should be emphasized again in this connection that, during the growing season, the fusiform initials of the lateral meristem or cambium have large elongated vacuoles; the volume of the protoplasm is, in comparison,

relatively small. When growth is active, the vacuoles stain faintly, or not at all, in Neutral Red, and they do not form precipitates in most of the species investigated. The vacuoles of the daughter cells are derived directly from those of the initials. At each division, the elongated vacuole of the initial is split longitudinally by the expanding phragmoplast. The original form of the vacuome may be retained in the differentiating derivatives of the fusiform initials or it may be dissected into smaller vacuoles or may even be modified temporarily into various "myelin" forms. The vacuoles of the differentiating and fully differentiated cells frequently stain intensely and uniformly. They do so regardless of their size and form, but those having acid contents tend to form more or less copious, deeply colored precipitates.

During the so-called resting season, when the temperature is close to or below zero degrees Centigrade and the protoplasm is inactive, the vacuome of the fusiform initials commonly is dissected into a varying number of smaller vacuoles. During transitions from the inactive to the streaming condition and *vice versa*, the form of the vacuome fluctuates more or less, and various elongated, reticulate and "myelin" patterns may be formed temporarily. The vacuoles stain intensely and uniformly in most cases, and they do so regardless of variations in their size and form. The intense staining of the vacuoles during the resting season is not due merely to dehydration of the vacuome at the end of the growing season, as shown by the fact that in *Fraxinus americana* the volume of the vacuome remains relatively constant. There appears to be an actual accumulation of stainable material in the vacuoles during the resting season.

Although the ratio of volume of vacuome to volume of protoplasm and nucleus is, in general, somewhat lower in the ray initials than in fusiform initials, the former cells are conspicuously vacuolated. The form of vacuome is not constant. In the Coniferæ, as shown by the writer, it fluctuates not only from species to species and from season to season, but from cell to cell. "Myelin" forms are of more frequent occurrence than in the fusiform initials, but they are no more characteristic of ray initials than of the fully differentiated xylem ray parenchyma. During the differentiation of the derivatives of the ray initials, there may or *may not* be a marked increase in the volume of the vacuome. The vacuoles, both of the ray initials and of their derivatives, commonly stain intensely and uniformly, regardless of their size and form. Those having acid contents tend ultimately to form more or less copious precipitates.

The objection may be raised that the cambium is a relatively specialized type of meristem and that its vacuoles are derived originally from the vacuome of the terminal meristems. This raises the question whether DANGEARD and GUILLIERMOND are justified in placing so much emphasis upon the occurrence of pseudomitochondrial forms of the vacuome in terminal meristems and upon the "origin" of typical vacuoles by hydration of these metachromes. It is admitted that reversible changes occur at certain stages in the life cycle,—*e.g.*, in ripening and germinating seeds, etc. Thus, there appear, *a priori*, to be as valid arguments for emphasizing transitions between large vacuoles and pseudomitochondrial or "myelin" forms as *vice versa*. Indeed, WENT maintained, as previously stated, that all vacuoles

of the higher plants are derived from divisions of the vacuoles of the egg or embryo-sac.

In order to secure more specific information concerning this question it seemed advisable to examine the terminal meristems of various Pteridophyta, Gymnospermæ, and Angiospermæ. The results of an investigation of this character are presented by ZIRKLE (1932). Although the curious pseudomitochondrial forms of the vacuome evidently are not artifacts produced by the techniques used in their demonstration, there is no such constancy in the occurrence of these tenuous forms in primordial meristems as hypothesized by DANGEARD and by GUILLIERMOND. The vacuome of terminal meristems, as of the cambium, fluctuates considerably in form, in volume, and in its staining reactions in Neutral Red. The large apical cells of certain of the Pteridophyta are as highly vacuolated as are the fusiform initials of the cambium. Furthermore, in the case of the Gymnosperms and Angiosperms, the form of the vacuome varies in the promeristem much as it does in the ray initials of the lateral meristem. Not infrequently the "myelin" forms of the vacuome appear some distance back from the promeristem. They obviously are derived from the conspicuous, more or less spherical vacuoles of the truly apical cells. The form and volume of the vacuome may vary more or less, not only in different groups of plants, but in different growing points of the same plant,—*e.g.*, root *vs.* shoot, short shoot *vs.* long shoot, resting buds *vs.* elongating shoots, growing points of germinating seeds *vs.* growing points of mature plants, etc. It is significant, in addition, that the viscosity, the intensity and uniformity of vital staining, and the formation of precipitates may or *may not* be correlated closely with variations in the size and form of the vacuoles.

The present status of available information concerning the plant vacuome may, accordingly, be summarized as follows:

All plant cells, with the possible exception of spermatozoids, are vacuolated, as maintained by WENT, by the DANGEARDS, and by GUILLIERMOND and his students. This is as true of terminal and lateral meristems as of their derivative tissues. Therefore, the classical conception of non-vacuolated meristems should be abandoned.

The vacuome assumes many diverse forms, certain of which resemble mitochondria and the GOLGI apparatus of animals. The similarity between these "myelin" forms of the vacuome and mitochondria appears to have been over-emphasized. Forms which closely resemble the chondriome are of comparatively infrequent occurrence even in meristematic tissue, and they may be distinguished readily from mitochondria both in living and in fixed material. On the contrary, the similarities—in form, in vital staining, and in fixation by standardized techniques—between certain "myelin" forms of the vacuome and the GOLGI apparatus are much closer, as has been demonstrated by GUILLIERMOND, PARAT, and their co-workers. Although these similarities are extremely suggestive, it has not been demonstrated, as yet, that the two categories of structures actually are homologous.

The varied changes in the form of the vacuome are reversible. In the higher plants each type of the vacuome may be observed to develop as a modification of some previously existing form. Thus, large vacuoles become

dissected into smaller ones, and the latter coalesce to reform larger vacuoles. Similarly, vacuoles of varying form and size metamorphose into "myelin" patterns and *vice versa*. Such reversible changes in the form of the vacuome are of normal occurrence, not only in ripening and germinating seeds and in connection with the phenomenon of aggregation, but also in the terminal and lateral meristems and even in their differentiating and fully differentiated derivatives. Many of the reversible fluctuations that occur in specific tissues are seasonal, whereas others are correlated with cellular changes that take place during growth or ontogeny. *From the point of view of the whole life cycle of the plant, the vacuome is a perpetuated structure of varying form.* It is divided more or less passively,—*e.g.*, by the phragmoplast, at each successive cell division. The vacuoles of the germinal cells are derived directly from those of the somatic tissue.

WENT and the DANGEARDS maintain that the vacuome is self-perpetuating, and that vacuoles never originate *de novo*. Such an assertion is difficult to prove or to disprove. That vacuolation of the protoplasm may be induced experimentally is claimed by many physiologists, but a careful analysis of their investigations reveals no reliable proof of the neoformation of true vacuoles. In many cases the so-called vacuoles obviously are artifacts produced by coagulation and other irreversible changes in seriously injured or dying cells. In other cases, the vacuolation is evidently due to enlargement of preëxisting vacuoles, to the dissection of the vacuome, or to various combinations of such phenomena. KLEMM's (1895) experiments are commonly cited as direct proof of neoformation; but they are not convincing to anyone familiar with the phenomena of aggregation and with the precipitation of basic vital dyes in vacuoles which contain aromatic substances. The segregation of vacuolar material into colored and colorless portions is of not infrequent occurrence. Nor is PFEFFER's (1890) much quoted experiment with the plasmodia of Myxomycetes as conclusive as has commonly been assumed. Its significance is dependent upon an accurate definition of vacuoles. The abnormal vacuoles induced by crystals of Asparagin, as well as the food-vacuoles and contractile vacuoles of the lower organisms may not be homologues of the vacuoles of the higher plants. Their exact status in the theory of the vacuome remains to be determined. GUILLIERMOND visualizes the protoplasm as constantly building diverse colloidal substances which are not miscible with the cytoplasmic colloids. These by-products of the protoplasm, being of the same semi-fluid consistency as mitochondria, are physically made to assume the same patterns as the latter. The viscous, pseudomitochondrial bodies possess a strong power of imbibition and hydrate readily into liquid vacuoles. Therefore, vacuoles must originate *de novo*. It is significant, however, that GUILLIERMOND's arguments in favor of neoformation are largely speculative. No direct and conclusive proof of the *de novo* origin of vacuoles is presented even in the case of algæ and fungi. Although the writer has kept living cells of many types under continuous observation for relatively long periods of time and has witnessed a host of reversible changes in the form and volume of the vacuome, he has never encountered the slightest evidence of neoformation. If the vitally stainable substances are elaborated by the protoplasm, rather than by the

vacuome, the available evidence suggests that they must accumulate in the existing vacuoles, at least in the great majority of cases.

The problem of permeability and of the penetration of vital dyes into the vacuome is so extraordinarily complex, as demonstrated by the intensive investigations of OSTERHOUT, CROZIER, Miss IRWIN, Mrs. BROOKS, and others, that the cytologist should proceed with considerable caution in discussing the phenomena of the vital staining of the vacuome. In the case of *Valonia* and *Nitella*, the problem appears to have been approached largely from the point of view of permeability. It is evident, however, from the extensive investigations of the DANGEARDS, of GUILLIERMOND, and of the writer that the vital staining of the vacuome in most of the tissues of multicellular plants involves a more or less pronounced accumulation or concentration of dye in the vacuoles. That the concentration of dye is due to the presence of specific substances is suggested by much cumulative circumstantial evidence. The viscous, refractive, markedly acid vacuoles of the higher plants—which stain intensely in basic vital dyes and form copious deeply colored precipitates—apparently contain aromatic substances. Not only do these vacuoles give positive results with tests for aromatic substances,—*e.g.*, tannins, but those tissues which are used commercially, owing to their high yields of such substances, contain an abundance of these vacuoles; whereas tissues which give unusually low yields contain relatively few, if any, of them. In certain cases, the contents of the vacuoles are not soluble or miscible in saccharine solutions containing Neutral Red. The writer has succeeded in extracting the vacuolar material from the living cells and in observing its staining reactions. *It concentrates the dye and forms precipitates much as it does when inclosed within the protoplasm and protoplasmic membrane.* In the vacuoles of many of the lower plants the staining appears to be due to the presence of a putative nitrogenous substance that is designated as metachromatin. Similarly, there is considerable evidence to indicate that the concentration of dye in vacuoles of ripening seeds is due to an accumulation of proteins. Furthermore, the vacuoles of the meristems of perennial Coniferæ and Dicotyledons commonly stain intensely during the winter, but faintly or not at all during the active growing season. That such seasonal variations in vital staining may be due to the accumulation and depletion of reserve materials seems probable. There appears to be a marked increase in viscosity during the resting period, and many of the cells do not plasmolyze even in extremely concentrated solutions of sucrose. An additional phenomenon of considerable theoretical significance in connection with vital staining with Neutral Red is the occurrence of both bluish-magenta (acid) and orange (alkaline) vacuoles within the same protoplast. Such differences in vital staining must be due to differences in the contents of the two categories of vacuoles.

Although there is considerable evidence which indicates that the accumulation of basic vital dyes in the vacuome is correlated with the presence of specific substances in the vacuoles, the generalizations of the DANGEARDS that all plant vacuoles are characterized by containing metachromatin or similar categories of vitally stainable nitrogenous substances, and that variations in the size, form, and vital staining of vacuoles are due to hydration

and dehydration of these colloidal substances, are largely speculative, as admitted by PIERRE DANGEARD. In parrying GUILLIERMOND'S criticisms, PIERRE DANGEARD argues that the vacuoles of embryonic and meristematic tissues are typically alkaline and that, in the acid vacuoles of fully differentiated tissues, the characteristic metachromatic staining is masked by an accumulation of aromatic substances. In the coniferous species and in many of the dicotyledonous ones investigated by the writer, the vacuoles of the meristematic elements, *when stainable,* are slightly alkaline, and the markedly acid, tannin-containing vacuoles of the xylem and phloem metamorphose from alkaline ones. On the contrary, in certain of the Dicotyledons,—*e.g., Fraxinus americana,* the vacuoles of the cambial initials, when stainable, are acid, and transitions from acidity to alkalinity and *vice versa* occur at times in the differentiating derivatives. It should be emphasized, in this connection, that there are no reliable bio-chemical data available at present for determining whether all acid vacuoles of the higher plants contain aromatic substances, and all alkaline vacuoles, nitrogenous ones. The so-called phenomenon of metachromasy in staining with basic vital dyes is apparently a color change of hydrogen ion indicators, and is not indicative, necessarily, of the presence of metachromatin or other active or labile nitrogenous material.

That changes in the viscosity, volume, form, and vital staining of the vacuome are not due merely to the hydration and dehydration of comparatively constant amounts of specific vacuolar colloids is indicated by the writer's investigations. The contrast between (1) intensely and uniformly staining, viscous, pseudomitochondrial forms of the vacuome and (2) the faintly staining, precipitate forming, large, "typical," liquid vacuoles of fully differentiated tissues appears to have been over-emphasized. Large vacuoles may contain viscous contents that stain intensely and uniformly without forming precipitates. When growth is active, the vacuoles of the promeristem and cambium may stain faintly, if at all, whereas during the resting season they stain intensely and uniformly, regardless of their size and form. That such fluctuations in vital staining may be due largely to seasonal accumulation and depletion of reserve materials, rather than solely to hydration and dehydration, is indicated by the fact that in *Fraxinus americana* and other species the volume of the vacuome does not vary conspicuously. Furthermore, the transitions between "myelin" and other forms of the vacuome, or *vice versa,* may or may not involve striking variations in the volume and staining reactions of the vacuome. The reversible changes in the vacuome appear to be due to a complex of varying physico-chemical factors, affecting both the protoplasm and the contents of the vacuoles. Not until these factors have been isolated and studied quantitatively under controlled experimental conditions will it be possible to formulate an accurate definition of the vacuome in physiological terms. It is evident, however, that the classical conception of the plant vacuole is inadequate both from morphological and physiological points of view.

SUMMARY AND CONCLUSIONS

1) A technique has been perfected for investigating the living cells of the cambium and of its derivative tissues. Cambial initials have been kept alive and actively streaming for 500 hours in saccharine solutions and in "White Russian Oil."

2) In applying this technique in a reconnaissance of the vacuome, particular attention has been devoted to the generally neglected, but fundamentally important, problem of determining to what extent the visible structure of the living cells is modified during the process of removing the tissue from its normal environment to an abnormal one.

3) By reducing the critical time factor—the interval between the cutting of the sections and the microscopic examination of the uncut cells—to less than 60 seconds, it is possible to study the normal form of the vacuome and to observe the various morphological changes that are induced by traumatic stimuli and by such controllable factors as temperature, illumination, vital dyes, the p_H, osmotic pressure and composition of culture media, etc.

4) Normal cambial initials are conspicuously vacuolated. Indeed, certain of them are as highly vacuolated as plant hairs, which are commonly cited as illustrations of extreme specialization of the protoplast in fully differentiated cells. The classical conception of non-vacuolated meristems, and the various physiological generalizations that have been deduced therefrom, should be abandoned.

5) There is no such constancy in the occurrence of viscous, pseudomitochondrial forms of the vacuome in meristems and in the origin of typical liquid vacuoles by hydration of such metachromes, as hypothesized by P. A. DANGEARD and by GUILLIERMOND.

6) The form of the vacuome in cambial initials, as in many of their derivatives, is not constant. It fluctuates, not only from species to species, but also from season to season.

7) The various transitions in the form of the vacuome are reversible. Tenuous, threadlike, reticulate, and other "myelin" forms metamorphose from large vacuoles and *vice versa*. In other words, the vacuome is a perpetuated cell constituent of markedly varying form. It is divided more or less passively at each cell division.

8) The "myelin" or pseudomitochondrial forms of the vacuome may be distinguished readily from the chondriome both in living and in fixed material.

9) The problem of vitally staining the vacuome does not appear to be solely a question of permeability. The concentration of basic dyes in the vacuoles of the higher plants is correlated, evidently, with the presence of specific substances in the vacuome.

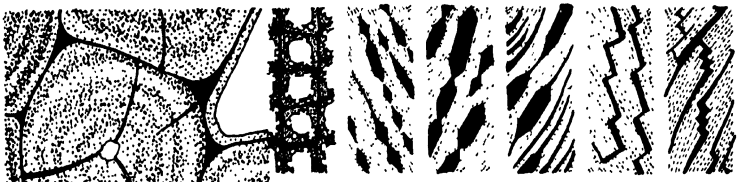
10) In the arborescent Conifers and Dicotyledons investigated by the writer, two distinct categories of vacuoles may be distinguished by their reactions in Neutral Red: (1) relatively alkaline (reddish-orange) and (2) markedly acid (bluish-magenta). The latter contain aromatic substances and commonly tend to form copious, deeply colored precipitates. Both types may occur within the same protoplast.

11) In the Conifers, the vacuoles of the cambial initials, when stainable, are usually of the alkaline type, and the acid vacuoles of the differentiated tissues metamorphose from alkaline ones. In certain of the Dicotyledons, on the contrary, the vacuoles of the cambial initials are acid, and alkaline vacuoles may metamorphose from acid ones.

12) There is no constant correlation between the volume and form of the vacuome and its staining reactions in Neutral Red. The contrast in vital staining between (1) viscous, pseudomitochondrial forms of the vacuome in meristems and (2) "typical" vacuoles in differentiated tissues appears to have been over-emphasized. Large vacuoles frequently stain as intensely and uniformly as the smallest ones.

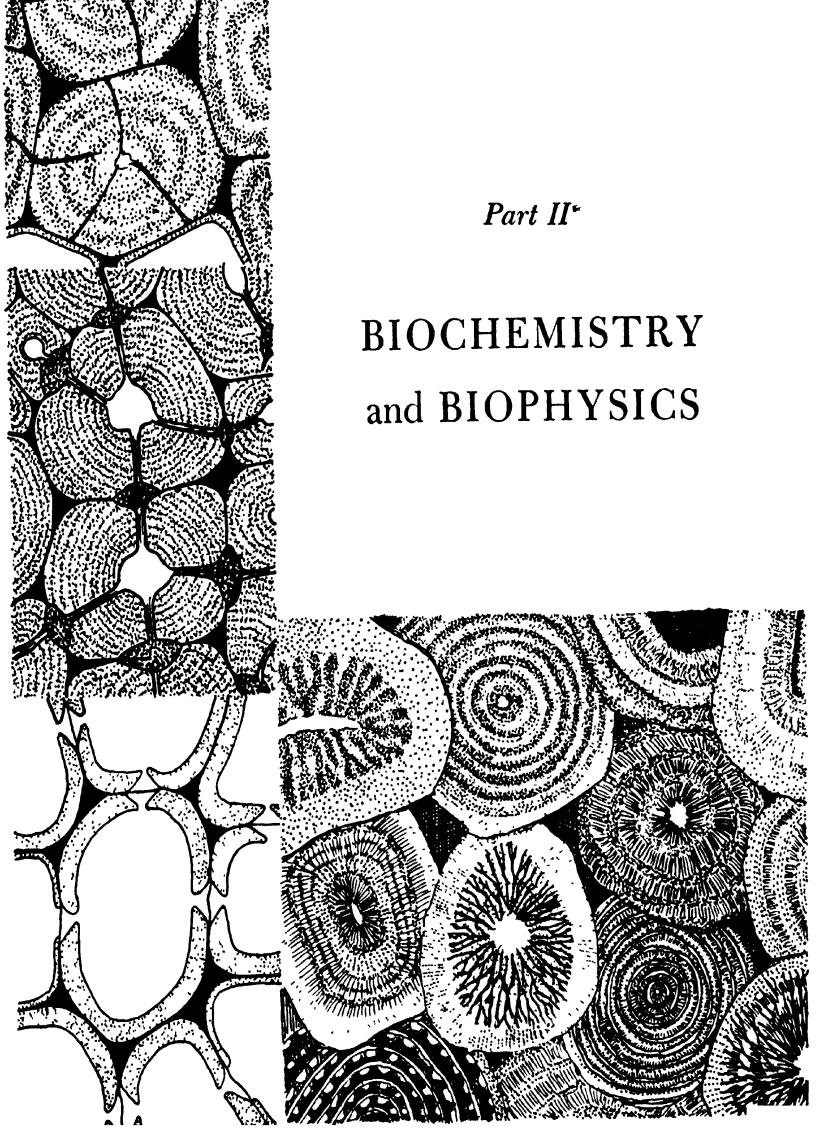
13) Fluctuation in vital staining may be due largely to ontogenetic or seasonal accumulation and depletion of specific substances in the vacuoles rather than solely to hydration and dehydration of such substances.

14) Reversible changes in the form of the vacuome appear to be due to a complex of varying physico-chemical factors, affecting both the protoplasm and the contents of the vacuoles, rather than solely to the hydration and dehydration of relatively constant quantities of specific vacuolar colloids.



Part II

BIOCHEMISTRY and BIOPHYSICS



Chapter 6

THE CAMBIUM AND ITS DERIVATIVE TISSUES

X. STRUCTURE, OPTICAL PROPERTIES AND CHEMICAL COMPOSITION OF THE SO-CALLED MIDDLE LAMELLA*

in collaboration with THOMAS KERR

MUCH has been written during the last hundred years concerning the structure, the development, and the physical and chemical properties of the cell wall. A careful study of this literature reveals many varied and more or less contradictory points of view. For example, there is no consensus of opinion concerning the use of such terms as *intercellular substance, middle lamella, primary wall, secondary wall, tertiary wall,* etc. Each of them is employed in several fundamentally different senses and to designate entirely different structures. It is essential to clarify this situation, since the existing confusion results in serious discrepancies, not only in descriptive morphological work, but also in physiological, biophysical, and biochemical investigations.

What Constitutes the So-Called Middle Lamella?:—VON MOHL and the earlier botanical investigators believed that the cells of the xylem and of other tissues are bound together by a refractive intercellular substance which differs from the cell walls in its solubilities and other properties. Subsequently, with the discovery of cell division and an altered viewpoint regarding the origin and development of cells, this refractive layer was referred to by HOFMEISTER, SACHS, and many other botanists, as the middle lamella. The cytological investigations of TREUB, STRASBURGER, TIMBERLAKE, and ALLEN ultimately led to the conclusion that the middle lamella originates between the halves of the split cell-plate and is, in fact, the first-formed membrane or partition wall, more or less modified in thickness and in chemical composition during tissue differentiation.

Most botanists have differentiated the middle lamella by its refractive character, its differential staining, or its solubility in macerating agents. DIPPEL (1867-69) demonstrated, however, in the first edition of "Das Mikroskop" that the refractive layer, which appears more or less homogeneous in white light, consists of three distinct layers, *i.e.,* two anisotropic layers and an intervening isotropic layer. He designated the anisotropic layers as primary walls and the isotropic central layer as intercellular substance, the three layers combined constituting the middle lamella. More recently there has been a tendency, among certain investigators, to limit the term middle lamella to the putative isotropic layer. Thus, RITTER (1925) has defined the middle lamella as "the isotropic peripheral layer of

* Reprinted from the Journal of the Arnold Arboretum 15: 327-349, 1934.

the cell wall, including the irregular masses of isotropic material commonly found where three or more cells adjoin." VAN ITERSON (1927) similarly differentiates the central isotropic layer as the original or "true middle lamella" and refers to DIPPEL'S middle lamella as the "compound middle lamella."

MANGIN (1891, 1892) demonstrated that the middle lamella of soft tissues is composed of pectic substances, and that "pectose" is intimately associated with cellulose in cell walls which have not been modified by lignification, suberization, etc. He inferred, from macerations of meristematic tissues, that the original partition membrane is of pectic composition and that cellulose is first deposited during the process of secondary thickening. In recent years, RITTER (1925), and others have emphasized the fact that a large proportion of the lignin in wood is located in the middle lamella. Furthermore, SCHORGER (1926) and RITTER (1930) consider that the middle lamella is actually composed of lignin.[1]

In view of the existing confusion concerning what constitutes the so-called middle lamella and regarding its physical and chemical constitution, it seemed desirable to the writers to undertake an extensive investigation of various tissues in an endeavor to clarify the situation. In order to have a clear conception of the so-called middle lamella in the mature xylem, it is necessary to consider the conditions in the cambium and the changes which take place during tissue differentiation. The results of a detailed study of the cambium and of its differentiating and fully differentiated derivatives are recorded in the following pages.

Material and Methods:—In our work on the cambium, the following species were studied:

1. *Pinus Strobus* L.
2. *Juniperus virginiana* L.
3. *Thuja occidentalis* L.
4. *Robinia Pseudo-Acacia* L.
5. *Cladrastis lutea* (Michx. f.) Koch
6. *Fraxinus americana* L.
7. *Acer rubrum* L.
8. *Catalpa speciosa* Warder
9. *Liquidambar Styraciflua* L.

Most of the original observations were made on *Pinus Strobus*, but every phase of the work was checked and rechecked on other species. In dealing with the cambium, it is essential to secure freshly collected material from living trees. This is due to the fact that cambial walls contract so much during dehydration that permanent preparations present a caricature of the original structure.

Transverse and longitudinal sections of the wood of more than 800 genera of gymnosperms and angiosperms were examined in polarized light. The following species were selected for subsequent microchemical investigation.

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[1] It should be noted in this connection, in view of various criticisms of botanical workers, that not only did SCHACHT, STRASBURGER, DIPPEL, and many other botanists subject sections of tissues to chemical treatments, but also that they were cognizant of the fact that the middle lamella of woody tissues is insoluble in concentrated sulphuric acid and in SCHWEIZER'S reagent.

1. *Pinus Strobus* L.
2. *P. radiata* D. Don
3. *Taxodium distichum* (L.) Richard
4. *Sequoia sempervirens* Endl.
5. *Libocedrus decurrens* Torr.
6. *Trochodendron aralioides* Sieb. & Zucc.
7. *Ulmus americana* L.
8. *Tilia glabra* Vent.
9. *Quercus agrifolia* Née
10. *Q. Douglasii* Hook. & Arn.
11. *Prosopis juliflora* DC.
12. *Citrus Limonia* Osbeck
13. *Betula papyrifera* Marsh.
14. *Populus grandidentata* Michx.
15. *P. trichocarpa* Torr. & Gray
16. *Nyssa sylvatica* Marsh.
17. *Robinia Pseudo-Acacia* L.
18. *Liquidambar Styraciflua* L.
19. *Catalpa speciosa* Warder
20. *Fraxinus mandshurica* Rupr.
21. *Rhizophora Mangle* L.
22. *Myodocarpus simplicifolius* Brong. & Gris
23. *Protomegabaria Stapfiana* Hutch.
24. *Homalium guianense* (Aubl.) Warb.
25. *Brackenridgea Hookeri* A. Gray
26. *Tetramerista glabra* Miq.

The last six species are tropical forms which were used for comparison with the species of warm-temperate and cold-temperate regions. Sections were made either from freshly collected wood or from seasoned wood soaked in cold water. No softening agents were employed, as these change the chemical properties of the cell walls.

In studying the optical properties of wood, thin and truly transverse sections of straight-grained specimens are essential. Most of the critical work with polarized light was done on sections 5 μ in thickness. Thicker or slightly oblique sections obscure the finer details and give much distorted images. Chemical tests were made on sections 5 to 15 μ in thickness, depending upon the ease with which the material could be cut.

The Cambium:—Since 1900, most botanists have visualized the cambium as an extremely delicate and very thin-walled tissue. Such a conception is due largely to the study of fixed and dehydrated material and to unfamiliarity with the investigations of SANIO, SCHACHT, STRASBURGER, DIPPEL, and others who worked with freshly cut and undehydrated material. SANIO (1873) demonstrated that the radial walls of the fusiform initials in the cambium of *Pinus sylvestris* are of considerable thickness and that those of adjacent cells are separated by an amorphous intercellular layer or "Zwischensubstanz." SANIO and others erred, however, in their assumption that the occurrence of a "Zwischensubstanz" is an unusual phenomenon in meristems and involves the splitting of an originally entire and homogeneous partition membrane. If they had devoted more attention to the study of ray initials and to the cambia of dicotyledons, they might have recognized the fact that each initial is enclosed, *on all sides,* within a wall of its own which is separated from the walls of adjoining cells by more or less intercellular material. The cambial wall expands as the initial increases in size. The intercellular material is a plastic colloidal substance that passes readily into a semi-liquid phase, thus facilitating those movements and adjustments of cells which are such characteristic features of the actively growing cambium.[2]

[2] For a discussion of these and other phenomena and a description of techniques employed in studying the living cambium, the reader is referred to preceding papers of this series.

In other words, the wall of the cambial initials is a discrete morphological structure which, once formed, maintains its identity under all conditions of growth and development, whereas the intercellular layer is passively molded into various forms and possesses few of the attributes of a true membrane.

DIPPEL (1898) concluded, in the second edition of "Das Mikroskop," that the walls of the cambium are isotropic. He figured (Part II, page 573) a transverse section of the cambium and its derivatives as seen in polarized light with crossed nicols. The walls of the initials and of the enlarging derivatives are dark, whereas the "primary walls," formed during the later stages of the differentiation of the xylem and phloem, are brilliant. This was in line with DIPPEL's contention that the cambial walls—which are incorporated in the isotropic "intercellular substance" of mature tissues—are composed of "pectose," whereas the subsequently formed "primary walls" consist largely of cellulose.

In view of the accuracy of DIPPEL's delineations of polarized light pictures of mature tissues, which are much superior to those of recent workers, it is difficult to understand how he could have been led so far astray in his treatment of the cambium. The walls of both ray initials and fusiform initials are birefringent. Furthermore, an examination of transverse and longitudinal sections of the cambia of a wide range of gymnosperms and angiosperms indicates that all cambial walls are anisotropic, but that the intensity of double refraction, angles of extinction, etc. vary somewhat from plant to plant. The anisotropy is not due to "rod" or "plate" double refraction, since the walls are birefringent even when they are saturated with a liquid of the same refractive index as their own. The intercellular substance, on the contrary, is truly isotropic and is dark at all angles and in all planes of section. PLATE 4, FIG. 5, a highly magnified portion of a thin tangential, longitudinal section of the cambium of *Pinus Strobus*, shows the anisotropic walls of two adjacent fusiform initials and the dark isotropic intercellular material between them.

It should be noted, in this connection, that cambial walls are characterized by having more or less numerous plasmodesmata which may be uniformly distributed or aggregated in thinner areas of the walls, *i.e.*, in so-called primary pit-fields. These primary pit-fields are more closely approximated in the radial walls of the fusiform initials of most dicotyledons than they are in the case of gymnosperms. Thus, the walls have a beaded appearance in sectional view. The intercellular material is much reduced in amount in the region of the primary pit-fields and between the tangential walls of the fusiform initials of gymnosperms, but its presence may be clearly demonstrated by adequate modern techniques.

Our investigations of the cambium indicate that the isotropic intercellular material is composed largely of polyuronides and contains little or no cellulose, and that the anisotropic cambial walls contain both cellulose and polyuronides. When freshly cut sections of the living cambium are subjected to standard chemical treatments used in the extraction of pectic compounds, *e.g.*, ammonium oxalate or dilute acids followed by dilute alkalis, the intercellular material dissolves, leaving the dissociated cambial

walls, which are anisotropic and give a typical blue color with chloro-zinc iodide or iodine and sulphuric acid. The dissociated cambial walls are insoluble in 30% sodium hydroxide, or after prolonged treatments in hot dilute acids followed by alkalis, but dissolve completely in such standard solvents of cellulose as 72% sulphuric acid or SCHWEIZER's reagent. When freshly cut sections of the cambium are used, the walls are also completely soluble in 72% sulphuric acid which dissolves both cellulose and polyuronides, but when treated with SCHWEIZER's reagent, there remains a swollen residue of the cambial walls which is isotropic and no longer produces a blue color with iodine and sulphuric acid. This residue stains intensely with ruthenium red and disappears in standard solvents for pectic compounds.

The physical properties, chemical solubilities, and colorations with ruthenium red, chloro-zinc iodide, and iodine-sulphuric acid indicate, therefore, that cambial walls are composed of a mixture of cellulose and polyuronides, and that they are separated by intercellular material which consists largely, if not entirely, of polyuronides. In other words, our observations and analyses are in substantial agreement with those of MANGIN (1893), CARRÉ and HORNE (1927), and many others who have dealt with other types of soft, unlignified tissues. Furthermore, we have worked, during the last two years, in close coöperation with Professor ERNEST ANDERSON, who has extracted the polyuronides from the cambium and young phloem of *Robinia Pseudo-Acacia* and has determined that they are of a pectic nature.

The walls of cambial initials are characterized, from a physiological point of view, by their capacity for growth and expansion and for undergoing various reversible changes, *e.g.*, seasonal variations in thickness and in colloidal properties. During the resting season, the cambial walls tend to be thicker and to have the general properties of a firm gel, whereas during the active growing season, they appear to be thinner and more pliable.

Tissue Differentiation:—The specific changes that the cambial walls undergo during the differentiation of the xylem and phloem vary markedly, depending upon the particular types of tissue cells that are to be formed. In the case of those parenchymatous elements of the phloem, which retain their capacity for growth and enlargement, the original cambial walls are but slightly modified in form and thickness during tissue differentiation, and no true secondary walls are formed.

On the contrary, in the case of tissue cells which undergo irreversible changes and form layers of true secondary wall thickening, the cambial walls and the intercellular material tend to become thinner and considerably modified in form during the process of cell enlargement. Vestiges of the original primary pit-fields and intervening thickenings may persist, however, in parts of cells which are not subjected to excessive expansion. Such vestiges are of common occurrence in the radial walls of the tracheids of most conifers and of the terminal wood parenchyma of many dicotyledons.

The enlargement of those derivatives of the cambium which form tracheids, fiber-tracheids, libriform fibers, and bast fibers involves an increase in the cross-sectional area of the cells and elongation due to apical sliding

growth. The cambial wall expands as the cell increases in size, but layers of secondary thickening are not deposited until the cell has attained its mature diameter. As the cambial walls increase in surface area and decrease in thickness, the layer of intercellular material becomes so tenuous that it is difficult to differentiate it by the use of ordinary microscopical methods, except in the angular spaces where three or more cells adjoin. Lignification is initiated during the earlier stages of secondary wall formation, and, in the species studied by us, is first visible in the cambial walls and the intercellular substance. It spreads centripetally through the successively formed layers of the secondary wall. The lignification of the cambial walls is usually very intense, except in the pit-membranes and crassulæ; whereas that of the secondary layers is extremely variable, particularly in dicotyledons. The difficulty of differentiating the cambial walls from the intercellular material is accentuated by lignification, which alters the staining reactions and the indexes of refraction of the three adjoining layers, and makes them appear as a single homogeneously refractive unit. Furthermore, although the cambial walls are clearly anisotropic during the period of cell enlargement, their anisotropy tends to be obscured after the initiation of secondary wall formation and of lignification.

During the transformation of sapwood into heartwood the walls may absorb more or less "Gerbstoffe" which further alters their solubilities, optical properties, and staining reactions. Similarly, during the seasoning of sapwood, the walls may become saturated with various substances that are contained in the vacuoles of the living cells or are produced by the dying protoplasts. Therefore, critical observations should be made, if possible, upon freshly cut sections of living sapwood.

The Mature Secondary Xylem:—As will be shown in a subsequent paper of this series, the secondary wall is exceedingly complex, from a morphological point of view. It varies greatly not only in different tissue cells, but also in homologous walls of different plants.

In the case of normal tracheids[3] (TEXT FIG. 1), the secondary wall consists of three layers:[4] (1) a relatively narrow outer layer (c), (2) a narrow inner layer (e), and (3) an intervening layer (d) of variable thickness. The narrow inner and outer layers are characterized by having their fibrils of cellulose oriented more nearly at right angles to the longer axis of the cell, whereas the intervening layer is characterized by having them arranged either longitudinally or diagonally. The secondary walls project inward and are not in contact with the cambial walls in those parts of the cell where bordered pits are formed (TEXT FIG. 2). The narrow inner and outer layers of the secondary wall come together in the rim formed about the pit-aperture.

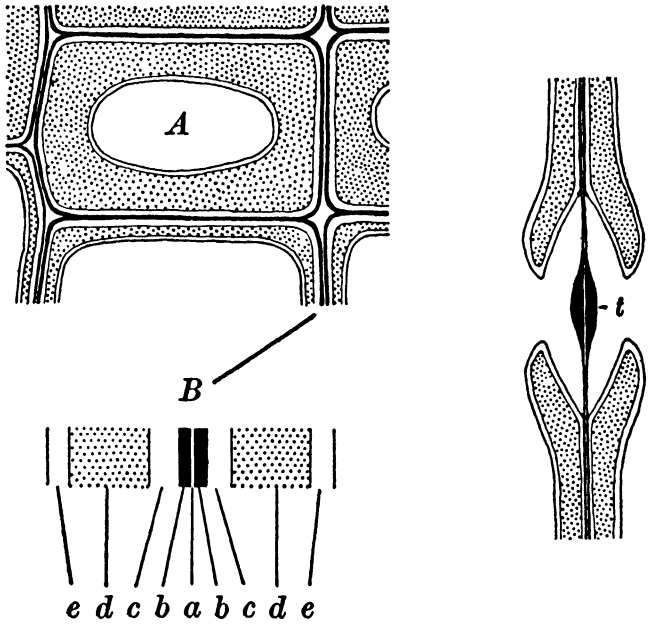
When unstained sections of wood are examined in white light (PLATE 4, FIG. 1), the narrow outer layers of the secondary walls of adjoining tracheids tend to blend with the cambial walls and the intercellular substance, forming an apparently homogeneous refractive layer, *i.e.*, the so-

[3] Excluding "Rotholz" tracheids and other specialized and peculiar types.

[4] We are not concerned here with the submicroscopic layering which is revealed by swelling the walls and by other drastic treatments.

called middle lamella of DIPPEL and of other botanical writers. That this middle lamella is actually a compound structure may be determined by a careful study of the layering in the bordered pit-pairs (compare PLATE 4, FIG. 1 and TEXT FIG. 2) or by transferring the sections to liquids of varying indexes of refraction.

When unstained transverse sections of wood are examined in polarized light with crossed nicols, the images vary considerably, depending upon a number of fluctuating factors. The narrow inner and outer layers of the secondary wall exhibit double refraction and are brilliant (PLATE 4, FIG. 2),



TEXT FIGURES 1-2.— FIG. 1 (*left*): *A*, Diagrammatic transverse section of one entire tracheid and of parts of seven others; *B*, section of adjacent walls more highly magnified; *a*, truly isotropic intercellular substance, *b*, cambial or primary wall, *c*, outer layer of secondary wall, *d*, central layer of secondary wall, *e*, inner layer of secondary wall. — FIG. 2 (*right*): Diagrammatic bordered pit-pair in sectional view, showing pit-membrane, torus, and pit-apertures or openings in the secondary walls. Wall layers as in TEXT FIG. 1, *t*, torus or central thicker portion of the pit-membrane. The torus and the pit-membrane are composed of two cambial walls and of intercellular material.

whereas the central layer of the secondary wall is dark or less birefringent. In other words, the phenomena observed between crossed nicols are closely correlated with the orientation of cellulose micellæ. Where these are arranged parallel to the long axis of a tracheid, a layer appears dark in cross sections; where they are oriented nearly at right angles, a layer is brilliant. The brightness varies at intervening angles.

The closely approximated outer layers of the secondary walls of adjoining cells allow so much light to get by the crossed nicols that they fog or obscure (PLATE 4, FIG. 2) the three tenuous intervening layers, except in

extremely thin sections (PLATE 4, FIGS. 3, 4). But in the case of the latter sections, the cambial walls and the intercellular material appear as a single isotropic layer, *i.e.*, DIPPEL's intercellular substance, the so-called middle lamella of VAN ITERSON, RITTER, and others. The isotropic aspect of the cambial walls is due largely to the fact that they are so feebly anisotropic in comparison with the brilliant outer layers of the adjoining secondary walls, that they appear dark in contrast to them. Therefore, it is difficult to demonstrate the anisotropy of cambial walls when they are in contact with secondary walls, except under favorable circumstances. In the case of the mature xylem, the best material for this purpose occurs in those cells or portions of cells in which the cambial walls do not thin down excessively during tissue differentiation. Thus, the feebly anisotropic cambial walls are visible between the brilliant outer layers of the secondary walls. By treating thin sections of the mature xylem with 65% phosphoric acid or diluted SCHWEIZER's reagent, there is sufficient swelling so that it is possible to see the cambial walls as an anisotropic entity distinct from the outer layers of the secondary walls.

When woods are prepared for sectioning by the usual procedure of boiling in water and softening in hydrofluoric acid, the cambial walls and the intercellular material react as a unit—*i.e.*, the so-called middle lamella or primary wall of various botanical writers—and stain more intensely in ruthenium red and haematoxylin than do the three layers of the secondary wall which react as another unit. It is possible to differentiate this homogeneously-staining so-called middle lamella into its constituent layers, not only by optical methods but also by chemical methods and differential staining. Thus, when sections of freshly cut wood, or of seasoned wood soaked in cold water, are treated with solvents for removing polyuronides, the cambial walls may be stained without appreciably coloring either the secondary walls or the intercellular material (PLATE 5, FIG. 1). By accurately controlled delignification, followed by treatment in SCHWEIZER's reagent, it is possible to dissolve both the intercellular material and the secondary walls, leaving the cambial walls intact (PLATE 5, FIG. 2). Owing to the fact that they may be unlignified, such minute and delicate structures as tori and pit-membranes may be resolved into their constituent layers by the use of solvents for polyuronides.

As shown by HARLOW (1928), the residues which remain after treating sections of wood with 72% sulphuric acid and subsequently boiling in 3% sulphuric acid, vary considerably in different species, depending upon the intensity of lignification. In the case of the dicotyledons, the secondary walls of the tracheids and fiber-tracheids frequently dissolve or disintegrate into a finely granular residue, whereas the more intensely lignified walls of the vessels do not. In most of the Coniferæ, and in certain of the more heavily lignified dicotyledons, the secondary walls of the tracheary elements swell greatly but do not dissolve or disintegrate. Although the residues of the secondary walls may or may not be present after the action of 72% sulphuric acid, the compact remains of the so-called middle lamella persist. Such "middle lamella" residues always consist of at least three layers, and usually are composed of five layers (PLATE 5, FIG. 3). Five-layered struc-

tures occur where the residues of the heavily lignified outer layers of the secondary walls remain closely attached to the residues of the cambial walls and of the intercellular substance. They may be differentiated from three-layered residues by the structure of the bordered pits (compare (b) in PLATE 5, FIG. 3 with TEXT FIG. 2). If the residues are five-layered, the pits retain their bordered appearance.

Since lignin and pectic compounds are isotropic, the anisotropy of the cambial walls suggests that the cellulose which is present originally persists during tissue differentiation and during the physiological processes of lignification. But what becomes of the polyuronides? Are they replaced by, or transformed into, lignin, or are they merely masked by lignification, which modifies their chemical solubilities, staining reactions, and other properties? Since the so-called middle lamella is intensely lignified, and since the presence of lignin may alter the reactions of the polyuronides, these questions should be attacked by a study of delignified material. It is essential, however, in delignifying wood to use solvents that do not simultaneously remove both lignin and polyuronides. For example, hot sodium sulphite macerates, and removes pectic substances from the cambium and other soft tissues. Thus, the fact that sections of wood are quickly macerated by alternate chlorinations and treatments with hot sodium sulphite does not indicate necessarily that the intercellular layer is composed solely of lignin rather than of a mixture of lignin and polyuronides.

An extensive study of the delignification of wood by standard procedures indicates that delignification and maceration are not necessarily coincident reactions. When sections of various dicotyledons are given alternate treatments with chlorine water and 10% ammonium hydroxide in either aqueous or alcoholic solution at room temperature, until they no longer show traces of the MÄULE reaction, *they do not macerate,* although they dissolve completely in 72% sulphuric acid. The delignified sections *may be macerated,* however, by subsequent treatments with standard solvents for polyuronides. In other words, the isotropic intercellular substance of mature wood appears to be composed of two substances, lignin and polyuronides, which may be separated by their differential solubilities.

In delignified sections of wood, the cambial walls react much as they do in freshly cut sections of the cambium. In both types of sections, they persist as a non-cellulose residue after treatment with SCHWEIZER'S reagent (PLATE 5, FIG. 2). Furthermore, both types of sections are completely soluble in cuprammonium hydroxide after treatment in solvents of polyuronides. In other words, the cambial walls of the mature wood appear to be composed of a mixture of lignin, cellulose and polyuronides.

If the polyuronides originally present in the cambium persist in the mature xylem, and are more or less masked by lignification, it should be possible to extract substances of a pectic nature from wood. This has been accomplished by Professor ANDERSON. It should be emphasized in this connection, however, that, if the polyuronides of the so-called middle lamella are of a pectic nature whereas those of the secondary wall are hemicelluloses—as appears to be the case—the yields of the former when computed as percentages of the total dry weight of the wood must of neces-

sity be very low. This is due to the fact that the volume of the cambial walls and intercellular substance is very small in comparison with that of the thick secondary walls. In the cambium and other soft tissues, on the contrary, the intercellular substance and the cambial or primary walls form a high percentage of the total dry weight of the tissue. Therefore, the yields of pectic compounds from a given weight of such tissue is high in comparison with that of mature wood. It is evident accordingly that a transformation of pectic compounds into hemicelluloses and lignin is not essential to account for the fact that soft tissues give high percentages of pectic substances and low yields of hemicelluloses, whereas wood gives high percentages of hemicelluloses and lignin and low yields of pectic compounds.

Discussion:—The data recorded in preceding paragraphs indicate that the walls of the cambial initials contain cellulose and are truly anisotropic, and that these walls retain their cellulose and anisotropy during tissue differentiation. Similarly, there is considerable cumulative evidence to indicate that the polyuronides which are present in the walls of the cambial initials and constitute the bulk of the intercellular material, are carried over into the mature wood, and are not entirely replaced by, or transformed into, lignin.

RITTER's arguments for considering that the "middle lamella" is composed largely of "lignin" rather than of pectic compounds are the following: (1) When sections of mature wood are subjected to a standard process for isolating cellulose—*i.e.*, alternate chlorinations and treatments with hot sodium sulphite to remove lignin—the "middle lamella" dissolves, leaving the secondary walls, which are birefringent and are soluble in 72% sulphuric acid. (2) Conversely, when sections are treated with 72% sulphuric acid, the "middle lamella" remains intact, but may be dissolved by subsequent chlorinations and treatments with hot sodium sulphite. (3) When sections are treated with standard solvents of pectic compounds, the "middle lamella" is not dissolved. (4) Ruthenium red is not specific for pectic compounds.

We have shown that delignification and maceration, produced by chlorinations and treatments with dilute alkalis, are not necessarily coincident reactions. Sections of wood may be delignified without dissolving the so-called middle lamella. Such delignified sections may be macerated by the subsequent use of standard solvents for polyuronides. Where sections of wood are macerated by repeated chlorinations and treatments in hot sodium sulphite, the truly isotropic intercellular material dissolves, but the cellulose-containing cambial walls do not. In other words, all of the middle lamella —as defined by VAN ITERSON, RITTER, and others—does not dissolve when sections are macerated by these standard procedures for removing lignin. Furthermore, we have found that hot sodium sulphite alone is a rapid macerating agent for the cambium and other meristems. Thus, chlorination and hot sodium sulphite remove polyuronides as well as "lignin."

We have shown that the "middle lamella" residues left after treating wood with 72% sulphuric acid always consist of at least three layers and

usually of five layers. It should be emphasized, in addition, that lignin is not the only substance present in plant tissues that is insoluble in 72% sulphuric acid. DADSWELL (1931) has shown that much of the "Gerbstoffe" which fills the lumens and saturates the walls of the xylem cells of *Eucalyptus* and of other genera remains as a residue after standard treatments with 72% sulphuric acid. Our own observations upon sections of a wide range of angiosperms and gymnosperms indicate that, in addition to various types of "Gerbstoffe," coagulated protoplasm and nuclei and the contents of certain types of vacuoles may persist after standard treatments with sulphuric acid. Although the walls of the freshly cut cambium dissolve readily in 72% sulphuric acid, they do not do so after soaking in tannic acid or if they are allowed to absorb certain phenolic substances that are contained in the vacuoles of the living cells. Such facts as these suggest that in the case of wood, the so-called lignin residues may actually consist of a varying mixture of insoluble substances.

The ruthenium red reaction was developed by MANGIN (1893) during his extensive investigations of the distribution of pectic substances in plant tissues. Although it is not specific for pectic substances, it is extremely useful, when employed with proper precautions, in analytical work. Our investigations of a wide range of organic compounds of known chemical composition indicate that ruthenium red in dilute solutions stains three distinct categories of substances that are of common occurrence in the cambium and its derivative tissues: (1) coagulated protoplasm and nuclei, (2) certain lipoids, and (3) polysaccharides that contain glucuronic or galacturonic acid, *e.g.*, pectic compounds, gums, mucilages, hemicelluloses, oxycellulose, etc. In view of the fact that the first category of substances may be differentiated microscopically and that the second category are removed by preliminary treatments with lipoid solvents, an intense staining of cell walls with ruthenium red is strong presumptive evidence of the presence of polyuronides. Thus, the ruthenium red reaction is extremely useful, not only in studying the distribution of polyuronides in plant tissues, but also in chemical analyses, as a means of visually following the effects of successive steps in the extraction of such substances. We have utilized it effectively in our cooperative investigations with Professor ANDERSON as a means of modifying chemical techniques for the extraction of pectic compounds and hemicelluloses, and of securing larger yields of these substances from specific tissues.

RITTER'S data may be interpreted as demonstrating that the so-called middle lamella is strongly lignified, but they do not provide a reliable basis for concluding that it is composed of lignin rather than of cellulose, polyuronides, and lignin. Our observations, on the contrary, tend to support the views of SCHMIDT and his co-workers (1925, 1927), who have argued that lignin occurs in close association with polyuronides.

As stated in our introduction, there is no consensus of opinion concerning the use of such terms as intercellular substance, middle lamella, primary wall, etc. Serious discrepancies in morphological, biophysical, and biochemical investigations have arisen not only owing to differences in definition, but also as a result of inconsistencies in applying specific terms to

different tissues and even to the same tissue when different techniques are employed. It is essential to clarify the situation, if possible.

That DIPPEL and others are not consistent in their use of the terms, *intercellular substance, middle lamella,* and *primary wall,* is indicated by a careful study of their texts and particularly of their illustrations of different tissues (TABLE I). Thus, in dealing with the secondary xylem, DIPPEL (1898) obviously uses the term *middle lamella* in referring to a five-layered structure which consists of two cambial walls, two layers of secondary thickening, and a layer of intercellular material (TEXT FIG. 1). His isotropic substance—*i.e.*, VAN ITERSON's (1927) "original or true middle lamella" and

TABLE I· *Comparative Terminologies:—*

SOFT TISSUES		PROPOSED TERMINOLOGY FOR SOFT OR WOODY TISSUES (TEXT FIGURES 1 and 2)		WOODY TISSUES
		(a) **Middle Lamella** or		
Intercellular Substance (DIPPEL)	=	**Intercellular Substance**		
		(b)		
Primary Wall (DIPPEL)	=	**Cambial or Primary Wall**		
		(c) **Outer Layer of Secondary Wall**	=	Primary Wall (DIPPEL and others)
		(d) **Central Layer of Secondary Wall**	=	Secondary Wall (DIPPEL and others)
		(e) **Inner Layer of Secondary Wall**	=	Tertiary Wall (DIPPEL and others)

c + b + a + b + c = Middle lamella of DIPPEL and intercellular substance of earlier botanists. b + a + b = Intercellular substance (DIPPEL), middle lamella (VAN ITERSON and others), and primary wall (various botanists).

the middle lamella or primary wall of various other writers—actually consists of two feebly anisotropic walls and a layer of truly isotropic intercellular material. His primary wall is the strongly isotropic first-formed layer of secondary thickening. On the contrary, in dealing with the unlignified parenchyma of other tissues, DIPPEL frequently uses the term *primary wall* in referring to layers that are homologues of the cambial walls, and the term *intercellular substance* in designating the truly isotropic intercellular material. It should be emphasized, in this connection, that many of the most serious discrepancies are due, on the one hand, to confounding the cambial walls with the intercellular material, and, on the other hand, to confusing them with the narrow outer layer of the secondary wall.

The question arises, accordingly, as to what changes are advisable in

the definition and use of such terms as *middle lamella, intercellular substance,* and *primary wall.* Are they so firmly established in the literature that they should be retained and redefined, or has their varied use led to so much confusion that they should be replaced by new terms?

Our own conclusions, based upon a detailed study of the cambium and its derivatives and upon preliminary investigations of other meristems and their derivatives, are (1) that the term *primary wall* should not be applied to the first-formed layer of secondary thickening but should be used solely in designating the cambial wall and its homologues in other tissues, and (2) that the term *middle lamella* should be used synonymously with *intercellular substance* in referring to the truly isotropic layer of intercellular material. If some term of convenience is required in referring to those complexes of lignified layers which appear more or less homogeneous under certain specific conditions, VAN ITERSON'S term, *compound middle lamella* is available. It should be clearly recognized, in this connection, however, that the compound middle lamella will be five-layered or three-layered, depending upon the specific techniques used in examining the tissue.

Our reasons for advocating these changes in terminology are the following: There are two distinct and fundamentally different categories of cell walls. Meristematic elements and such of their derivatives as retain a potentiality for growth and enlargement have walls which are characterized by their capacity for growth and extension and for undergoing reversible changes, *e.g.*, in thickness. On the contrary, tissue cells which undergo irreversible changes and thus lose their potentiality for growth and enlargement may form a supplementary or *secondary wall* which tends to be more or less conspicuously laminated.[5] The term *primary* wall has been used in referring to the walls of meristematic elements, to the walls of their enlarging derivatives, and to the first-formed layer of true secondary thickening in lignified tissues. To continue to apply the term *primary wall* to two entirely different structures must inevitably perpetuate the existing confusion. Therefore, in view of the fact that the cambial wall—and its homologues in other tissues—is a discrete morphological structure which maintains its identity under all conditions of growth and development, it seems advisable to designate it as the *primary wall.* Any terminology which attempts to differentiate successively formed layers of secondary thickening as primary, secondary, and tertiary walls breaks down completely when applied to all types of cells, *i.e.*, to vessels, fibers, sclerenchyma, etc.

The term *middle lamella,* which was used originally as a substitute for *intercellular substance,* has been employed in the case of lignified tissues in referring to five-layered or three-layered structures and in the case of soft unlignified tissues in designating a layer of truly isotropic material. In view of the fact that there has been an increasing tendency to apply the term to a supposedly isotropic layer in lignified tissues, it seems desirable to restrict the term to the truly isotropic *intercellular substance* which separates the primary walls of adjoining cells.

[5] This differentiation of primary and secondary walls is similar to that used by BALLS in his study of the structure of the cotton hair.

The objection may be raised that the partition membrane is secreted between the halves of the split cell plate, that it originates as a single unit which has a "primary cleavage plane," and therefore that the two adjacent cambial walls and the subsequently formed intercellular material should be designated as the middle lamella or primary wall. The conception of a cell plate which originates from central thickening of spindle fibers and divides to form two protoplasmic membranes, and of a partition wall which is secreted between the halves of the split cell plate and possesses a predetermined plane of cleavage, rests upon entirely inadequate cytological evidence. It has been severely criticized in recent years by ROBYNS (1929) and others and is questioned by most of those who have worked with living cells and are familiar with the physico-chemical properties of living protoplasm. The colloidal properties of the spindle, the cell plate, and first-formed partition membrane are such that they tend to become more or less profoundly modified during fixation and in injured or plasmolyzed living cells. There is no reliable evidence at present to refute MANGIN's suggestion that pectic substances are secreted between the newly formed protoplasmic membranes and that mixtures of cellulose and pectic substances are not formed until subsequently.

In the case of cambial initials and of many other cells, a fraction only of the cambial wall originates during cytokinesis. A large proportion of the wall is formed by the growth and extension of its existing surfaces. Therefore, in view of the fact that each cambial wall is a discrete morphological structure which maintains its identity under all conditions of growth and development, we do not believe that terminology should be governed solely by phenomena which occur during cytokinesis.

SUMMARY AND CONCLUSIONS

1) A detailed study of the cambium indicates that each initial is enclosed within a wall of its own which is separated from the walls of adjoining initials by more or less intercellular material.

2) The cambial wall is composed largely of cellulose and polyuronides, and is truly anisotropic. It is characterized by its capacity for growth and extension and for undergoing reversible changes in thickness. It is also characterized by possessing plasmodesmata which may be uniformly distributed or aggregated in more or less conspicuous primary pit-fields.

3) The amorphous intercellular material, on the contrary, is composed largely, if not entirely, of polyuronides and is truly isotropic. It is characterized by its plasticity which facilitates those movements and adjustments of cells which are such typical features of the actively growing cambium.

4) In other words, the wall of the cambial initial is a discrete morphological structure which maintains its identity under all conditions of growth and development, whereas the intercellular material is passively molded into various forms and possesses few of the attributes of a true membrane.

5) In the case of those derivatives of the cambium which retain their capacity for growth and enlargement and for undergoing reversible changes, the cambial walls are but slightly modified during tissue differentiation, and no supplementary walls are formed.

6) On the contrary, in the case of tissue cells which undergo irreversible changes and form layers of true secondary thickening, the cambial walls and the intercellular

layer become thinner and considerably modified in form during the process of cell enlargement. Furthermore, their optical properties, chemical solubilities, and staining reactions are altered or masked by intense lignification.

7) It is possible to demonstrate, however, that the cambial walls retain their anisotropy during and after tissue differentiation, and, by accurately controlled delignification, to unmask the original chemical solubilities and staining reactions of both the cambial walls and the intercellular material. There is, in fact, much cumulative evidence to indicate that the original cellulose and polyuronides are not completely replaced by or transformed into lignin during tissue differentiation.

8) Thus, the putative "isotropic middle lamella" of the mature xylem is not a homogeneous layer, but consists of two lignified anisotropic cambial walls and an intervening, truly isotropic layer of lignified material.

9) Residues of the so-called middle lamella, obtained by the action of 72% sulphuric acid on mature wood, always consist of at least three layers, *i.e.*, the residues of two cambial walls and of the intercellular material.

10) In macerations produced by repeated chlorinations and treatments with hot sodium sulphite, which dissolves both lignin and pectic compounds, a portion only of the so-called middle lamella dissolves, *i.e.*, the truly isotropic intercellular material. The cellulose-containing cambial walls persist and adhere to the layers of secondary thickening.

11) Delignification of the so-called middle lamella and maceration are not necessarily coincident reactions. By carefully controlled chlorinations and treatments with 10% ammonium hydroxide at room temperatures, sections of wood may be delignified without dissolving the so-called middle lamella. Such delignified sections may be macerated, however, by subsequent treatments with standard solvents of pectic substances. In other words, the isotropic intercellular substance of mature wood appears to be composed of two substances, lignin and pectic compounds, which may be separated by their differential solubilities.

12) Serious discrepancies in the use of such terms as *intercellular layer, middle lamella,* and *primary wall* are due not only to differences in the definition of these terms, but also to inconsistencies in applying them to different tissues, and even to the same tissue when different techniques are employed.

13) 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 suggest (1) that if the term *middle lamella* is to be retained, it should be used synonymously with *intercellular substance* in referring to the truly isotropic layer of intercellular material and (2) that the term *primary wall* should no longer be applied to the first-formed layer of secondary thickening, but should be used solely in designating the cambial wall and its homologues in other tissues.

Chapter 7

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

in collaboration with THOMAS KERR

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.

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 ade-

* Reprinted from the Journal of the Arnold Arboretum 16: 273-300, 1935.

quate 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, 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 (63), 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

[1] For comprehensive reviews of the literature relating to this subject, the reader is referred to VAN ITERSON (1927, 1933) and FREY-WYSSLING (1935).

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.

THE PRINCIPAL LAYERS OF THE SECONDARY WALL

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, perfectly transverse sections of such cells are examined in polarized light between crossed nicols (PLATE 6, 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. 4) 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 (1867-69) 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 (PLATE 4, FIGS. 3, 4). 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 PLATE 4, FIGS. 2, 3 & 4).

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. 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 (PLATE 7, FIG. 18), 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 (PLATE 6, FIGS. 3, 4). 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 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 (PLATE 13, FIG. 8), 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 (PLATE 6). Thus, the central layer exhibits strong double refraction in longitudinal sections and isotropy or relatively feeble double refraction in transverse sections; 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 PLATE 7, 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 (1935), a major axis ($N\gamma$) 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 PLATE 7, FIGS. 17, 18, 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 PLATE 12, FIG. 3.

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 (1928) and by ASTBURY and his co-workers (1932). Furthermore, there is much cumulative evidence[2] 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.

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 intensified by differential staining (PLATE 7, FIG. 20). These subsidiary layers 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

[2] This evidence has recently been summarized and discussed by FREY-WYSSLING (1935).

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. These truly isotropic layers may be accentuated by differential staining and are clearly visible in ordinary light. They present some difficulties, however, when sections are examined in polarized light between crossed nicols. For example, the entire laminated structure in PLATE 7, FIG. 20, 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. 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.

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. A detailed discussion of these cells and of other non-fibrous types is reserved for subsequent papers of this series.

THE FINER VISIBLE STRUCTURES OF THE SECONDARY WALL

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.

PLATE 8, FIG. 2 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 (PLATE 10, FIG. 4). 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, 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.[3]

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 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. 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 (PLATE 9, FIG. 1) 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 PLATE 8, FIG. 2, 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, are so feebly birefringent that they appear to be comparatively isotropic.

[3] 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.

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 (PLATE 9, FIG. 2), 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 PLATE 8, FIG. 2 and PLATE 9, FIG. 2, 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 (PLATE 8, FIG. 2), or they may be diffused in finer radioreticulate patterns (PLATE 10, FIG. 3), 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 or to zones of varying intensities of lignification. 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 (PLATE 10, FIG. 2), and in the fiber-tracheids or libriform fibers of many dicotyledons. 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 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 more porous, feebly anisotropic lamellae.

It should be emphasized, in this connection, that the concentric structure of swollen cotton hairs—to which the work of BALLS 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 feebly anisotropic. During subsequent swelling (PLATE 10, FIG. 1), 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, of coniferous tracheids and of many fiber-tracheids 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 contents, 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 PLATE 10, FIG. 1, 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 libriform 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 (PLATE 8, FIG. 1) are of common occurrence in the fiber-tracheids and libriform fibers of dicotyledons.[4] In these 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* (PLATE 10, FIG. 5). In the case of the more heavily lignified zones of such central layers, 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 (1929), 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.[5] 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 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 noncellulosic 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. On the contrary, where the two optically different parts are segregated into coarser structural complexities, 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, 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

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

[5] 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.

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.

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. 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 PLATE 7, FIG. 18 and FIG. 20, the two cell walls are of a fundamentally different type. In the libriform fibers of various representatives of the *Flacourtiaceae,* 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 (PLATE 7, FIG. 19). 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, in 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. 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 PLATE 8, FIG. 2 are of not uncommon occurrence in the individual anisotropic layers of certain bast fibers; whereas in *Pandanus* (PLATE 12), 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, 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 (PLATE 12, FIG. 2), 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 (1931).

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* 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, or helical bands. The internal layers of cellulose—which may be slipped apart—expand more or less in unison 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 (PLATE 7) may be disrupted by the lateral expansion of the broader layers (PLATE 12) and in the case of entire, delignified fibers, may give rise to constricting rings and helical bands. 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. 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 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

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 (1931, 1934), 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 (1928) argues that it is possible to dissect the secondary wall by chemical means into concentric lamellae which may be slipped apart. 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.

"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 (1934) 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 (1934), 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 (1925), measured the width of fibrils and obtained values of some 0.3-0.5 μ. Balls and Hancock (1922), proceeding upon the assumption that lamellae[6] 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 (1935) tabulates the dimensions of fibrils as $0.4 \times 0.4 \times 100$ 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, dermatosomes have dimensions of $0.4 \times 0.4 \times 0.5$ μ; whereas Farr and Sisson (1934) state that ellipsoid bodies prepared from cotton have axes of 1.1 μ and 1.5 μ. Lüdtke (1928) believes that dermatosomes are held together by his "Fremdsubstanz"; whereas Farr and Eckerson (1934*a*) 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 evi-

[6] 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.

dence, 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, as in *Pinus, Poraqueiba,* and *Siparuna,* 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. In PLATE 10, FIG. 1 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 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 (1884) and of SCHWENDENER (1894) 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, 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 (1933) has summarized the modifying effects of oxidation and hydrolysis as follows: "The loss of strength and fall in viscosity which ac-

company 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 (1933). Ruthenium red behaves, in some respects, as a basic dye, and the staining of ellipsoidal particles, obtained by treating cotton with relatively strong acid, 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 (1931) or by Farr and Eckerson (1934*a*). 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, is based upon measure-

ments 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 (1892) 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 (1864*b*), 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.

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 (1933) has shown that certain misconcep-

tions regarding *Valonia* might have been avoided by an acquaintance with the work of CORRENS (1892) and others upon the visible structure of the walls of algae. PRESTON (1934) 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 (1930) and RITTER and STILLWELL (1934) 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 (1929), 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 (1928); 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 (1934) 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 structure, 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.

Chapter 8

THE ORIENTATION OF CELLULOSE IN THE SECONDARY WALL OF TRACHEARY CELLS *

in collaboration with MARY R. VESTAL

MUCH ATTENTION has been focused, in recent years, upon the study of the arrangement of chain molecules, micelles, and fibrils in the cell walls of the cotton hair, bast fibers, tracheids, and other types of commercially important fibers. Various methods of studying the arrangements of the structural units are employed by different investigators:

1) The study of visible fibrils and striations, based upon the assumption that the long axis of the micelles is oriented parallel to these structures.
2) The study of pit-orifices and of mechanically induced cracks, based upon the assumption that these structures are oriented parallel to the fibrillar axis.
3) The study of extinction angles, of dichroism, and of other phenomena in polarized light.
4) The study of X-ray diagrams.

Each of these methods yields significant data under favorable circumstances, but each is subject to serious limitations when applied to miscellaneous types of cell walls.

Even when coarse aggregations of fibrils and striations are clearly visible in surface views of unswollen secondary walls, it frequently is difficult to determine with certainty, whether a specific orientation occurs throughout the wall or in one of its layers only. Swelling the wall to reveal its finer structure is effective in dealing with the broad central layers—provided allowances be made for distortions due to longitudinal contraction and lateral expansion—but such treatments commonly disrupt and conceal the structure of the tenuous inner and outer layers.

In the case of thick secondary walls of the 3-layered type, the long axis of the slitlike pit-orifices is oriented parallel to the fibrillar axis of the central layer, but affords no evidence regarding the fibrillar arrangements of the inner and outer layers. In thin-walled cells, the orifices of the pits commonly afford no clue regarding the fibrillar orientations of any of the layers. Similarly, mechanical cleavages or seasoning cracks may afford valuable evidence in the case of layers which have pronounced radio-longitudinal or radio-helical porosities, but are difficult to interpret accurately in the case of other layers.

Phenomena visible under the polarization microscope are significant where material can be oriented so that the polarized light passes through single layers; but accurate interpretations are difficult where the light passes through several superimposed layers of varying thickness and of different fibrillar orientations. Thus, in the case of transverse sections of 3-layered

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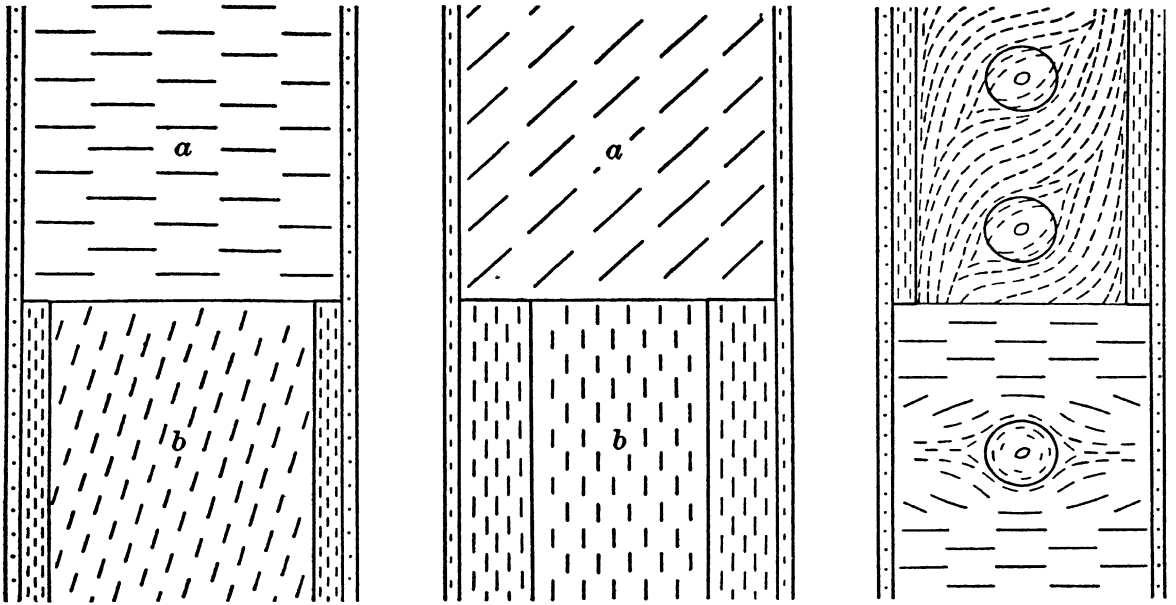


TEXT FIGURES 1-3.— FIG. 1 (*left*): Diagrammatic illustration of the orientations of cellulose in the outer layer (*a*) and the central layer (*b*) of the unpitted tangential wall of a normal earlywood tracheid. — FIG. 2 (*middle*): Diagrammatic illustration of the orientations of cellulose in the outer layer (*a*) and the central layer (*b*) of the unpitted tangential wall of a normal latewood tracheid. — FIG. 3 (*right*): Diagrammatic illustration of the orientations of cellulose in the outer layer and the central layer of the pitted radial wall of a normal earlywood tracheid.

secondary walls, it is possible to demonstrate that the fibrils of the central layer are, on an average, oriented more nearly parallel to the long axis of the cell than in the case of the inner and the outer layers; but it is difficult to determine in longitudinal sections whether the orientation of the latter layers is actually at right angles to the long axis of the cell or at some intervening angle.

Similar obstacles must be overcome in the interpretation of X-ray diagrams where the rays pass through a complex of layers of markedly varying thickness and of very different structural orientations.

In view of such technical difficulties as these, it is not surprising to find many contradictory statements in the literature concerning the structural arrangement of cellulose in the walls of specific cells. It obviously is essential to develop a technique which will enable the investigator to trace the details of fibrillar orientation throughout each of the successively formed parts of the secondary wall. The method recorded in the following pages appears to be of considerable value in the study of lignified tissues.

Method of Demonstrating the Fibrillar Orientation in Lignified Walls:—When sections of lignified tissues are chlorinated, rinsed in 95% ethyl alcohol, treated with dilute ammonia in strong ethyl alcohol, rinsed in alcohol, chlorinated, rinsed in alcohol, stained in a 2-4% aqueous solution of iodine-potassium iodide, and finally mounted under a cover glass in a drop of 60% sulphuric acid, dark brown crystals of iodine form within the layers of the secondary wall. These crystals aggregate in slender, elongated, crystalline complexes which vary in size and number, depending upon the duration and the intensity of the successive treatments and upon other factors. The crystals evidently originate within the elongated interstices of the cellulosic matrix and are oriented parallel to the long axis of the fibrils of cellulose. The crystals, or crystalline aggregates, are so conspicuous and so clearly visible, microscopically, that it is possible not only to detect such major variations in orientation of the cellulose as occur in passing from layer to layer of the secondary wall, but also to observe such fluctuations in orientation as occur within the limits of a single layer.

Orientation of Cellulose in Secondary Walls of Normal Coniferous Tracheids:—In the case of the normal 3-layered tracheids of conifers (PLATE 6, FIG. 2), it is possible, by varying the details of the technique, to induce crystals to form (1) within the central layer of the secondary wall, (2) throughout both the central layer and the outer layer, or (3) in the outer layer alone. We have not succeeded, as yet, in obtaining them within the tenuous inner layer of the secondary wall. This appears to be due, at least in part, to the fact that the iodine escapes, during the treatment, from the exposed surfaces of the wall.

Orientations of the outer layer.—The orientation of the cellulose in the outer layer of the secondary wall fluctuates more or less from specimen to specimen, from tracheid to tracheid of the same specimen, and in different parts of the same cell. In certain samples of wood that we have sectioned, the cellulose tends to be arranged at right angles to the long axis of the cell

in the unpitted parts of the earlywood tracheids (TEXT FIG. 1*a*), and to have a helical orientation in homologous parts of the latewood tracheids (TEXT FIG. 2*a*). In other samples of wood, the arrangement may be helical in both earlywood and latewood, or it may fluctuate from tracheid to tracheid throughout the annual ring. There is no evidence to indicate that specific orientations are characteristic of particular species. On the contrary, the available data suggest that the arrangement of the cellulose fluctuates considerably in different parts of the same tree.

Local deviations in the prevailing orientation of any specific tracheid are of common occurrence in pitted parts of the wall. Not only is there a circular arrangement of the cellulose in the embossed parts of the wall which form the borders of the pits (TEXT FIG. 3), but there is a modified orientation in the adjacent parts of the wall as well. Such local deviations in orientation are more extensive and pronounced in large thin-walled tracheids than in small, thick-walled ones.

Orientations of the central layer.—The arrangement of the cellulose in the central layer of the secondary wall also varies more or less from specimen to specimen, from tracheid to tracheid, and in different parts of the same cell. Not infrequently the fibrils of cellulose tend to be oriented more nearly parallel to the long axis of the cell in the latewood (TEXT FIG. 2*b*), than in the earlywood (TEXT FIG. 1*b*), but helical arrangements are of not uncommon occurrence in latewood, and the cellulose may, at times, be oriented parallel to the long axis of the cell in earlywood. The most striking deviations in orientation occur in pitted parts of the wall, particularly in the radial walls of the earlywood tracheids (TEXT FIG. 3). The fibrils do not have a circular or concentric arrangement in the embossed parts of the wall—as is the case in the outer layer of the secondary wall—but curve about and through the borders of the pits as indicated in TEXT FIG. 3. Thus, in the earlywood, the orientation of the cellulose commonly tends to deviate more from the vertical axis in the radial walls than in the unpitted or sparsely pitted tangential walls (TEXT FIGS. 1-3).

The orientation of the fibrils may fluctuate, at times, in the successively formed growth rings or lamellae of the central layer, but pronounced shifts in orientation are of relatively infrequent occurrence in the tracheids of conifers. Regularly recurring changes from right-handed to left-handed helices or *vice versa*, such as are hypothesized by various investigators, are rarely, if ever, encountered in the central layer of coniferous tracheids.

Correlations between the outer layer and the central layer.—There are four combinations of orientations that are of common occurrence in the secondary walls of normal coniferous tracheids:

1) The cellulose of the outer layer may be oriented at right angles to the longitudinal axis of the cell, and the cellulose of the central layer may be arranged parallel to this axis.

2) The cellulose of the outer layer may be oriented at right angles to the long axis of the cell, whereas that of the central layer has a helical arrangement.

3) The cellulose of the outer layer may have a helical orientation, whereas that of the central layer is arranged parallel to the long axis of the cell.

4) The cellulose of both the outer layer and the central layer may be helically oriented.

It is significant in this connection, however, that the helices of the central layer have relatively steep slopes and rarely deviate as much as 45 degrees from the longitudinal axis of the cell, whereas those of the outer layer usually have comparatively low slopes. Thus, even when both layers have helical arrangements, the orientations rarely, if ever, are parallel. In all the material of normal coniferous tracheids that we have examined, the differences in orientation in unpitted parts of the secondary wall are of such magnitude that they may be detected when very thin (5 μ) transverse sections of the cells are examined in polarized light between crossed nicols. In other words, the central layer in such sections is either isotropic (PLATE 6), or detectably less birefringent than the outer layer (PLATE 13). This is due, of course, to the fact that a layer is dark in transverse sections when its cellulose is oriented parallel to the long axis of the cell but attains its maximum birefringence when the cellulose is oriented at right angles to this axis.

It should be noted, in passing, that according to FREY (1926), the micelles of cellulose have a circular orientation in the borders of the pits, whereas SCARTH and his co-workers (1929) consider that the fibrils merely curve about the bordered pits, "instead of regularly circling round them." Both investigators are right or wrong, depending upon the part of the wall which is selected for observation. As we have shown, the orientation of the outer layer is circular and thus in agreement with FREY's contention, whereas that of the central layer is entirely in accord with SCARTH's view.

Orientation of Cellulose in the Secondary Walls of the Tracheary Cells of Dicotyledons:—

Normal 3-layered tracheids, fiber-tracheids, and libriform fibers.—The arrangement of the cellulose in secondary walls of normal 3-layered tracheids, fiber-tracheids, and libriform fibers of dicotyledons resembles that which occurs in the tracheids of conifers. Thus, the orientation of the outer layer of the secondary wall fluctuates from positions at right angles to the longitudinal axis of the cell, to various helical arrangements, whereas that of the central layer varies from helical to longitudinal. It is significant, however, that with the progressive reduction of bordered pits in fiber-tracheids and libriform fibers, the orientation—particularly of the central layer—tends to show less extensive local deviations, and the arrangement of the cellulose in the radial and tangential walls is more nearly uniform and comparable.

Aberrant types of fiber-tracheids and libriform fibers.—Deviations in the orientation of cellulose in the successively formed parts of the central layer appear to be of more frequent occurrence in thick-walled fiber-tracheids and libriform fibers of dicotyledons than in the tracheids of the latewood of conifers. Not infrequently the deviations are of such magnitude that they may be detected when thin transverse sections of the cells are viewed in polarized light between crossed nicols. In the so-called gelatinous fibers of dicotyledons, there are abrupt transitions from concentric to radial or radio-concentric structural patterns and *vice versa*. These abrupt changes in the structural pattern of the cellulosic matrix may or may not involve con-

comitant modifications in the orientation of the cellulose in relation to the longitudinal axis of the cell.

Vessels.—In the less specialized types of dicotyledonous woods, the vessel members resemble tracheids in size, form, and structure. They tend to be comparatively thin-walled, and to have secondary walls which are conspicuously 3-layered, except in heavily pitted parts where the vessel members of the same or of different vessels are in contact. The arrangement of the cellulose in such 3-layered secondary walls of vessels fluctuates much as it does in normal tracheids. Thus, the outer layer may have an orientation at right angles to the long axis of the cell, or it may have a helical arrangement of comparatively low slope. As in the case of normal tracheids, the central layer has an orientation which is either parallel to the long axis of the cell or steeply helical.

As the vessels of dicotyledons become more and more highly specialized, they tend to form secondary walls of a wider range of structural complexity and diversity. Not infrequently, they tend to lose their typical 3-layered structure and to form multi-layered walls or thick walls which are more or less birefringent throughout, when transverse sections are viewed in polarized light between crossed nicols. In other words, the orientation of the cellulose in the more highly specialized types of dicotyledonous vessels frequently deviates markedly from that which occurs in normal tracheids.

It should be emphasized, in this connection, that variations in the thickness of the secondary walls of normal 3-layered tracheids are due primarily to variations in the width of the central layer (PLATE 4)—*i.e.*, of a layer which is dark or feebly birefringent in transverse sections. On the contrary, fluctuations in thickness of the secondary wall of vessels commonly are due to variations in the width of layers which are intensely birefringent, when transverse sections are examined in polarized light between the crossed nicols. Thus, the more conspicuous differences between the secondary walls of tracheids and of specialized types of vessel members are due primarily to different orientations of cellulose in the successively formed parts of the secondary wall.

Discussion:—The secondary wall of tracheary cells and fibers is composed of a continuous and firmly coherent matrix of anastomosing fibrils of cellulose. Lignin and other non-cellulosic substances may be deposited in the elongated, intercommunicating interstices of this matrix, thus resulting in two continuous interpenetrating systems of different chemical composition. The threadlike parts of the two interpenetrating systems have parallel orientations. Therefore, the crystals of iodine which form within the elongated interstices of the cellulosic matrix after chlorination are oriented parallel to the long axis of the fibrils of cellulose. That such is indeed the case may be demonstrated by various corroborative lines of evidence.

1) Where aggregations of fibrils or so-called striations are clearly visible in surface views of unswollen secondary walls, the crystalline aggregates of iodine are oriented parallel to the long axes of these structures.

2) In the case of thick secondary walls of the 3-layered type—where the slitlike

pit orifices are oriented parallel to the fibrillar axis of the central layer—the crystals of the central layer are arranged parallel to the slitlike orifices of the pits.

3) In secondary walls having a pronounced radio-longitudinal or radio-helical structural pattern—where mechanical cleavages and seasoning cracks are oriented longitudinally or radio-helically—the crystals of iodine are arranged parallel to the cleavage planes and seasoning cracks.

4) In favorable material—where the angles of extinction and dichroic phenomena in polarized light are clearly visible and can be accurately measured—the orientation of the cellulose as determined by the crystal method is in close agreement with the data obtained by polarization techniques. In fact, the evidence secured by the crystal and polarization techniques is so strikingly complementary that our photographs of crystal orientations might be substituted for FREY's diagrams of micellar arrangements in homologous layers.

The crystal method is so significant in studying the details of fibrillar orientation in different parts of a single wall or layer that it is of interest to determine whether the technique may be modified for use in the study of unlignified secondary walls. This has been attempted by Doctor THOMAS KERR, who has succeeded in inducing crystals of iodine to form within the wall of the cotton hair. His modification of our technique consists in staining the secondary wall of the cotton hair with iodine-potassium iodide or with chloro-iodide of zinc, and subsequently treating the stained preparation with a syrupy, "supersaturated" solution of zinc chloride. Thus, it is evident that the crystal method may be modified for the study of both lignified and unlignified secondary walls.

CONCLUSIONS

1) Crystalline aggregates of iodine may be induced to form within the elongated interstices of the cellulosic matrix of the secondary wall. These elongated crystals are oriented parallel to the long axis of the fibrils of cellulose and therefore of the micelles and chain molecules.

2) The crystalline aggregates are so conspicuous and so clearly visible, microscopically, that it is possible not only to detect such major variations in orientation of the cellulose as occur in passing from layer to layer of the secondary wall, but also to observe such fluctuations in orientation as occur within the limits of a single layer.

3) In the case of normal, 3-layered tracheids, fiber-tracheids, and libriform fibers, the orientation of the cellulose in the outer layer and in the central layer of the secondary wall fluctuates more or less from specimen to specimen, from cell to cell, and in different parts of the same cell.

4) Although the orientation of the cellulose may deviate, at times, in the successively formed growth rings or lamellae of the central layer, there is no regular alternation of right-handed and left-handed helices as hypothesized by various investigators.

5) In the case of the large bordered pits of the earlywood of conifers, the cellulose has a circular orientation in the outer layer, but merely curves about the pits in the central layer.

6) The less specialized types of dicotyledonous vessels resemble normal tracheids in having a 3-layered secondary wall, whereas the more highly specialized types have walls of a much wider range of complexity and structural variability, owing to fluctuations in the orientation of the cellulose.

Chapter 9

THE SIGNIFICANCE OF CERTAIN WOOD-DESTROYING FUNGI IN THE STUDY OF THE ENZYMATIC HYDROLYSIS OF CELLULOSE *

in collaboration with MARY R. VESTAL

IN 1913, one of us (*10*) called attention to certain wood-destroying fungi which produce helically oriented cavities within the thick secondary walls of the latewood of *Pinus Taeda* L. Subsequently, in studying the comparative anatomy of a wide range of conifers, monocotyledons, and dicotyledons, we have encountered similar fungi, not only in the wood of many different species, genera and families of the higher plants, but also in material from diverse temperate and tropical environments. The fungi evidently are ubiquitous forms which attack the woody tissues of the gymnosperms and of the angiosperms when these tissues are cut and are exposed to the air.

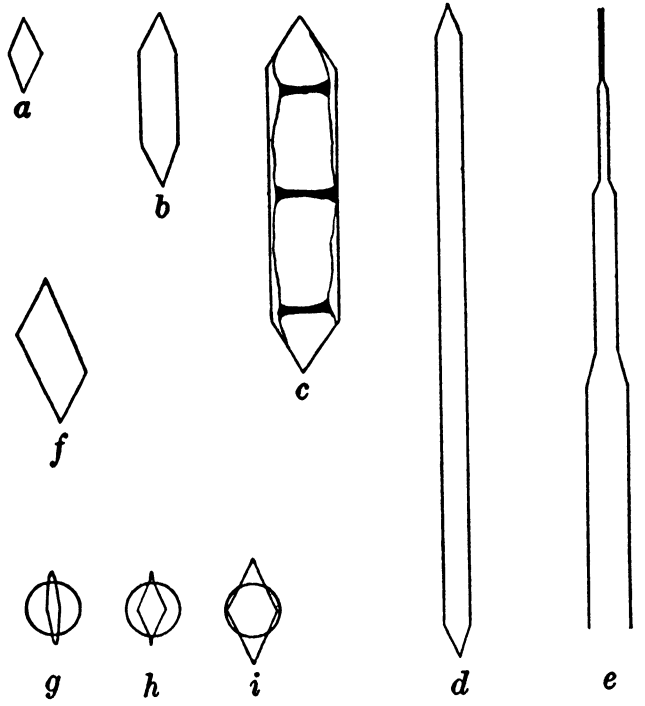
The fungi are characterized by the facts (1) that at least a part of their hyphae move forward within the secondary wall and (2) that their enzymes dissolve cavities which are oriented either helically or parallel to the long axis of the cell. The arrangement of the enzymatically-produced cavities suggests that hydrolysis proceeds along planes that are determined by the structural orientation of the cellulose, and, therefore, that such fungi may afford a means of securing significant information regarding predetermined planes of chemical reaction in the cellulosic matrix of the secondary wall. The results of a reconnaissance of woods that have been attacked by these fungi[1] are recorded in the following pages.

Form and Development of the Enzymatically-produced Cavities:— During their stages of elongation, the hyphae are extremely tenuous filaments which dissolve correspondingly minute, elongated cavities within the secondary wall (TEXT FIG. 3). These slender, cylindrical perforations subsequently are enlarged by further enzymatic activity which may continue until much of the secondary wall is dissolved. The process of lateral enlargement rarely progresses uniformly throughout the length of the cylindrical cavities, but tends to be accelerated in certain parts and to be retarded in others. Thus, as indicated in PLATE 11, FIGS. 8-13, localized enzymatic activity produces more or less numerous dilations which are oriented in a linear series and are connected by unaltered or less modified parts of the original elongated perforation.

* Reprinted from the Journal of the Arnold Arboretum 18: 196-205, 1937.

[1] We have found these fungi in 114 species, 88 genera, and 36 families of the gymnosperms and angiosperms.

Although the chambers vary considerably in size, they obviously are restricted to two principal geometric forms (1) biconical or (2) cylindrical with conical ends. In perfectly median longitudinal sections, the former cavities have a diamond-shaped outline (PLATE 11), whereas the latter have parallel sides and terminate in acute angles (TEXT FIG. 1, *b*, *c* and *d*). Of course, the outlines deviate considerably in other planes of section, and many complex forms arise during the fusion of cavities, either of the same

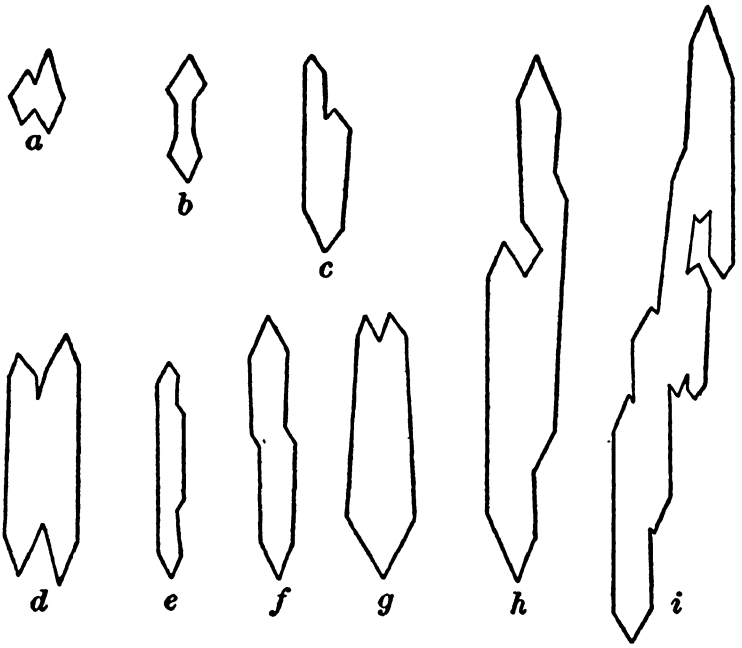


TEXT FIGURE 1.— Enzymatically-produced cavities as seen in median longitudinal planes of optical section. *a*, Biconical cavity; *b*, *c*, and *d*, cylindrical cavities with conical ends, *c*, containing remains of dilated hypha; *e*, progressive stages in the enlargement of a slender cylindrical perforation; *f*, cavity produced by the lateral fusion of two biconical cavities; *g*, *h*, and *i*, successive stages in the enlargement of a pit orifice.

linear series (TEXT FIGS. 1, *e* and 2, *b*) or of adjacent series (TEXT FIGS. 1, *f* and 2, *a*, *c-i*).

Such facts as these suggest that hydrolysis of the cellulose through enzymatic activity proceeds along two clearly defined sets of planes. During elongation of the tenuous hyphae, *terminal* enzymatic activity progresses primarily along one of the sets of planes producing attenuated, cylindrical perforations, whereas subsequent *lateral* enzymatic activity develops along either or both of these sets of planes and produces biconical cavities or cylindrical chambers with conspicuously conical ends.

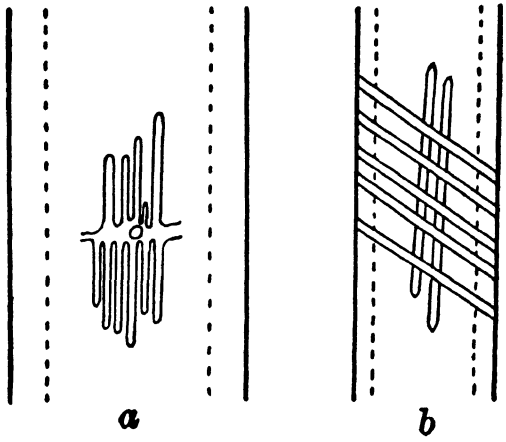
Orientation of the Planes of Enzymatic Hydrolysis:—As a result of varied optical and physico-chemical investigations, it is now generally recognized that, in the case of tracheids and fibers, the chain molecules of cellulose are oriented approximately parallel to the long axis of the anastomosing fibrils which constitute the secondary wall. That the long axis of the hyphal filaments and of the cylindrical perforations is oriented parallel to that of the fibrils and, therefore, to that of the chain molecules or micelles, may be demonstrated by various lines of evidence. (1) Where the fibrillar structure of the secondary wall is clearly visible, as is sometimes the case, it may be observed that the hyphae and the linear series of cavities are oriented parallel to the long axis of the fibrils. (2) In thick-walled tracheids, fiber-



TEXT FIGURE 2.— Complex types of cavities resulting from the fusion of primary forms.

tracheids, and libriform fibers of the normal 3-layered type, the slitlike orifices of the pits are oriented parallel to the fibrils of the broad central layer of the secondary wall. Since most of the enzymatically-produced cavities are confined to this layer, the pit orifices afford a reliable means of correlating the orientations of the fibrils and of the cylindrical perforations within the central layer of the secondary wall. (3) In thin, 5 μ, longitudinal sections of favorable material, it may be demonstrated that the positions of extinction of the cellulose in polarized light are oriented parallel to the sides of the cylindrical cavities. (4) When sections of lignified secondary walls are chlorinated, treated with an aqueous solution of iodine-potassium iodide, and subsequently with a drop of 60% sulphuric acid, dark brown crystals of iodine form within the elongated interstices of the cellu-

losic matrix. These elongated crystals, or crystalline aggregates, are visible microscopically and are oriented parallel to the long axis of the fibrils. By means of these crystals, it is possible not only to detect such major variations in the structural orientation of cellulose as occur in passing from layer to layer of the secondary wall, but also to observe such minor fluctuations in orientation as occur within the limits of a single layer. The linear series of cavities and the sides of the individual cylindrical chambers are oriented parallel to the long axis of the crystals, and therefore of the fibrils and chain molecules.



TEXT FIGURE 3.– *a*, Early stage of the growth of hyphae within the central layer of the secondary wall; *b*, helically oriented perforations in the outer layer of the secondary wall in contrast to the more nearly vertical arrangement in the central layer.

These and other lines of corroborative evidence indicate that the hyphae, the cylindrical perforations, and the linear series of enzymatically-produced cavities are oriented parallel to the long axis of the cellulosic fibrils. Where the chain molecules, the micelles, and the fibrils are helically oriented, the hyphae and the cavities have a helical arrangement (PLATE 11, FIGS. 12, 13) and where they are oriented more nearly parallel to the long axis of the cell, the hyphae and the perforations have a similar arrangement (PLATE 11, FIGS. 8-10). Furthermore, where the orientation of the cellulose changes in different parts of the secondary wall, the arrangement of the hyphae and of the enzymatically-produced cavities fluctuates accordingly (TEXT FIG. 3, *b*). In other words, one set of the predetermined planes of enzymatic activity is oriented parallel to the long axis of the fibrils, and, therefore, of the chain molecules of cellulose. The second set of planes obviously is oriented at an acute angle to this axis, and it is essential to measure the angle and to determine, if possible, whether it is variable or constant.

Unfortunately, there are inherent optical and other difficulties to be overcome in measuring this angle with consistent accuracy. In the first place,

there is the difficulty of turning the lines of the eyepiece into exact coincidence with the two legs of the angle to be measured. The smaller the cavity, the greater this source of error becomes. In the second place, there is considerable uncertainty in determining whether a particular cavity is being viewed in a truly median longitudinal plane of optical section. This difficulty is accentuated by the fact that, in the case of helically oriented structures, the chambers are curved, and furthermore by the fact that many of the larger cavities are not truly cylindrical or biconical, *i.e.*, they are not perfectly circular in transverse sections. Another common source of error, particularly in dealing with the larger cavities, is local deviations in the orientation of the cellulose, which lead to the formation of asymmetrical cavities. It is significant, in addition, that the critical angles become smaller when the wall contracts, *e.g.*, in drying, and enlarge when the wall swells. Thus, the angles may be modified during the processes of drying, and subsequently of resoaking, softening, and dehydrating the material for microscopic examination.

The measurements recorded in TABLE I were obtained from nine species and genera of seven different families, including one gymnosperm. Although the individual measurements for particular species, and the averages for different species, fluctuate through a range of variation of from 5 to 6 degrees, it is reasonable to assume that many of these variations are due to

TABLE I: *Measurements of the angle of intersection of the two principal planes of enzymatic activity:—*

Plant	Min.	Av.	Max.
Ilex formosana Maxim.	15 0	19.5	21.8
Myodocarpus fraxinifolius Brongn. & Gris.	16.9	19.5	22.1
Iryanthera macrophylla (Benth.) Warb.	18 9	20 5	21.8
Laurelia aromatica Juss.	18 3	22.7	24.3
Adinandra sp.	21 0	22.8	24 9
Cussonia Barteri Seem.	19 9	23 2	26.0
Osteophloeum platyspermum (A. DC.) Warb.	20.3	23.2	25.6
Pinus echinata Mill.	18 8	23.7	27.5
Brackenridgea Hookeri A. Gray	22.9	25.6	28.1
	Average	22.3	

Basis of individual averages, 20 measurements.

inherent difficulties in accurately measuring the angles of intersection of the two sets of planes. Thus, there appear to be two predetermined sets of planes of enzymatic activity in the secondary walls of tracheary cells and fibers, (1) oriented parallel to the long axis of the chain molecules and fibrils and (2) oriented at an angle of from 20-25 degrees to this axis.

The second set of planes is not correlated with any visible structures of the cellulosic matrix and, therefore, is determined by submicroscopic ones. The fact that the orientation of these planes is modified during the swelling of the secondary wall,—*i.e.*, where the spacing of the glucose residues is altered—suggests that these planes of hydrolysis are determined by specific configurations in the unit cell of cellulose.

It is possible to isolate fibers that have been attacked by these fungi, and subsequently to treat them with reagents—*e.g.*, sulphuric acid, phosphoric acid or cuprammonium hydroxide—which dissect the wall into "fusiform bodies" and other minute anisotropic fragments. By observing the phenomena in close proximity to the enzymatically-produced cavities, it may be observed that the fusiform bodies are dissected from the wall along planes that are parallel to those of these cavities. In other words, the chemical changes induced by these inorganic reagents progress along planes that are oriented parallel to the predetermined planes of enzymatic activity. As the fusiform bodies are cut free from the wall, they tend to be more or less rapidly deformed by the swelling effects of the reagents used in their production. When the walls themselves are swollen during the treatment—*i.e.*, where the spacing of the chain molecules is altered—the angles between the two intersecting sets of planes are increased, and the shape of the enzymatically-produced cavities becomes correspondingly modified. Under such circumstances, the original cavities contract longitudinally and expand laterally. Thus, the orientation of the oblique surfaces is gradually modified.

The illustrations of partially acetylated fibers published by HESS (1928), KANAMARU (1934), and others indicate that acetylation of cellulose tends to proceed along similar planes, *i.e.*, planes parallel to the long axis of the chain molecules or fibrils, and planes set at an acute angle to this axis. A careful study of these planes of chemical action during hydrolysis and acetylation should yield significant data regarding the submicroscopic configurations of cellulose.

Miscellaneous Data Concerning the Fungi and Their Hyphae:—In view of the fact that we have not succeeded in finding any descriptions of these fungi in the literature or information concerning their identity, it seems advisable to record the following data regarding them, even though our observations, thus far, are based solely upon the study of sections of thoroughly seasoned tissues. There are two types of hyphae in a majority of the specimens that we have examined: (1) delicate, colorless filaments and (2) coarse, dark brown hyphae which connect with them. Both types of hyphae are septate, and both are devoid of obvious clamp-connections. The abundance and distribution of the hyphae vary greatly from specimen to specimen. In certain cases, the colored hyphae are confined largely to the lumina of the rays and wood parenchyma—as is true for the "blue stain" fungus—whereas in others they occur chiefly in the lumina of the vessels, fiber-tracheids or libriform fibers. The colorless hyphae perforate the secondary walls and move forward within them. As the enzymatically-produced cavities enlarge, the hyphae tend to become more or less conspicuously dilated (TEXT FIG. 1, *c*) and, in dried material, frequently are encrusted both internally and externally with a granular substance which stains deeply with Haidenhain's haematoxylin.

The hyphae which move forward within the secondary wall usually attack the walls of the tracheids, fiber-tracheids or libriform fibers, and occasionally of the vessels, but rarely, if ever, of ray parenchyma or of wood parenchyma. Furthermore, they tend to develop primarily within the cen-

tral layer of the secondary wall; although in the case of certain specimens, they may perforate the outer layer as well (TEXT FIG. 3, *b*). The fungi frequently dissolve the central layer of the secondary wall, leaving the inner and the outer layers intact. Such facts as these suggest that the enzymatic activity may be retarded or inhibited in walls and layers which are very intensely lignified.

In a few of the specimens, which have abundant hyphae within the lumina of the fiber-tracheids, and few, if any, hyphae within the secondary wall, the enzymes attack the inner surface of the wall, and the hydrolysis progresses centrifugally through the central layer. Although this type of enzymatic activity produces less obviously symmetrical cavities, it tends to proceed along two clearly defined sets of planes, *i.e.*, parallel to the long axis of the fibrils and at an angle of 20-25 degrees to this axis. The latter planes of enzymatic hydrolysis are most clearly visible in walls where the orifices of the bordered pits are undergoing enlargement (TEXT FIG. 1, *g, h, i*).

It should be emphasized, in conclusion, that these fungi are so significant from experimental and physico-chemical points of view that an effort should be made to isolate them, to grow them in pure cultures, and to obtain reliable information concerning their identity.

CONCLUSION

1) There are certain fungi whose hyphae perforate and move forward within the secondary walls of tracheary cells and fibers.

2) The cavities produced by these fungi are of two geometrical forms, *i.e.*, (1) cylindrical with conical ends or (2) biconical, and are of remarkably constant angularity, regardless of the particular group of gymnosperms or angiosperms in which they occur.

3) It is evident that the enzymatic activity of these fungi progresses along two predetermined sets of planes, (1) oriented parallel to the long axis of the fibrils and chain molecules of cellulose and (2) at an angle of from 20-25 degrees to this axis.

4) These fungi evidently are ubiquitous forms which attack the vascular and fibrous tissues of the higher plants when they are cut and are exposed to the air.

5) The fungi are so significant from experimental and physico-chemical points of view that an effort should be made to isolate them, to grow them in pure cultures, and to determine their identity.

Chapter 10

THE SIGNIFICANCE OF X-RAYS IN STUDYING THE ORIENTATION OF CELLULOSE IN THE SECONDARY WALL OF TRACHEIDS *

in collaboration with EARL E. BERKLEY

MUCH EVIDENCE has accumulated from varied lines of research which indicates that the orientation of cellulose fluctuates more or less conspicuously in passing from the first-formed to the last-formed part of the walls of plant cells. In the case of normal tracheids of gymnosperms and dicotyledons, the secondary wall is commonly a three-layered structure; the first-formed and the last-formed lamellae of this wall have cellulose that is transversely oriented or arranged in helices of relatively low pitch, whereas the intervening lamellae have cellulose that is oriented longitudinally or in helices of comparatively steep pitch.

Although admitting changes of orientation in the cell wall of *Valonia* and of various tissue cells, PRESTON (1934, 1939) maintains that there is but a single spiral arrangement of cellulose throughout the secondary wall of coniferous tracheids. The chief evidence presented in support of such a divergent view consists of a small photograph obtained by X-raying a tracheid isolated from wood by maceration. Neither this photograph nor its enlargement exhibit a clearly defined diffraction pattern.

In the case of cotton, ramie and other plant fibers, significant data are obtained by X-ray analyses of aggregates or bundles of cells. Thus, in view of the fact that the structurally simpler types of wood are composed largely of longitudinally oriented tracheids, it is reasonable to anticipate that pertinent data may be obtained by X-raying small pieces of secondary xylem. That such is indeed the case has been demonstrated by STILLWELL (1933), SISSON (1935, 1938) and others. It should be emphasized in this connection, however, that in selecting and preparing material for X-ray analysis and in interpreting diffraction patterns, it is essential to be fully cognizant of the numerous morphological, histological, chemical and other variables in the material that is being studied. For example, certain serious misconceptions concerning the composition of the primary wall of the cotton hair might have been avoided by an accurate visualization of the significance of such variables (BERKLEY 1939).

It is advisable, therefore, before discussing X-ray analyses of wood to evaluate the evidence that has accumulated from varied lines of research regarding the visible structure of the secondary wall of tracheary cells.

* Reprinted from the American Journal of Botany 29: 231-241, 1942.

Visible Striations and Fibrillar Orientations in the Unswollen Walls of Tracheids:— PRESTON (1934) states that "in general no indication is given, visible under an ordinary microscope, of the direction of the cellulose fibrils in the walls" of tracheids. This is an untenable premise. Striations, such as are characteristic of *Valonia* and of various tissue cells, are visible in the secondary walls of many tracheids, provided that the cells are not mounted in media of unfavorable indices of refraction. Freshly cut, moist, longitudinal sections of the wood of living trees fortunately are suitable for microscopic examination and may be studied without staining, chemical pre-treatments, or the use of mounting media. Detailed investigations of such sections demonstrate that the first-formed and the last-formed parts of the secondary wall have striations that are transversely oriented to the long axis of the cells or are arranged in helices[1] of relatively low pitch (45°-90°), whereas the intervening part of the wall exhibits striae that are longitudinally oriented or arranged in helices of comparatively steep pitch (0°-45°). As in *Valonia* and other plant cells, these striations are due fundamentally to the fibrillar structure of the cellulosic matrix and may be differentiated without difficulty from slip planes, cracks, pressure ridges, parallel scratches and other abnormalities mechanically induced by the edge of a knife in sectioning.

It is evident, accordingly, that sections from freshly cut wood of living trees may be utilized as controls or bases of comparison in studying the effects of various chemical and other treatments upon the orientation of the cellulosic fibrils in tracheary cells. When such sections are immersed for a few minutes in chlorine water, stained in iodine-potassium iodide and mounted under a cover glass in a small drop of diluted sulphuric acid, crystals of iodine may be induced to form in the elongated porosities of the cellulosic matrix (BAILEY and VESTAL, 66). The long axis of these slender crystals[2] is oriented parallel to the long axis of the porosities and, therefore, *pari passu* to the long axis of the fibrils. In other words, where the visible striae or fibrils are longitudinally oriented, the crystals are arranged parallel to the long axis of the cell (PLATE 6, FIG. 2); where the striae are transversely oriented, the crystals are arranged at right angles to the long axis of the cell, and where the striations have a helical orientation, the crystals are arranged in a helix of identical pitch. Since the dark-colored crystals are equally conspicuous in transverse, longitudinal and diagonal sections of wood, they provide a useful and reliable means of studying fluctuations in the orientation of cellulose in the successively formed parts of the secondary wall. Evidence that may be obtained by this technique cannot be brushed aside upon the mere assumption that the deviations in orientation are in-

[1] The term, helical, is used in a descriptive sense without rigid geometrical implications. The tracheids of conifers rarely are perfectly cylindrical, but tend to have more or less conspicuously flattened sides. The pitch of the helix changes not infrequently in passing from one surface of the cell to another.

[2] The size and the number of the crystals may be altered by varying the details of the technique. Numerous minute crystals are desirable for accurate visual study of the fibrillar orientations in adjacent lamellae, but larger crystal complexes are advantageous for photographic illustration.

duced by swelling. In the first place, the crystals form in dilutions of sulphuric acid—50 per cent or less—which produce no detectable swelling in heavily lignified cell walls. In the second place, the visible striae in untreated sections have similar orientations. In the third place, even the swelling produced by stronger acid—50 per cent to 60 per cent—does not induce changes of from 45° to 90° in the fibrillar orientations of the inner (last-formed) and outer (first-formed) layers of such heavily lignified secondary walls. Nor can the swelling produce abrupt transitions in adjacent lamellae from right-handed to left-handed helices or *vice versa.*

Effects of Drying upon Fibrillar Orientations:— Many investigations in the past have been based upon the study of cells or tissues that at some stage have been dried below the fiber saturation point. For example, the customary procedure in preparing fibers for X-ray analyses involves a thorough drying. Both primary and secondary walls contract in drying along planes at right angles to the long axis of the fibrils. The contraction of the walls, in turn, produces more or less extensive changes in the form and the dimensions of the cells; the type and the magnitude of which depend upon a wide variety of factors. Obviously the orientation of the fibrils may be modified during the process of drying. Therefore, microscopic observations and X-ray analyses of desiccated material provide reliable evidence regarding the orientation of fibrils, crystallites and chain molecules in *dry* specimens, but do not *necessarily* provide an accurate picture of the orientations in cells that have never been dried below the fiber saturation point. It is essential in each particular case to obtain some clue regarding the changes that may have occurred during drying.

Longitudinal sections of the freshly cut wood of living trees may be mounted under a cover glass and kept under continuous microscopic observation throughout the entire process of desiccation. As the sections dry, the visible striations of the secondary wall of the tracheids become increasingly conspicuous. In the case of the normal wood of coniferous species and of the vesselless dicotyledon, *Trochodendron,* there are minor readjustments during drying, but there are no extensive changes in the orientation of the fibrils except possibly in the corners of rectangular or hexagonal cells. Therefore, X-ray analyses of wood which has not become excessively warped or deformed during drying should yield fairly close approximations to the orientations of cellulose in unseasoned wood.

Effects of Delignification and Maceration upon Fibrillar Orientations:— Numerous investigations in the past have been based upon the microscopic study of pulp or of cells that have been isolated by maceration. Furthermore, in obtaining sharply defined X-ray diffraction patterns of the cellulose in wood, it is necessary to remove most of the non-cellulosic constituents, particularly the lignin. Therefore, it is essential to determine what effects delignifying and macerating reagents have upon cellulose. In the case of the woods that were selected for investigation, it is possible to delignify and macerate longitudinal sections and to study the microscopically visible effects of specific reagents upon the fibrillar orientations of the cellu-

lose. As in drying woods, minor readjustments of form and structure occur during delignification[3] and maceration, but there are no pronounced changes in the fibrillar orientations. Walls having layers with transverse, longitudinal, right-handed or left-handed helical orientations, retain these orientations throughout the processes of delignification and of maceration. This is of considerable significance since it justifies the study of fibrillar orientations in single cells or in pieces of cell wall. Thus, the complications of accurately focusing through adjacent cells or cell walls may be avoided. It indicates, in addition, that data obtained by the micro-dissection of isolated cells (SEIFRIZ and HOCK 1936) cannot be ignored or rejected upon the assumption that the fibrillar orientations were extensively modified during maceration.

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Correlations Between Fibrillar Orientations and Anisotropy:— Physical evidence has accumulated in recent years which indicates that the chain molecules and crystallites of cellulose are oriented within fibrils with their long axes parallel to the long axis of the fibrils. Isolated macro-fibrils exhibit positive optical anisotropy and sharply defined extinction angles. Thus, the optical anisotropy of the various layers of the cell wall should correlate closely with the visible fibrillar orientations within them. Where the striae or fibrils of the central layer of the secondary wall of tracheids are longitudinally oriented, this layer should exhibit no birefringence under the polarizing microscope in transverse sections of the wood; it should show intense birefringence in longitudinal sections, and more or less reduced birefringence in sections cut at varying diagonals to the long axis of the cell. Conversely, where the fibrils of the inner and outer layers are oriented at right angles to the long axis of the cell, these layers should exhibit intense birefringence in transverse sections of wood, more or less reduced birefringence in diagonal sections, and no birefringence in those parts of longitudinal sections where plane surfaces of the walls can be viewed edge on. Where the layers have helical orientations, they should be more or less birefringent in both transverse and longitudinal sections, depending upon the pitch of the helix, and should exhibit no birefringence on one side of the cell in certain specific planes of diagonal section. Where a layer has a helical orientation of approximately 45°, this layer should be dark on one side of the cell and intensely birefringent upon the opposite side in sections cut at an angle of 45° to the long axis of the cell.

All of these theoretical predictions have been fully verified in woods of conifers and dicotyledons (BAILEY, 73) but there are certain of them—those involving absence of birefringence—which require unusually favorable material and great care in sectioning for convincing proof. To demonstrate the absence of birefringence from longitudinally striated layers[4]

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[3] That the removal of lignin and cellulosans from wood causes no fundamental change in the X-ray diffraction pattern of cellulose is indicated by the work of PRESTON and ALLSOPP (1939).

[4] Wall layers having a perfectly uniform orientation of fibrils throughout all of their constituent lamellae are of exceptional, rather than of typical, occurrence in tracheids.

in transverse sections of wood, it is essential in the first place to find specimens in which the tracheids are straight and are uniformly oriented with their long axis parallel to the vertical axis of the tree. Such specimens are by no means of common occurrence. In the second place, the plane of sectioning must be oriented with precision at right angles to the long axis of the tracheids and the sections must be cut with a very thin, perfectly smooth and properly oriented knife edge which does not produce serious distortions of the fibrils in sectioning. In the case of layers having fibrils arranged in planes at right angles to the long axis of the tracheids, it is essential to obtain accurately oriented longitudinal sections from wood composed of large tracheids with flattened parallel sides. In such sections only is it possible to eliminate the complicating effects of wall curvatures. It should be noted in this connection that no critical evidence can be obtained under the polarizing microscope from sections of the quality illustrated by PRESTON (1939).

Cellulose swells considerably in planes at right angles to the long axis of the fibrils, but only slightly in a plane parallel to this axis. Therefore, if the inner and the outer layers of the secondary wall of the tracheids have fibrillar orientations which deviate markedly from that of the central layer, these layers should exhibit characteristic and predictable differences in swelling anisotropy. Where the fibrils of the outer layer are transversely oriented or are arranged in helices of low pitch—*i.e.*, nearly at right angles to those of the central layer—the outer layer should not increase in circumference during swelling, but should act as a constricting cylinder about the laterally expanding central layer. Numerous evidences of this constricting behavior of the outer layer may be observed in the swelling of isolated tracheids and of transverse sections of wood (BAILEY, 73).

Correlations Between Fibrillar Orientations and Planes of Enzymatic Hydrolysis:— There are certain ubiquitous fungi whose hyphae penetrate and move forward within the secondary walls of tracheary cells. Enzymatic hydrolysis progresses along two sets of predetermined planes, one of which is oriented parallel to the long axis of the fibrils of cellulose (BAILEY and VESTAL, 67). The hyphae and the elongated cavities which they produce are oriented with their long axes parallel to the long axis of the fibrils. Where the hyphae penetrate the inner and the outer layers of the secondary walls of tracheids, they are oriented transversely or in helices of low pitch, whereas in the central layer of such walls, they are oriented longitudinally or in helices of comparatively steep pitch with respect to the long axis of the tracheids.

Unreliable Indexes of Fibrillar Orientations:— Various investigators have attempted to determine fibrillar or micellar orientations by measuring the extinction angles of cell walls in surface view. Such measurements lead to serious misconceptions, however, when derived from walls that are composed of two or more layers of fluctuating thicknesses and different fibrillar orientations. The problem is further complicated in the case of many tracheids where the orientation of the fibrils fluctuates considerably in

the successively formed lamellae of the individual layers of the secondary wall or even within different parts of the same lamella.

In the case of thick secondary walls of the three-layered type, the long axis of the slitlike pit orifices is frequently oriented approximately parallel to the fibrillar orientation of the central layer, but affords no evidence regarding the fibrillar orientations of the inner and outer layers of the cell wall. In thin-walled tracheids, the pit orifices commonly afford no reliable clues concerning the fibrillar orientations of any of the wall layers. Similarly, planes of mechanical cleavage may afford useful evidence in the case of radio-longitudinally or radio-helically striated layers, but are difficult to interpret accurately in the case of other types of wall layers.

Conclusions Regarding Visible Orientations:— It is evident from the preceding discussion that the fibrillar orientations of cellulose fluctuate more or less markedly in passing from the first-formed to the last-formed lamellae of the secondary walls of tracheids, and that the most reliable means at present of demonstrating such deviations in orientation are microscopically visible striations and crystals that may be induced to form in the elongated porosities of the cellulosic matrix. The close correlation between fibrillar orientation, optical anisotropy, swelling anisotropy, and predetermined planes of hydrolysis supports the view that the chain molecules and crystallites of cellulose are oriented within fibrils with their long axes parallel to the long axis of the fibrils. If the chain molecules are so oriented in the fibrils of tracheids, it should be possible under favorable circumstances to correlate salient features of X-ray diffraction patterns with visible fibrillar orientations.

Selection and Preparation of Wood for X-Raying:— In obtaining X-ray diffraction patterns from wood, it is essential to reduce the number of morphological, histological, chemical and other variables to a minimum, and in any case to obtain reliable evidence regarding the significance of such variables in the material that is being analyzed. In dealing with tracheids it is advisable to use wood which is devoid of vessels and contains a low percentage of parenchyma. Straight-grained secondary xylem from the outermost part of the stems of large conifers and of the vesselless dicotyledon, *Trochodendron,* provides favorable material for X-ray analyses. In such specimens, the tracheids are of large size, thus reducing the ratio of tapered ends within the limits of a unit area. Furthermore, the tracheids tend to be arranged in more uniform seriations, and the curvature of the growth rings is greatly reduced. The problems of X-ray analysis may be further simplified by separating the early wood from the late wood. To facilitate this procedure, it is desirable to select wood having very sharply and abruptly defined layers of dense late wood. However, before dissecting small cubes of wood into their constituent layers of early wood and late wood, it is essential to cut transverse, radial and tangential sections for microscopic study and as permanent records of the specific combinations of morphological and histological variables in each specimen that is analyzed.

In the X-ray analyses reported here, small pieces of early wood and late

wood were used, which had tangential surfaces of 4 × 6 millimeters and radial thicknesses of from 1 to 2.5 millimeters. These specimens were first treated successively with hot alcohol, benzene, ether, chloroform and acetone for the extraction of fats, waxes, terpenes, resins, etc. The specimens were subsequently delignified by alternating treatments with chlorine water and dilute alcoholic ammonium hydroxide.[5] The use of the latter reagent at room temperature, instead of hot sodium sulphite, enables one to remove the bulk of the lignin from wood without macerating the tissue. Much care must be exercised in drying the delignified specimens to prevent undue warping and splitting. Un-delignified specimens should be retained as controls, since the more conspicuous features of the diffraction patterns of cellulose frequently may be detected in spite of the amorphous fogging produced by lignin and other amorphous constituents.

In obtaining X-ray diffraction data from wood, it is desirable to compare the diffraction patterns obtained by passing the X-ray beam (*a*) parallel to the long axis of the wood rays, *viz., radially,* (*b*) at right angles to the long axis of both the tracheids and the wood rays, *viz., tangentially,* (*c*) at right angles to the long axis of the tracheids and at 45° to the long axis of the wood rays, *viz., diagonally,* and (*d*) parallel to the long axis of the tracheids, *viz., longitudinally.* Furthermore, in making accurate analyses, it is advisable to obtain micro-photometric readings from the original negatives, since such data frequently reveal intensity maxima which may be overlooked in a visual examination.

A detailed discussion of the data will be presented in a subsequent paper dealing with the more purely physical aspects of the problem. Thus, the corroborative microphotometric data, with one exception, are omitted from this paper and illustrations of diffraction patterns are reduced to an essential minimum.

Diffraction Patterns of Late Wood:— The conventional procedure in dealing with cotton, ramie and other fibers is to orient the cells with their long axis at right angles to the beam of X-rays. PLATE 13, FIG. 7 illustrates a diffraction pattern obtained by passing a beam of X-rays through the late wood of *Pinus longifolia* Roxb. at right angles to the long axis of the tracheids and parallel to the long axis of the wood rays. This diffraction pattern closely resembles that of ramie, which is considered to be indicative of a very uniform orientation of chain molecules approximately parallel or in a spiral of about 3° to the long axis of the cell. This particular sample of wood was selected for X-raying because the broad central layer of the secondary walls of the tracheids exhibits unusual regularity in the orientation of visible striations and of crystals (PLATE 6) parallel to the long axis of the tracheids. Thus, there is a perfect correlation between the X-ray diffraction pattern and the fibrillar orientation of cellulose in the central layer of the secondary wall.

It is significant in this connection, however, that the fibrils of the tenuous inner and outer layers of the secondary wall are oriented either transversely or in helices of relatively low pitch. Therefore, these layers

[5] 150 cc. ammonium hydroxide, sp. gr. 0.90, to 850 cc. 95 per cent ethyl alcohol.

are intensely birefringent in transverse sections of the wood (PLATE 13, FIG. 6), whereas the central layer exhibits no birefringence even in relatively thick sections. The fibrillar orientations of the inner and outer layers of the secondary wall obviously produce no detectable effects in the X-ray diffraction pattern. This is due to two sets of factors. In the first place, the fibrillar orientation of the inner and outer layers fluctuates considerably not only in different cells, but also in different parts of the same cell. In other words, the angle of inclination is not constant as in the central layer, but fluctuates between 60° and 90°. Therefore, the diffractions produced by the inner and outer layers are diffused over broad arcs rather than concentrated in localized interference spots. In the second place, recent work with cotton (BERKLEY 1939) has demonstrated that the orientation of the cellulose in the tenuous primary wall of the cotton hair cannot be detected in X-ray patterns after the secondary wall attains any considerable thickness. The inner and outer layers of the secondary walls in the late wood of *Pinus longifolia* are too tenuous in comparison with the breadth of the central layer for both sets of diffractions to be detected simultaneously. For similar reasons, the walls of the wood rays produce no visible images.

PLATE 13, FIG. 9 illustrates a diffraction pattern obtained by passing a beam of X-rays through the late wood of *Sequoia sempervirens* Endl. at right angles to the long axis of the tracheids and parallel to that of the wood rays. This particular sample of wood was selected for X-ray analysis because it exhibits the following combination of visible structural characters. The inner and the outer layers of the secondary wall are unusually thick[6] and the fibrils of these layers exhibit considerable uniformity of orientation at right angles to the long axis of the tracheids. The fibrillar orientation of the central layer is helical, the pitch fluctuating within relatively narrow limits, *viz.*, between 30° and 40°. Therefore, all three layers of the secondary wall exhibit anisotropy in transverse sections of the wood (PLATE 13, FIG. 8), but the inner and outer layers are more intensely birefringent than the central layer.

In the X-ray pattern (FIG. 9) there are four pairs of conspicuous diffraction maxima in both the 002 and 10$\bar{1}$ diffraction rings. The long axes of the tracheids were arranged vertical to the page; therefore, reading clockwise from the top of the pattern, the pairs of maxima may be observed at approximately 0° and 180°, 52.5° and 232.5°, 90° and 270°, 127.5° and 307.5°, each pair presumably representing a set of fibrils or crystallites in the cell wall. The two pairs of interference spots at 52.5° and 232.5° and at 127.5° and 307.5° are indicative of a helical orientation of chain molecules with a pitch of approximately 37.5°. The two pairs result from the same spiral but from opposite tangential walls of the tracheids. The somewhat less conspicuous maxima of both the 002 and 10$\bar{1}$ diffraction rings at 0° and 180° are indicative of a transverse orientation of chain molecules, *viz.*, approximately at right angles to the long axis of the tracheids. The diffraction maxima at 90° and 270° suggest a third orientation of chain

[6] The apparent thickness of the layers in polarized light is deceptive. Accurate measurements of the average breadth of the layers must be made under high magnification with non-polarized light.

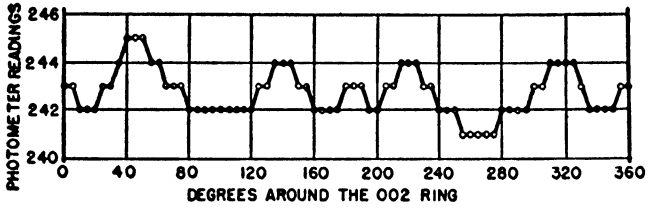
molecules parallel to the long axis of the cells. The helical and transverse orientations of chain molecules obviously correlate closely with the visible fibrillar orientations of the central layer and of the inner and outer layers of the secondary walls of the tracheids, since they occur in X-ray diffraction patterns obtained by passing the X-ray beam perpendicular to the long axis of the tracheids but in the radial, the tangential, and diagonal directions to them (PLATE 13, FIG. 9 & PLATE 14, FIGS. 12, 13). On the contrary, there are no visible longitudinal orientations in the tracheids which may be correlated with the diffraction maxima at 90° and 270°. Furthermore, the form, arrangement, and wall structure of the ray cells is such that these diffraction maxima cannot be attributed to them.

For the solution of such a paradox attention must be devoted to a study of the effects of variations in the shapes of cells and the thickness of the different wall layers upon X-ray diffraction patterns. Most of the tracheids in the wood of *Sequoia sempervirens* are rectangular (PLATE 13, FIG. 8). The broad, flattened, tangential walls are oriented at right angles to the beam of X-rays, whereas the radial walls are oriented parallel to the beam when it is passed perpendicular to the long axis of the tracheids and parallel to the long axis of the wood rays. Similarly the tangential walls would be parallel and the radial walls perpendicular to the beam when it is passed in the tangential direction. Curved surfaces, the parts between the radial and tangential walls, are reduced to a minimum in this sample. Three of the four pairs of interference spots in FIGURE 9 obviously are due to the diffraction of X-rays in passing through the tangential walls of the tracheids. The diffraction maxima at 90° and 270° on the other hand are apparently due to X-rays which pass through the radial walls of the tracheids and are diffracted by the transverse cellulose in the outer and inner layers of the secondary wall. These spots are more pronounced, as would be expected from the more uniform diffracting surface presented by the tangential walls (PLATE 13, FIG. 8) when the X-ray beam was passed perpendicular to the long axes of both the tracheids and the wood rays, *i.e.*, tangentially (PLATE 14, FIG. 12). Diffractions produced by the curved parts of the walls and by diagonally oriented radial walls tend to produce relatively broad arcs and thus to bridge the gaps lightly between the various sets of interference spots. That the diffraction phenomena in FIGURE 9 may be accounted for without resorting to a hypothetical system of longitudinally oriented chain molecules can be demonstrated by the absence of the spots at 90° and 270° in FIGURE 13, which was obtained by passing the beam of X-rays through the specimen perpendicular to the long axis but diagonal to the radial and tangential surfaces of the tracheids. If the interference spots had resulted from longitudinally arranged cellulose, they should not have disappeared in this pattern. Their disappearance in the diagonal pattern indicates that they were caused by a diffraction of the X-rays by the end on position of the transverse cellulose in the outer and inner layers of the secondary wall.

Diffraction Patterns of "Compression Wood":— PLATE 13, FIG. 11 illustrates a diffraction pattern obtained by passing a beam of X-rays through the "compression wood" of *Taxodium distichum* (L.) Rich. at right angles

to the long axis of the tracheids and parallel to that of the wood rays. As in PLATE 13, FIG. 9, there are diffraction maxima indicative of helical and transverse orientations of chain molecules, but there are no detectable interference spots to indicate parallel arrangement. The tracheids of this particular specimen of "compression wood" are characterized by their large size, relatively thin (3.5 μ) secondary walls and somewhat compressed cylindrical form. The fibrillar orientation of the outer layer is dominantly transverse. As in other tracheids of the "Rotholz" type (HARTIG 1896), the inner layer is composed of radio-helical bands, having a pitch of 44°-48° to the long axis of the tracheids. In this layer, the long axis of the fibrils is oriented parallel to that of the helical bands. Thus, both the inner and the outer layers are conspicuously birefringent in transverse sections of the wood (PLATE 13, FIG. 10). The isotropy or feeble birefringence of the intervening layer is due, not to a longitudinal orientation of fibrils, but to the fact that this layer is composed largely of non-cellulosic constituents.

The transverse cellulose of the outer layer of the secondary wall will account for the pair of interference spots at 0° and 180° of FIGURE 11. The two pairs of interference spots at approximately 50° and 225° and



TEXT FIGURE 1.— Photometric readings from same film as PLATE 14, FIG. 15.

at 132.5° and 310° can be ascribed to the radio-helical bands of the thick inner layer of the secondary wall. There are no maxima at 90° and 270° such as occur in FIGURE 9. That the absence of such maxima is correlated with the more cylindrical shape of the tracheids is indicated by the fact that other samples of "Rotholz" (FIGURE 14) having cylindrical tracheids exhibit similar diffraction patterns regardless of variations in the thickness of the secondary wall and of its constituent layers.

PLATE 14, FIG. 15 illustrates a DEBYE-SCHERRER type of diagram obtained from the "Rotholz" of a young stem of *Pinus ponderosa* Lawson. Such diffraction diagrams are commonly interpreted as resulting from random orientation of cellulose crystallites in the cell wall. It is significant in this connection, however, that the fibrillar orientation as indicated by visible striations, iodine crystals and optical and swelling anisotropy is dominantly transverse in the outer layer of the secondary wall of the tracheids and is prevailingly helical in the inner layer. Furthermore, the tracheids are very short and exhibit—both in radial and tangential views—a high ratio of pronounced curvatures and other deformations, particularly in their tapered upper and lower thirds. *A priori* one would expect the straight parts of the tracheids—*i.e.*, those oriented parallel to the long axis of the stem—

to produce diffraction maxima as in FIGURES 11 and 14, whereas the curved and deformed parts would tend to produce a ring-type of diffraction pattern, since the fibrils are oriented at varied angles to the beam of X-rays. The microphotometric readings recorded in TEXT FIGURE 1 demonstrate that maxima do occur at 0° and 180°, 45° and 225°, and 140° and 320° which are barely detectable visually in PLATE 14, FIG. 15.

Diffraction Patterns of Early Wood:— The fibrillar orientations in the thin-walled tracheids of early wood fluctuate considerably, not only in different cells, but also in different parts of the same cell, in different lamellae of the same wall layer, and in different parts of the same lamella. The widest range of variability occurs in the radial walls of the tracheids and is correlated with the occurrence of bordered pits and numerous contacts with ray cells. In general, the fibrillar orientations tend to be steeper in the central layer than in the inner and outer layers and in tangential walls than in radial ones (BAILEY and VESTAL, 66). The thickness of the secondary wall and *pari passu* of the central layer varies considerably in different samples of early wood and between the first-formed and last-formed tracheids of the early wood. Furthermore, it is significant that not infrequently there is less regularity in the form of the tracheids in early wood than in late wood. Many of the large, thin-walled tracheids of the early wood are of hexagonal form. Therefore, there commonly is less uniformity in the orientation of radial walls parallel to the long axis of the rays in early wood than in normal late wood.

In view of such facts as these, one should expect early wood to produce broad arcs or rings of diffraction when X-rayed at right angles to the long axis of the tracheids. FIGURES 16 and 17 illustrate such diffraction patterns of the early wood of *Trochodendron aralioides* Sieb. & Zucc. and of *Sequoia sempervirens* Endl. Owing to the tenuous character of the central layer in the tracheids of the early wood of *Trochodendron,* the diffractions produced by the steeper fibrillar orientations (0°-45°) of this layer tend to blend into those produced by the lower fibrillar orientation (45°-90°) of the inner and outer layers. On the contrary, in the case of the early wood of *Sequoia* where the central layer is thicker and has a higher ratio of fibrillar orientations between 20° and 45°, the broad arcs of diffractions produced by this layer may be differentiated from those due to the fibrillar orientations (45°-90°) of the inner and outer layers.

Discussion:— There is a close correlation between the position of the long axis of the visible fibrils in the secondary walls of tracheids and that of the cellulose molecules in the crystallites as shown by the X-ray diffraction patterns that are produced from such walls. In other words, the evidence presented on the preceding pages supports the widely held view that to a large extent the long chain molecules and, therefore, the crystallites of cellulose are oriented within fibrils with their long axis parallel to the long axis of the fibrils. The X-ray data afford no support for PRESTON's assumption of a single spiral orientation of cellulose throughout the secondary

wall of coniferous tracheids. On the contrary, they demonstrate that the orientation of the chain molecules fluctuates markedly in different layers of the secondary walls of coniferous and dicotyledonous tracheids.

With the exception of PLATE 13, FIG. 7, the X-ray diffraction patterns taken perpendicular to the long axis of the tracheids are clearly indicative of more than one orientation of chain molecules and correlate closely with evidence obtained by the study of visible fibrillar orientations, of optical anisotropy, of swelling anisotropy and of predetermined planes of hydrolysis. In the case of the late wood of *Pinus longifolia,* the uniformly longitudinal orientation of fibrils in the broad central layer of the secondary wall correlates perfectly with the isotropy of this layer in transverse sections of the late wood (FIG. 6) and with the X-ray diffraction pattern (FIG. 7) produced by this layer. The inner and outer layers having fibrillar orientations of from 60° to 90° are intensely birefringent in transverse sections (FIG. 6), but are so tenuous in comparison with the thickness of the central layer that they produce no conspicuous diffraction arcs in the X-ray photograph (FIG. 7). If it be assumed from the X-ray diffraction pattern that there is a single longitudinal orientation of cellulose throughout the secondary wall, the inner and outer layers in FIGURE 6 should not exhibit birefringence. If one assumes that there is a single spiral orientation of cellulose throughout the secondary wall and that the appearance of layering is due to considerable angular dispersion of chain molecules within the central layer,[7] this layer should exhibit some birefringence in transverse sections, and the X-ray diffraction pattern should not indicate such uniform longitudinal orientation of chain molecules.

It should be emphasized again in conclusion that, in selecting and preparing plant material for X-raying and in interpreting X-ray diffraction patterns, it is essential to be fully informed concerning the numerous morphological, histological, chemical and other variables in the material that is being analyzed. Where the layers of cell walls vary considerably in thickness, X-ray diffraction patterns may afford no evidence concerning the orientation of chain molecules in the more tenuous layers. Thus, X-ray diffraction patterns of mature cotton hairs give no clue concerning the orientation of cellulose in the primary wall. A ring-type of diffraction pattern is not indicative necessarily of a truly random orientation of chain molecules, but may be due (*a*) to deviations in the orientation of cellulose in the successively formed lamellae of cell walls, (*b*) to deviations of orientation in different cells of a particular specimen, (*c*) to curvatures and other deformations of the shape and orientation of cells within a specific specimen or (*d*) to a combination of such variables. Furthermore, certain variations in the type of diffraction pattern may be due to the shape and the arrangement of cells within a particular specimen, rather than to peculiarities of the orientation of cellulose within the walls of the cells.

[7] This is contradictory, since the angularly dispersed chain molecules are not in alignment with a single spiral orientation. In PRESTON'S (1939) revised diagram the alignment of chain molecules in surface view of the central layer does not correspond with the orientations figured in sectional views of this layer.

Therefore, in dealing with the tracheids in wood, it is necessary to obtain reliable clues regarding the following variables:

1) The ratio of parenchymatous cells to tracheary tissue and the types of diffractions which they produce.
2) The shape and arrangement of the tracheids in transverse sections of the wood.
3) The shape and arrangement of the tracheids in radial and tangential longitudinal sections of the wood.
4) The comparative thickness of the wall layers.
5) The fibrillar orientations in the successively formed lamellae of these layers.
6) Fluctuations in the fibrillar orientations in different tracheids of the same specimen and in different surfaces of the same cells.
7) Morphological changes that may occur during chemical pretreatments and drying.

SUMMARY

The most reliable means at present of studying the fibrillar orientations of cellulose in individual layers of tracheids are microscopically visible striations and crystals that may be induced to form in the elongated porosities of the cellulosic matrix of unswollen walls. The over-all average or dominant arrangement of the crystalline cellulose may be best obtained from X-ray diffraction patterns.

Careful drying, delignification and maceration of wood produce minor changes in the orientation of fibrils but no pronounced deviations in orientation, such as from longitudinal to transverse or from right-handed to left-handed helices, etc.

There is a close correlation between fibrillar orientation, optical anisotropy, swelling anisotropy, predetermined planes of hydrolysis and X-ray diffraction patterns. This indicates that the chain molecules and crystallites of cellulose are oriented within fibrils with their long axis parallel to the long axis of the fibrils.

The fibrillar orientations of cellulose in the secondary walls of tracheids fluctuate markedly, not only in wood from different parts of the same tree but also in different layers of the same cell wall and frequently also in different lamellae of the same layer.

The fibrillar orientations of the inner and outer layers fluctuate between transverse and helices of comparative low pitch (45°-90°), whereas those of the central layer usually deviate between longitudinal and helices of relatively steep pitch (0°-45°). Uniform orientation of cellulose throughout the successive lamellae of a wall layer is exceptional rather than typical of tracheids.

The use of the polarizing microscope and of X-ray diffraction patterns alone may lead to serious misconceptions regarding the orientation of cellulose in cell walls, unless the numerous morphological, histological, chemical and other variables in plant materials are accurately visualized and accounted for.

Chapter 11

THE WALLS OF PLANT CELLS *

IN ANY discussion of the cell walls of plants, it is essential to differentiate between two distinct categories of structures. Meristematic cells and such of their derivatives as retain a capacity for growth and for increase in volume are provided with a wall capable of expansion and of increase in surface area. This wall is also capable of undergoing various reversible changes, for example, in thickness. Many tissue cells, particularly those in which the mechanical functions are emphasized, form a supplementary wall that is incapable of surface expansion. Such cells are unable to grow or to increase in volume unless the protoplast can escape from its indurated envelope. A wall of the former category will arbitrarily be designated as a *primary* wall; the latter type of wall will be referred to as a *secondary* wall. The secondary wall of plant cells provides a more favorable medium for physical, chemical, and microscopic investigations; and, because of its economic and industrial importance in textile fibers and pulp and as a source of cellulose and its derivatives, it has been intensively studied during the past one hundred years. In other words, much more is known about the chemical composition, the microscopic structure, and the optical behavior, and other physical properties of secondary walls than is known about primary walls.

Under high magnification the secondary wall of plant hairs, of fibers, and of sclerenchyma in general, frequently exhibits many diverse structural patterns; these range from concentric to radial patterns, or to various complex combinations of such simpler patterns (PLATE 8). When the wall is expanded by the use of suitable swelling reagents, finer and finer details of the characteristic structural patterns become successively visible under the microscope (PLATES 9 and 10). In all cases the secondary wall resolves itself ultimately into a system of fine threads, or microfibrils, which are variously aggregated and more or less extensively coalesced. Where the microfibrils are oriented approximately parallel to the long axis of a cell, they appear in expanded transverse sections of such a cell as more or less circular specks or granulations (PLATE 10). The concentric, radial, ramifying, and other structural patterns of the secondary wall are due, therefore, to varying densities or porosities in different parts of the wall. In the denser (darker) parts, the microfibrils are more numerous per unit area and are more extensively coalesced, whereas in the more porous (lighter)

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parts they are less closely aggregated and the area of interfibrillar capillary spaces is larger.

In the case of a heavily lignified secondary wall, it is possible to remove the cellulose and leave a firmly coherent residue of lignin; and, conversely, it is possible to remove the lignin and leave the coherent matrix of cellulose. The cellulosic matrix (PLATE 9, FIG. 1) and the lignin residue (PLATE 9, FIG. 2) exhibit positive and negative images of the same original, unswollen structural pattern (PLATE 8, FIG. 2). It is evident that the lignin residue may be interpolated within the porosities of the cellulosic matrix. Such facts as these indicate not only that lignin occurs within the elongated porosities of the cellulosic matrix, but also that the microcapillary spaces in cellulose are intercommunicating. It is evident, accordingly, that in dealing with the secondary wall of plant cells we are concerned with two continuous interpenetrating systems, (*a*) a matrix that is composed of coalesced microfibrils[1] and (*b*) a system of elongated microcapillaries that are interconnected.

The microfibrils of the secondary wall are commonly composed largely of cellulose, but in certain types of plants may be formed of other substances (*e.g.*, of chitin, as in *Phycomyces*, whose structure has been carefully investigated by E. S. CASTLE). The microcapillaries of the secondary wall may be filled with liquid, or with lignin, cutin, suberin, hemicelluloses,[2] or a great variety of other organic compounds, and even at times with crystals, such as silica.

It is advisable to raise the question, how may one be certain that the structural patterns visible under the microscope are not artifacts, produced for example by stresses and strains in an expanding disk-shaped section? There are four lines of evidence which should be emphasized in this connection:

1) Specific, complex structural patterns whose coarser details are clearly visible in unswollen material are characteristic of particular types of cells and of specific types of plants.

2) Fragments of the secondary wall, whatever their shapes and sizes, exhibit, when swollen, their own particular part of the general structural pattern from which they were removed.

3) During the earlier stages of formation of the secondary wall, it may be observed that the cellulose is deposited in the form of coalesced fibrils.

4) In the case of cotton, the hairs elongate for a certain number of days; then the secondary wall is formed during a period of from twenty to forty days. By collecting material each successive day after a known date of flowering, KERR (1937) has shown that two lamellae of varying porosity are formed during each 24 hours, a denser lamella being deposited during the day and a more porous one during the night. The width and the porosity of these lamellae fluctuate more or less with variations in environmental conditions. In the cotton fields near Raleigh, North Carolina, characteristic patterns of lamellae are frequently formed which are closely correlated with specific variations in temperature. Thus, the hairs of different cotton

[1] The term microfibrils is used not necessarily to designate actual discrete entities, but rather as a convenient expression for describing the elongated parts of a continuous, porous matrix.

[2] A. G. NORMAN (1937) has suggested that in the case of the "cellulosans" the chain molecules of hemicellulose may be associated with chain molecules of cellulose within the micelles.

plants which have grown during the same period of varying environmental influences exhibit identical patterns of lamellae, and it is possible to cross-date *daily* growth rings, much as the *annual* rings in the stems of western yellow pine may be cross-dated. In addition, ANDERSON and KERR (1938) have shown that lamellation of the secondary wall of the cotton hair may be eliminated by growing plants under constant light and temperature. By varying these environmental factors, lamellae may be induced to form as desired.

Since the concentricities of secondary walls are not artifacts, they provide a convenient means of determining the approximate diameters of the microfibrils. If the diameter of the unswollen wall is carefully measured and the number of constituent lamellae in a swollen section is counted, a fairly close approximation of the diameter of the lamellae in their unswollen state can be obtained by dividing the diameter of the unswollen wall by the number of constituent lamellae. The finer types of lamellae grade down to 500 Å or less in diameter. Since such lamellae are composed of a single layer of coalesced microfibrils, these threads, in their unswollen condition, must likewise approach 500 Å or less in diameter.

The evidence presented thus far indicates that in the field of microscopically visible structures, the secondary wall of plant cells is composed of a continuous system of coalesced microfibrils which is perforated by a continuous system of intercommunicating microcapillaries. The cellulosic matrix is an extraordinarily porous structure, yet it exhibits a very high tensile strength. It is of interest to compare the visible structure of cellulose with pictures of its structure which have been postulated in the truly submicroscopic field. For many years following the publication of NÄGELI's micellar hypothesis, the micelles or crystallites of cellulose were considered to be discrete entities separated on all sides by intermicellar spaces. Subsequently, evidence obtained largely by x-ray analyses was interpreted as indicating that these micelles are composed of chain molecules which are oriented parallel to one another, with specific and characteristic spacings between the individual chains of the aggregation.

Recently, FREY-WYSSLING has profoundly modified this classical conception, in order to harmonize the conflicting evidence from various fields of physical, chemical, and biological research. He now regards cellulose as a continuous matrix of overlapping chain molecules, which is perforated by a continuous system of intermicellar capillaries. In other words, micelles are no longer to be regarded as discrete crystallites, but merely as anastomosing parts of a coherent porous matrix. There is much that can be said in favor of such a view, since it allows for chain molecules of greater length, for the coherence of purified cellulose, and for various optical, physical, and chemical properties of cell walls in general. It should be recognized, however, that according to such a modification of NÄGELI's hypothesis, the crystallite or micelle loses its individuality.

FREY-WYSSLING's modified conception of the submicroscopic structure of cellulose resembles so closely the finer visible structure of swollen secondary walls that a question arises whether there are two distinct sizes of fibrils, or rather a series of these structures which grade down in size from macrofibrils to microfibrils and to micelles 60 Å or less in diameter. Although reliable factual data for conclusively answering these questions are

not available at present, it seems likely that the latter alternative may ultimately prove to be the correct one.

It should be noted in passing that in cases where the orientation of the macrofibrils or microfibrils, and of the chain molecules, has been accurately determined [3] in the same material, the chain molecules are oriented approximately parallel to the long axis of the fibrils. Apparent deviations in the orientation of chain molecules or of micelles within the secondary wall are commonly due to fluctuations in the orientation of fibrils, rather than to aberrations in the alignment of chain molecules or of micelles within the fibrils.

Not all of the visible concentricities of the secondary wall are due, necessarily, to fluctuations in density or porosity of the cellulosic matrix. Thus, the broader concentricities that are of common occurrence in tracheary and sclerenchymatous cells are due largely to: (*a*) varying amounts of lignin, polyuronide hemicelluloses, or other organic substances that are deposited within the porosities of successively formed parts of the secondary wall; (*b*) the occurrence of non-cellulosic layers in certain specific types of cells (PLATE 7, FIG. 20) and (*c*) variations in different layers of the secondary wall, in the orientation of the microfibrils and *pari passu* of the chain molecules of cellulose (PLATE 6, FIG. 3 and PLATE 7, FIG. 18).

In many cases fluctuations in the porosity of the cellulosic matrix, in the amounts of organic substances that are deposited or formed within the capillary spaces, and in the orientation of the chain molecules and microfibrils, occur simultaneously within the limits of a single secondary wall. Much of the controversy in recent years and many of the existing discrepancies in the literature about the physico-chemical structure of plant cell walls, and particularly that concerning the orientation of chain molecules and of micelles within them, might have been avoided if the significance of the numerous biological variables in the material under investigation had been clearly visualized and accurately interpreted. Where a wall is composed of layers which differ markedly in thickness (PLATE 6, FIG. 3), or of multiple layers of more nearly uniform thickness but of widely fluctuating fibrillar orientations (such as certain types of sclerenchyma), it is frequently difficult to obtain an accurate picture of its structure by examining the wall in surface view with polarized light, or by x-ray analyses of entire cells. Thin transverse, longitudinal, and diagonal sections provide the most favorable material for preliminary investigations. Not only may such sections be examined under a polarizing microscope both before and after extraction of non-cellulose constituents with suitable solvents, but they may also be swollen, in order to reveal variations in density or porosity of the cellulosic matrix and in the swelling anisotropy of successive layers. Furthermore, crystals of iodine may be induced to form in the elongated porosities of the cellulosic matrix (PLATE 6, FIG. 2). Since these crystal aggregates are oriented approximately parallel to the long axis of the fibrils, they provide a convenient means of studying variations in the orientation

[3] For example, in the case of *Valonia*, compare the work of CORRENS and others with that of ASTBURY and his coworkers; or in the case of cotton, compare that of ANDERSON and KERR with that of BERKLEY.

of cellulose in different parts of a secondary wall. Additional evidence may be obtained by observing changes in the orientation of "slip planes" and of predetermined planes of hydrolysis (PLATE 11). The orientation of pit apertures and of planes of mechanical cleavage (PLATE 12, FIG. 1) must be utilized with extreme caution, particularly in dealing with thinner types of secondary walls.

In the three types of cells illustrated in PLATE 6 and PLATE 7, the evidence which may be obtained from these varied lines of investigation is in close agreement, and clearly indicates that the secondary walls have the general types of structure that are shown in the diagrams. The central layer of PLATE 13, FIG. 10 and the dark layers of PLATE 7, FIG. 20 are isotropic in longitudinal and diagonal sections of the cells, as well as in transverse sections, and are soluble in reagents which do not dissolve cellulose. On the contrary, the broad, apparently isotropic layers of PLATE 6, FIG. 3 and PLATE 7, FIG. 18 are strikingly birefringent in longitudinal sections, and the tenuous layers that are birefringent in transverse sections become isotropic or feebly anisotropic. The intensity of birefringence fluctuates in diagonal sections with changes in the angle of obliquity. This is true regardless of whether the sections are examined under the polarizing microscope before or after treating the sections with solvents for non-cellulosic constituents. It may be determined by direct observation, and by the various lines of corroborative evidence outlined in the preceding paragraph, that the microfibrils and microcapillaries of the tenuous layers are oriented transversely or in helices of relatively low slope, whereas those of the broad layers are arranged longitudinally or in helices of very steep slopes. In native cellulose, the major axes of swelling anisotropy are oriented at right angles to the long axis of the micelles and microfibrils. In other words, a layer of the secondary wall expands considerably in planes at right angles to the long axis of the microfibrils, but only slightly in planes parallel to this axis. Thus, in walls having the type of structure illustrated in PLATE 6, FIG. 3 and PLATE 7, FIG. 18, the broad central layers expand laterally. The tenuous layers, which envelop these layers externally, have a nearly transverse orientation of microfibrils and are unable to increase markedly in circumference. Therefore, they tend to rupture during swelling of the secondary wall, as shown in PLATE 12, FIG. 3.

Although the cellulosic matrix of the secondary wall appears to be a continuous rather than a discontinuous system, it exhibits predetermined planes of hydrolysis and of cleavage. The enzymatic hydrolysis of cellulosic walls (PLATE 11) which is caused by the activity of certain remarkable wood-destroying fungi, progresses along two predetermined planes, as does the acetylation of cellulose and its hydrolysis by mineral acids. One of these sets of planes is oriented parallel to the long axis of the microfibrils and, *pari passu*, of the micro-capillaries, micelles, and chain molecules. The other is oriented at an angle of 20° to 25° to this axis. The latter set of planes of hydrolysis and of chemical reaction is not correlated with any visible structure of the cellulosic matrix, but it does correspond rather closely with certain spacings within the crystal-lattice of cellulose. These

spacings are between the easily hydrolyzable ether-linkages of the cube-center and cube-side chains. They fluctuate around 14 A, and form planes which intersect the long axis of the chain molecules at angles of from 22° 40′ to 25° 04′. Although the submicroscopic and the visible planes are oriented at similar angles, it is not evident why the chemical reactions should progress along these specific planes. If it be assumed that the spacing between the ether-linkages is of most favorable magnitude for the insertion and activities of enzyme molecules, it is not clear why the reactions due to acetylation, or to hydrolysis by phosphoric or sulphuric acids—where smaller molecules are concerned—should progress along the same planes. Nor is it evident why the enzymatic hydrolysis should progress so commonly along a single plane rather than in a zigzag manner, since at any specific ether-linkage it would seem that hydrolysis might progress in various directions, either upward or downward at angles of from 22° to 25°. Therefore, the most that may be concluded at present is that there are predetermined planes of chemical reaction in native cellulose, certain of which are closely correlated with visible orientations in the cellulosic matrix and others which must be caused solely by molecular configurations. Similarly, in mechanically-induced cracking of the cell wall, there are predetermined planes of cleavage, some of which may be correlated with visible weaknesses in the cellulosic matrix, and others which are caused by submicroscopic factors. One of these sets of planes of structural weakness is oriented parallel to the long axis of the microcapillaries and microfibrils and is much accentuated in cell walls having concentric (PLATE 10, FIG. 2) or radial lamellae (PLATE 8, FIG. 2) of strikingly different porosities. Such walls tend to split concentrically, radio-longitudinally or radio-helically (PLATE 12, FIG. 1) even in ordinary drying; walls of more uniform texture or of ramifying patterns do not.

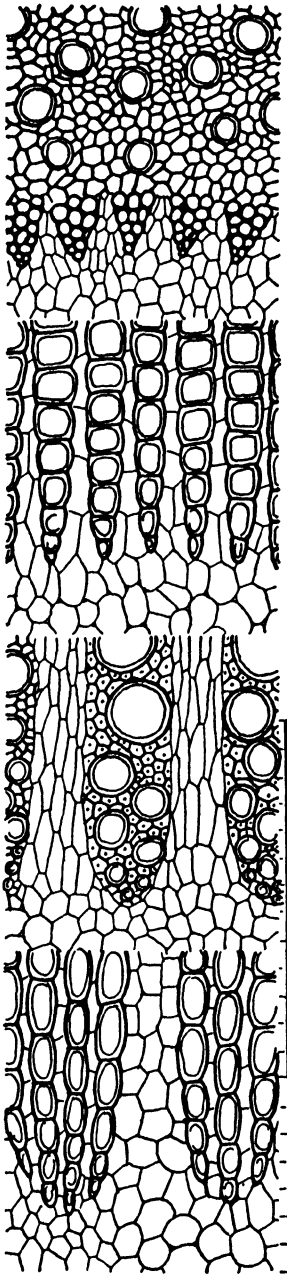
By more or less drastic chemical and mechanical treatments, the secondary wall may be dissected along these predetermined planes of cleavage and hydrolysis into fragments of varying shapes and sizes—dermatosomes, ellipsoid particles, fusiform bodies, elongated shreds or fibrils, etc. During the last 100 years a succession of investigators have hypothesized that certain of these fragments are the elementary units from which the cell wall is constructed. That such fragments are heterogeneous is indicated by their particle double refraction, by their dichroism, and by other physical evidence. Furthermore, it should be clearly recognized that many cell walls and wall layers are much less than one micron in diameter and that the narrower types of lamellae grade down to 500 A or less in diameter. Obviously, these structures cannot be constituted of units 10,000 A or more in diameter.

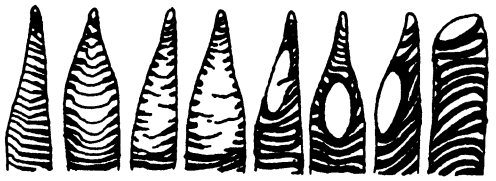
As previously stated, much less is known about the primary wall of plant cells than about the secondary. Evidence is accumulating, however, which indicates that the primary wall has a fundamentally similar physical structure. Statements in the literature that the primary wall is amorphous or isotropic, that it is not composed of typical cellulose, that it is constituted of unoriented cellulose, or that it is composed of cellulose which is invariably

oriented at right angles to the long axis of the cell or to the direction of major expansion are incorrect and based upon errors of observation or of interpretation.

The primary wall, like the secondary one, is composed of a continuous system of more or less extensively coalesced microfibrils of anisotropic cellulose, or at times of chitin, mannan, etc. The elongated porosities of the cellulosic matrix commonly contain pectic compounds and hemicelluloses, and not infrequently lignin, cutin, suberin, waxes, and many other organic substances. The primary wall frequently is lamellated, and, as in the case of the secondary wall, the orientation of the microfibrils, micelles, and chain molecules varies greatly in different types of cells and in different lamellae or parts of the same wall. Growth and expansion of the primary wall appear, in many cases at least, to involve the pulling apart of the fibrils in the previously formed lamellae and the deposition of new layers of coalesced fibrils. In fact, I suspect that the classical controversy regarding growth by intussusception versus growth by apposition will ultimately be settled largely in favor of apposition.

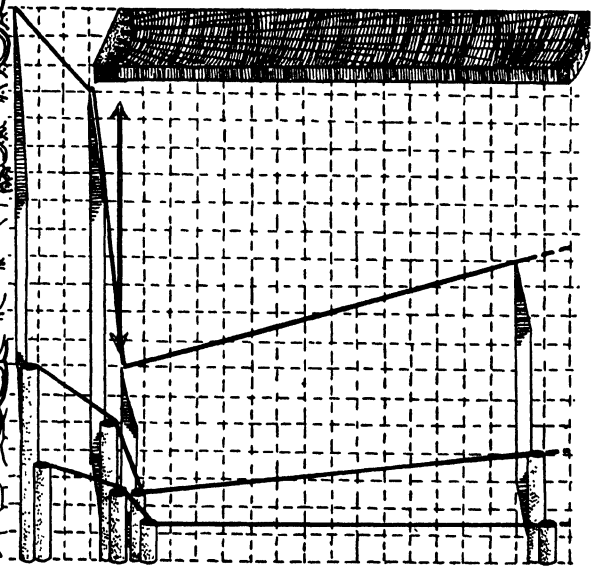
Although we are gradually obtaining a much clearer picture of the complex structures of the walls of plant cells, we have learned very little during the last 100 years about the actual processes by which these remarkable structures are formed by the protoplast. In this direction lies an unexplored field for future research.





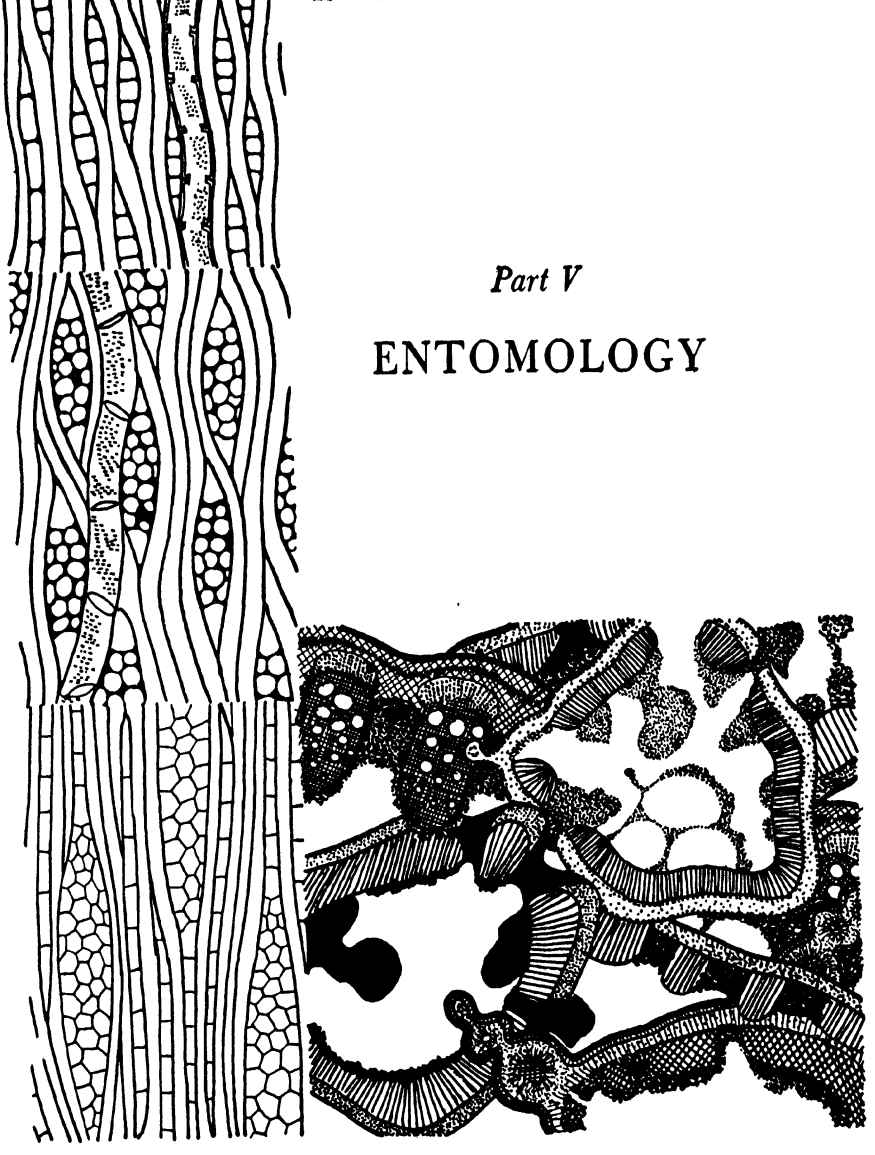
Part III

PHYLOGENY



Part V

ENTOMOLOGY



Chapter 17

NOTES ON NEOTROPICAL ANT-PLANTS*

I. CECROPIA ANGULATA

In a previous paper (43) the writer discussed the significance of the anatomical peculiarities of a number of Ethiopian ant-plants. So many features of unusual interest were encountered in studying these plants, that it seemed desirable to extend the scope of the investigation, and to include certain neotropical myrmecophytes for comparative purposes. With this end in view, the writer spent the summer of 1920 at William Beebe's Tropical Research Station in British Guiana, where the following ant-plants (*Tococa aristata* Benth., *Triplaris surinamensis* Cham., *Tachigalia paniculata* Aubl., *Cordia nodosa* Lam., and *Cecropia angulata,* sp. nov.) grow in close proximity to the laboratory.

Since the publication of Schimper's (1888) much quoted investigations, *Cecropia adenopus* Mart. has been considered one of the most classical illustrations of myrmecophytism. Schimper interpreted the "Müllerian food bodies" and the "prostomata" of this ant-plant as adaptations for enlisting the services of an aggressive army of Aztecas, which protect their host against the attacks of the destructive, leaf cutting, Attine ants; a conclusion that has been assailed by von Ihering (1907), Ule (1906), Rettig (1904), Fiebrig (1909), and other critics of the Belt-Delpino theory of myrmecophily. In view of the important rôle that has been assigned to *C. adenopus,* in discussions concerning the significance of ant-plant symbioses, the writer welcomed the opportunity of studying a somewhat similar species of this interesting genus. The results of the investigation are summarized in the following pages.

Taxonomy:— One of the first difficulties encountered in studying a neotropical biocœnose, in which representatives of the higher plants, ants, coccids, and other insects are closely associated, is the identification of the organisms concerned in the complex. Of course, the principle of significant figures must be considered in biology, as in physics and mathematics. The most detailed field observations and carefully planned experiments may lose much of their significance if an investigator fails to secure adequate information concerning the identity of the plants and animals with which he is working. In most cases, therefore, it is advisable to prepare museum specimens which may be preserved as a record for verification by other investigators. Typical herbarium specimens of all of the plants to be discussed in this and in subsequent papers have been deposited in the Gray Herbarium of Harvard University. Specimens of the insects have been preserved in the collections of Professor W. M. Wheeler, who col-

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laborated with the writer in the investigation of the myrmecophytes of the Kartabo region. The ants were identified by him, the coccids by Mr. HAROLD MORRISON of the United States Bureau of Entomology, and certain parasitic Hymenoptera by Professor C. T. BRUES.

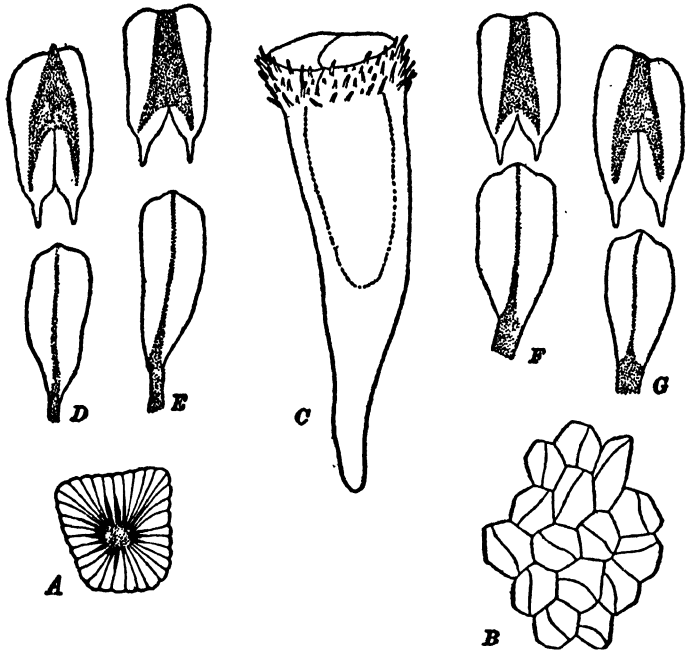
In dealing with *Cecropia,* one is concerned with a group of plants which present many taxonomic difficulties. The leaves of mature plants frequently are much too large for herbarium sheets of standard dimensions, and the inflorescences are difficult to season for museum purposes. Thus many of the descriptions of species are based upon the study of more or less fragmentary material. Furthermore, there appears to be as yet no consensus of opinion as to which of the foliar and floral characters are of the greatest diagnostic value. In view of these facts, the writer devoted considerable attention to the investigation of the variability of the morphological characters of the Kartabo species of *Cecropia.*

There proved to be two distinct species, a myrmecophytic species, with a well developed trichilium and numerous food bodies, and a non-myrmecophytic species, which is entirely devoid of these structures. The latter is considered by Dr. E. H. SNETHLAGE to be a variety (*decurrens*) of *C. sciadophylla* Mart. The former does not agree with any previously described species, and, owing to its association with ants, was studied more intensively.

During the earlier juvenile stages the leaves of this species are small, ±6 cm. long, ±2 cm. wide, simple, lanceolate, finely serrate, pilose above and densely albido-tomentose below. The subsequently formed leaves increase rapidly in size, forming first three, then five, and ultimately nine to eleven lobes. As the juvenile leaves develop lobes, they lose their marginal serrations and become sharply asperate upon the upper surface. The transitions from the juvenile to the typical adult foliage are gradual, and may be deferred until relatively late stages in the ontogeny of the plant. Thus the large deeply lobed leaves of tall saplings may retain many of the juvenile characters, that is, asperate upper surface, chartaceous texture, conspicuous white tomentum on the under surface, acuminate lobes, etc. The leaves of adult individuals are huge, 56-98 cm. long, 56-84 cm. wide, coriaceous, glossy glabrous above, and are provided with only a microscopic layer of closely appressed white hairs in the areoles of the lower surface. They are divided to within 4-7 cm. of the base into 9-11 cuneate-obovate or spatulate lobes which have undulate margins.

Although at first sight most of the vegetative characters appear to be extremely variable, many of them are relatively stable during specific stages in the ontogeny of the plant. For example, the leaves of adult individuals always are deeply lobed (PLATE 19) and undulate on the margins, never scabrous on the upper surface or conspicuously tomentose below; whereas the leaves of juvenile plants always are hairy or sharply asperate above, and are densely albido-tomentose on the under surface. It is evident, therefore, that in distinguishing species of *Cecropia* it is essential to compare leaves from plants of similar age classes, and, so far as possible, to avoid generalizations based upon highly variable, transitional types of foliage, such as occur on saplings.

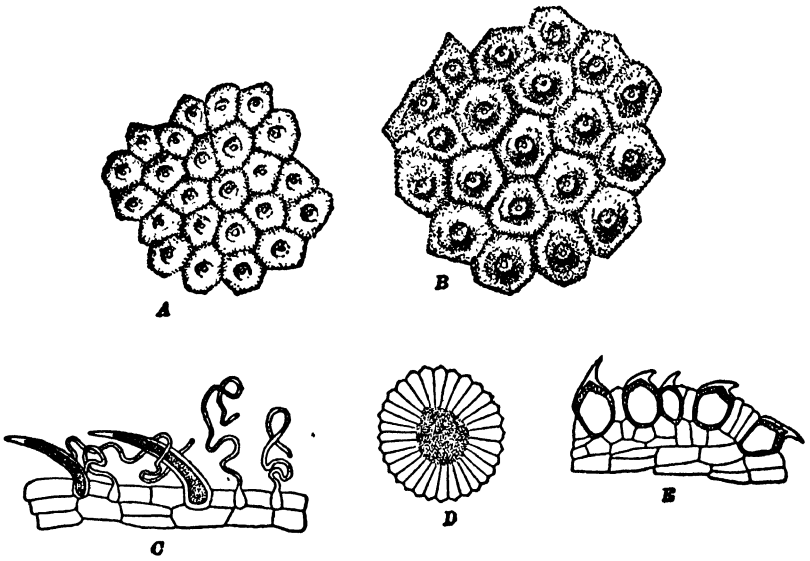
Certain of the floral characters are variable, whereas others are relatively stable. The bracts which envelop the young inflorescences are coated during the later stages of their development with dense, rufous tomentum, and are frosted with long white hairs. The male and female aments vary in size during different stages of their ontogeny, but the former (TEXT FIG. 1*A*) retain their juvenile angularity, whereas the latter (TEXT FIG. 2*D*) become nearly cylindrical at maturity. The peduncles vary considerably in length, but are asperate in both sexes. The female inflorescences are characterized by having one or more perigones in the sutures between the short,



TEXT FIGURE 1.— *Cecropia angulata*. *A*, cross section of ♂ ament; *B*, surface view of portion of ♂ ament, showing pentangular and hexangular, apically cleft perigones; *C*, ♂ perigone, showing collar of short, stiff hairs, and position of perigonial chamber; *D*, lower stamen of pair; *E*, upper stamen of pair; *F*, upper stamen of pair from another perigone; *G*, lower stamen of this pair.

stout, conferruminate pedicels (TEXT FIG. 3*C*). The clavate, pentangular or hexangular, apically cleft male flowers are not jacketed by a dense mat of long, interlacing trichomes, but are provided with a collar of short, stiff hairs (TEXT FIG. 1). The perigonial chamber is confined to the upper half of the flower, and the caudate anthers are borne on short filaments which become extraordinarily broad and membranaceous at the time of dehiscence. The filaments, connectives, and anthers vary considerably in size and shape (TEXT FIG. 1*D-G*); for example, the upper anther of each pair tends to be somewhat smaller, and to be attached nearer its base to a longer filament. The connective may or may not project beyond the apex of the anther.

The pentangular or hexangular perigones of the female aments (TEXT FIG. 2) are characterized by having three distinct types of trichomes. They are jacketed both internally and externally by dense mats of long, interlacing hairs (TEXT FIG. 3). In addition, they have a crown of extremely short, stout bristles on the margins of their exposed apical surface, and a fringe of longer bristles which project into the constricted upper portion of the perigonial chamber (TEXT FIG. 2). The glabrous, almond-shaped ovary is mottled, dark gray, and bears a cylindrical style which terminates in a comose stigma (TEXT FIG. 3). The style is provided with short, stout bristles, such as occur on the apical portion of the perigone. During the development of the seed, the perigone enlarges and becomes somewhat

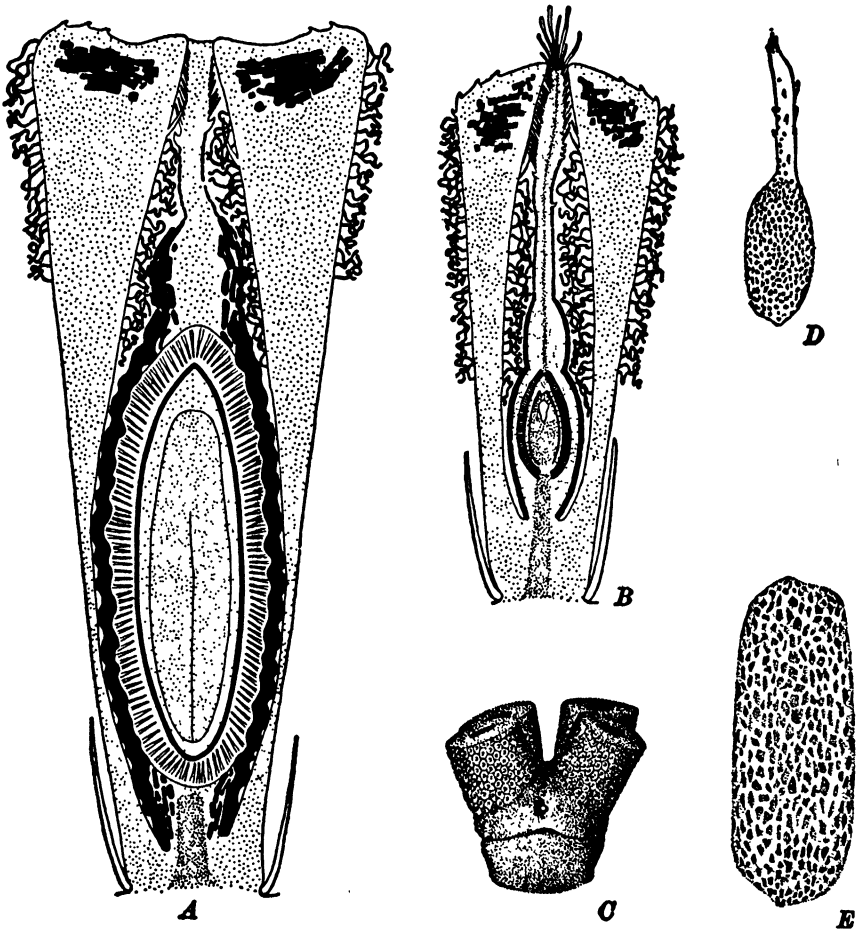


TEXT FIGURE 2.— *Cecropia angulata*. *A*, surface view of portion of ♀ ament, showing pentangular and hexangular perigones at fertilization; *B*, surface view of portion of ♀ ament, showing enlargement of perigones during earlier stages in formation of seed; *C*, two types of trichomes from inner wall of perigone; *D*, cross-section of ♀ ament at fertilization; *E*, trichomes from outer apical portion of perigone (similar bristles occur on style).

modified in form and structure (TEXT FIGS. 2, 3). The white tomentum is carried outward, so that the lower two-thirds of the flowers are not held together by interlacing hairs, and an annular depression is formed in the exposed outer surface of each perigone. At maturity the scurfy, dark reddish brown, oblong seeds (TEXT FIG. 3*E*) have a more or less symmetrical, lozenge-shaped, triangular or rectangular contour in cross-section.

Owing to the variability of many of the floral characters during the enlargement of the aments, there is a considerable element of uncertainty in comparing inflorescences which are not in equivalent stages of differentiation. In the case of the male aments, the anthers, and especially the filaments, do not attain their most characteristic size and shape until just before

dehiscence. Furthermore, there are two distinct stages in the differentiation of the female aments, one before and one after fertilization. The changes in the size and form of the perigones during the maturation of the ovules, and subsequently during the enlargement of the seeds, are reflected in the aments, whose external characters become correspondingly modified. The



TEXT FIGURE 3.— *Cecropia angulata.* *A*, longitudinal section of ♀ perigone and nearly mature seed, showing embryo and various seed coats; *B*, longitudinal section of ♀ perigone, style, ovary, and ovule, showing various types of trichomes; *C*, basal portion of ♀ inflorescence, showing perigone at point of contact of conferruminate pedicels; *D*, ovary and style; *E*, mature seed.

significance of these developmental stages does not appear to have been appreciated fully by a number of students of the species of *Cecropia.* The aments, perigones, stamens, seeds, etc., are described and are used for diagnostic purposes without reference to their variability or to their particular stage of development. Until more is known concerning the variability of the various species of *Cecropia,* and until their more stable

diagnostic characters have been isolated and fully described, it is desirable to give rather comprehensive descriptions of new species. The following description of the myrmecophytic species of the Kartabo region is based upon the study of typical adult specimens. The juvenile characters have been referred to previously.

Cecropia angulata, sp. nov.—Arbor 10-25 m. alta, caulibus ramisque juvenibus argute asperatis formicosis (Azteca). Folia ampla rubella vel viridia 56-98 cm. longa 56-84 cm. lata profunde 9-11-lobata, lobis cuneato-obovatis vel spatulatis, apice obtusis vel rotundatis saepissime mucronatis, margine undulatis, lobo medio 36-60 cm. longo 13-24 cm. lato, nervis secundariis 15-20, lamina supra glabra nitente (post exsiccationem castanea obscurata) infra in nervis venis venulisque glabrata, in areolis solis tenuiter (microscopice) albido araneoso-tomentosa et in disco centrali breviter griseo-villosa, petiolo cylindrico (post exsiccationem costato) 50-120 cm. longo glabro vel albido-tomentoso ad basin incrassato et cum trichilio albido (tardius castaneo) instructo. Stipulae vaginantes maximae castaneae paullo griseo-tomentosae. Amentae ♂ 14-18 rectae rigidae *angulatae* 5-12 cm. longae 4-7 mm. crassae in pedicellis tenuibus 5-17 mm. longis gestae et in spatha castanea griseo-tomentella fusiformi ad basin constricta 14-16 cm. longa inclusae. Amentae ♀ 4 rectae rigidae vel apice paullo curvatae cylindricae ante anthesin 9-12 cm. longae 7-8 mm. crassae (tardius 13-17 cm. longae 11-13 mm. diametro) in pedicellis crassis conferruminatis 3-7 mm. longis gestae et in spatha castanea griseo-tomentella quadrangulata obtusa 10-12 cm. longa inclusae. Pedunculi argute asperati compresso-cylindrici, ♂ 9-12 cm. longi 8-11 mm. crassi, ♀ 8-10 cm. longi 7-10 mm. crassi. Perigonia ♂ clavata pentangularia vel hexangularia 2-3 mm. longa, filamentis in maturitate membranaceis 0.5-1.4 mm. longis 0.3-0.5 mm. latis, antheris caudatis 0.8-1.0 mm. longis 0.4-0.5 mm. latis. Perigonia ♀ pentangularia vel hexangularia cum albido tomento vestita ad anthesin 1.5-2.0 mm. longa deinde usque ad 3.0-3.5 mm. elongata. Stylus cylindricus ovarium aequans vel eo longior. Stigmata comosa. Fructus maturus furfurosus rubidus 2.0-2.5 mm. longus.
Kartabo region, British Guiana: *I. W. Bailey* (1920), nos. 3, 4, 5, 6, 8, 9, 14, 15, and 17; Kangaruma, British Guiana: *H. A. Gleason* (1921), no. 198.

The fistulose stems of *Cecropia angulata* are inhabited by four distinct species of *Azteca;* a majority of the plants are colonized by a black species, *A. constructor* Emery, a considerable number by a yellowish species, *A. alfari* Emery, and an occasional isolated individual by either *A. instabilis* F. Smith or *A. trigona* subsp. *mediops* Forel. The following observations upon the habits of the guest ants are based upon a study of the first two species.

Of the coccids which are associated with *Azteca, Pseudococcus rotundus* Morrison is the commonest species. *Akermes quinquepori* Newstead and *Pseudococcus bromeliae* Bouché are of more or less sporadic occurrence.

Are Cecropias Protected by their Guest Ants?:— In studying the remarkable leaf cutting and fungus growing habits of the neotropical Attine ants, Belt (1874) became much impressed by the efficiency of these insects in defoliating large numbers of native and introduced plants, and he was led to wonder how tropical vegetation has survived their devastating attacks. He inferred that the leaves of many plants are distasteful to the ants or are unsuitable for their purposes, and that other plants are provided with special means of defense. He concluded that the so-called bull's-horn Acacias and other myrmecophytes are protected by their guest ants, which drive away their leaf cutting relatives. Müller (1880-81) and Schimper (1888) endeavored to prove that the myrmecophytic species of *Cecropia*

are protected by the ants which inhabit their fistulose stems and branches. They found that plants which were colonized by Aztecas were not molested by Attine ants, whereas uninhabited individuals were more or less completely defoliated by them.

That BELT, MÜLLER, and SCHIMPER tend, on the one hand, to exaggerate the destructiveness of the leaf cutting ants, and on the other hand to overemphasize the protection afforded by the guest ants, has been suggested by VON IHERING (1907), RETTIG (1904), ULE (1906), FIEBRIG (1909), WHEELER (1913), and other critics of the theory of myrmecophily. Several of these investigators call attention to the fact, previously noted by MÖLLER (1893), that the leaf cutting ants feed upon a great variety of plants, and show no particular preference for the foliage of *Cecropia*. Thus, although the Attine ants frequently are troublesome pests in gardens and orchards, their feeding habits under normal conditions are such that they are not likely to exterminate indigenous species. According to VON IHERING'S computations, 183 nests of leaf cutting ants consume no more foliage during a year than does a single cow. In many cases the myrmecophytes grow in regions, such as swamps and periodically inundated areas, where the fungus growing Attas do not occur. Furthermore, during the earlier stages of its development *Cecropia* is not inhabited or protected by its putative guardians. In addition, it has been shown that plants which are not inhabited by Aztecas may remain unmolested by Attas for long periods, and that trees which are inhabited may be seriously injured by phytophagous insects and sloths. Of course it must be admitted in this connection that the discrepancies between the conclusions of MÜLLER and SCHIMPER and those of ULE, VON IHERING, and others, may be due to the fact that they were concerned with different species of Attine ants. Furthermore, it may be argued that the juvenile plants do not need the protection of the Aztecas, since they are immunized by various protective devices.

In the forests of the Kartabo region there are numerous colonies of the common leaf cutting and fungus growing ant, *Atta cephalotes* L. The writer found, as MÖLLER had previously done in the case of *A. discigera* Mayr., *A. hystrix* Latr., and *A. coronata* Fabr., that this ant utilizes the leaves of a great variety of plants in the construction of its fungus gardens. In virgin and second growth forests, it seldom works for any considerable length of time upon a particular type of plant, but continually shifts its activities from one species to another. Owing to this fact and to the rapid recovery from injury by plants in moist tropical environments, the effects of its attacks upon a given species appear to be more or less evanescent. Its normal leaf cutting habits, however, may be considerably modified under unusual or abnormal conditions. It frequently exhibits a strong preference for plants to which it has not previously been accustomed. Thus its attacks upon certain exotic plants in gardens and orchards at times may prove to be singularly persistent and destructive.

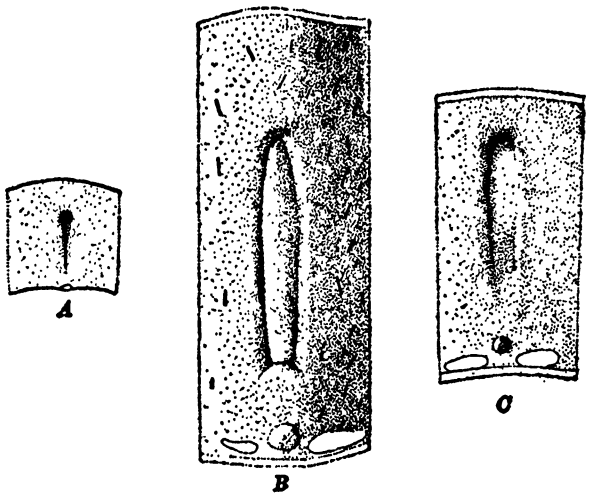
In many cases *Cecropia angulata* is not colonized by its guest ants until it has attained a considerable size. This is largely due to the fact that the young, fecundated queens, which initiate the new colonies, are killed by a Hymenopterous parasite, *Conoaxima aztecicida* Brues. Such plants are no

more subject to defoliation than are the inhabited individuals. In order to determine whether the leaves of the juvenile *Cecropia* are distasteful to the leaf cutting ants, or are unsuitable for their purposes, a number of young plants were placed in close proximity to a large nest of *Atta cephalotes*. Leaves of adult Cecropias and of various other plants were used as controls. Although the ants worked upon this material in a more or less sporadic fashion, and showed a strong preference for certain types of leaves, they devoted no more attention to the mature than to the juvenile foliage of *Cecropia angulata*. In view of the fact that both BELT and SCHIMPER admit having seen young myrmecophytes defoliated by Attas, there appears to be little evidence in favor of the suggestion that juvenile ant-plants are less susceptible to the attacks of phytophagous insects than are the adult individuals.

In defending the theory of myrmecophily, SCHIMPER placed great emphasis upon the discovery, in the Corcovado near Rio de Janeiro, of a species of *Cecropia* which is devoid not only of ants, but also of prostomata and Müllerian food bodies. He inferred that this *Cecropia* possesses no adaptations for attracting a defending army of Aztecas, because it is protected by a waxy coating which prevents the leaf cutting ants from climbing its stems. *Cecropia sciadophylla* Mart. var. *decurrens* Snethlage is not colonized by Aztecas, but, as will be shown later, is provided with conspicuous and highly differentiated prostomata. The external surfaces of the plant are scabrous and afford a firm foothold for ants, as evidenced by the fact that several species of these insects were seen climbing its stems and branches. This species of *Cecropia* is no more subject to defoliation by Attas than is *Cecropia angulata*. That the leaves may be utilized in the construction of fungus gardens is shown by the fact that, when cuttings from plants of various ages are placed near nests of *Atta cephalotes*, the ants frequently transport a portion of the foliage into their subterranean dwellings.

SCHIMPER's critics place considerable emphasis upon the fact that the myrmecophytic Cecropias may be inhabited simultaneously by two or more distinct genera of ants. To infer from this, however, that the Aztecas are indifferent to the presence of other ants is somewhat misleading. Although species of *Cecropia* were found that were inhabited by *Cryptocerus*, *Crematogaster*, stingless bees, etc., these insects were always confined to the older and lower internodes, which had been abandoned by the Aztecas. In spite of this marked segregation of the colonies in different levels of the stem, fierce conflicts may be waged for possession of the intervening internodes. Thus, the internodal cavities, where the colonies come in contact, frequently are filled with the corpses of dead warriors. The Aztecas are dominant, and in general do not tolerate the presence of other ants, in those portions of the stems and branches which are provided with leaves and food bodies. SCHIMPER found that when leaf cutting ants were deposited upon the terminal portions of a *Cecropia*, they were quickly seized by the Aztecas, if their presence was discovered, and were hurled to the ground. How then may one account for the defoliation of trees which are inhabited by Aztecas? When a *Cecropia* is touched or shaken, the angry and aggressive

Aztecas rush out of their nests and swarm over the whole plant, but under normal conditions only a limited number of workers are visible on the stems and bases of the petioles. Furthermore, there are periods during which the entire colony is quiescent, that is, has withdrawn into its nest. It is well known that these periods of quiescence are not the same in the case of different species of ants. Thus, certain ants are nocturnal, others are active at temperatures when other species are inactive, etc. In other words, as suggested by MÜLLER and MÖLLER, a *Cecropia* may be defoliated by leaf cutting ants during periods when the Aztecas are quiescent.



TEXT FIGURE 4.— Prostomata or internodal diaphragms of various species of *Cecropia*. *A*, *C. adenopus*, after SCHIMPER; *B*, *C. sciadophylla* var. *decurrens*; *C*, *C. angulata*.

Prostomata:— Above the insertion of every leaf in *Cecropia adenopus* there is a shallow groove, which terminates just below the next node in a roundish depression (TEXT FIG. 4). As the external depression corresponds to an internal one, the wall of the fistulose stem is very thin at this place, and is a mere diaphragm in a tube. SCHIMPER showed that this diaphragm is devoid of hard and tough elements (fibrovascular bundles, collenchyma, lignified parenchyma, etc.), such as occur in the adjoining portions of the stem. He inferred from this that the diaphragm originated as an adaptation for facilitating the entrance of ants. At the phylogenetic commencement of symbiosis, the ants bored an entrance through the groove, evidently because the wall of the stem was somewhat thinner there. In accordance with a custom that is almost invariably followed and is connected with the domestic arrangements in their nests, they tended to penetrate into the internodal cavity at its upper extremity (at the apex of the groove). All features which facilitated their entrance at this place were retained in the struggle for existence, and were accentuated through natural selection. This led eventually to the differentiation of a thin, weak diaphragm or prostoma (VON IHERING). In other words, although SCHIMPER

admitted that the groove is due to the pressure of the axillary bud, he maintained that its terminal portion is a highly specialized adaptation, acquired through the action of natural selection. On the contrary, RETTIG and FIEBRIG assert that the prostoma is merely the youngest or less highly differentiated portion of the groove, and that it is produced by the pressure of the axillary bud and other growth phenomena in the elongating internodes. The former investigator is of the opinion that the ants are deterred from excavating in the lower portion of the groove, not by mechanical obstructions, but by the occurrence of "laticiferous vessels" which are absent in the prostoma. It is to be emphasized in this connection, however, that RETTIG's and FIEBRIG's statements do not necessarily invalidate SCHIMPER's conclusion that the diaphragm is an adaptation which originated as a modification of a previously existing structure.

Under most growth conditions *Cecropia angulata* does not form a shallow groove which terminates in a conspicuous circular pit (TEXT FIG. 4). The more or less fusiform depression or rill is somewhat deeper in the upper than in the lower portions of the internode, but the differentiation of specialized or mechanical types of tissue is retarded throughout its extension (PLATE 20, FIG. 1). As the walls of the internode increase in thickness, after the initiation of secondary growth, this fusiform diaphragm of delicate parenchyma is slowly reenforced by tougher and denser tissues (PLATE 20, FIG. 3). The metamorphosis begins at the base of the groove, and gradually extends upward, but the ants excavate their exits before these changes have progressed very far. It is evident, therefore, that in the case of the myrmecophytic *C. angulata,* the whole groove is a potential prostoma. The exact location of the aperture is determined, not by the presence or absence of resistant tissues or of "latex" vessels, but by the relative thickness of different portions of the diaphragm. That the whole groove is a potential prostoma, and that the ants merely excavate their exits and entrances in its thinnest and most easily perforable portion, are indicated by the behavior of young queens in juvenile plants. In many instances, several queens attempt to occupy the same internode, and as many as five entrances were found cut at different levels of a single groove. Not only do the queens cut through the basal portions of the diaphragms under such conditions, but they may even excavate entrances in portions of the internodes which are not provided with preformed depressions.

It may be argued that in *C. angulata* the whole groove has been modified as an adaptation to ants. Such an assumption is not warranted, however, when important facts in the anatomy of the non-myrmecophytic *C. sciadophylla* var. *decurrens* are taken into consideration. Although this species is not inhabited by Aztecas, it is provided with more tenuous and highly specialized diaphragms than is *C. angulata* (TEXT FIG. 4; PLATE 20, FIG. 2). The internodal groove is very broad and deep, and the diaphragm, which is entirely devoid of tough tissues and secretory vessels, is composed of extremely thin layers of delicate parenchyma. Such facts as these suggest that the so-called prostoma of *C. adenopus,* and of other myrmecophytic species of *Cecropia,* is not an adaptation for attracting ants, but is merely a

structural peculiarity, produced by the pressure of the axillary bud, which is utilized by the ants in their parasitism upon the plants.

Feeding Habits of Guest Ants:— The *Azteca* colonies are initiated by young fecundated queens which cut entrances into the fistulose stems of juvenile plants. Although I examined hundreds of plantlets of *C. angulata,* none of any considerable size were found that had been entered by but a single queen. The queens are so numerous that many of the successive internodes become inhabited, and, as already stated, one not infrequently finds that several queens have penetrated into the same internodal cavity. So far as it was possible to determine, however, a large proportion of these queens perish before they have produced a brood. A very considerable number are killed by the parasitic *Conoaxima aztecicida,* others are killed by their rivals in conflicts for possession of a given internode, and, as soon as the workers become numerous, they cut through the nodal partitions and kill all but one of the surviving queens.

When a young *Azteca* queen enters an internodal chamber, she covers the entrance aperture with a layer of triturated pith. The entrance subsequently becomes occluded by callus, which continues to grow internally, and finally projects some distance into the medullary cavity (PLATE 20, FIG. 4). Thus the queen is sealed within the internode during the period when she is initiating the new colony. In view of the fact that the queen is unable to leave her nest in search of food during a period of two months or more, MÜLLER, VON IHERING, and FIEBRIG infer that she must feed upon medullary tissue and the inwardly projecting callus or "stomatome." It should be noted in this connection, however, that such an assumption is based upon two more or less fallacious premises: (1) that the queen must feed during her period of isolation, and (2) that tissues which are gnawed or excavated by ants actually are eaten by them. Most students of the Formicidae are familiar with the fact that female ants are able to do without food, except such as is stored in their own bodies, for the greater part of a year, while they are founding their colonies. Furthermore, it is well known that many ants tend to gnaw into and smooth the walls of their nests, regardless of whether they are composed of living tissues or of inert materials. I was unable to find any evidence that the *Azteca* queens feed upon the tissues in the young internodes of *C. angulata.* The so-called stomatomes, upon which MÜLLER and VON IHERING place so much emphasis, are not uncovered and cut back by the queens until just before the first workers are ready to emerge from the nest, nor do they excavate the medullary tissue to any considerable extent, except when in search of material with which to block up the newly formed entrance aperture. Although VON IHERING is of the opinion that the luxuriant growth of callus is due to the stimulus of some substance excreted by the queens, I found that homologous structures may be produced by purely mechanical injuries.

With the advent of the first workers, the entrance to the primordial chamber is reopened, and the young colony either migrates to a higher internode or cuts through the nodal partitions into adjoining cavities. VON IHERING states that the Aztecas always abandon the primordial chamber

and never perforate its upper and lower walls. Such is not invariably the case in C. *angulata,* for primordial chambers were frequently found in direct communication with internodes which were not provided with prostomal openings. Regardless of its exact mode of origin, the permanent domatium soon becomes stocked with food bodies by the young workers. These small beadlike structures (PLATE 20, FIG. 6), which are packed with fat and protein, are formed in large numbers in a curious cushion or mat of hairs, situated at the base of each petiole (PLATE 19). The ripe food bodies are so assiduously collected by the ants that it is almost impossible to find one *in situ,* except in young uninhabited plants. Indeed, the ants frequently trim away the surrounding hairs and dig out the immature food bodies. SCHIMPER interpreted these so-called Müllerian corpuscles, and similar structures which occur on the leaflets of certain myrmecophytic species of *Acacia,* as metamorphosed glands or highly specialized allurements for attracting ants. RETTIG and others, however, have called attention to the fact that such glands occur on plants that are not frequented by ants, and it is difficult for the adherents of myrmecophily to account for such occurrences without resorting to the purely gratuitous assumption that they are survivals from former symbioses. ULE is of the opinion, in addition, that the expenditure of carbohydrates and nitrogenous substances, contained in these corpuscles, is not compensated for by the protection which the ants afford to the plants.

Although most investigators agree that the food bodies are an important item of food in the diet of the Aztecas, it has been suggested that there are other potential sources of food in *Cecropia.* Thus, VON IHERING and FIEBRIG maintain that the imagines feed upon the succulent medullary tissues in immature internodes. It is true that the ants cut away the softer portions of the pith down to a hard, smooth peripheral layer of medullary tissue, but I found no evidence to indicate that this is not purely a process of house cleaning, such as occurs in many ant nests. In the moist, warm interiors of plants, ants have to contend with luxuriant growths of fungi which obstruct the cavities and interfere with the brood, unless they are held in check. The ants trim away the hyphae and cut back the substratum upon which these organisms tend to grow. FIEBRIG records having seen Aztecas busily engaged in excavating the pith, and in casting fragments of medullary tissue from their entrances, but such observations cannot be interpreted as evidence that the ants actually feed upon the tissues that they are removing.

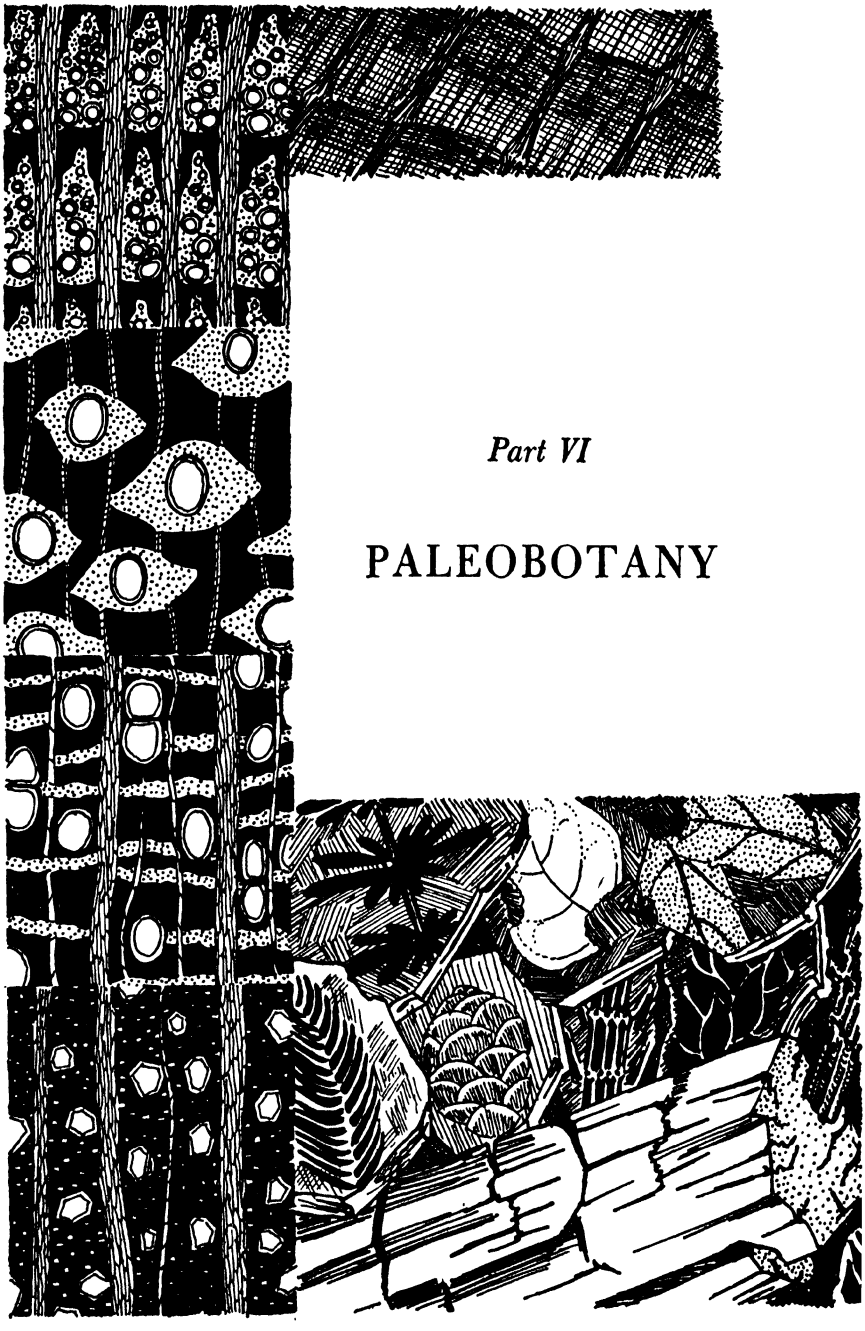
Most students of the myrmecophytic species of *Cecropia* have found coccids associated with the ants which inhabit the fistulose stems. Their presence has been variously interpreted. BELT, MÜLLER, and ULE consider that they are tended by the ants which feed on their sugary exudations, but FIEBRIG states that the insects "in keinen direkten Verhältnis zu diesen Ameisen stehen." Having found a very close and significant relation between ants and coccids in most Ethiopian ant-plants, I devoted particular attention to the investigation of their behavior in C. *angulata.* I did not succeed in finding a single large, ant-inhabited specimen which did not contain numerous coccids. When a tree is split open the ants are as

solicitous for the welfare of the coccids as they are for that of their eggs, larvae, and pupae. They seize them in their mandibles and carry them about until some unopened portion of the plant is found where they may be deposited in safety. In artificial nests, the workers spend hours in tending and stroking the coccids, and in feeding upon their sugary exudates. In view of these facts, it cannot be doubted that the miniature milch cows are an important source of liquid carbohydrates for the ants.

As in many of the African myrmecophytes, the ants excavate pits in the walls of their domatia which enable the coccids to reach and feed upon the softer tissues of the *Cecropia*. Such excavations are essential, owing to the fact that the internodal, medullary cavity is entirely jacketed by a dense, horny layer of sclerenchyma. In the African ant-plants, the ants cut through to the cambium and induce the formation of a nutritive callus. In *C. angulata* the pits are not located in the sides of the internodal chamber, but in the nodal diaphragms. At the time when the ants begin their excavations, the nodal partition consists of five distinct layers (PLATE 20, FIGS. 5, 7). The soft, internal layer, which is provided with strands of conducting tissue and which is fed upon by the coccids, is separated from the external layers of porous, medullary tissue by two layers of dense, thick walled tissue. The ants remove the two external layers and cut circular pits in the underlying sheets of horny sclerenchyma (PLATE 20, FIGS. 8, 9). The coccids sit in these pits and thrust their setae into the succulent tissue which is thus exposed. That the pits are not made by the coccids, as suggested by VON IHERING, is indicated, not only by the fact the delicate sucking mouth parts of these insects are not adapted for excavating in dense tissues, but also by the fact that I have actually observed the ants in the process of excavating them.

SUMMARY AND CONCLUSIONS

The theory of myrmecophily, as modified by SCHIMPER, interprets the structural peculiarities of myrmecophytic species of *Cecropia* as adaptations for enlisting the services of an aggressive army of Aztecas which protect their hosts against the attacks of the leaf cutting Attine ants. Cleverly conceived and suggestive as this Neo-Darwinian hypothesis undoubtedly is, it appears to be based upon a series of plausible deductions or teleological inferences, and is open to serious criticism. The distribution and feeding habits of the Attine ants in luxuriant, tropical forests are such that the ants are not likely to exterminate indigenous species. They show no strong preference for the foliage of *Cecropia*, and rarely attack either inhabited or uninhabited trees. Although Aztecas tend to prevent other ants from visiting the terminal portions of the adult Cecropias, they do not protect the juvenile individuals. That the curious prostomata and Müllerian corpuscles of *Cecropia adenopus* and of other myrmecophytic species of *Cecropia* are not allurements, acquired through natural selection, is indicated by the fact that such structures occur on certain species of *Cecropia* and other plants which are not frequented by ants. The *Azteca-Cecropia* associations, and ant-plants in general, are extremely interesting cases of parasitism, which illustrate the remarkable adaptiveness of ants in availing themselves of the potentialities of given environments. This is shown, not only in the utilization of the preformed food bodies, prostomata, and internodal cavities, but more strikingly in the structural modifications produced within the plants for the growth and tending of coccids. Thus, the ants are able to obtain food from the plant in two ways, fats and proteins directly from the Müllerian corpuscles, and carbohydrates vicariously through the coccids.



Part VI

PALEOBOTANY

Chapter 18

THE CAMBIUM AND ITS DERIVATIVE TISSUES

VII. PROBLEMS IN IDENTIFYING THE WOOD OF MESOZOIC CONIFERAE *

MUCH emphasis has been placed upon the occurrence of supposedly intermediate or transitional types of structure in the wood of Mesozoic Coniferae. Although most paleobotanists agree that such genera as *Protopiceoxylon, Protocedroxylon, Planoxylon, Araucariopitys,* etc., are generalized or transitional forms, striking differences of opinion have arisen concerning their classification and phylogenetic significance. One group of investigators considers them to be forerunners of the *Pinaceae*[1] (*Protopinaceae*) which exhibit evidences of an Araucarian or Cordaitean ancestry, whereas another group of workers asserts that they are primitive Araucarian conifers (*Araucariopityeae*) which retain vestiges of a Pinaceous ancestry.

The principal argument for regarding such conifers as generalized or transitional forms is the occurrence of so-called Araucarian pitting in combination with structures that are considered to be characteristic of the *Pinaceae.* The chief argument for segregating them into new hypothetical genera and subfamilies is the tacit assumption that similar combinations of structural characters do not occur in living representatives of the Coniferae.

The writer does not propose to enter the controversy concerning the relative antiquity of the *Pinaceae* and *Araucariaceae,* but to raise the question whether these putative transitional Mesozoic forms fall within the range of structural variability of living coniferous genera.

Tracheary Pitting:— Before attempting to discuss this question, it is essential to determine first what is meant by 'typical Araucarian' pitting, as contrasted with the 'ordinary Coniferous' type. There appears to have been a general consensus of opinion among paleobotanists that in the former type the bordered pits are contiguous and flattened and, when in more than one vertical series, typically 'alternate'; whereas in the latter type the bordered pits tend to be more or less widely spaced and, when in more than one vertical row, characteristically 'opposite.'

The tracheary pitting of the transitional Mesozoic Conifers is not typically Araucarian but rather a mixture of putative Pinaceous and Araucarian types. Thus, in *Cedroxylon transiens* Gothan, the bordered pits may be spaced or contiguous, opposite or alternate, or arranged in stellate clusters. According to SEWARD (1919), 'the Abietineous features predominate over the Araucarian, the latter being limited to the local occurrence of polygonal and alternate bordered pits.' Similar patches of alternating pits

* Reprinted from the Annals of Botany 47: 145-157, 1933.

[1] PILGER'S (1926) nomenclature for the Coniferae is used throughout this paper.

occur in *Protopiceoxylon extinctum* Gotham. In *Araucariopitys americana* Jeffrey, the pits are mostly uniseriate and either widely spaced or contiguous; rarely biseriate, and then either opposite or alternate. They are stated to be contiguous and alternate in the spring-wood of *Thylloxylon irregulare* Gothan, and *Planoxylon Hectori* Stopes, but to be more or less widely spaced in the summer-wood. In *Metacupressinoxylon cedroides* (Holden) Torrey, they are described as uniseriate, spaced, or biseriate alternating.

The bordered pits of these plants are considerably larger than those which occur in living Araucarians, at least in comparable material of mature stem-wood, and certain of them possessed a well-developed torus. These are Pinaceous rather than Araucarian attributes. Furthermore, the bordered pits, when alternating, commonly tend to be less closely compressed than in typical Araucarian pitting.

It should be emphasized, in this connexion, however, that the distinctions between the two contrasted types of tracheary pitting are not as infallible diagnostic criteria as has commonly been hypothesized. They cannot be relied upon in dealing with the zone of transitional tracheides—*i.e.*, metaxylem and first formed secondary tracheides—since mixed types of pitting occur in such regions and since, as the writer (53) has shown, the primary wood of the Coniferae is of a singularly modified and extremely specialized type. Pool (1928) and others have called attention to the fact that separate and circular pits may occur in the secondary xylem of Araucarians. Contiguous and more or less flattened pits are of not infrequent occurrence in mature wood of other representatives of the Coniferae. Even the occasional occurrence of alternate pitting is not considered by Seward (1919) to be an infallible criterion of Araucarian affinity. It is evident, accordingly, that the distinctions between Araucarian and Pinaceous types of tracheary pitting are quantitative rather than qualitative. In other words, the tracheary pitting of the Mesozoic fossils is to be regarded as transitional because the proportions of contiguity, flattening, and alternation presumably are higher than in living representatives of the *Pinaceae*. Is such, indeed, the case?

Our systems of classifying and identifying the woods of the higher plants have developed largely through trial and error and, as the writer (28, 50) has shown, are subject to constant correction and revision. This is due to the fact that comparatively little is known concerning the limits of variability of the diagnostic criteria used in the construction of keys. The extent to which structural characters may vary in different parts of a single tree, or in trees grown under different environmental conditions, does not appear to have been appreciated fully by most of those who have concerned themselves with the identification either of commercial woods or of fossils. As will be shown in succeeding paragraphs, the variability of the tracheary pitting in the genus *Cedrus* affords an interesting verification of these criticisms.

Recently the writer had occasion to study the structure of the secondary xylem in *Cedrus Libani* Bar., *C. Deodara* Loud., and *C. atlantica* Man. Samples of wood were secured from branches, stems, and roots of trees

growing in California, Massachusetts, and Lincolnshire, England, and from the outer portions of the stems of older trees grown in their native habitats —*i.e.*, India, Palestine, Algeria, and Morocco. Although the writer anticipated considerable variation in the tracheary pitting of *Cedrus*, he was surprised to find that all the principal types of pitting illustrated in PLATES 21 and 22 occurred, not only in the genus as a whole, but within the limits of a single tree, and even of a single specimen.

The particular admixtures of different types of pitting and the proportions of contiguity, flattening, and alternation vary markedly from specimen to specimen. In wood from the branches and young stems of trees growing in parks and botanical gardens of North America and England, the tracheary pitting tends to be of the 'ordinary Coniferous' type—*i.e.*, in each tracheide there are one or two vertical series of more or less widely spaced bordered pits (FIGS. 15, 17, 20, 22, and 23). Alternate pitting is absent or of sporadic occurrence, though contiguity and flattening of the *Araucariopitys*-type may be present. In specimens from the outer portions of the stems of what appear to have been large, old, slow-growing trees, the Pinaceous pitting may occur in combination with varying proportions of 'Araucaroid' (FIGS. 27 and 30), and of intermediate and clustered types (FIGS. 16 and 21). The combinations of pitting in certain specimens closely resemble those that characterize *Cedroxylon transiens*, *Protopiceoxylon extinctum*, and other Mesozoic fossils. The most extreme cases of contiguity, flattening, and alternation encountered by the writer are in certain samples of root wood. In these, the 'Araucaroid' pitting may be greatly accentuated in the tracheides of the spring-wood (FIGS. 25 and 26), and is in marked contrast to the more widely spaced pits (FIG. 28) that occur in the transitional zone and summer-wood. In other words, these particular combinations of tracheary pitting approximate those that are considered to be salient diagnostic features of *Planoxylon*, *Thylloxylon*, and *Protocedroxylon araucarioides*.

It is evident, accordingly, that the tracheary pitting of the genus, *Cedrus*, is extraordinarily plastic and variable, and is of fully as 'transitional' a character as any that occurs in Mesozoic fossils. Furthermore, preliminary investigations indicate that similar admixtures of Pinaceous and Araucarian pitting occur in *Keteleeria* and other living representatives of the *Pinaceae*, but that in stem-wood of *Pinus*, *Picea*, *Pseudotsuga*, *Larix*, *Tsuga*, and *Abies*, contiguity, flattening, and alternation usually are of more limited and sporadic occurrence than in the root wood of these genera or in the stem-wood of *Cedrus*.

So-Called Rims or Bars of Sanio:— The so-called rims or bars of Sanio are regarded by certain paleobotanists as the most reliable of all diagnostic criteria in distinguishing woods of Pinaceous and Araucarian affinities. The reputed absence of these structures in *Protocedroxylon*, *Protopiceoxylon*, *Planoxylon*, and other Mesozoic Coniferae is held to be a sufficient justification for classifying such genera as Araucarians rather than as *Protopinaceae*. Unfortunately, there is, in all probability, no more confused and contradictory chapter of paleobotanical literature than that which deals with

these structures. They have not been defined accurately either from the morphological or the biochemical point of view, and structures of entirely different developmental history, as well as various artifacts, have been confused with them.

In 1924, in reviewing the work of SIFTON (1922) and HALE (1923), the writer (52) called attention to the fact that controversies concerning the structure, distribution, and diagnostic value of the so-called rims or bars of Sanio had reached an *impasse,* which could be clarified only by detailed investigations of the cambium and its differentiating derivatives. During the last ten years, he has undertaken a number of such investigations in connexion with the work which has been described in preceding papers of this series. These investigations indicate that the developmental history of the rims or bars of Sanio, as of the torus, is morphologically and biochemically an extremely complicated story. Only the salient features need be mentioned here, as a detailed discussion will be given in a separate paper.

In the cambium of Gymnosperms and Dicotyledons the radial walls of the initials are not in close contact throughout their radial extension, but are separated by an intercellular layer[2] of varying thickness. This intercellular material differs from the wall of the cambial cells in its optical and other physical properties, in its chemical solubilities and reactions with dyes, and in its physiological behaviour. It is evidently a rather plastic, colloidal substance that passes readily into a liquid or semi-liquid phase, thus facilitating those movements and adjustments—*i.e.,* sliding growth—which are such characteristic features of the actively dividing and growing cambium. The so-called primordial pits are more or less irregularly distributed areas where the radial walls of adjacent initials are in closer contact—*i.e.,* separated by less intercellular material. There is no evidence, however, to indicate that the 'primordial pits' are permanent structures whose position remains unaltered during the life of the cambial initials. Nor is there evidence for believing that protoplasmic connexions[3] occur in these areas. On the contrary, the size, number, and orientation of the primordial pit areas appear to vary—particular areas disappearing and others being formed *de novo* during periodic movements and displacements of the intercellular substance.

In the case of the Araucarians, the so-called primordial pit areas are eliminated during the earlier stages of the development of the tracheides. Thus, the radial walls and intercellular layer are of relatively uniform thickness at the stage when the differentiation of the pit-membranes of the bordered pits is initiated. In the case of the *Pinaceae,* as of the *Taxaceae, Podocarpaceae, Cephalotaxaceae, Taxodiaceae,* and *Cupressaceae,* the primordial pit areas are not completely obliterated in most cases during the earlier stages of the differentiation of the tracheides, though their size and form may be modified more or less profoundly and their number augmented

[2] The conception of the middle lamella that has arisen since the discovery of the cell-plate is not applicable to the cambium.

[3] Detailed investigations of 'plasmodesma' lead the writer to question whether true protoplasmic connexions occur in any of the tissues of the higher plants, with the possible exception of sieve-tubes and other highly specialized cellular systems.

by subsequently formed ones. The pit-membranes of the bordered pits differentiate within these areas, so that the distribution of the bordered pits appears to be determined by the size, form, and spacing of the primary pit areas (PLATES 21 and 22, FIGS. 15-24, 28, 29). In the fully differentiated secondary xylem, the intercellular material that persists and is re-deposited between the primary pit areas, may be uniformly distributed (FIGS. 15, 17, 20, 21, 29), or it may be aggregated about the upper and lower margins of the primary pit areas (FIGS. 16, 22, 23, 28). It is these transverse, thicker portions of the intercellular substance that were originally designated as 'bars of Sanio' by GERRY (1910).

Where tracheary pitting is of the type illustrated in FIGS. 25, 26, and 30, there is obviously no room for the persistence of either 'primary pit areas' or 'bars of Sanio.' Therefore, in searching for these structures in woods which have an Araucarian type of pitting, it is the unpitted or less abundantly pitted portions of the radial facets upon which attention should be focused. Furthermore, it is essential in differential staining and de-staining to guard against the production of artifacts which simulate the structures that are being searched for.

Although the writer's investigations support those of GERRY (1910) and POOL (1928) and justify their conclusion that 'true bars of Sanio' do not occur in the wood of the *Araucariaceae,* it should not be inferred from this that these structures may be used indiscriminately in the identification of fossil woods. For such an assumption presupposes that the primary pit areas and bars of Sanio are preserved in visible form under all conditions of fossilization—a clearly fallacious inference. It should be emphasized, in this connexion, that even in the wood of living representatives of the *Pinaceae, Taxodiaceae, Cupressaceae, Podocarpaceae,* and *Taxaceae,* the primary pit areas and bars of Sanio frequently may be demonstrated only after delicately controlled differential staining. The primary walls and intercellular layer vary, not only in thickness, but also in chemical composition —*i.e.,* degree of lignification, in optical properties, and in the amount of material adsorbed during the transformation of sapwood into heartwood. Only the coarser and more refractive bars of Sanio are visible in unstained sections, and even these may be obscured in heartwood. Similarly, they may be visible in one portion of a fossilized specimen and entirely invisible in others. That the reputed absence of bars of Sanio in the *Protopinaceae* is due, in part, to their having been obscured during fossilization and, in part, to a failure to recognize them when visible, is indicated by the writer's investigations of *Protopiceoxylon.*

Protopiceoxylon:— The genus *Protopiceoxylon* was instituted by GOTHAN (1907, 1910) for some Lower Cretaceous wood from King Charles Land. Three species have been described, *P. extinctum* Gotham, *P. arcticum* Seward, and *P. Edwardsi* Stopes. The salient diagnostic feature of the genus—and the one in which it is supposed to differ, not only from other genera of the *Protopinaceae,* but also from all other gymnosperms, both living and extinct—is the occurrence in the secondary xylem of 'normal' resin canals in the vertical direction only. *Pinoxylon dacotense* is excluded by

both GOTHAN and SEWARD on the grounds that it is not possible to determine from KNOWLTON's (1898-99) description whether the vertical canals are normal or traumatic. Recently, through the courtesy of Mr. CHARLES B. READ of the U. S. Geological Survey, the writer has had the privilege of examining sections of KNOWLTON's type specimen. The structural details, even of the tori and pit-membranes, are beautifully preserved. The vertical resin canals are of the so-called 'normal' type, and the specimen so closely resembles those that have been classified as *Protopiceoxylon* that it obviously cannot be referred to a separate genus.

In GOTHAN's photomicrograph of a radial section of *Protopiceoxylon extinctum,* the pitting in certain of the tracheides is of the type illustrated in PLATE 21, FIGS. 20-22), whereas in others it is of the type shown in PLATE 22, FIG. 30. The paired and clustered pits of the former elements appear to have been formed over primary pit areas as in FIGS. 20-22. Similar combinations of opposite, clustered, and alternate pitting occur in KNOWLTON's specimen. Bars of Sanio are clearly visible between the pairs of opposite pits in certain of the tracheides, though they have been obscured by processes of silicification throughout the summer-wood and most of the spring-wood.

Protopiceoxylon obviously is not an Araucarian Conifer. Is it an extinct Protopinaceous genus, transitional between *Cedroxylon* and *Piceoxylon?* The only remaining argument for considering it to be such is the assumption that a similar distribution of resin canals does not occur in living representatives of the *Pinaceae.*

Keteleeria:— Comparatively little is known regarding the anatomy of this interesting representative of the *Pinaceae.* References concerning the structure of the xylem are few in number and extremely contradictory. PIROTTA (1889-90) states that resin canals are irregularly distributed in the secondary xylem of the root of *K. Fortunei* Carr., but that he did not succeed in finding any in the wood of the stem. JEFFREY (1905) concludes that '*Keteleeria* in its anatomy obviously belongs with *Abies,* although it has the habit of *Cedrus;* the wood of both vegetative and reproductive axes is entirely without resin canals.' HOLDEN (1913) likewise expresses the opinion that '*Keteleeria* has the wood structure of *Abies.* Ray tracheides are entirely absent, even in such primitive regions as the first annual ring, cone-bearing branch, cone axis, and are not recalled after wounding, though there is an abundant formation of traumatic resin canals.' HUTCHINSON (1917), who accepts HOLDEN's conclusions, figures a transverse section of a young branch of *K. Fortunei;* there are no resin canals in the xylem. KANEHIRA (1921), in describing the wood of *K. Davidiana* Beissn., states: 'Vertical resin ducts small, distributed evenly or in groups . . . ray tracheides present, without spirals.' In KANEHIRA's (1921, 1926) photomicrographs of the wood of *K. Davidiana,* the resin canals are widely spaced, but are arranged in tangential series. Therefore, the objection may be raised, as was done in the case of *Pinoxylon dacotense,* that the canals are of traumatic origin.

Although KANEHIRA's selection of illustrative material may have been

somewhat unfortunate—he figures a similar type of structure in *Picea morrisonicola* Hay.—his diagnosis of the mature stem-wood of *K. Davidiana* is accurate. The distribution of the vertical resin canals is of the so-called 'normal' type, and is the same as in corresponding stem-wood of *Picea, Pseudotsuga, Larix,* and *Protopiceoxylon;* but in *Keteleeria Davidiana* and *Protopiceoxylon* there are no horizontal canals, except such as may occur sporadically in the immediate vicinity of certain types of injuries. In *Keteleeria,* as in *Picea,* the innermost layers of the secondary xylem may at times be entirely devoid of resin canals, or they may have only vertical canals of the aggregated or so-called traumatic type.

It should be noted in this connexion, however, that THOMSON and SIFTON (1925) have raised the question whether any of the resin canals in the secondary xylem of the *Abietoideae* are of 'normal' origin. They present considerable evidence to show that even the isolated or diffused vertical resin canals of *Picea* are induced by traumatic or other abnormal environmental stimuli. From this point of view, the various genera of the *Abietoideae* form a graded series of increasing (or decreasing) sensitivity to environmental stimuli. In such super-sensitive plants as *Picea, Pseudotsuga, Larix,* and *Keteleeria Davidiana,* resin canals are always present in the adult wood, owing to the cumulative overlapping effects of successive abnormal stimulations. Furthermore, THOMSON'S and SIFTON'S investigations suggest that the sensitivity of the tissues in the cambial region may vary in different parts of a tree and during different stages of its development.

In any case, it is evident that *Protopiceoxylon* falls within the range of structural variability of *Keteleeria,* not only as regards the peculiar distribution of its resin canals, but also as concerns its tracheary pitting, tori, bars of Sanio, rays, and other structural features. The specific combinations of anatomical structures that characterize such Protopiceoxyla as *P. extinctum, P. arcticum, P. Edwardsi,* and *Pinoxylon dacotense* may be duplicated in different specimens of the wood of *Keteleeria.* Furthermore, the pith of *Keteleeria* may, at times, be provided with sclerenchymatous diaphragms, as in certain of GOTHAN'S type-specimens of *P. extinctum,* or it may be devoid of them, as in *P. Edwardsi.* Indeed, it is a question whether the various types of *Protopiceoxylon* may not eventually be duplicated in a set of specimens secured from different parts of a single tree.

Discussion and Conclusions:— During the last twenty-five years, at least fourteen new genera of Mesozoic Coniferae have been instituted for woods of supposedly intermediate or transitional structure. This increasing assemblage of hypothetical genera forms one of the main whirlpools in the stream of controversy concerning the relative antiquity of the *Araucariaceae* and *Pinaceae.* As noted in the introduction, the principal argument for regarding these Mesozoic Conifers as generalized or transitional forms is the occurrence of so-called Araucarian tracheary pitting in combination with structures that are not essentially Araucarian. The chief argument for segregating them into new genera and subfamilies, or families, is the tacit assumption that similar combinations of structural characters do not occur

in living representatives of the Coniferae. Both these premises are unreliable, at least in so far as the genera listed in the first of the following categories are concerned.

I.	II.
'Abietineous' ray-pitting.	Non-Abietineous ray-pitting.
Planoxylon Stopes.	*Anomaloxylon* Gothan.
Protocedroxylon Gothan.	*Brachyoxylon* Hollick & Jeffrey.
Protopiceoxylon Gothan.	*Paracedroxylon* Sinnott.
Thylloxylon Gothan.	*Protobrachyoxylon* Holden.
Araucariopitys Jeffrey.	*Paracupressinoxylon* Holden.
Metacupressinoxylon Torrey.	*Paraphyllocladoxylon* Holden.
	Telephragmoxylon Torrey.
	Xenoxylon Gothan.

The tracheary pitting and other structural characters of *Protocedroxylon, Protopiceoxylon, Thylloxylon, Planoxylon,* etc., fall within the range of variability of living representatives of the *Abietoideae.* If these fossils are to be classified as *Protopinaceae* or *Araucariopityeae,* then so must fragments of the wood of *Cedrus, Keteleeria,* and other extant genera; clearly a *reductio ad absurdum.* Furthermore, preliminary investigations suggest that the tracheary pitting of most, if not all, of the genera listed in the second category of putative transitional forms falls within the range of variability of living representatives of the *Podocarpaceae, Taxodiaceae,* and *Cupressaceae.*

This paradoxical situation and many others have arisen owing to a dearth of extensive and reliable information concerning the limits of structural variability in living representatives of the Coniferae. They cannot be fully clarified until large collections of authentic specimens are assembled, not only from different genera, species, and geographical races, but also from different parts of the tree and from trees grown under different environmental conditions. The information available at present is so fragmentary and unreliable that it is not possible to construct dependable keys for distinguishing even the commercial woods of many of the genera of Coniferae, to say nothing of species. Furthermore, although the normal adult stem-wood of the *Araucariaceae* may be readily distinguished from that of the *Pinaceae,* and this, in turn, from that of the remaining families of the Coniferae, there are no reliable criteria, as yet, for separating many of the *Taxodiaceae, Cupressaceae,* and *Podocarpaceae.*

In view of such facts as these, a number of pertinent questions arise concerning the wisest procedure in dealing with fossil woods. The writer is of the opinion that sufficient material from diverse sources has been examined to indicate that the *Pinaceae* may be distinguished by the character of their ray-pitting. It is true that the so-called Abietineous type of ray-pitting, as originally defined by Gothan, is not an infallible criterion, since superficially similar types of ray-pitting occur, at times, in certain of the *Cupressaceae* and other families. But, as will be shown in a separate paper, the rays of the *Pinaceae* are characterized by having numerous pits which communicate with the intercellular spaces. Furthermore, the pits between adjacent parenchymatous cells of the rays are of a fundamentally different type from those that occur in the *Taxodiaceae* and *Cupressaceae.*

Within the *Pinaceae* there are three groups of genera whose wood may be distinguished with a considerable degree of certainty. These are:

1) *Pinus.*
2) *Picea, Pseudotsuga,* and *Larix.*
3) *Keteleeria, Cedrus, Pseudolarix, Abies,* and *Tsuga.*

It may be objected that the wood of *Pseudotsuga* is characterized by having tertiary spiral thickenings in the tracheides of both spring-wood and summer-wood; that of *Keteleeria,* by the distribution of its resin canals; and that of *Cedrus,* by its peculiar tori; and that these genera should be placed in separate categories. Unfortunately, tertiary spirals occur, at times, in both spring-wood and summer-wood of certain species of *Picea.* The adult wood of *Keteleeria* has been studied in one species only. Although there is some evidence to indicate that the torus (PLATE 21, FIGS. 18, 19) may eventually provide reliable criteria for differentiating the wood of *Cedrus, Tsuga,* and other genera of the *Abietoideae,* it is not possible, as yet, to state that such is actually the case. Therefore, the wisest procedure in dealing with woods of obvious Pinaceous affinities is to refer them, *for the present,* to one of the following form-genera, rather than to attempt to classify them under extant or hypothetical extinct genera.

1. *Pinuxylon.*— For woods which fall within the range of structural variability of the genus *Pinus.*

2. *Piceoxylon.*— For woods which fall within the range of structural variability of *Picea, Larix,* and *Pseudotsuga.*

3. *Cedroxylon.*— For woods which fall within the range of structural variability of *Keteleeria, Pseudolarix, Cedrus, Tsuga,* and *Abies.* (Here should be included *Planoxylon, Pinoxylon, Protopiceoxylon, Thylloxylon, Protocedroxylon* or *Metacedroxylon, Araucariopitys,* and *Metacupressinoxylon.*)

It should be emphasized in conclusion that, in the present status of our knowledge concerning the variability of diagnostic criteria, specific names as applied to fossil woods have no significance other than as aids in designating particular specimens. The word 'species' must be used in an entirely different sense from that in systematic botany. The failure to recognize this fact has led to much confusion and to numerous misleading generalizations.

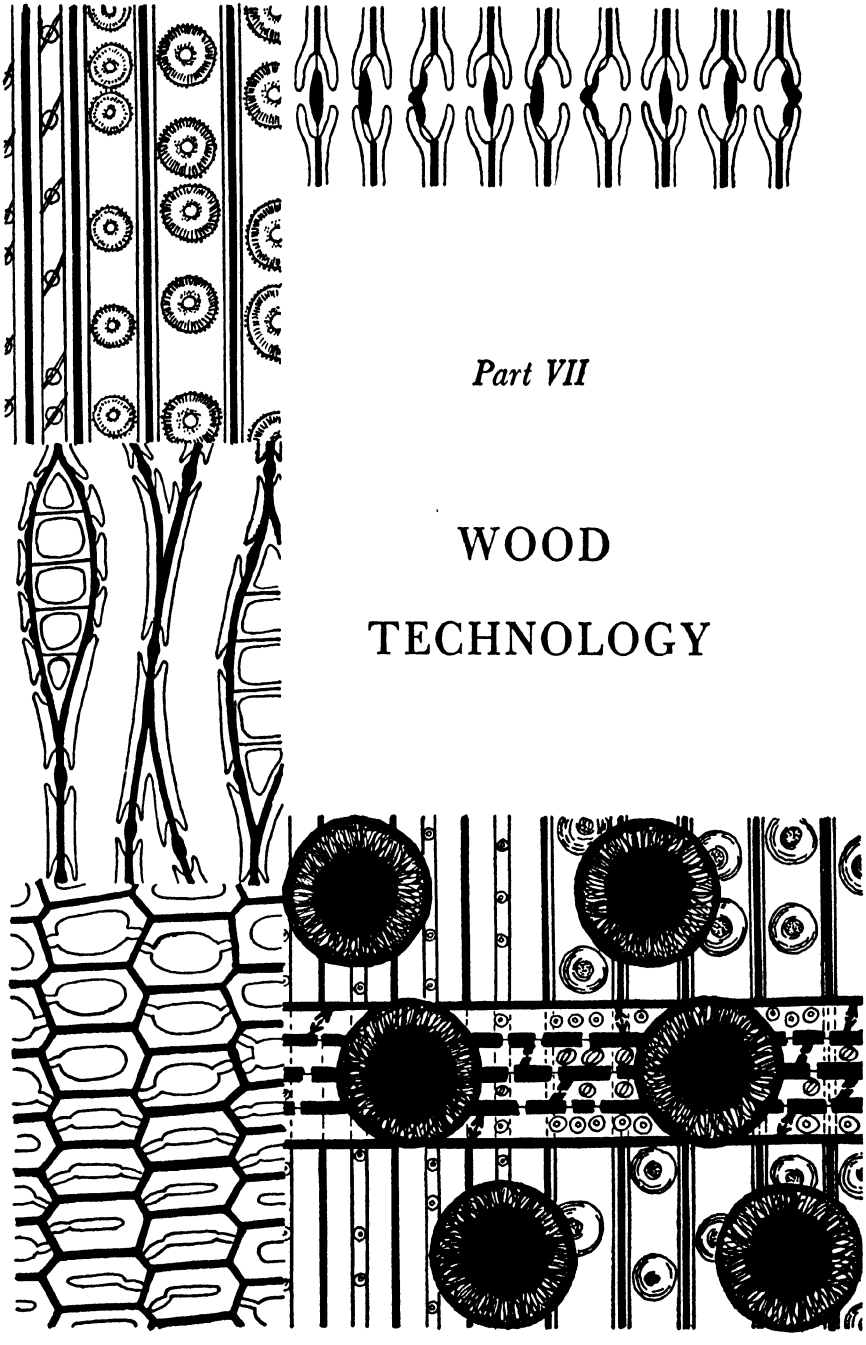
SUMMARY

1) The tracheary pitting of the genus *Cedrus* is plastic and variable, and of fully as intermediate or transitional a character as any that occurs in the so-called *Protopinaceae* or *Araucariopityeae.*

2) The distribution of the resin canals in the adult wood of *Keteleeria Davidiana* is of the same type as occurs in *Protopiceoxylon* and *Pinoxylon dacotense.*

3) Such hypothetical transitional genera as *Protocedroxylon, Protopiceoxylon, Planoxylon, Thylloxylon,* etc., fall within the range of structural variability of *Cedrus, Keteleeria,* and other genera of the *Pinaceae.* If they are to be classified as *Protopinaceae* or *Araucariopityeae,* then so must fragments of the wood of *Cedrus, Keteleeria,* and other genera of the *Pinaceae.*

4) This paradoxical situation and many others have arisen owing to a dearth of extensive and reliable information concerning the limits of structural variability in living representatives of the Coniferae. They cannot be fully clarified until large collections of authentic specimens are assembled, not only from different genera, species, and geographical races, but also from different parts of the tree and from trees grown under different environmental conditions.



Part VII

WOOD TECHNOLOGY

Chapter 19

THE PRESERVATIVE TREATMENT OF WOOD

II. THE STRUCTURE OF THE PIT MEMBRANES IN THE TRACHEIDS OF CONIFERS AND THEIR RELATION TO THE PENETRATION OF GASES, LIQUIDS, AND FINELY DIVIDED SOLIDS INTO GREEN AND SEASONED WOOD*

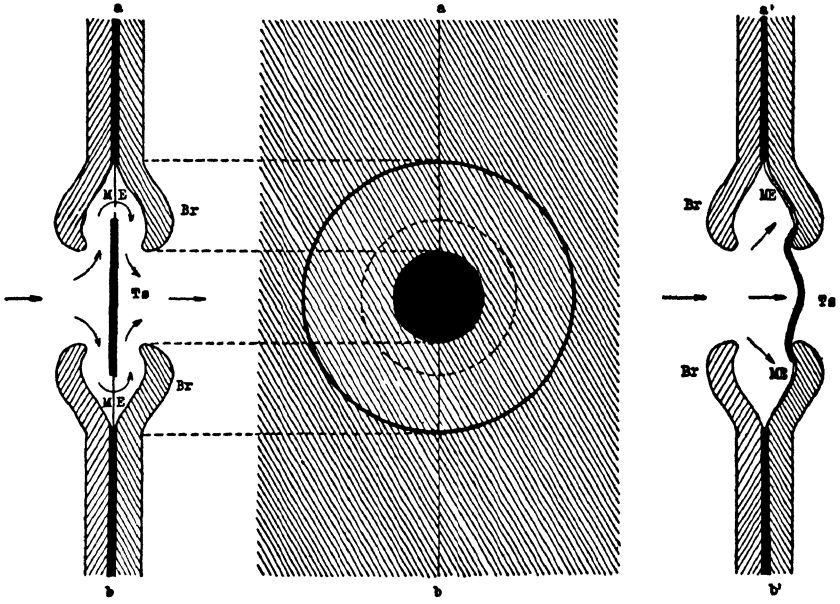
THE XYLEM or woody portion of arborescent plants has three important functions. It conducts large quantities of water, with gases and salts in solution, from the roots to the cambium and leaves, provides a strong and rigid stem which lifts the foliage to a position where it can secure desired amounts of air and light, and serves as an important reservoir for the storage of elaborated food substances. The structure of wood has been evolved to fulfill these functions and varies greatly in different species and even in the same individual with variations in the expression of these functions. In the less complex woods the same elements serve in more than one capacity, but in those which are more highly organized a division of labor takes place and certain elements act principally as conductors of water, whereas others have almost, if not entirely, ceased to act in that capacity. It is quite clear, therefore, in studying the water conducting passages of wood, as a means of securing information in regard to the behavior of preservative fluids when injected into timber, that one must consider, to some extent at least, other functions which this tissue is called upon to perform.

If we turn to the microscopic examination of the wood of coniferous trees or "softwoods," which is less complex than that of the higher seed plants or "hardwoods," we find that the woody tissue is composed largely of minute vertically arranged elements or tracheids which are devoid of living contents. These cells, resembling minute tubes with closed tapering ends, are a fraction of a centimeter long and serve the double purpose of conducting fluids and providing strength and rigidity to the stem. In accomplishing the latter object the cells are provided with a thick and heavily lignified wall, the so-called secondary wall, which is completely enveloped by the thin primary wall. These layers or walls surround an elongated cavity or *lumen* through which fluids pass on their way to the leaves. It is quite obvious, however, that provision must be made for enabling fluids to pass easily and rapidly from the cavity of one cell into that of adjoining cells. The dense secondary wall, although hygroscopic, is not easily permeable to solutions and is therefore provided with numerous pores or minute openings called *bordered pits*. Since these structures are of fundamental importance in the penetration of liquids through woody tissues it is essential to examine them with considerable care.

A circular area of the secondary wall surrounding the minute opening

* Reprinted from the Forestry Quarterly 11: 12-20, 1913.

or pit orifice is embossed or pulled away from the primary wall forming a saucer shaped blister which projects into the cavity of the cell. In surface view (*see* TEXT FIG. 1) this area forms a halo or border (Br) about the opening in the secondary wall. The bordered pits of adjacent cells are exactly opposite to one another and form a row of lens shaped cavities which communicate with the cavities of the two cells by means of the two openings in the adjacent walls. Communication between the two cells is interrupted, however, by the primary walls which in this region become very thin and form a delicate membrane (Me) which divides the lens



TEXT FIGURE 1.— Middle figure: Piece of radial wall of a "fiber," showing a bordered pit in surface view. Part of the dark-colored torus or thickened portion of the pit membrane may be seen through the opening in the wall. This pit orifice is surrounded or bordered by the embossed portion of the secondary wall. — *At left:* Section cut at a-b, showing embossed wall seen in the middle figure and that of cell directly under it. The membrane and torus occupy a median position. — *At right:* Section showing the valvelike action of the torus. — Br, bordered area, ME, membrane, Ts, torus.

shaped cavity into two plano-convex cavities. The dividing membrane possesses a circular thickened area (Ts) called the *torus* which acts as a valve. Before sufficient pressure can be brought to bear upon the delicate membrane to rupture it, the torus is forced to one side or the other sealing more or less effectively one of the openings in the secondary walls.

The necessity for these delicate and complicated valve-like structures is seen when it is taken into consideration that water in passing from the roots to the leaves of a tall tree must pass through a large number of minute cells which are less than a centimeter in length. Not only must the

aqueous solutions pass through a large number of cell walls, but at times they must do so comparatively rapidly. The bordered pit is designed, therefore, to expose a relatively large surface of very thin, permeable membrane without seriously impairing the strength and rigidity of the secondary wall.

Detailed Structure of the Pit Membrane:— It is a commonly accepted fact among physiologists and anatomists that in conifers the wood cells or tracheids are entirely enclosed in the thin primary membrane. In the region of the bordered pits the "primary wall" may be greatly reduced in thickness but, although very permeable to water containing salts and gases in solution, it is supposed to be impervious to undissolved gases and finely divided solids, and more or less impervious to heavy oils and other heavy or viscous liquids. This conclusion, that the primary wall or membrane is unperforated even in the region of the bordered pits, is based upon two lines of evidence. Careful microscopic examinations have failed to reveal perforations in the pit membranes. Russow, Nägeli, Strasburger and others discovered that the pit membrane was not homogeneous throughout, but frequently composed of fine alternating radial bands of thinner and thicker wall substance. They concluded, however, that the membrane was unperforated. In addition, numerous experiments in injecting freshly cut green wood with water containing powdered solids and heavy liquids, *e.g.*, Sachs' (1887) cinnabar and mercury experiments, have led to the same conclusion, *that the pit membranes are entire* in the wood of a living tree.

This fact afforded, apparently, a satisfactory explanation of the difficulty in forcing air through green wood; the use of salt solutions, but not creosote, in the Boucherie and Kyanizing processes; the necessity for preliminary steaming or seasoning in the Bethell process, etc., etc.

The question may well be raised at this point, since Tiemann's (1910) "slit" hypothesis cannot be accepted as conclusive, what structural change, if any, produces the difference in the behavior of green and seasoned wood. It occurred to the writer that the delicate pit membranes were ruptured by the shrinkage of the cell walls in drying. To determine conclusively by microscopic examination whether the pit membranes are ruptured in a given piece of seasoned timber is difficult, since it is not easy to eliminate the possibility that the ruptured condition was produced by the process of sectioning. However, by embedding material in nitro-cellulose and subsequently cutting sections with a very sharp microtome knife the writer was able, in large measure, to overcome this difficulty, and to determine that in many pieces of seasoned wood the pit membranes had been ruptured by the shrinkage of the cell in drying. In order to test this point experimentally the writer injected the thoroughly air-dried wood of several conifers with an aqueous mass containing very finely divided particles of carbon held in suspension. Obviously this dark colored liquid could penetrate only when actual openings existed in the cell walls. Subsequent examination of the material revealed the interesting fact that the penetration from one cell to another occurred entirely through the bordered pits. Plate 23, fig. 5, a longitudinal section of air-dry *Sequoia* heartwood, illustrates the penetra-

tion of the carbon particles from one cell to another. The tracheid in the centre of the photomicrograph is entirely filled with the dark colored mass. The carbon particles are passing through the chain of bordered pits into the adjacent cell cavity on the right. It may be noted that the bordered pit in the centre of the photomicrograph is partly filled with a resinous substance and slight penetration occurs. At the extreme left is a so-called "medullary" ray which, with carbon particles of this size, remained unpenetrated. PLATE 23, FIG. 6 illustrates a tangential section of the "summer-wood" of an ordinary long-leaf pine paving block. Since the heavy tar oils had penetrated almost exclusively the dense bands of "summer-wood," it is significant, in view of WEISS' (1912) theory, that the penetration from one cell to another takes place by means of the bordered pits and not by cracks or "slits" in the thick secondary walls.

As a "control" for these experiments the writer tested the penetration of the carbon mass with pieces of freshly cut green sapwood. A deeper and easier penetration was secured in green material of white pine, pitch pine, spruce, hemlock, larch, and cedar than could be secured in the same material after thorough kiln or air drying. In both cases the material was subjected to similar temperatures (65° F.), pressures, and duration of treatment. At first negative and positive pressures of approximately one atmosphere were used. In order to obviate the possibility that the membranes were ruptured by these pressures, two series of tests were made. In the first, experiments were made to determine at what pressure the pit membranes could be broken. With pressures up to 250 lb. per sq. in., the maximum capacity of the pressure cylinder used, the membranes of all conifers remained unruptured. This was undoubtedly due to the valve-like action of the torus which has been described earlier in this article. A second series of experiments was then made to test the penetration of the carbon mass under very slight hydrostatic pressure. HARTIG showed that if a stick of freshly cut green sapwood several inches long was held in a vertical position and a drop of water placed upon the top it disappeared very quickly and appeared at the lower end of the stick. The writer repeated this experiment, connecting the upper end of the stick to a rubber tube of a few cubic centimeters capacity. If a sufficiently dilute solution of the carbon mass was used the water passed rapidly through the stick carrying the carbon particles with it. PLATE 23, FIG. 4, a longitudinal tangential section of freshly cut green white pine sapwood, shows the carbon passing from one tracheid to the next, through the membranes of the bordered pits. On the left two tangential pits filled with carbon are seen in surface view. PLATE 23, FIG. 8 illustrates a radial section of the same wood under high magnification. The carbon particles are seen to penetrate the membrane in a rim about the torus and in lines radiating outward from it. Thus, the writer was forced to the conclusion that *the pit membranes in the tracheids of living coniferous trees are not entire* as has previously been supposed, *but are perforated by extremely minute openings,* which are located in the thinner radii of the membrane.

The next step was to subject coniferous woods to a microscopic examination to determine if these perforations were visible under the highest powers

of the microscope. This proved a difficult undertaking owing to the minute size of the structures under observation. Special methods of sectioning and staining were essential owing to the peculiar structure of the bordered pits. However, fairly satisfactory results were finally secured by cutting exceedingly thin sections (2 micra). In this way it was possible to cut away one of the embossed areas of the secondary wall exposing to view the membrane and torus. By careful staining and the use of very high magnification the detailed structure of the membrane could in many cases be successfully studied. PLATE 23, FIG. 7 illustrates in a diagrammatic manner the structure of the pit membrane. In the center is the thickened portion of the membrane or torus. Surrounding the torus is the thin, permeable portion of the pit membrane. This is seen to be composed of alternating bands of denser and thinner membrane substance. The perforations are located in the thin, lighter colored radii of the membrane. They are extremely variable in size and may in certain species increase in size or coalesce to form larger and more conspicuous openings. PLATE 23, FIG. 9 illustrates an unusually coarse type of perforation which sometimes occurs in the wood of the larch.

It may well be asked in view of the perforated structure of the pit membranes, why it is that gases cannot be forced easily through long pieces of green wood and through many specimens of thoroughly re-soaked, dry wood. This is, I believe, due to capillary or surface tension phenomena combined with the valve-like structure of the bordered pits. If we have an aqueous solution on both sides of the pit membrane shown in PLATE 23, FIG. 2 and the molecules or minute particles of water are set in motion by a slight pressure they are free to pass easily and rapidly from one cell to another through the perforations in the pit membrane. Now let us suppose that we force air into either of the cells. The water will be gradually driven out until the air comes in contact with the pit membrane. As soon as this happens the surface tension of the water in the minute openings of the membrane resists the further penetration of the air. When additional pressure is applied to force the water from the perforations this membrane is forced to the side and the torus seals, more or less effectively, the opening in the secondary wall. (*See* PLATE 23, FIGS. 1 and 3.) If a very short piece of wood is used so that the air has to penetrate only one or two membranes it is sometimes possible to pump air through green wood. On the other hand, if there are numerous membranes to be encountered, capillarity or surface tension and the valve-like structure of the tori effectively prevent the penetration of air, even under heavy pressures. The fact that re-soaked dry wood is, in many cases, somewhat less impervious to air than unseasoned material is probably due to the rupturing of the pit membrane during the process of drying.

SUMMARY AND CONCLUSIONS

1) Wood is a highly specialized and complex plant tissue, designed primarily to conduct aqueous solutions and to give strength and rigidity to stem and branches.

2) It is extremely variable in different species and even in different parts of the

same individual due to variations in the functions which it is called upon to perform.

3) Coniferous woods or "softwoods" are composed largely of minute cells or tubes with closed ends. Liquids in passing through this tissue travel primarily in the cavities of the cells and pass from cell to cell by means of delicately constructed valves or bordered pits in the cell walls.

4) The membranes of the bordered pits are not always entire as has been previously supposed, but possess (in all species examined) numerous minute perforations whose presence may be demonstrated by careful microscopic examination and by experimental means.

5) When wood is thoroughly dried no structural modification, such as the rupturing of the cell walls, is essential in order to account for the penetration of gases and preservatives into seasoned wood.

6) In green wood the bordered pits and membranes are very permeable to aqueous solutions, but are comparatively impervious to undissolved gases, and to oils, and other heavy or viscous liquids. This is due undoubtedly to capillary or surface tension phenomena and the valve-like action of the torus.

7) Dry wood (except when the cells are clogged with resins or other secretions) is very permeable to gases, since water is no longer present to resist the passage of the gases through the perforations in the pit membranes.

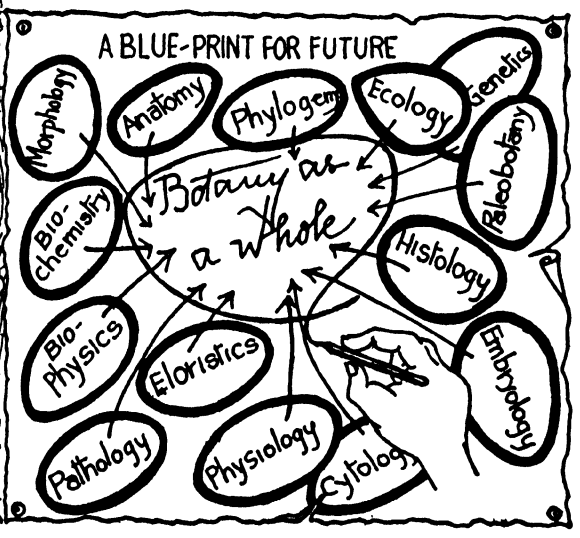
8) Whenever preservatives are injected rapidly into green or seasoned wood the penetration takes place primarily through the cavities of the cells, and the preservatives pass from one cell to another through the bordered pits.

9) Rupturing of the pit membranes was found in some specimens to be a concomitant of the process of drying, and may account for the fact that in certain cases resoaked dry wood is less impervious to air than green material.

10) The impregnation of wood by modern commercial methods is a complicated chemical, physical, and anatomical problem, since any given phenomenon may be the result of numerous interacting chemical, physical, and anatomical factors.

Part VIII

COOPERATION in SCIENTIFIC RESEARCH



Chapter 20

THE ROLE OF RESEARCH IN THE DEVELOPMENT OF FORESTRY IN NORTH AMERICA*

in collaboration with HERMAN A. SPOEHR

Introduction:— In any discussion of forestry and of programs of research in the general field of forest production,[1] it is essential to emphasize the fact that a forest is an exceedingly complex biological unit. It comprises not only a more or less diversified aggregation of trees, but numerous species of shrubby and herbaceous plants, fungi, insects, herbivorous animals and a complex soil fauna and flora. In other words, it consists of a very large number of mutually interacting organisms which are affected by, and themselves affect, a complex of environmental factors.

In the case of a normal virgin forest, the biological complex is in relatively stable equilibrium and such changes as are induced by climatic and geological fluctuations are in general very gradual. With man's advent, this natural equilibrium is upset by logging, fire, grazing and other disturbances which initiate numerous and protracted chains of highly involved and extraordinarily variable reactions.

The task of the silviculturist is to control or modify these reactions in order to secure a sustained maximum yield of valuable forest products. Frequently he must also bear in mind the effects of different types of forest management upon soil erosion and stream-flow, upon public health and recreation and upon the welfare of the grazing and other subsidiary industries. For economic and other reasons to be outlined on succeeding pages, the silviculturist is forced to deal with relatively natural units of vegetation and cannot, in most cases at least, simplify his problems to the extent that the farmer has succeeded in doing. He can, in general, modify environmental factors and regulate the future growth on timberlands only through intelligently supervised gross treatments of the forest vegetation itself, *i.e.*, by "thinnings," by modifying and properly timing his methods of harvesting, by varying his technique in disposing of brush, by controlling fire and grazing, etc.

Such gross treatments have highly diversified and far-reaching effects upon the biology of the forest, not only upon the soil and the trees but also upon the minor vegetation, insects, fungi, and other elements of the complex. The latter effects cannot safely be ignored since they in turn may later profoundly influence the future growth of the forest. Thus, in de-

* Reprinted from "The Rôle of Research in the Development of Forestry in North America" (Introduction, Chapters I, II, V, & VI), Macmillan, New York City, 1929.

[1] Forestry may be divided into (1) those phases which are concerned with the culture and care of forests, *forest production* or *silviculture*, and (2) those which are concerned with the harvesting and use of forest products, *forest utilization*.

veloping rational systems of silvicultural management, the forester must take into consideration the whole complex of interactions in the forest and, to do so effectively, he must rely upon the cooperation of various sciences.

The primary object of the following discussion is to determine how and to what extent certain categories of the natural sciences may be of service to silviculture during different stages of its development. Before passing on to a detailed treatment of this subject, it is necessary to comment briefly upon various salient characteristics of these sciences themselves.

All of natural science is observational. During the earlier stages of its development, it concerned itself with describing and comparing the more obvious, complex and grosser aggregates of matter and units of energy. Through the analysis of large volumes of descriptive data, it succeeded in establishing many valid correlations between groups of phenomena. Subsequent search for actual causal relationships has led to the investigation of smaller and less complex aggregates or units, and natural science has resolved itself into a series of subdivisions, each of which deals with particular groups of phenomena and has developed its own specific observational technique. Thus, in the biological field, morphology has passed successively from a consideration of external form (taxonomy and systematic biology) to the study of gross internal structure (anatomy and histology) and finally to the description of the visible structure of living matter (cytology). Similarly chemistry has progressed from the investigation of compounds to that of molecules, atoms and electrons.

Physics and chemistry in passing from the investigation of visible to invisible units were forced to develop highly refined and exact quantitative experimental methods. In other words, they have substituted an intensive analytical technique for an extensive observational one. Only in the study of heredity and in certain aspects of bacteriology has biology succeeded in originating quantitative experimental methods which are comparable to those of physics and chemistry. Most of the biological sciences are still concerned with the investigation of visible phenomena and with the establishment of correlations through cumulative circumstantial evidence; the transition from gross morphology to cytology has involved the use of the microscope, but no radical change in the point of view of the investigator.

It should be emphasized in this connection that plants and animals are so numerous and diversified, and vital phenomena are so complex and so extraordinarily variable, that all of the biological sciences still have important rôles to play in the future. The compilation, codification and analysis of descriptive data and the formulation of valid correlations are not only of great practical significance in the development of the biological arts, but are indispensable in the visualization and definition of those fundamental problems which biology seeks to solve. Nor should it be inferred that this work when well done is of an inferior intellectual quality. The descriptive method requires capabilities and disciplines which are by no means inferior to those used in the exact sciences. In fact, the successful employment of cumulative circumstantial evidence—*e.g.*, DARWIN and the Theory of Evolution—demands qualities which are rarer and often more finely discriminating than those employed in the exact sciences. Thus, although

most biological research and experimentation, except when concerned with the simpler physico-chemical reactions in organisms, is essentially descriptive and empirical from the point of view of modern physics and chemistry, it does not follow that it is inferior or less useful.

It is evident accordingly that there are two distinct methods of investigating complex biological phenomena, one the extensive observational method of the descriptive sciences and the other the intensive analytical method of the basic experimental sciences. The former sciences work to establish valid correlations between visible aggregates of variables. The latter sciences aim to resolve these aggregates into their constituent variables and to study these individual variables under accurately controlled experimental conditions. Each line of attack has distinct advantages and limitations, and each requires particular abilities and disciplines and a specific psychological outlook upon the part of the investigator. On the one hand, the descriptive sciences are able to make rapid progress in the study of the visible forms and activities of living matter and in establishing correlations between them, but they are handicapped in determining the underlying fundamental whys and wherefores. On the other hand, the accumulation of reliable data through the methods of the basic experimental sciences is a tedious and time-consuming process, and no valid generalizations are possible until a certain essential total of experimental evidence is accumulated.

Both lines of attack appear to be essential in the ultimate solution of the fundamental problems of biology. The descriptive sciences are able to formulate and roughly define these problems, but they are dependent upon the basic experimental sciences for the necessary means of analyzing them accurately. Although physics and chemistry have developed methods for investigating certain of the simpler physico-chemical reactions in plants, they have not succeeded as yet in perfecting adequate techniques for analyzing the more complex vital phenomena. Until such techniques are available, attempts on the part of plant science to solve highly complex and variable biological problems through the use of quantitative experimental methods are of doubtful value. Furthermore, there is a serious question whether the methods of the descriptive and of the exact sciences can be combined successfully in the hands of a single investigator. Occasionally an exceptional individual may be able to master modern physics and chemistry and one or more of the descriptive sciences, but most investigators are unable to do so and tend to become more or less superficial in all fields. Thus, much of the so-called "modern fundamental" biological research is unfortunately an aimless puttering with quantitative methods and is unproductive from the point of view both of the descriptive sciences and of the basic experimental ones. The phenomena to be investigated are so complex and variable that, in many cases at least, they can be analyzed accurately only through the active cooperation of a group of experts in physics, chemistry and biology.

The utilization of scientific data in the arts is dependent (1) upon the stage of development of the art, (2) upon the stage of development of the sciences and (3) upon economic factors. The descriptive method coupled with simple empirical experimentation is of maximum service during the

pioneer stages of a biological art, since it affords the most economical and rapid means of establishing such correlations between dominant factors or between groups of phenomena as are of practical significance. Eventually this line of attack tends to exhaust its most promising possibilities and during the later stages of the development of the art there is an increasing necessity for truly fundamental experimental research.

Agriculture as contrasted with Silviculture:— The natural production of plant food, suitable for man's consumption, on wild or uncultivated land is extremely low. Therefore, agriculture is essential for the development of even relatively primitive civilizations, and it is significant in this connection that all of the more important plant foods were discovered and brought into cultivation by prehistoric man.

Prolonged investigation of the earth's terrestrial vegetation indicates that first class food-producing plants are limited in number. Indeed, at present the bulk of the world's food is produced by a relatively few species, and increased production is secured largely by bringing successive areas of wild land under cultivation of selected or "improved" races of these species, rather than by the domestication of endemic wild species. Of course, the growth of selected races or of hybrids in pure cultures beyond the limits of the native habitats of their progenitors is, from the biological point of view, a highly unnatural process, and necessitates considerable expenditures for controlling environmental factors, *e.g.*, by drainage, irrigation, tillage, use of fertilizers and protective sprays, rotation of crops, etc.

Each agriculturally used species produces one specific primary product. The market requirements for these primary products are relatively highly standardized and stable, and there is little evidence to indicate that they will not remain so. The possible substitution of synthetic products for natural plant foods must at best be relegated to the distant future.

In the case of forest products, as contrasted with plant foods, the accumulation[2] of utilizable material on wild lands is very high. Furthermore, logged-over areas commonly are capable of yielding additional crops, particularly in more moist environments and in regions where protection is afforded against fire and grazing. In fact, successive crops of considerable magnitude and value may be secured without special cultural treatment. Thus, silviculture of an intensive type becomes essential only after sources of wild forest products become exhausted and when costs of imported material become excessive.

The world's list of trees which now produce, or may in the future yield, valuable raw material is very extensive. A sustained industrial utilization of forest products has been, and is still being, secured largely through the harvesting of the accumulated material of one after another of these wild species, rather than by extending the cultivation of a few highly domesticated species as in agriculture. Many species of trees are capable of yielding a variety of different types of material, *e.g.*, fire wood, posts, ties, lumber,

[2] Wood is chemically a relatively stable substance and accumulates in a tree from year to year. On the contrary, seeds, nuts, fruits, etc., are perishable and must be harvested at the end of each growing season.

pulp, distillates, chemical extracts, etc. Furthermore, the raw products of different species are to a considerable degree potentially interchangeable. In addition, there are substitutes for wood in most of its varied uses. Thus, the market requirements for specific forest products are much less stable and assured than is the case in agriculture.

Such facts as these raise the question, will silviculture, after the world's virgin forests have been logged over, tend to develop along lines closely parallel to those of agriculture? This question is of vital significance in any general discussion of forestry and of future programs of research in the field of silviculture.

There are five points which should be emphasized in this connection:

1) In the future, forests will be grown mainly on lands which cannot be used advantageously for agriculture; *i.e.*, on less fertile soils and steeper slopes where cultivation, in the agricultural sense, is relatively difficult.

2) Even on potentially tillable lands the character, size and longevity of forest plants renders difficult the application of agricultural methods.

3) The value of forest crops will rarely justify any considerable expenditure for directly controlling environmental factors by tillage, irrigation, use of fertilizers, sprays, etc. Thus, the forester will in general be restricted to the use of relatively inexpensive and more or less indirect methods. He will be forced to control forest growth and to modify environmental factors largely through his treatment of the forest vegetation itself.

4) Forests are widely distributed natural units of vegetation; and, as previously indicated, may be made self-perpetuating and highly productive through relatively inexpensive treatments.

5) Industrial and other requirements for wood are so varied and relatively plastic that it will be possible to market the products of a wide range of arborescent species of forest plants. This is due, of course, to the fact that the wood of most species has a wide range of possible commercial uses.

It is evident, accordingly, that silviculture will be concerned, at least for an extended period, with the modification of relatively natural units of vegetation and with the restoration of more or less natural arborescent growth on devastated areas, rather than as in agriculture with extending the culture of a limited number of highly domesticated species under comparatively artificial conditions. That such is indeed the case is indicated by a strong reaction among silviculturists, particularly those of Northern and Western Europe, against the more artificial systems for regenerating and managing forests. In fact, a number of the ablest European foresters are of the opinion that silviculture should aim to reproduce as natural units of forest vegetation as possible, since such systems of forest management are held to be the most productive in the long run, to obviate serious deterioration of the soil and to reduce losses due to insects and plant diseases. Of course, this does not mean that certain species of trees may not be grown ultimately in more or less pure cultures as such units of vegetation occur naturally, especially in the temperate regions. Nor does it necessarily eliminate the possibility of successfully establishing selected races or desirable exotics, but it renders difficult and uncertain the task of so doing. It does indicate that stereotyped systems of silviculture, such as have been developed in certain portions of Central Europe, cannot be practised extensively in other phytogeographical regions. Each system, if actu-

ally efficient, is adapted to meet the requirements of a specific, local complex of factors, and, therefore, must be modified more or less profoundly when applied in a different region.

Most agricultural crops are grown on short rotations and by cultural methods which have become relatively highly standardized. Even the longer lived nut- and fruit-bearing species yield annual harvests after a brief preliminary period. In other words, the character of the investments and returns in agriculture is such that the art is practised extensively by individuals on relatively small holdings of land. On the contrary, long rotations in silviculture, coupled with uncertainties concerning future market requirements and other economic variables, tend to inhibit the development and practice of the art by individuals and by any except the most far-sighted communities. Thus, silviculture is practised during relatively late stages in the development of civilization, and, even in regions where sources of wild forest products have been exhausted, by a few of the most progressive countries only. Indeed, ZON and SPARHAWK estimate that at present "only 10-15 per cent of the world's timberland is being handled as a renewable, continuously productive resource." [3]

Research and its Applications in Silviculture as contrasted with Agriculture and Medicine:— Medicine is of necessity closely dependent for efficient development upon scientific research, and has reached a stage where future progress depends largely upon the results of investigations in the more basic, experimental aspects of the physical, chemical and biological sciences. It is inherently an art in which there is a wide range of possibilities for the direct application of specific, fundamental, scientific discoveries.

The higher animals, particularly man, in contrast to terrestrial plants are not attached to, or directly dependent for part of their nutritional requirements upon, the soil. Their motility and habit of ingesting food facilitate their observation and treatment under relatively rigidly controlled environmental conditions. Furthermore, their highly specialized and clearly differentiated respiratory, digestive, circulatory, etc., systems simplify the task of studying and of directly modifying internal physiological processes. Of even greater significance, however, is the fact that life and health are matters of vital importance to most members of the human race. *Therefore comparatively large expenditures are available for the treatment of single individuals.* The strong altruistic appeal of medicine is, of course, an additional factor in providing liberal funds for research and its applications in alleviating the suffering of mankind in general.

Agriculture is in the midst of a prolonged period of expansion, the extension of known cultural methods to successive areas of wild land, which has been rendered possible largely through the development of farm machinery and through improvements in the transportation, storage and utilization of agricultural products.

As previously stated, all of the more important food-producing species

[3] Forest Resources of the World. McGraw-Hill. Vol. 1, page 35, 1923.

of plants were discovered and brought into cultivation by prehistoric man. This was accomplished by a long process of "trial and error." Subsequent improvement of these plants through selection and breeding, and the development of more efficient cultural techniques have been attained largely through empirical investigation. The rôle of the basic sciences has in general been that of *ex post facto* interpretation, clarification and modification, rather than the actual origination of new cultural methods. It should be noted in this connection that, although genetics is providing a scientific basis for the arts of animal and plant breeding, the bulk of the numerous, improved races and varieties, *now in use,* was developed by essentially empirical methods.

In agriculture, as contrasted with medicine, expenditures for cultural treatments are determined by strictly economic, rather than humanitarian, factors. The farmer in most cases must deal with individuals *en masse, i.e.,* with aggregates of plants which are attached to, and are dependent for their existence upon, specific edaphic complexes. He can affect internal physiological processes only indirectly through relatively limited and gross methods of modifying external environmental factors, *e.g.,* by tillage, irrigation, fertilizers, rotation of crops, etc. Thus, the range of possible direct applications for specific researches in the fundamental sciences is considerably restricted.[4] Only in the field of genetics has agriculture a conspicuous advantage over medicine.

There are obviously three salient means of increasing the future yields of agricultural products on cultivated lands: (1) by the selection and breeding of improved stocks or races of crops, (2) by eliminating or reducing losses due to pests and diseases and (3) by more efficiently controlling external environmental variables and intelligently regulating their effects upon internal physiological processes. As previously noted, genetics is rapidly providing a more scientific means for approaching the first of these objectives. Bacteriology, entomology and phytopathology, through an intensive study of the life histories of bacteria, fungi and insects, are contributing materially toward a more effective control of pests and diseases, but, in so doing, are in general restricted to the external application of fungicides and insecticides, the reduction of external sources of infection and the development, in cooperation with genetics, of disease resistant types of crops. Although soil science and plant physiology are making some important progress in their attack upon the third objective, their advance is comparatively slow and falls far short of fulfilling earlier expectations. This is due to the fact that the plant and the soil in which it grows form two extraordinarily complex, interacting systems of physical, chemical and biological variables. It is extremely difficult to isolate specific individual factors and to devise adequate methods for studying them under accurately controlled experimental conditions; or, having done so, to determine their exact effects upon other variables in the complex. Only a beginning has

[4] This statement refers to those phases of agriculture which are concerned with the *growing* of crops. The situation is entirely different as regards research in the *utilization* of agricultural products. Animal husbandry is in many respects more clearly comparable to medicine.

been made in the development of methods for the study of the water relations of plants, probably the most important single factor. The same is essentially true for the temperature relations. Such fundamentally significant physico-chemical phenomena in the plant as photosynthesis, the synthesis of carbohydrates, fats and proteins, respiration, osmosis, etc., are still imperfectly understood. Relatively little is known concerning the actual functions of various tissues, the physico-chemical reactions involved in their differentiation, and the translocation of salts and of elaborated products within them. Furthermore, there is a host of complex organic substances, *i.e.*, latex, oleoresins, glucosides, tannins, gums, alkaloids, etc., whose rôle in the vital activities of plants is at present entirely obscure, and a large number of physiologically important substances which have never been subjected to thorough chemical study.

Not only are soil science and plant physiology seriously handicapped in their possible service to agriculture by the present inadequate state of knowledge concerning the complex of physico-chemical reactions in the plant and concerning the complex of interacting physical, chemical and biological factors in the soil, but so also are plant pathology, entomology and genetics. The phenomena in the plant and in the soil are so complex and variable that efficient progress toward elucidating them can be made only through a prolonged and sustained attack by physics, chemistry and biology working in close cooperation. To be successful such an attack should not be shackled by demands for practical applications. Unfortunately, agriculture does not afford as urgent a spontaneous stimulus for the development of truly fundamental research in plant physiology as does medicine in the general field of animal physiology. The range of possible direct applications for specific scientific discoveries is considerably restricted by economic factors. Therefore, in the absence of a strong humanitarian appeal, it is difficult to justify the expenditure of public funds for protracted basic researches, which may not yield practical results until a certain *essential total* of biophysical, biochemical and biological information is accumulated.[5]

Silviculture is of necessity a more plastic and adaptable art than is agriculture, due to the fact that it is concerned with essentially natural and varied, rather than with highly artificial and stereotyped, units of vegetation, and therefore must modify or revise its methods to meet the requirements of varying complexes of natural and economic factors. There are at present no sound, well established, fundamentally scientific generalizations for determining in advance the best cultural treatment of a new unit of forest vegetation. Even in those regions, *e.g.*, Europe and Japan, where silviculture is most intensively practised, it has developed almost entirely through an efficiently systematized empiricism. *Thus, the extension of silvicultural management over the earth's vast area of wild forest land must be preceded by a comprehensive descriptive survey and analysis of widely fluctuating natural and economic variables, and by an intelligently formulated program of empirical experimentation*—a task, the magnitude and

[5] It should be emphasized here that whereas medicine is concerned with one organism (man) in health and in disease, agriculture is forced to deal with a variety of crop plants, each of which must be studied intensively.

importance of which are not generally recognized by biologists. In other words, from the point of view of the world as a whole, silviculture, in contrast to agriculture, is in the incipient, pioneer stages of its development.

In all probability the basic sciences can contribute most effectively to the extension of silviculture by helping to systematize and accelerate the process of accumulating essential data, and by aiding in their analysis and interpretation, rather than by attempting prematurely to originate fundamentally scientific, silvicultural techniques. The forester must first of all be equipped with reliable information concerning the identity of the numerous organisms with which he is called upon to deal, their complete life histories, their distribution in relation to salient environmental factors and their natural association in varying mixtures. Such information is now available for limited areas only, and the descriptive sciences, *i.e.*, systematic botany, systematic entomology, mycology, morphology, ecology, meteorology, physiography, etc., should contribute materially in its accumulation. However, in order to do so efficiently, they must in many cases develop a broader and less stereotyped outlook and devote more attention than heretofore to an accurate visualization of the forester's problems.

Although the rôle of the descriptive sciences in a reconnaissance of the world's forests is obvious and relatively simple, that of the basic experimental sciences, *e.g.*, physics, chemistry, physiology, soil science,[6] genetics, etc., is more or less obscure and difficult. The latter sciences should, of course, endeavor to devise reliable quantitative methods for measuring both external environmental variables and internal physiological factors, and for accurately investigating their interactions. But the phenomena to be analyzed are so complex and so extraordinarily variable, and the time factor in experimentation with arborescent plants is so large, that real progress toward attaining these objectives must inevitably be very slow. It should be emphasized in addition that, owing to their size and longevity, trees must be studied *in situ;* they cannot readily be grown to maturity in the laboratory as are many agricultural plants. *In fact, a special physiological technique must be elaborated for their investigation.* Therefore, there is a grave question as to how far the forester may profitably count on the basic experimental sciences for assistance during the pioneer stages of the development of silvicultural management on wild forest lands.

Even in regions where silviculture is most intensively practised, the forester can modify internal physiological reactions and external environmental factors only indirectly in most cases through relatively inexpensive gross treatments of the forest vegetation itself, *e.g.*, "thinnings," improvement cutting, modifying and properly timing methods of harvesting, varying technique in planting and in disposing of brush, etc. In other words, the range of possible direct applications for specific scientific researches is greatly restricted in silviculture as contrasted with medicine or even with agriculture.[7] Thus, forest entomology and forest pathology cannot in most

[6] Fundamental physico-chemical aspects.

[7] Those phases of animal husbandry which are concerned with the grazing of animals on uncultivated lands are in certain respects more nearly comparable to silviculture.

cases resort to a wholesale use of insecticides and fungicides, and plant physiology and soil science usually cannot justify periodically recurring heavy expenditures for fertilizers, irrigation, tillage, etc. Furthermore, although genetics occupies at present by far the most strategic position of any of the basic experimental sciences, it is not evident as yet how materially it can contribute to an increased production of forest products. It certainly cannot do so as directly and easily as is possible in agriculture.

In the first place, the length of the life cycle in trees is a serious obstacle. The forest geneticist must concern himself mainly with the selection of promising races or varieties and with the production of vigorous first generation hybrids rather than with an elaborate program of breeding which involves an intensive study of many successive generations of plants. As in horticulture, he must depend largely upon vegetative propagation for eliminating variability. In the second place, the forester must proceed with considerable caution in attempting, as in agriculture, to grow selected races or hybrids in pure cultures, *since the more artificial a unit of forest vegetation becomes the greater the necessity for a compensating control over environmental factors.* It should not be inferred from this, however, that exotic species or selected races may not be substituted for existing elements of a specific "forest type" provided this can be accomplished economically and without unduly disturbing the natural equilibrium of edaphic and biotic factors. In fact, most of the so-called wild species of trees when carefully analyzed will resolve themselves into aggregates of more or less numerous morphologically and physiologically distinct races. The isolation, and exchange and trial under varying climatic, edaphic and biotic influences, of the most promising of these races will ultimately become an international problem of considerable magnitude, and one in which systematic botanists, geneticists and silviculturists may profitably cooperate.

It is evident that, not only is the forester forced to deal with more complex and variable phenomena than is the farmer, but his means of controlling them are less tangible and direct. Therefore, the basic experimental sciences can, in the long run, be of maximum service to silviculture by a concerted attack which aims to advance the general status of knowledge concerning forest phenomena, rather than by isolated and uncoordinated investigations of specific practical aspects of the forester's problems.

The situation in the field of forest production is in marked contrast to that in the field of forest utilization, where specific scientific researches, particularly those dealing with the physico-chemical properties of forest products, have a wide range of possible immediate and direct practical applications.

Plant physiology and soil science, and indirectly plant pathology, genetics, plant anatomy and other divisions of plant science, as previously stated in our discussion of agriculture, are seriously handicapped at present by inadequate information concerning physico-chemical reactions in the plant and in the soil, and by a lack of accurate quantitative methods for studying them under controlled experimental conditions. The situation is merely intensified in silviculture, since relatively less is known regarding trees and forest soils and less progress has been made in devising methods

for investigating them. *Thus, any coordinated program for basic scientific research should center about a nucleus of biophysical and biochemical investigations.*

It should be emphasized again in this connection that silviculture must depend mainly upon critical observation in the field and upon empirical experimentation during the pioneer stages of its development. This fact should be clearly recognized and frankly admitted in order to obviate unwarranted expectations both upon the part of foresters and of scientific investigators concerning the rôle of truly fundamental research, and to prevent misguided efforts. From a strictly utilitarian point of view, a comprehensive program of research in the basic experimental sciences can be justified only through its ultimate, rather than its more immediate, practical applications. This renders difficult the expenditure of industrial or public funds for such researches, since they must be supported over a more or less prolonged period before tangible results may be expected. However, the time factor in forest experimentation is so long and the phenomena to be investigated are so complex and so extraordinarily variable that a clearly visualized and adequately financed program of basic scientific research should be initiated as soon as possible if it is to contribute materially during the later stages of the development of the art of efficiently managing the earth's timberlands.

Can Research in the Basic Experimental Aspects of Forestry be Developed and Handled Adequately by Existing Agencies?:— In the preceding pages the endeavor has been made to outline some of the salient factors which have determined the development of the art of forestry. Many of these considerations are of a general nature and apply to almost the entire world. For reasons already stated, America occupies a unique position in regard to potentialities which may contribute to the solution of universal forestry problems. The situation in America has, therefore, been subjected to special analysis. Herein the chief consideration has been given to the institutions and activities which concern research in various phases of forestry problems, more particularly those affecting, directly or indirectly, forest production. It has seemed important to point out certain fundamental differences between forestry and other fields of endeavor in which research has been applied to extend knowledge. Forestry must be visualized as a huge and intricate structure. For its construction many workers of different types are necessary.

On the one hand, the fact that immediate modes of procedure are of the greatest urgency and, on the other, that any truly fundamental attack on the problems of forestry entails, first of all, the accumulation of much information of a basic nature involving very extended periods of time, lead to the conclusion that two distinct types of research are essential. First, the securing of more extensive information of a descriptive nature, and the coordination and application thereof through empirical methods, constitutes an immediate necessity which can be met more effectively through existing agencies.

But advancement of forestry through research is faced with a further

necessity. In order to discover principles which have a wider and more universal significance, to become familiar with the grounds or the reasons for the phenomena observed, it is essential to analyze more thoroughly and synthesize more securely than is possible by empirical or descriptive methods. Such an undertaking entails the close cooperation of various scientific disciplines and involves the laborious and time-consuming features of exact experimental procedure. Forestry is in a less fortunate position than many arts in that the scientific substratum upon which it must build is for the most part but poorly developed or non-existent. The stones which must be used to construct this foundation are widely scattered and few, and of such poor quality that some cannot be used with safety while others are in such form that they must be hewn and reshaped to meet the needs. These stones cannot be loosely assembled; they must support each other so that the foundation may be laid deep and secure.

The problem is huge; it has its own characteristics which demand appropriate treatment. The undertaking is in its infancy; it deserves and needs the most modern and effective means conceivable. There are two features of basic forestry research which with others make a special means of attack necessary. These two features are the complexity of the problems and the long time required to attain reliable results from experimental researches. The two are in many respects interrelated. *They are probably more pronounced in forestry research than in any other scientific endeavor yet undertaken.* These features, of themselves, preclude any individualistic or circumscribed approach. Isolated and sporadic efforts, attempts to advance one section of the biological structure without adequate support of the exact sciences, the dissipation of resources and energy in scattered endeavors can produce no advance. On the contrary, with the realization of certain common interests and aims, different disciplines and viewpoints when brought to bear upon the problems can, through mutual support and criticism, clarify these problems and increase the efficacy of each.

In almost every human endeavor, increased complexity has resulted in specialization. But such specialization of itself is of little value. It is only when it is demonstrated that the specialist can make a better contribution through intensive efforts in a restricted field that specialization becomes justified. Such is undoubtedly the case in the sciences which constitute the foundation of forestry. It needs no further emphasis, however, that no single one of these sciences can grapple successfully with as complex problems as are presented in forestry. No single mind can be in possession of all the facts and concepts of the various special sciences which must be considered in many of the forestry problems. Not only does each generation of scientific workers stand upon the shoulders of the preceding generation, but each specialist gains support and inspiration in his work from other specialists. As a consequence of this situation, the advance in our understanding of forestry problems will be in proportion to the extent to which the various special sciences work together toward a common aim.

The question naturally arises can this type of fundamental research be undertaken and developed effectively by existing agencies? This question brings to consideration first of all the Federal and State forest services.

These agencies have for the most part been established for definite and specific purposes. The predominant and pressing demand in these institutions is for practical results and modes of immediate action. As has been repeatedly stated, it is not only essential that this work be done, but there is also a very urgent demand for this work. The dissemination of information gained from experience and investigation and the guiding of the policy of owners of many types of forest tracts by Federal and State forestry agencies constitutes a public service which can hardly be overestimated. This is, in fact, the direct function and responsibility of these agencies. The broad basis of the Federal Service, the more intimate relations of the State agencies, together with the possible effective cooperative relations of the two, admirably fit these institutions for direct contact with those problems with which the art of forestry can effectually cope in the immediate future. With the growing recognition of the value of the results produced, increased burdens will fall upon the Federal and State agencies. Augmented financial support will inevitably be consumed largely in this manner. Under such circumstances no scientific undertaking, no matter how ideally it has been inaugurated, is proof against pressure exerted by economic and political exigencies. In a democracy this is apparently unavoidable. The incessant urge for applications of scientific research tends to circumscribe its outlook and to prevent the broadest and most fundamental development.

In this connection, there is one aspect of the investigations in the basic and exact sciences as applied to forest production, the significance of which can hardly be over-emphasized. That is that such investigation will of necessity be of the long time type; not only years but decades will be required before reliable conclusions can in all probability be drawn. A first requisite for such an undertaking is the assurance of a stable and continuing policy. The support for obtaining public funds or facilities for utilizing public institutions is found, on final analysis, to come from the body politic itself. Such support is forthcoming and to a measure assured as long as comprehensible and practical results are visible. At the same time it must be borne in mind that forestry is an eminently practical matter, highly susceptible to economic disturbances. Under these circumstances it is often an impossibility to continue on a course of costly and apparently random and impractical scientific investigations.

Similar considerations apply also to the future research activities of the forest industries. Too close an analogy cannot be drawn between the great manufacturing industries, such as the electrical and telephone industries, and the timber producing industry. The gap between discoveries in the so-called exact sciences and their application in the case of the former industries is much narrower than is the case in forest production. For instance, in the electrical industries, the basic sciences upon which these depend have reached a very much higher stage of development and are relatively much simpler than is the case with forest physiology, soil science, bio-chemistry and bio-physics. Nor, in all probability, are the forest industries on as sound a financial footing as are many of the electrical industries. Fundamental research for the former entails commitment to programs running through decades and generations far beyond the safe prediction for

continuance of administrative policy. This is not the case in most of the researches in physics and chemistry undertaken by the electrical industries, in which definite results are attainable much more rapidly. It is, of course, true that placing silviculture upon a thoroughly scientific basis will ultimately remove many of the hazards and uncertainties which now threaten it. However, to accomplish this will require a prodigious amount of work, which considering the uncertain economic aspects of the situation, the forest industries are not likely to feel warranted in undertaking.

The situation in the forestry schools for research in the basic experimental sciences is, in its broadest aspects, not very different from that existing in the Federal and State institutions and in the industries. This is due largely to the fact that the type of training given in the schools has, to a considerable measure, been determined by the latter agencies which are the main employers of forest school graduates. The schools have been concerned primarily with training practitioners and have not the equipment, personnel or point of view to inaugurate sound and effective research in the basic experimental sciences. As has been emphasized in the preceding chapter, the forest schools and the Federal Forest Service are at present confronted with the difficult task of developing the more intensive lines of descriptive and empirical research in forest production which are essential for the future welfare of the whole forestry movement in North America. The magnitude and complexity of this task is such that it should absorb their combined energies and resources for some time to come. *Furthermore, it is the descriptive sciences and genetics, rather than the basic experimental sciences, which can cooperate most effectively with silviculture in the present stage of its development.*

What has been stated in the preceding paragraphs relative to the adaptability of the Federal and State forest services, industries and forestry schools for research in the basic experimental sciences applies more particularly to the problems of *forest production.* In the field of *forest utilization,* conditions are rather different. Here there are a number of institutions, notably the forest products laboratories and industrial laboratories, which are equipped for intensive experimental researches of different phases of the utilization of forest products, such as pulp and paper, chemical extractives, alcohol, wood preservation, etc. This offers a situation more closely analogous to the engineering industries. For example, basic experimental researches in the chemistry of cellulose, lignin, oleoresins, and other substances may, within a relatively short time, yield results which would profoundly influence many industries which use the substances, or develop entirely new industries. This is, however, due to the fact that those aspects of chemistry that are concerned herein are relatively highly developed, and because in the carrying out of the industrial processes all conditions essential thereto can be controlled. Such intensive basic experimental researches should be encouraged and strongly supported by the existing agencies, not only because they are of value for direct application to the utilization of forest products, but also because they are significant in a better understanding of the physical and chemical properties of forest products and bear indirectly upon many problems of silviculture. Thus, although in the immediate

future the work of the regional experiment stations should be primarily of a descriptive and empirical experimental character, the Federal Forest Products Laboratory could well devote an increasing portion of its activities and resources to truly fundamental investigation in the exact sciences. Much of the work of an immediately applicable and practical nature should be performed by the industries themselves, since it is no more than can be expected from the history of other industries that those which derive their profits from forest resources should also support the researches dealing with the practical and immediate aspects of their problems.

While, then, the Federal and State forest services, forest schools and industries do not offer the medium for comprehensive programs of research in those phases of the basic experimental sciences, upon which the art of forest production must ultimately rest, the question arises whether such programs can be handled adequately by the existing scientific departments of the universities.

As has been indicated in the second chapter of this discussion, the descriptive plant sciences in general, and ecology, plant physiology and soil science in particular, are seriously handicapped at present by the lack of detailed, specific and reliable information concerning the host of interacting physico-chemical reactions in the living plant and in the soil. These phenomena are so complex, so diversified and so extraordinarily variable that in most cases they can be analyzed effectively only through the concatenated and sustained efforts of groups of physical, chemical and biological experts working in close cooperation. The older and relatively stereotyped analytical methods must be refined and perfected, and new techniques developed if real progress is to be made. Science has become dissociated into so many highly technical and specialized sub-divisions that no single investigator can acquire a sound working knowledge of more than a limited portion of the whole field. Attempts upon the part of biologists to master modern chemistry and physics, in addition to one or more of the descriptive sciences, almost inevitably lead to superficiality and tend to encourage a dangerous tinkering with chemical and physical tools and techniques which are but imperfectly understood. Nor can the biologist solve his problems merely by hiring young chemists or physicists as assistants. The roots of the undertaking must strike deeper than this. The personal interests and convictions of the workers must be enlisted and retained. Can the difficulty be solved through inter-departmental cooperation? Such cooperation cannot be coerced. It must arise through some strong natural stimulus or not at all.

Before attempting to answer this question, it is essential to discuss certain aspects of the present status of science and of scientific research in our universities. The general tendency for science to dissociate into increasingly numerous subdivisions, each with its own particular interests, aims and points of view, has been especially strong in the case of biology. That this tendency has been more pronounced in biology than, for example, in chemistry, is reflected in the organization of numerous biological departments in educational institutions and of the various national scientific societies. Whereas chemistry has succeeded in retaining a considerable de-

gree of cohesion and unity, biology has broken up into a relatively large number of more or less independent units. The problem of stimulating cooperation between individuals in these separate compartments is by no means a simple one, even in the case of those subdivisions of biology which have developed to a stage where problems of mutual concern can be visualized accurately and where methods and techniques for investigating these problems have been perfected. Thus, although the results which may be attained through cooperative activities between systematic botany, genetics, cytology, pathology, entomology, bacteriology, etc., and between these sciences and agronomy, horticulture and silviculture are fairly obvious, instances of effective inter-departmental cooperation in research, as contrasted with education, are by no means as numerous as might, *a priori*, be expected.

Traditions of an intensely individualistic nature in our universities, as in their European prototypes, have tended to inhibit the development of an atmosphere of mutually beneficial cooperative relations in research. It is not surprising, therefore, that under these self-centering influences and frequent lack of confidence and sympathetic understanding between the theoretical and applied aspects of biology, successive subdivisions, such as bacteriology, plant pathology, economic entomology, genetics, etc., tend to split off from botany and zoology and to become incorporated in separate departments. Although many of the latter units have a common bond of interest in the biological arts, each of them is concerned primarily with its own development. Its energies and resources are absorbed in its own specific and relatively circumscribed activities in education and research. Comparatively little headway has been made as yet in the difficult task of reassembling these dissociated units for concerted attacks upon those complex biological problems that require concatenated efforts for their solution. Nor is it entirely clear how this may be accomplished most wisely and effectively in existing educational institutions. In the case of the more difficult ecological and physiological aspects of biology, it certainly cannot be accomplished without the active and sympathetic cooperation of physics and chemistry. But at present the gap between physics and chemistry and biology is so wide that it cannot be bridged without some intermediate means of support. On the one hand, the average biologist is unable to resolve his problems into terms that are clearly comprehensible to the chemist or physicist, and, on the other hand, the average chemist or physicist is unable to visualize the numerous variables in biological phenomena. To meet the needs of the biologist, new aspects of physics and chemistry must be developed and refined, and special methods and techniques must be perfected. The existing departments of chemistry and physics have neither the time nor the inclination to assume such burdens. They are absorbed in their own problems and educational activities, and, not infrequently, they systematically discourage their abler students from acquiring an interest in the complexities of biological phenomena.

It has been emphasized repeatedly that the type of research in the basic experimental sciences which is here being advocated as necessary for forestry requires, first of all, a well coordinated group attack through the concurrent efforts of specialists in various scientific disciplines. In the case of forestry

the undertaking must be of a truly basic nature and of wide scope; at least there must be no barrier which may prevent development into fields which at the outset were unforeseen. Such an undertaking cannot recognize artificial divisions of science nor consider academic departments and administrative conveniences which in educational institutions have taken on the aspect of fundamental demarcations. It is of the utmost importance that the interests and growth of a group research project should never become subservient to any other motive but the advancement of the aims for which it was organized. A complex research project, which of necessity must run over many years, if allocated to existing departments will ultimately come into conflict with the traditions and regulations of an educational institution. There will always be differences of opinion within a university regarding its function and a certain competition between the purely pedagogic and research interests. The university has a great variety of duties; aims of university education will always be a subject of dispute; experimentation in university administration is unavoidable.

In other words, the policies of educational institutions are by no means fixed. In State institutions these are subject to political influences from various sources. Such influences may have the most significant consequences. In the privately endowed institutions, the policies vary radically with changes in the administration. A congenial and earnest research atmosphere with ample support and sympathy for creative work can suddenly change to conditions under which such work is clearly impossible. Such changes may arise from the inevitable shifts in the administrative as well as the faculty personnel; examples are all too numerous in our American universities. A research project intimately connected with the departments of a university must, under such conditions, share the fate of that institution or of some particular department. Nor would it be well for the existing departments of a university to have the course of their free development too strongly influenced by special research projects.

The apportioning of different branches of the basic researches of forestry to scientific departments in *various* universities would have even graver consequences. Not only would the aforementioned administrative problems be multiplied, but the very keystone of the principles of close coordination would be removed. The resulting situation would be quite analogous to the present one. In biology, it is not chiefly more research which is required but sounder research resulting from the direct criticism and assistance which the other sciences, especially the exact sciences, can lend to the methods and conceptions employed in biology.

Somewhat similar considerations to those which have been mentioned in relation to educational institutions apply to botanical gardens and arboreta. These institutions have been organized for and have had as their chief function the collection, classification and display of plants. Through botanical exploration, gardening and landscape architecture, and exhibition of exotic and cultivated plants, the gardens have rendered a most valuable service to general education, and have fostered an appreciation of the economic and esthetic aspects of plant life. These activities have been of very great value, especially to city dwellers who find herein one of the few re-

maining means of contact with nature. The more formal and technical activities have centered about systematic botany, and the botanical gardens have thus become also the great depositories for herbaria and collections of fossil plants. These may be of considerable aid in various aspects of forestry, and through cooperation with the scientific staff of experts in different groups of plants much benefit can be derived. Through exploration work, the introduction of exotics, the production and testing of hybrids, and cooperative investigations on the floristic aspects of forest types, the botanical gardens may render valuable assistance in the general field of forestry research.

For investigations in the basic experimental aspects of plant science, botanical gardens and arboreta have not proved to be a medium of permanent value. While this has been due to a variety of causes it is necessary here to discuss only such factors as bear upon the adaptability of these agencies for researches basic to forestry. A matter of first consideration in this connection is the fact that such researches are predominantly of an experimental nature. For this purpose, a prerequisite of inestimable value is an experimental viewpoint and atmosphere. Without doubt this is a subtle and perhaps even Platonic and transcendental quantity. It is none the less a real element, the *sine qua non* in the development of experimental science. While physics and chemistry have fostered and cherished this element, the plant sciences have not consistently applied it and consequently have not developed the rigorous, exacting and critical attitude that is essential for highly refined and exact experimental work. Moreover, the experimental viewpoint in no small measure conflicts with the traditional activities of botanical gardens and arboreta. Those institutions are already committed to definite tasks and through several hundreds of years have acquired their own precedents and atmosphere. Much of this is unquestionably of great service to mankind, but it is not conducive to the progressive and unhampered development essential to modern experimentation. The botanical gardens and arboreta have already a set pattern into which their activities fit, a scheme of organization and function. There are certain duties which must be discharged; in most cases an elaborate and ever enlarging collection of prepared and living material must be cared for and a constantly growing overhead provided for this natural growth and the new accessions.

Experimental research cannot remain within fixed boundaries; it should have every degree of freedom. No matter how well defined and specific the original purpose, research in the basic sciences, if it is virile, will grow in directions which are entirely unpredictable. The development of new ideas and of productive workers may lead such research into channels reaching far beyond the original plan. New physical, chemical and biological laboratories and equipment become essential; original lines of endeavor prove fruitless and are abandoned. Flexibility of organization and freedom of development are in the highest degree essential for a rational program of research in the experimental sciences basic to forestry. No greater incumbrance is conceivable than one which would limit this freedom. In some of the older European institutions where the botanical gardens have

been intimately affiliated with the laboratories for plant physiology and plant bio-chemistry, the increasing care demanded for the maintenance of botanical collections and displays has greatly hampered the normal development of these sciences on the one hand, and, on the other hand, the emphasis upon experimental work has resulted in the botanical gardens falling into a lamentable condition of neglect.

The value of the ground and of the living material in botanical gardens and arboreta does not permit the use of these for extensive experimentation. For this purpose there must be available extensive areas which may be put to a variety of uses immaterial of their appearance. Special forest areas in arboreta or affiliated with botanic gardens are of too restricted a nature to permit extensive experimentation of a character required by fundamental forestry research. Such facilities, when required, can undoubtedly be secured advantageously through cooperative arrangements with Federal and State agencies, yielding an extensive range of conditions. Such arrangements would have the added advantage that they would form a means of contact with the workers in the extensive empirical and descriptive investigations pursued by the Federal and State agencies and constitute an avenue through which the two types of investigation could maintain mutually beneficial relations.

It should not be inferred from the preceding discussion that forestry cannot be helped very materially through the allocation of additional funds to specific departments of universities or to botanical gardens and arboreta. As has been stated repeatedly, the descriptive sciences in general and plant pathology, entomology and genetics in particular, have an obvious and important rôle to play in the development of research in forest production. Even the older and essentially descriptive aspects of ecology, physiology, soil science, chemistry and physics may render valuable aid in the investigation of specific practical problems of silviculture. For such purposes, additional funds are required in the immediate future. However, it is evident that a concatenated and sustained attack upon the more fundamental experimental aspects of forest production cannot be handled adequately by existing scientific departments. For this purpose, new agencies are essential.

New Agencies Required for Research, Particularly in the Fundamental Physiological and Ecological Aspects of Forestry:— Many types of agencies have been organized to promote research, discovery and the application of knowledge to human needs. These aims have been realized in universities, special research institutes, museums and botanical gardens, scientific academies, governmental laboratories, industrial organizations and various combinations and modifications of these. Such agencies differ in their adaptation to particular needs and circumstances. Certain undertakings can be carried out more effectively in one type than in another. In fact, one of the most important points throughout this discussion has been that the type of agency and organization should be adapted especially to the nature of the research it is to pursue. This is particularly true where a large and complex group of problems, such as we are here dealing with, is concerned.

It should be recognized that the trend toward the creation of special research units of varying sizes, both within and without educational institutions, is in part an expression of dissatisfaction with the status, organization and accomplishments of research, and particularly with its subordination to teaching activities and to various administrative expediencies. Many of the older departments in universities have become to no small measure amassers and accumulators of knowledge rather than developers and refiners. The stereotyped outlook of such departments and their absorption in the task of instructing an ever increasing number of undergraduates frequently does not provide a congenial and stimulating environment for research, and hence the urge for the research professorship or the segregation of certain aspects of the field into a new department.

Just as those phases of biology which are especially significant to the biological arts tend to split off from botany and zoology and to become incorporated in separate departments, so the more intensive research activities strive to emancipate themselves from too intimate association with the formalistic and pedagogic interests. There is accordingly a spontaneous urge to incorporate the supposedly more virile and successful investigators in independent or semi-independent compartments of their own. It is, indeed, a natural concomitant of the increasing size and complexity of our educational institutions, of those traditions of an individualistic nature which are so highly cherished and of the general trend of specialization in science.

There are, however, inherent difficulties and dangers in this continued process of differentiation, dissociation and specialization in our universities, and there is obviously a serious question as to how far it may wisely and profitably be allowed to progress. Our universities have distinct educational duties and functions. There is a limit in size and in complexity of organization beyond which the primary aims of an educational institution become obscured in a multiplicity of divers and highly specialized interests, and beyond which productive work becomes constrained by the exigencies of administrative entanglements. A number of our educational institutions are rapidly approaching, if they have not already exceeded, this limit. In any case, they have developed so rapidly and have acquired such extensive resources that attention should now be focused upon the task of refining and of increasing the efficiency of the major projects and lines of endeavor, rather than upon expansion into new fields of activity.

In the general field of forestry, educational institutions, through their forestry schools, have already committed themselves to the task of training practitioners of the art. In addition, they are now faced with the burden of training investigators for the forest experiment stations and of developing research in those special phases of the descriptive sciences which are indispensable concomitants thereof. These tasks have already been alluded to; they are complex and difficult. They involve a large number of pressing problems of vital significance to the future welfare of the whole forestry movement. Intensive and sustained efforts will be required for their solution, since they necessitate fundamental changes in existing points of view, readjustments between individuals and departments, revisions of curricula, etc. Here also the tendency towards specialization seems imminent. Plant

pathology, entomology, genetics, etc., are tending to become dissociated from botany and zoology and to affiliate themselves, as separate departments, with agriculture. Should forest pathology, forest entomology, forest genetics, etc., in turn dissociate themselves from the agricultural aspects of these subjects or from botany and zoology, and become incorporated in the forest schools or in separate departments of their own? If so, how may the danger of premature specialization be avoided in the training of investigators? If not, how may effective interdepartmental cooperation be stimulated?

Furthermore, properly trained men are a paramount necessity for complex research undertakings involving the basic experimental aspects of forestry. Sound training in the fundamentals of science must as a rule be obtained at the universities. Therefore, if these complex research projects are to obtain an adequate personnel of investigators, the universities should be so organized as to be able to train these men most effectively.

In view of the complexity and difficulty of such special tasks as these, it would appear to be unwise for the universities to assume in addition the much more exacting and onerous task of initiating those pioneer researches in the basic experimental aspects of forest production which will require new agencies and a long sustained and well coordinated group attack for their efficient development. To attempt to do so will further complicate a general situation which is already highly involved and will inevitably prove to be a serious distraction in the solution of existing problems and administrative responsibilities.

May not the right type of research institute have a legitimate and essential rôle to play in safeguarding the universities from the danger of over-specialization? May not such an institution function as a valued collaborator rather than as a competitor in the general research field? *The activities of research institutes need not and should not be divorced completely from those of the universities.* In most cases, the research institute should be located in a university environment where stimulating contacts with other scientific workers and possibly with graduate students may be established, and where excessive expansion and needless duplication of effort and equipment may be avoided. At the same time the research institute can be assured of all necessary independence from purely academic and pedagogic control. Under such circumstances, there is no ground for the fear that the universities will lose their best men to the research institutes. The latter should have a distinctly quickening influence on the research activities of the universities, and what these contribute to the research institutes in men should be repaid by the latter in kind so that ultimately there is an interchange which is beneficial to both.

These statements are made not without the realization that special research organizations have been subjected to much criticism, some of which is thoroughly sincere and deserves careful consideration. It is argued that the creation of special research agencies robs the scientific departments of universities of their ablest investigators, that stimulating contacts with students and with a general university environment are essential for productive theoretical research, that research is a matter for individuals with their coteries of disciples and assistants, that real cooperation between sci-

entific peers is unattainable and merely restricts the freedom and effectiveness of the individual, that independent research organizations inevitably tend to expand and to become increasingly complex and unwieldy and thus to duplicate the activities of universities, that research institutes have not been productive of important original contributions to science in spite of their large resources and elaborate equipment, etc.

In principle, some of these criticisms have already been discussed. However, it must be admitted that serious mistakes have been made in the development of research institutes. In certain cases, there has been an unfortunate tendency to launch them prematurely, without a clear visualization of objectives, on too large a scale, with too circumscribed aims or without consideration of programs, and *particularly with greatly exaggerated expectations and pronouncements concerning their future contributions.*

To comb the field of science for the most outstanding investigators and to enlist their services through comparatively large salaries, relief from teaching activities and elaborate equipment may have grave consequences. It may not only tend to arouse misconceptions and even hostility among scientific colleagues, but to create a staff whose remaining period of productive research is relatively brief and *especially to produce an unwholesome urge for quick and spectacular results.* If the staff consists of permanent appointees without provision for a considerable inflow and outgo of younger investigators the activities of the institute sooner or later become unduly rigid and stereotyped. Under such conditions the broader educational aspects, which every research institution should foster, are lost and thus also one of its greatest means of influence. Nor may effective cooperation be expected from the association of specialists who are selected without regard to their personal points of view, eccentricities and prejudices.

It should be emphasized in this connection, however, that there are many types of research institutes which vary greatly in size and complexity, in organization, in the specific aims for which they are created and in their degree of isolation from or affiliation with universities. To single out a particular type of organization as the basis for generalized criticisms of the whole institute idea is obviously unfair.

In many respects the two agencies for scientific research, the university and the research institute, overlap. They should, in fact, be side by side, not as competitors, but as close cooperators. The research institute in no sense relieves the university of the responsibility for research activity. Certainly the training of young people in the advanced aspects of science can be most effectively carried out in an atmosphere of scientific inquiry and by men familiar at first hand with the development of science. Consequently the research institute does not change this aspect of the university's function, if anything the research institute should make its own contribution to the educational work.

Research institutes, to be effective, should not remain stereotyped. They must have many degrees of freedom and a flexible organization; funds must be mobile and not completely petrified in buildings and budgets. New discoveries necessitate ready adjustment in different lines of activity. Therefore, original lines of attack must be abandoned and new ones in-

augurated. It is difficult for universities to follow such a policy of freedom. They must always meet their responsibility of educating and training the oncoming generation in the fundamental subjects. Of necessity, they must have greater stability and be encumbered by larger equipment. These are the features that differentiate the two agencies. Certain fields of investigation can be developed most effectively in universities. Others of a more complex nature, demanding a concatenated attack of long duration and flexible organization are best pursued in a research institute.

What, then, are some of the means of approach to the development of forest production through research in the more fundamental aspects of the underlying sciences?

It should be emphasized again in this connection that, whereas many of the more descriptive aspects of the natural sciences have progressed to a stage where cooperation with silviculture is both possible and desirable, *i.e.*, where problems of mutual interest are clearly definable and where the necessary tools and techniques for their solution are readily available, the basic experimental aspects of plant science occupy at present an entirely different position. It is a striking fact, observed by many European and American foresters, that silviculture, which deals primarily with living trees, has available comparatively little in the form of exact information concerning the normal functioning of arborescent plants. The tree as a subject for intensive physiological study has been neglected. Thus, a knowledge of the normal physiology of trees and of the forest remains one of the great *desiderata* of silviculture. It clearly falls into the general domain of plant physiology and of ecology, but the latter subdivisions of science are poorly developed from the basic experimental point of view. Without the support of sound physics and chemistry, they are relatively ineffective weapons of attack. Thus far, plant physiology has dealt primarily with the smaller plants and with lower organisms. While this has been of some service to agriculture, the problems of silviculture require a different mode of attack, not only for the individual arborescent species, but for the whole forest complex. *A new science of forest physiology, involving both physiology of the tree and of the forest, must be developed.*

The study of the tree as a physical system and the determination of the substances thereof and of the chemical reactions which take place within the tree are some of the primary prerequisites for a physiological treatment of its functioning. To obtain special physical and chemical information of this nature requires serious and long continued study. New tools and techniques must be developed and refined. Such tasks, first of all, demand well-trained physicists and chemists, not merely temporary assistants who supplement the efforts of the plant physiologist. But that is not all. The tree is a complex system. The functioning of the tree can not be studied intelligently without consideration of its structure. The latter itself constitutes a difficult and intricate study. Consequently the anatomist and cytologist must join forces with the physicist, chemist and physiologist in the whole attack. The normal physiology of the tree involves not only a consideration of its structure, composition and functioning but of the influence of external factors as well. The effect of air-temperature, light, hu-

midity, etc., must all be analyzed, the whole complex of environmental factors subjected to the same basic treatment. Here again physics plays a dominant rôle. Moreover the all-important and hidden portions of the tree, the root systems, have their own environment, the soil. The latter presents a whole group of problems which have their purely physical, chemical and biological aspects. None of these can be entirely segregated, they are all interrelated. The intricate relations of the microorganisms within the soil, with the accompanying physical and chemical changes, constitute an environmental factor of foremost significance in a consideration of the normal physiology.

The physiological approach, moreover, offers a rational means of advance to other basic aspects of silvicultural problems. Many phases of forest pathology and forest entomology must await a clearer understanding of the normal functioning of trees. A thorough knowledge of the physiology of the host is indispensable to a rational treatment of parasitological problems. Not only would forest pathology and forest entomology make use of knowledge of the internal physiology of trees, but the research activities on the influence of external factors and of those in forest soils could contribute materially to the solution of the more complex problems of forest production. Furthermore, an intensive study of the abnormal functioning of trees will in many cases throw much light upon their normal physiological activities. Similarly, opportunities exist for the development of essential cooperative relations between genetics, physiology and pathology.

If we are to construct a picture of the normal and abnormal functioning of trees, it must necessarily be made up of many parts. Impressionistic rendering of some of these is hardly in keeping with the scientific accuracy of the rest. On the exact rendering and on its congruity depends the value of the picture to foresters and to others. The greatest advantage lies in bringing together groups with different viewpoints, enabling each to discuss freely and informally his own aspect and to construct thus a truer picture of the whole phenomenon than is possible when this is viewed from one restricted field. The difficulties of realizing such an idea are, however, by no means few. It cannot be accomplished in an atmosphere where traditions are of individualistic nature and consequently antagonistic to such an undertaking. Much depends upon the leadership of the various groups as well as on their personnel. Moreover, simply placing men together does not create cooperation. The personal interests and convictions of the workers must be enlisted. There are a host of purely physical and chemical problems which can engage the attention of the best talent, whose understanding of the general aims must be enlisted in order to be of service to the other sciences. Such cooperation need in no wise hamper individual initiative and personal effort, nor stifle original ideas. It should, on the contrary, give greater opportunity for both the development and expression of ideas by individual workers.

It is evident that a special undertaking of this character should not be farmed out in disjointed fragments to the existing departments of one or more universities. Nor would it be wise for a single university to have the course of its free development too strongly influenced by assuming the

entire responsibility for a specific project of this magnitude and complexity. Furthermore, as shown in the preceding chapter, it is of utmost importance that the interests and growth of this group research project should never become subservient to any other motive but the advancement of the aims for which it was organized. A matter of paramount importance is a clearly visualized and assured policy continuing over a long period of years. It is difficult to safeguard the maintenance of such a project in universities, since the interests, objectives and policies of our educational institutions are subject to sudden and more or less profound fluctuations with changes of administration and of other factors.

The writers have, therefore, been forced to the conclusion that in the long run the interests both of forestry and of educational institutions can be served best if the task of initiating, developing and guiding researches in the more basic experimental aspects of forest production is assumed by some special administrative agency. The Kaiser Wilhelm Gesellschaft may serve as an example of such an institution. It should be free to accept funds from various sources for consistent and carefully planned projects.

This institution should function not only as a central administration for funds contributed for the special purpose of furthering researches in the basic experimental aspects of forestry, but as an indispensable correlating and coordinating agent in the disbursement of such resources. One of its first and most important tasks should be a careful study of the status of different basic sciences in their relation to forestry problems. Especially in view of the long time feature of such researches, the most careful consideration must be given to the problems to be studied and to the most advisable means of approach. This cannot be done from a single viewpoint, but the problems must be considered from the viewpoints of the various sciences which may be called upon to assist in the attack. This must be the first step in a cooperative effort and should result in clarifying the picture of the problems. Only through the subsequent intensive cooperative researches can the feasibility of the plan of attack be discovered. In sciences as young and undeveloped as those here considered, such changes are unavoidable. This emphasizes the necessity for freedom of development in any direction, and of cautious and centralized cooperative planning or modes of attack.

Correlations must also be established ultimately with the descriptive and empirical investigations in forestry conducted by Federal, educational and other agencies. New methods and techniques can be devised and old ones tested in order to determine the scientific reliability and degree of usefulness of these methods, which can then be put into the hands of the empirical workers. Reliable instrumentation depends upon sound physical research, and the significance of a vast amount of ecological and other observational investigation is vitiated by faulty instrumentation and a disregard of the physical principles upon which the instruments used depend. The institution must stand for the most thorough and basic development of all the subjects for which it assumes the responsibility. It should be equipped to serve in the capacity of counselling and assisting in matters pertaining to the applicability of methods and concepts of the basic experimental sci-

ences to the descriptive and empirical investigations of forestry. This, of course, in no sense implies the supervision of the latter investigations. Nor is the idea to exercise any coercion on the part of the institution to inaugurate a universal plan of investigation to be followed by other forestry agencies. On the contrary, it should be the function of this agency rather to carry out those activities which existing agencies are obviously incapable of performing, but for which there is a fundamental need.

With the aims and viewpoints established and major projects mapped out, the drawing of specialists from educational and other institutions into such an undertaking through cooperative arrangements would be mutually helpful. Highly specialized researches would thus be strengthened, while the desultory tendency of such researches in biology would be stabilized through the major activities of the institution.

These activities should not involve the creation of a single, large, isolated research institute, but rather the development ultimately of several smaller research units which should be located in university centers and affiliated more or less informally with existing scientific departments. Research thrives best and is most productive in compact semi-independent units of moderate size where the investigators are closely and informally associated, but where they are able to maintain contacts with general scientific and intellectual interests.

The objectives of a special research organization must extend beyond the securing of knowledge regarding fundamental problems. The influence and value of such an institution are largely determined by the service which it renders. It is with this in mind that the creation of the type of research institutes mentioned is being urged. In order to justify themselves, these research units must not only advance knowledge in certain fields, they must also serve a special educational function. However, owing to the necessarily highly specialized nature of their work, neither direct means of education nor attempts at practical applications of the results of researches can be undertaken without great sacrifices. Their educational function lies in another and more neglected field. The research units should become centers of investigation for various branches of basic experimental science applicable to forestry. There are at present very few men in America or abroad who through training and experience are suited for this work. Younger men must be selected and trained. The nucleus of the research institutes must of necessity be small and carefully chosen. As research developments in the forest experiment stations, forestry schools and other institutions warrant, an exchange of young men should evolve, by means of which the work and experience of the research institutes can be carried to these institutions. Such training cannot be obtained in a few years; it is not a matter of courses, of lectures nor of stereotyped education, but rather of a highly specialized type, obtainable only through years of intimate contact with these disciplines and with the men who are developing them.

Such research institutes should, moreover, offer facilities for workers from other forestry institutions to spend some time in obtaining intensive training and in pursuing researches in the basic experimental sciences on

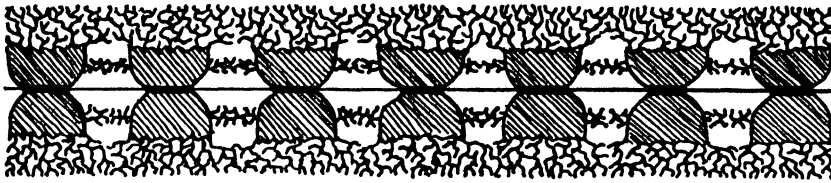
special aspects of forestry problems. Such an interchange of mature men should result ultimately in a general stimulation and quickening of forestry research the world over.

It is impossible to forecast the directions in which thorough research undertakings in the basic experimental sciences will develop. Nor does it seem wise to outline specifically the work which should be begun. This depends upon a number of factors involving the selection and training of an adequate personnel, etc. It is, therefore, of the greatest importance that the research institutes be developed with extreme caution, that they start on a small scale and that they be allowed to grow as the needs present themselves and especially as the proper personnel becomes available. Such beginnings, however, should carry the positive assurance that adequate means for growth are available as the researches demonstrate the need and justification for expansion. This should include not only developments within the research institutes themselves but it should also offer facilities for the training of younger workers, and particularly for conducting co-operative researches with agencies for descriptive and empirical investigations on the one hand, and with special researches in educational institutions on the other.

From the forests, man has drawn much of his power and inspiration. That it is essential to perpetuate these natural resources needs no special pleading. The object of this discussion has been to indicate the most effective means of meeting the needs of forestry through the application of scientific investigation. These needs are varied, complex and exacting–probably far more so than any which science has yet attempted to supply. Other endeavors in applying the scientific method to human needs, as in industry, medicine and even agriculture, which on first thought might be considered as yielding valuable analogies, on careful examination disclose many fundamental differences.

An attempt has been made to indicate in what manner the more immediate needs of silviculture may be met. A more thorough understanding of the exceedingly complex and intricate biological interrelations of the forest requires the combined efforts of various scientific disciplines. This is inevitably a long and arduous task, for new viewpoints and conceptions must be brought to light, new methods devised. In short, the science of trees and of forests must be developed in its most basic aspects.

It is a challenge to America. Here, if anywhere, are both the will and the financial means for accomplishing it. Beyond that, America has an even more precious heritage. Nature's own husbandry, as found in the remaining primeval forests, will ever be man's best teacher.



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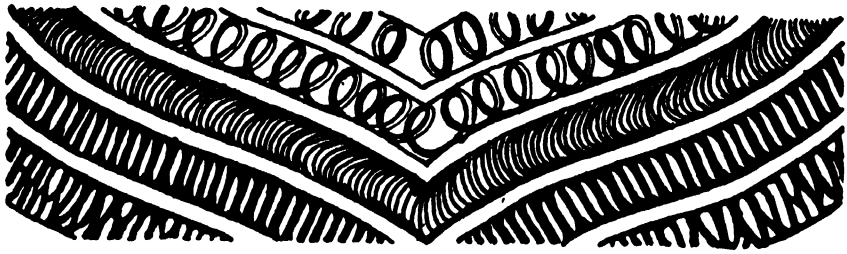

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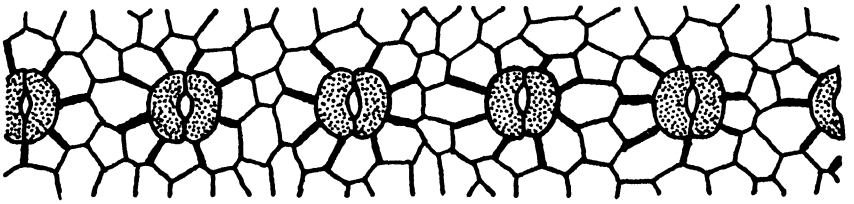

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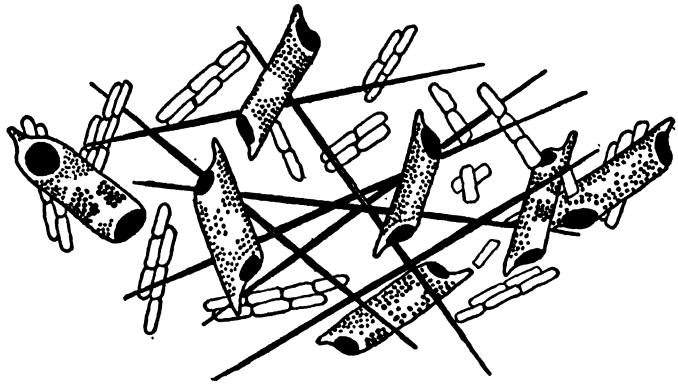

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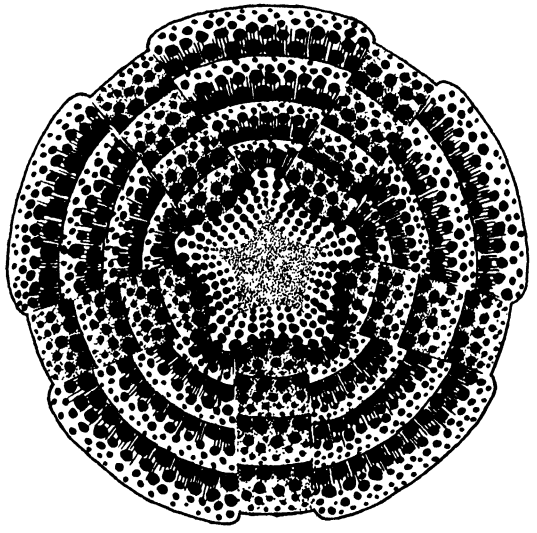

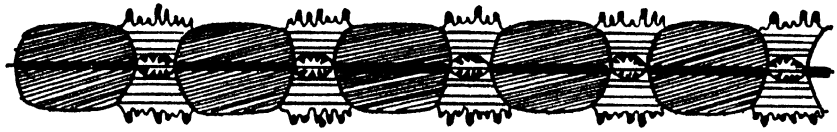

Explanatory Notes regarding the Sectional Title Pages, Head Pieces and Vignettes designed and drawn by Professor B. G. L. Swamy: — The motifs for the ornamental embellishments used in this book have been inspired, in very large measure, by Professor BAILEY's writings. The following method of execution has been followed in regard to the sectional title pages: the vertical column (*left hand side*) — from below upwards — conveys a salient trend in the phylogenetic specialization of diverse cell types, tissues or structural patterns of xylem, while the horizontal columns above and below concern the very subject matter of the articles reprinted in the respective parts of this book. Each of the triangular enclosures in the vertical and upper horizontal columns of the sectional title page for Part VIII idealizes a particular discipline of the plant sciences — from below upwards — wood anatomy, developmental anatomy, embryology, palynology, cytology, nodal anatomy, taxonomy and phytogeography. The "blue-print" occupying the lower horizontal column emphasizes the plea for closer coöperation, understanding, and integration among these and other branches of the plant sciences.

While the external appearance of plants or parts thereof have been widely exploited for decorative purposes, in arts and crafts, through the ages, an attempt has been made here to translate some of the histological or anatomical features of plants into a variety of ornamental designs. Anatomical characteristics of the secondary xylem have been the source for some of my drawings (pages ix, xiii, xxv). I have used the tracheary cells of the primary xylem (pages xx, 249), and the histological features of the leaves (pages xix, 254), etc. In some cases, cell types have been rendered in an "aesthetic arrangement" (pages xxiv, 256). Also included are idealized versions of a transection of an oak stem with depressed segments, a winteraceous pollen tetrad, and a three-flowered cluster of *Sarcandra irvingbaileyi* (page iii), etc.

The closing vignette (last page) illustrates — from below upwards — the history of the phylogenetic origin and specialization of the vessel member in the angiosperms. The successive stages in its morphological modification have been arranged in an "herb" pattern, a decorative motif frequently found in paintings of the Mughal school.

PRESIDENCY COLLEGE,
MADRAS, INDIA

Autumn, 1954

PLATES

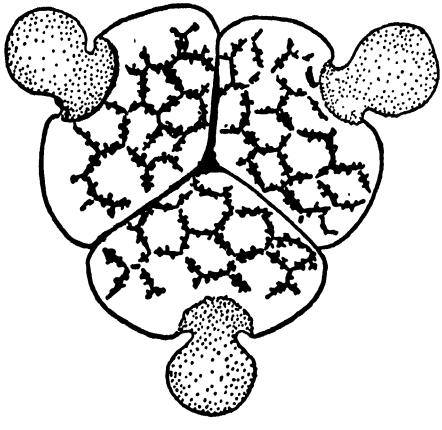

Plate 1

Fig. 17: *Pinus Strobus.*— Early telophase from longitudinally dividing fusiform initial, seen in radial, longitudinal extension. × 790.

Fig. 18: *Same.*— Widening of central spindle and beginning of cell plate formation. Longitudinally dividing fusiform initial, seen in radial, longitudinal extension. × 790.

Fig. 19: *Same.*— Later stage than that shown in Fig. 18, showing disappearance of connecting fibers and movement of nuclei toward the cell plate. × 790.

Fig. 20: *Same.*— Later stage than that shown in Fig. 19, showing daughter nuclei, cell plate, and kinoplasmasomes. × 790.

Fig. 21: *Same.*— Later stage than that shown in Fig. 20, showing movement of kinoplasmasomes in opposite directions toward ends of protoplast, and daughter nuclei in original positions at center of cell. × 370.

Fig. 22: *Same.*— Section of kinoplasmasome showing fibrillae and cell plate. × 1860.

Fig. 23: *Robinia Pseudo-Acacia.*— Fusiform initial in radial, longitudinal extension, showing cell plate, kinoplasmasomes, and daughter nuclei. × 790.

Fig. 24: *Same.*— Less advanced stage of cytokinesis. × 790.

Fig. 25: *Same.*— Beginning of cell-plate formation. Note transverse position of polar axis of division figure. × 790.

Fig. 26: *Same.*— "Resting" nucleus of fusiform initial, seen in radial, longitudinal extension. × 790.

Fig. 27: *Pinus Strobus.*— Cytokinesis in obliquely dividing fusiform initial, seen in tangential, longitudinal extension. One daughter nucleus out of plane of section. × 790.

Fig. 28: *Same.*— Early telophase in transversely dividing fusiform initial, seen in tangential, longitudinal extension. × 790.

Fig. 29: *Same.*— Later phase than that of Fig. 28, showing extension of spindle and beginning of cell plate formation. × 790.

Fig. 30: *Same.*— Thickening of connecting fibers in equatorial region of spindle. Ray initial. × 790.

Fig. 31: *Same.*— Later stage than that shown in Fig. 30, showing cell plate. × 790.

Fig. 32: *Same.*— Later stage than that of Fig. 31, showing extension of cell plate and "drawing in" of fibers and nuclei. × 790.

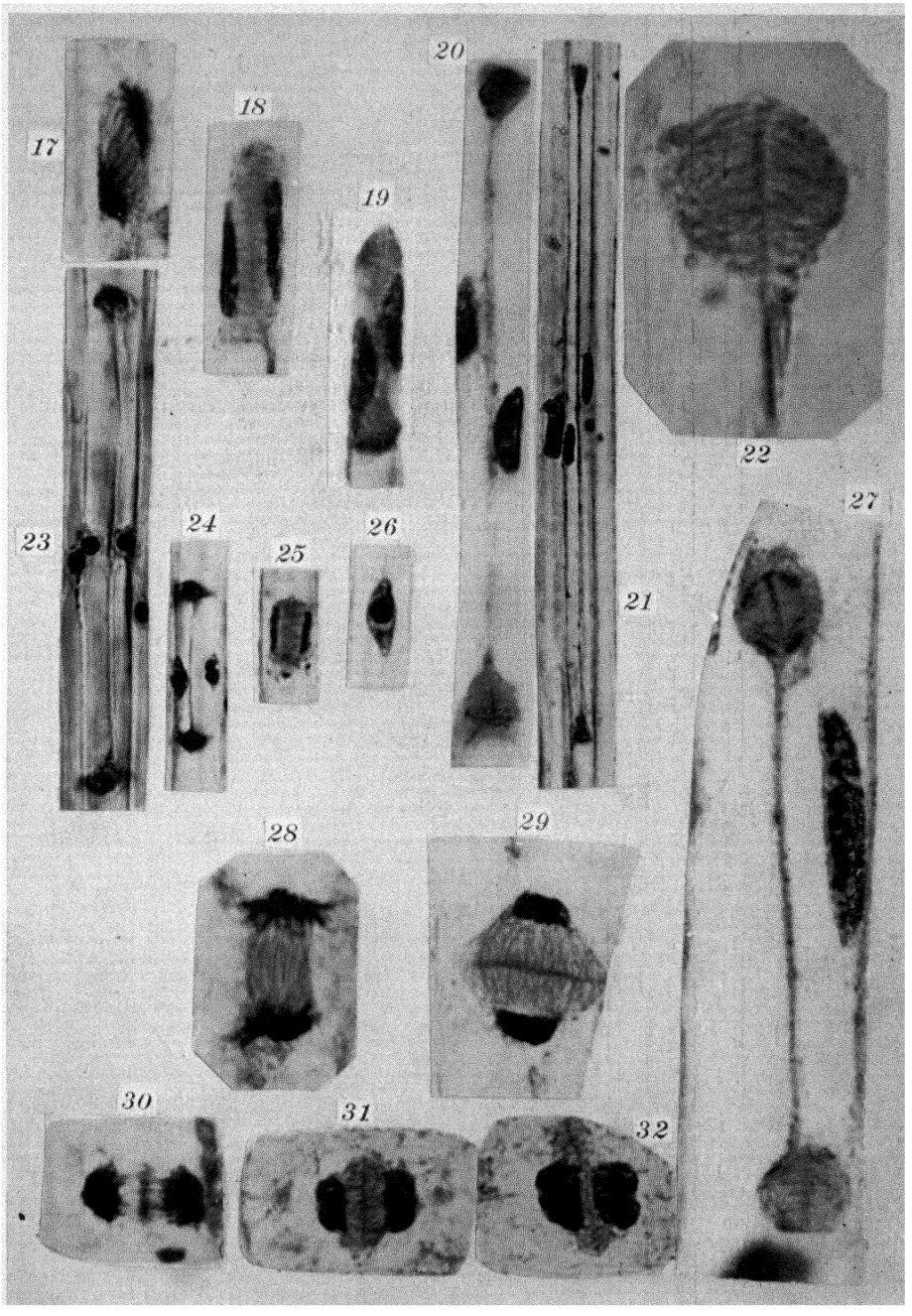
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Plate 2

Fig. 1: *Pinus Strobus*.— Tangential longitudinal section of non-stratified cambium. The cells are so long that only a portion of each fusiform initial is shown in the photomicrograph. × 110.

Fig. 2: *Myristica philippensis*.— Tangential longitudinal section of non-stratified cambium of less highly differentiated type of dicotyledon. × 110.

Fig. 3: *Fraxinus americana*.— Tangential longitudinal section of non-stratified cambium of highly specialized type of dicotyledon. × 110.

Fig. 4: *Robinia Pseudo-Acacia*.— Tangential longitudinal section of stratified cambium of highly specialized type of dicotyledon, showing parallel horizontal series of fusiform initials. × 110.

Fig. 5: *Heritiera littoralis*.— Tangential longitudinal section of immature phloem, showing stratified elements. × 110.

Fig. 6: *Diospyros virginiana*.— Tangential longitudinal section of stratified cambium. Fusiform initial (*A*) has divided radio-longitudinally. × 110.

Fig. 7: *Same*.— Portion of cambium more highly magnified. Cells *A* and *B* have formed radio-longitudinal, anticlinal partitions. Cell *C* is dividing periclinally. The kinoplasmasomes are approaching the ends of the cell. × 148.

Fig. 8: *Liquidambar Styraciflua*.— Tangential longitudinal section of non-stratified cambium, showing oblique anticlinal division of fusiform initial. × 450.

Fig. 9: *Same*.— Oblique anticlinal division. × 450.

Plate 3

Fig. 7: *Thuja occidentalis*.— Portion of fusiform initial, showing transitional stage in the breaking up of a single, large, elongated vacuole. In the upper portion of the figure, the vacuome is passing into the vermiculate or "myelin" form. Compare with Fig. 14. × 780.

Fig. 8: *Same*.— Portion of fusiform initial, showing the protoplasm invading the vacuole. Protoplasm in more or less discontinuous phase. × 780.

Fig. 9: *Same*.— Portion of fusiform initial, showing transitional stage in the breaking up of a single, large, elongated vacuole. Protoplasm continuous, vacuolar material more or less discontinuous. × 575.

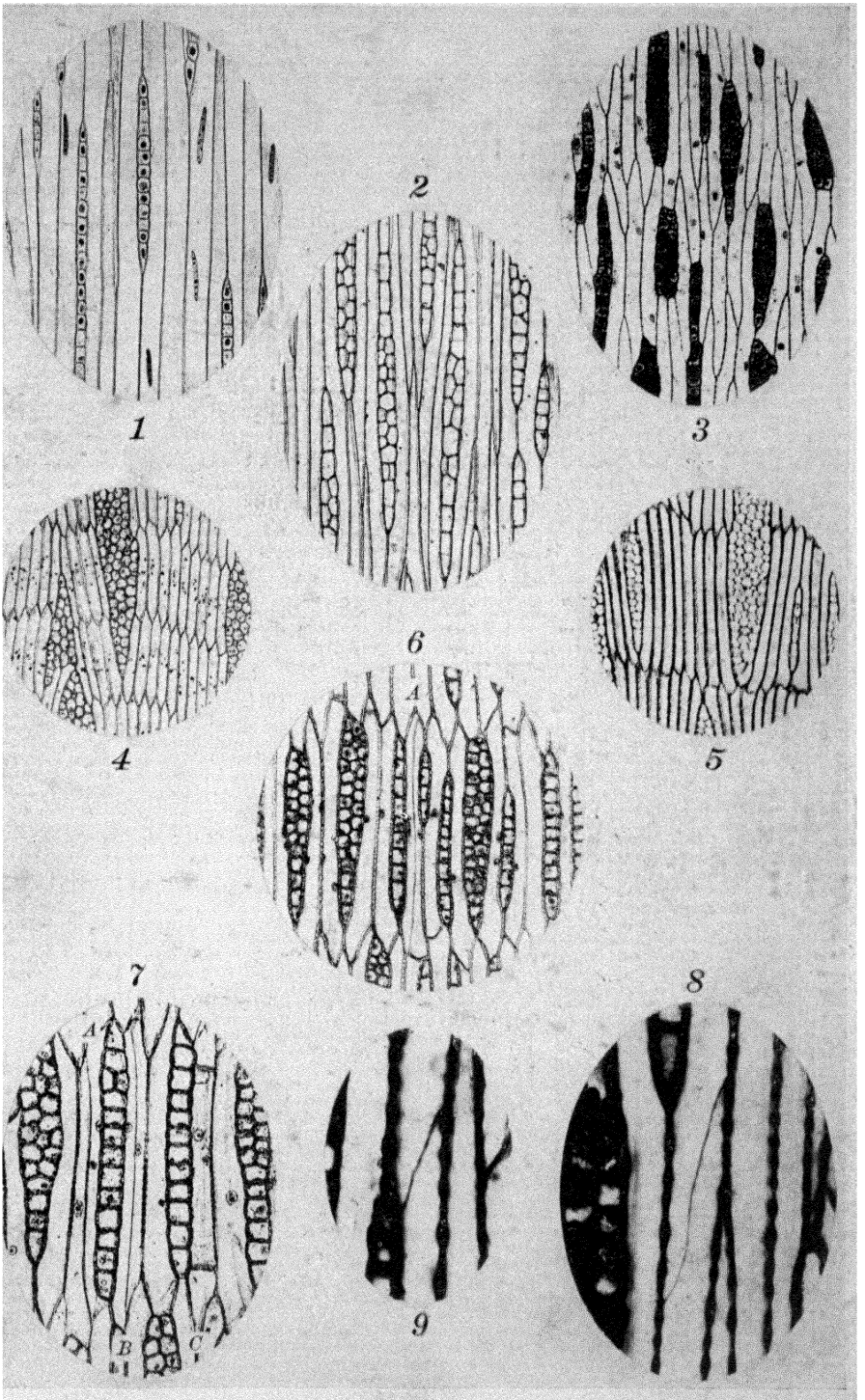
Fig. 10: *Fraxinus americana*.— Tangential longitudinal section of cambium, showing fusiform initials which are as highly vacuolated as are plant hairs. × 300.

Fig. 11: *Same*.— Portion of fusiform initial, more highly magnified, showing large continuous vacuole, nucleus, nucleolus, and strands of protoplasm. × 780.

Fig. 12: *Thuja occidentalis*.— Three adjacent cells of the phloem parenchyma, showing three forms of vacuome. × 575.

Fig. 13: *Same*.— Tangential longitudinal section of cambium, showing portions of two adjacent fusiform initials. Continuous elongated vacuole at the left; transition towards vermiculate or "myelin" form of vacuome at the right. × 575.

Fig. 14: *Same*.— Cell of phloem parenchyma, showing typical vermiculate or "myelin" form of vacuome. × 780.



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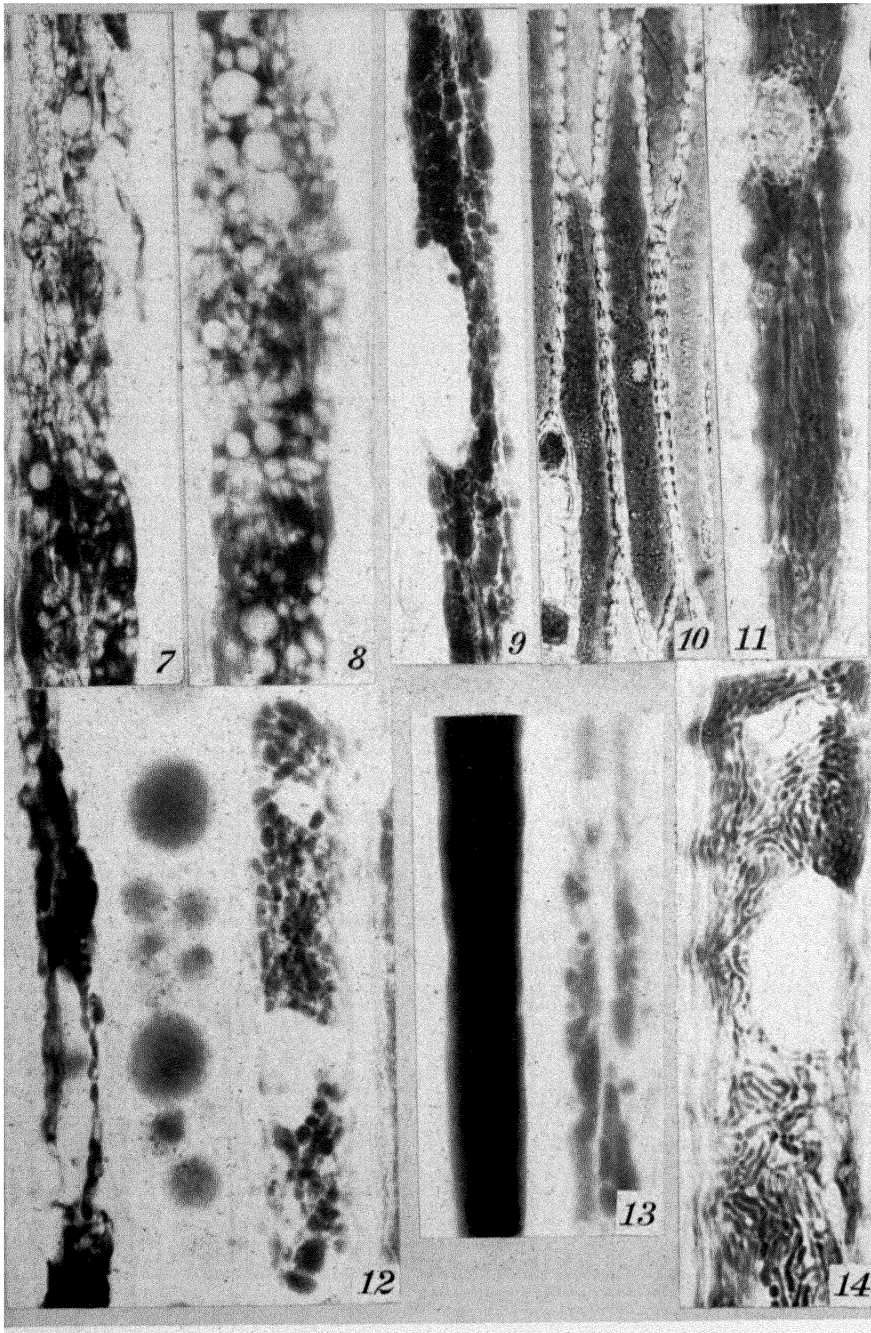

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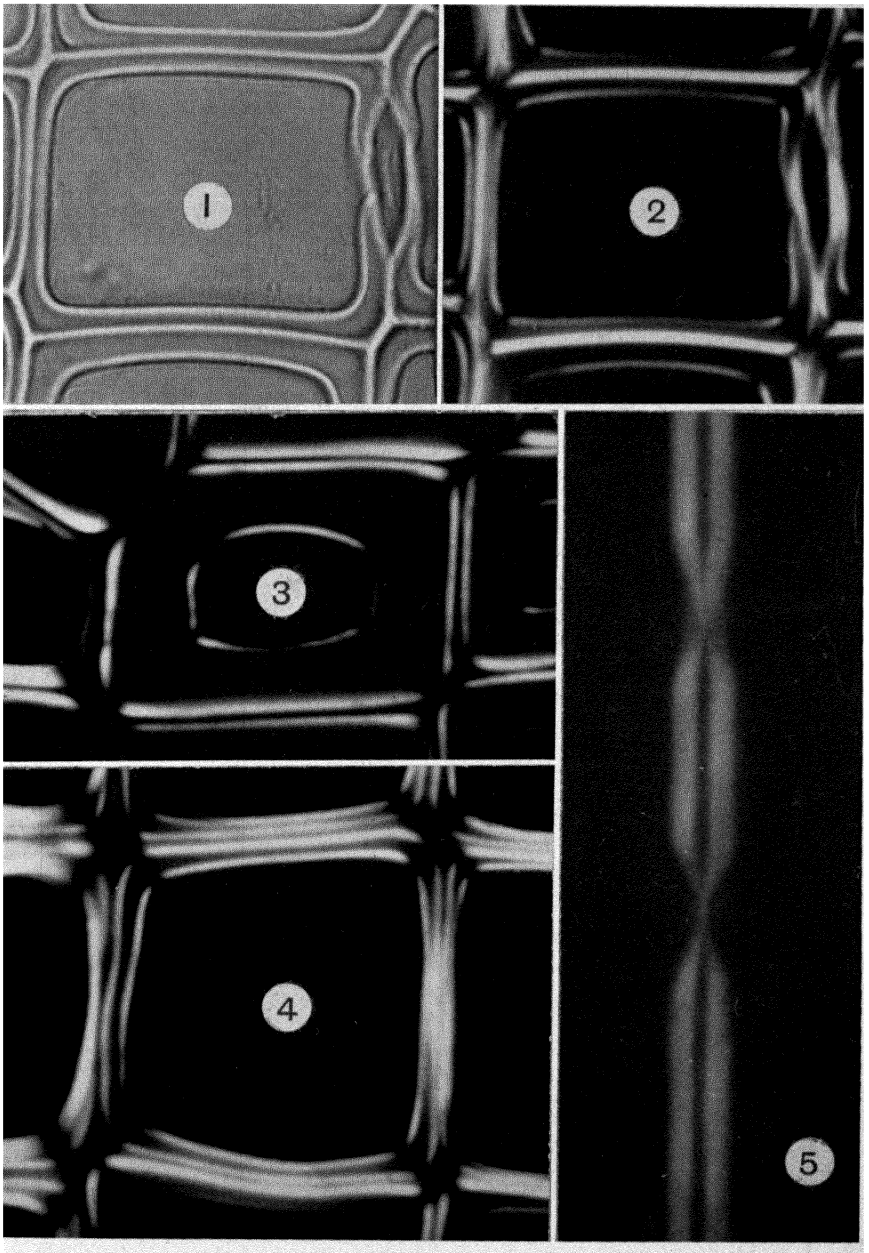


— Plate 4 —

Plate 4

Fig. 1: *Sequoia sempervirens.*— Transverse section of the xylem, unstained and photographed in white light. The inner and outer layers of the secondary wall are more refractive than the wide central layer. The outer layers of adjacent secondary walls blend with the intensely lignified cambial walls and intercellular substance, forming an apparently homogeneous middle lamella which actually consists of five layers. Section of bordered pit at the right. × 1400.

Fig. 2: *Same.*— Photographed in polarized light between crossed nicols. In a section of this thickness, 15 microns, the isotropic intercellular substance and feebly anisotropic cambial walls are completely fogged or obscured by the brilliant outer layers of the secondary wall. (Compare Fig. 3 for section 5 microns in thickness.) × 1400.

Fig. 3: *Trochodendron aralioides.*— Transverse section of the latewood, showing one entire tracheid and parts of seven adjoining ones. The thick secondary walls are composed of three layers, a narrow, brilliant, outer layer, a brilliant, narrow, inner layer, and a wide intervening dark layer. The brilliant outer layers of adjoining secondary walls are separated by a narrow dark layer, *i.e.* the so-called middle lamella which actually consists of two feebly anisotropic cambial walls and a truly isotropic layer of intercellular material. × 1460.

Fig. 4: *Same.*— Transverse section of the earlywood, showing one entire tracheid and parts of seven others. Details of structure as in Fig. 3 except for the difference in thickness of the central layer of the secondary wall. × 1460.

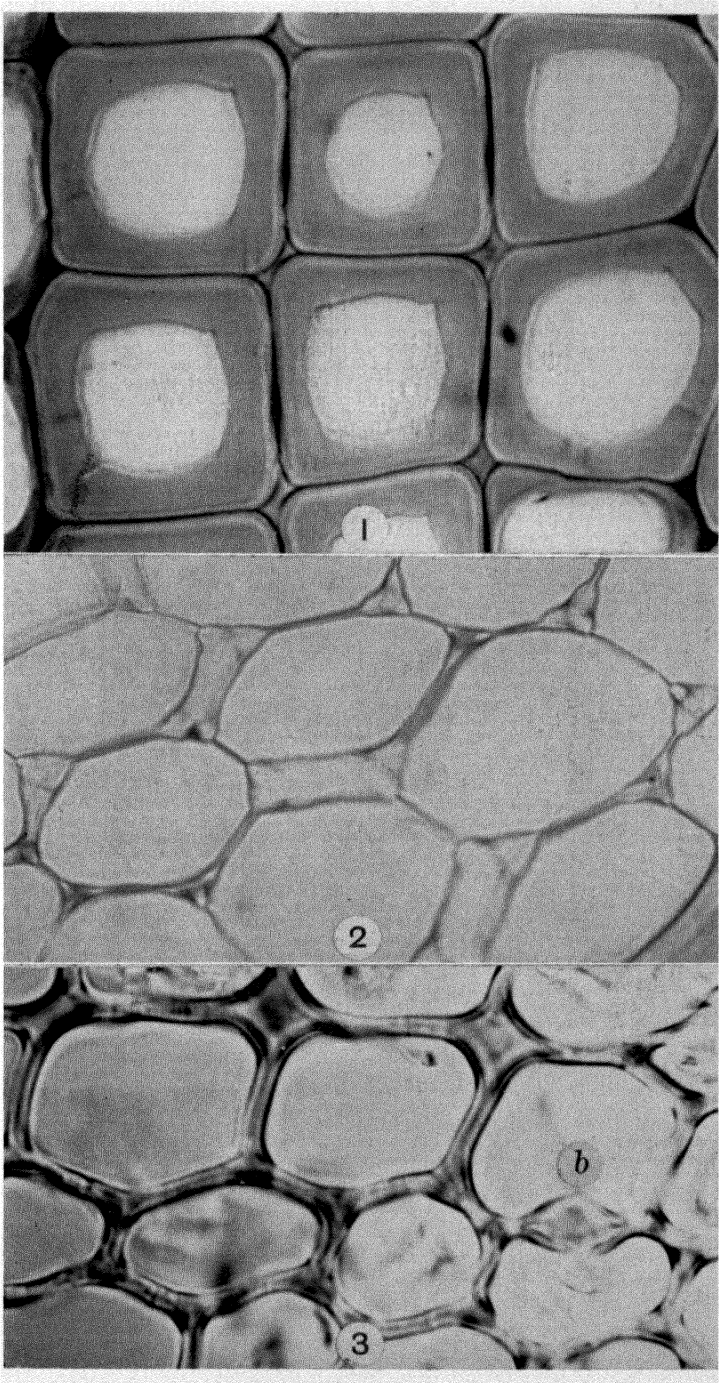
Fig. 5: *Pinus Strobus.*— Tangential section of the cambium, showing anisotropic walls of two adjacent fusiform initials and the isotropic intercellular material. The thinner areas of the walls are the so-called primary pit fields. × 1880.

Plate 5

Fig. 1: *Trochodendron aralioides.*— Transverse section of the xylem after repeated extractions with solvents of polyuronides and after staining with ruthenium red. The cambial walls are deeply stained, whereas the 3-layered secondary walls are not. × 1000.

Fig. 2: *Taxodium distichum.*— Transverse section of the xylem after five successive treatments with chlorine water and 10% ammonium hydroxide and extraction with Schweizer's reagent. The intercellular substance and the secondary walls have dissolved leaving the cambial walls only. × 875.

Fig. 3: *Pinus radiata.*— Transverse section of the xylem after standard treatment with 72% sulphuric acid, showing five-layered "middle lamella" residue. The five-layered character of the residue may be accurately determined by the structure of the bordered pit at *b*. × 945.



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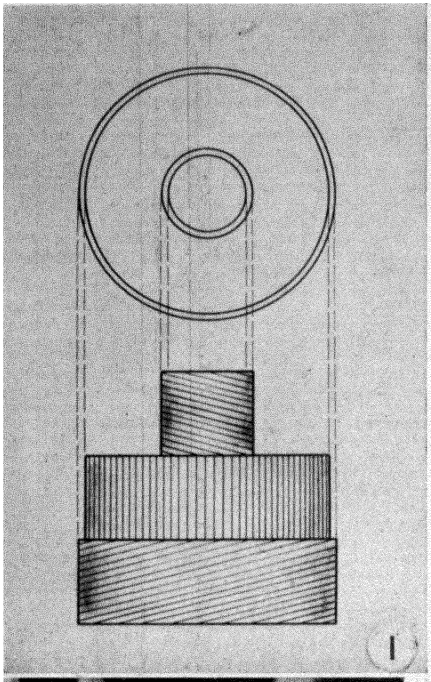
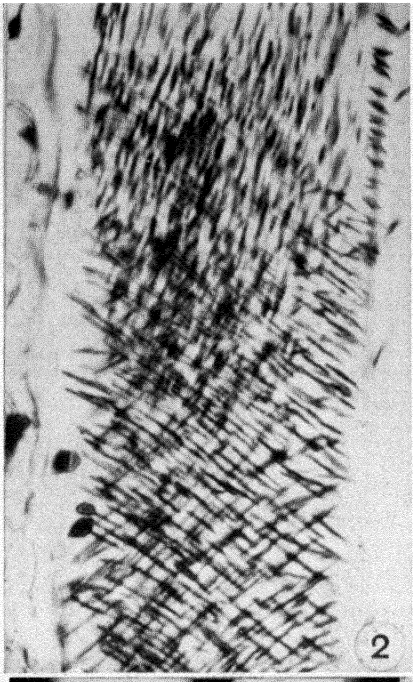
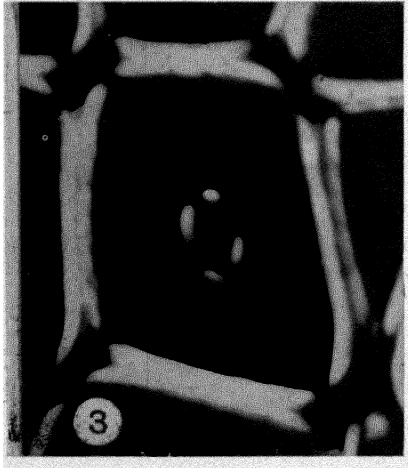






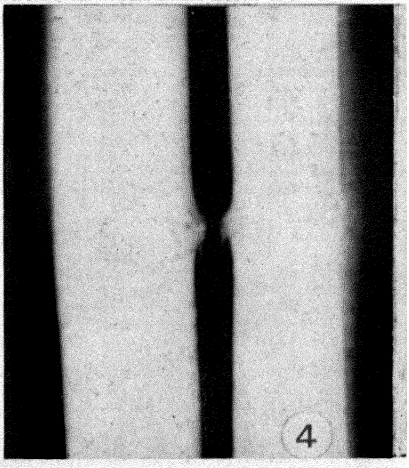
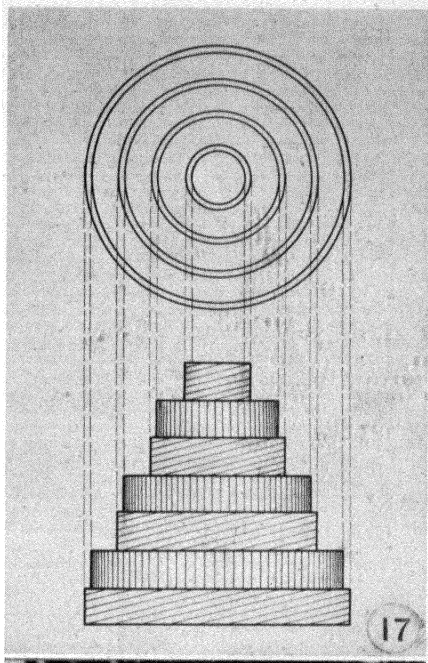


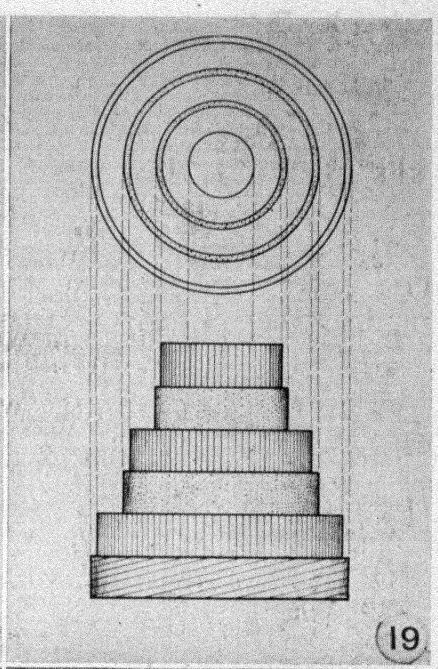
FIG. 1: Diagram illustrating changes in the orientation of cellulose in a 3-layered secondary wall.

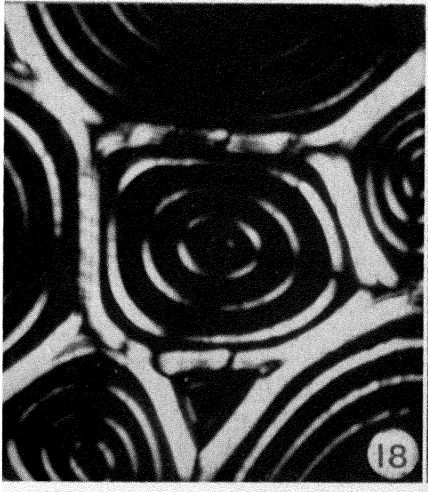
FIG. 2: Longitudinal section through parts of the secondary walls of two adjacent cells, showing three orientations of visible crystals of iodine in the micro-capillaries. × 900.

FIG. 3: Transverse section of 3-layered secondary walls, photographed in polarized light between crossed nicols. × 1750.

FIG. 4: Longitudinal section of such walls in polarized light. × 1750.







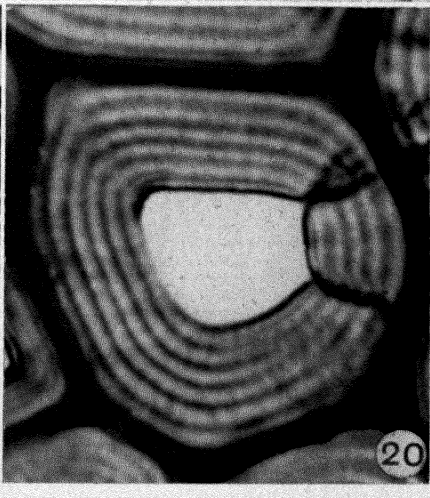


FIG. 17: Diagram illustrating changes in the orientation of cellulose in a many-layered secondary wall.

FIG. 18: Transverse section of such walls, photographed in polarized light between crossed nicols. × 1150.

FIG. 19: Diagram illustrating a many-layered secondary wall. The stippled layers are non-cellulosic and truly isotropic.

FIG. 20: Transverse section of such walls, differentially stained and photographed in ordinary light. × 1540.

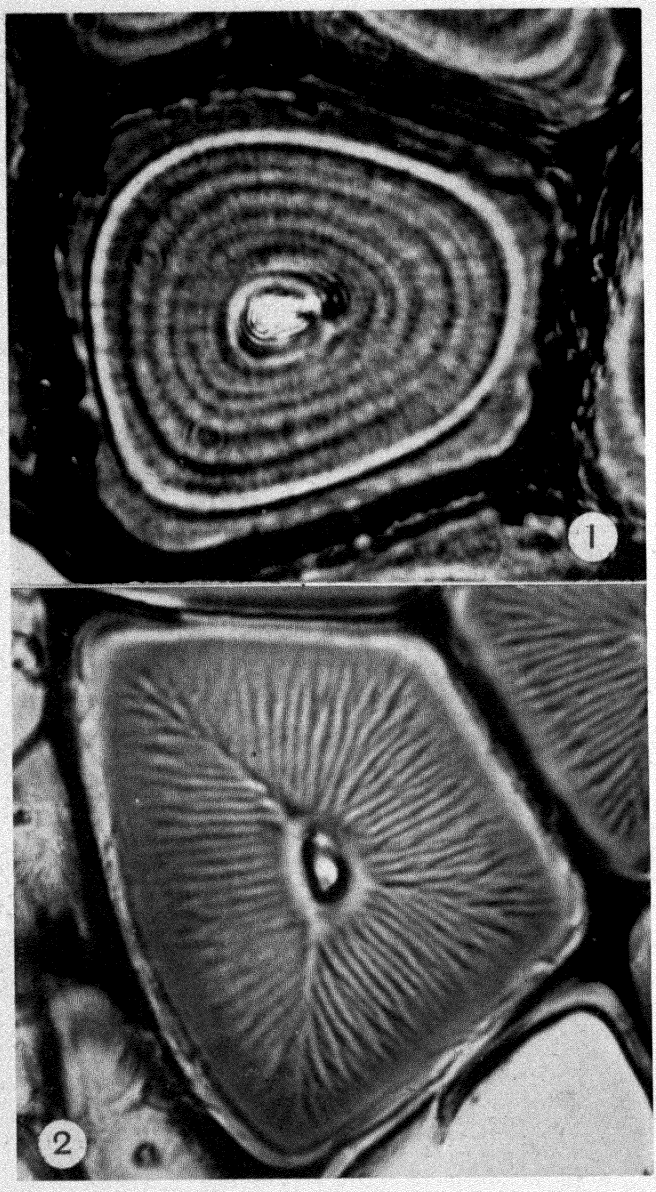


FIG. 1: *Tetramerista glabra.*— Transverse section of an unswollen fiber-tracheid. The broad, central layer of the secondary wall has a coarsely radio-concentric pattern which is complicated by zones of varying intensities of lignification. × 2000.

FIG. 2: *Siparuna bifida.*— Transverse section of a fiber-tracheid. The broad, unswollen central layer of the secondary wall is radially striated. × 2000.

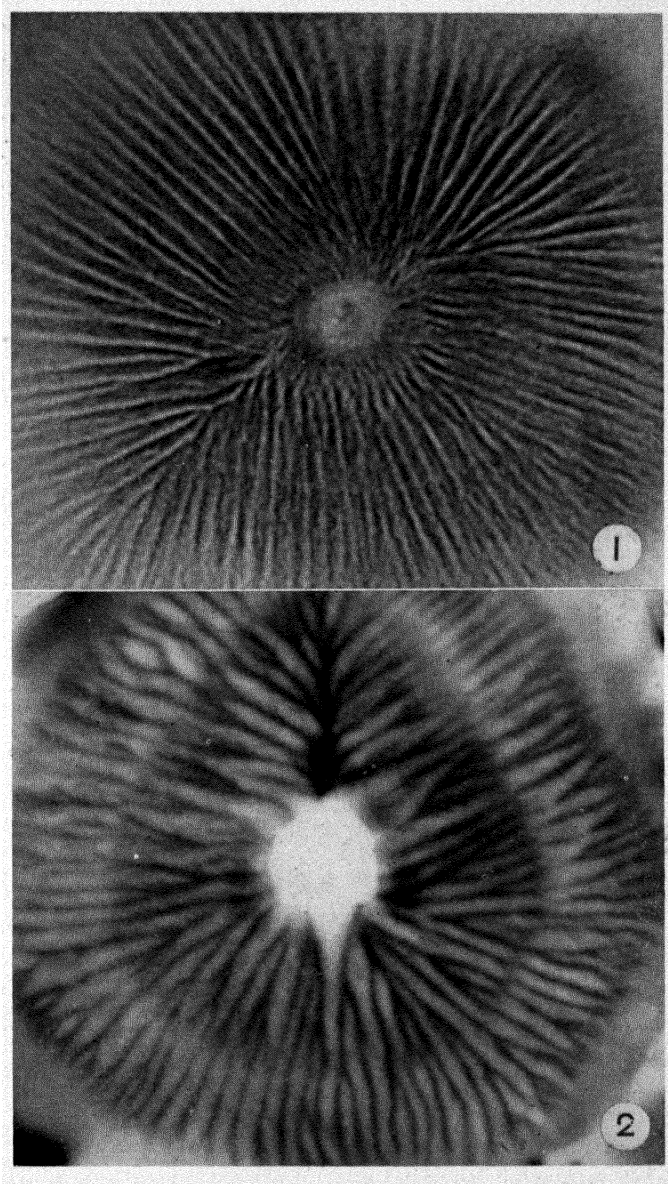


FIG. 1: *Siparuna bifida.*— Transverse section of a delignified fiber-tracheid, after treatment with diluted Schweizer's reagent and staining with congo red. The denser, darker radii of the purified cellulose correspond to the lighter radii of FIG. 2, PLATE 8. Owing to swelling the total enlargement of the structural pattern is approximately × 3600.

FIG. 2: *Same.*— Transverse section of a fiber-tracheid after standard treatment with 72% sulphuric acid, showing radially striated lignin residue of the central layer of the secondary wall. The darker, denser radii of lignin correspond to the darker radii of FIG. 2, PLATE 8. Total enlargement of structural pattern is approximately × 3000.

Plate 10

Fig. 1: Expanded transverse section of the concentrically lamellated secondary wall of a cotton hair. The swollen microfibrils of cellulose (dark) and the microcapillaries (light) are seen in cross section. In the denser lamellae, the microfibrils are more closely aggregated and more extensively coalesced than in the lighter, more porous lamellae. Owing to swelling the microfibrils and lamellae have a total enlargement of approximately × 7500.

Fig. 2: Coherent lignin residue from a structurally similar, concentrically lamellated secondary wall of a coniferous tracheid. The microfibrils of lignin are dark whereas the porosities left by the removal of the cellulose are light. Figs. 1 and 2 are obviously positive and negative images of the same structural pattern and the lignin residue may be interpolated within the porosities of the cellulosic matrix and vice versa. Total magnification is approximately × 9000.

Fig. 3: Expanded transverse section of a secondary wall having a complex radio-ramifying structural pattern. Total magnification approximately × 5850.

Fig. 4: Tangential longitudinal section of the unswollen secondary wall illustrated in Fig. 2, Plate 8, showing slightly helically oriented striae. × 1900.

Fig. 5: Transverse section of swollen secondary wall, showing abrupt transition between radial and concentric aggregations of microfibrils. Total magnification × 9000.

Plate 11

Fig. 8: *Brackenridgea Hookeri.*— Longitudinal section of the xylem, photographed in polarized light between crossed nicols, showing enzymatically produced cavities. × 990.

Fig. 9: *Laurelia aromatica.*— Longitudinal section of the xylem, showing cavities in the secondary wall. × 990.

Figs. 10 and 11: *Brackenridgea Hookeri.*— Longitudinal section of the xylem, showing cavities in the secondary wall. × 990.

Fig. 12: *Pinus rigida* var. *serotina.*— Longitudinal section of the xylem, showing helical orientation of enzymatically produced cavities in the secondary wall. × 300.

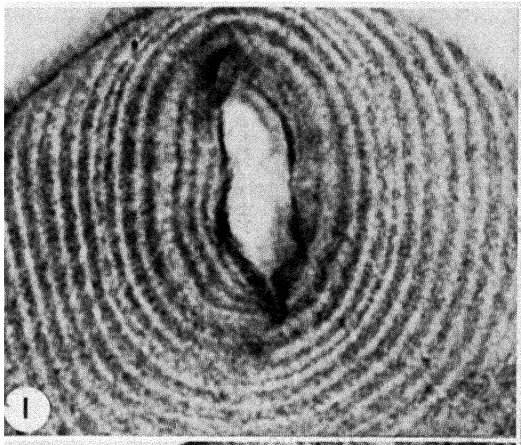
Fig. 13: *Osteophloeum platyspermum.*— Longitudinal section of the xylem, showing helical orientation of cavities in the secondary wall. × 990.

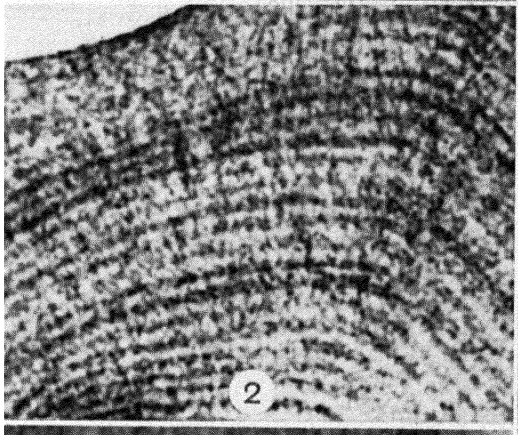
Plate 12

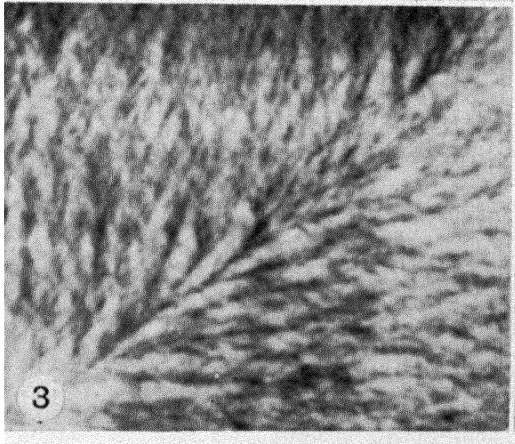
Fig. 1: Longitudinal section of a secondary wall, showing orientation of planes of mechanical cleavage. × 700.

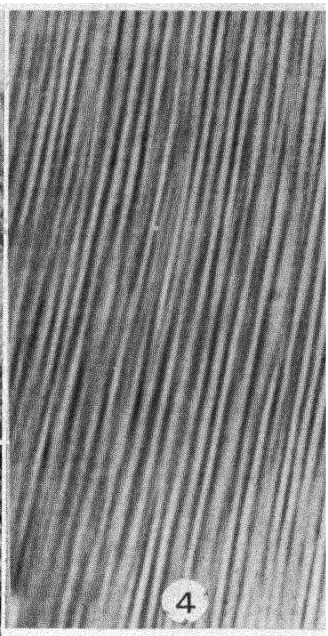
Fig. 2: Isolated, delignified, libriform fiber swollen in diluted Schweizer's reagent, showing beadlike swelling of the central layer of the secondary wall. The outer layer of the secondary wall is resolved into a series of constricting rings and helical bands. × 650.

Fig. 3: Swollen transverse section of the many-layered secondary wall illustrated in Fig. 18, Plate 7. The tenuous layers with helical orientation of low pitch have been ruptured by the lateral expansion of the broad intervening layers. × 1300.









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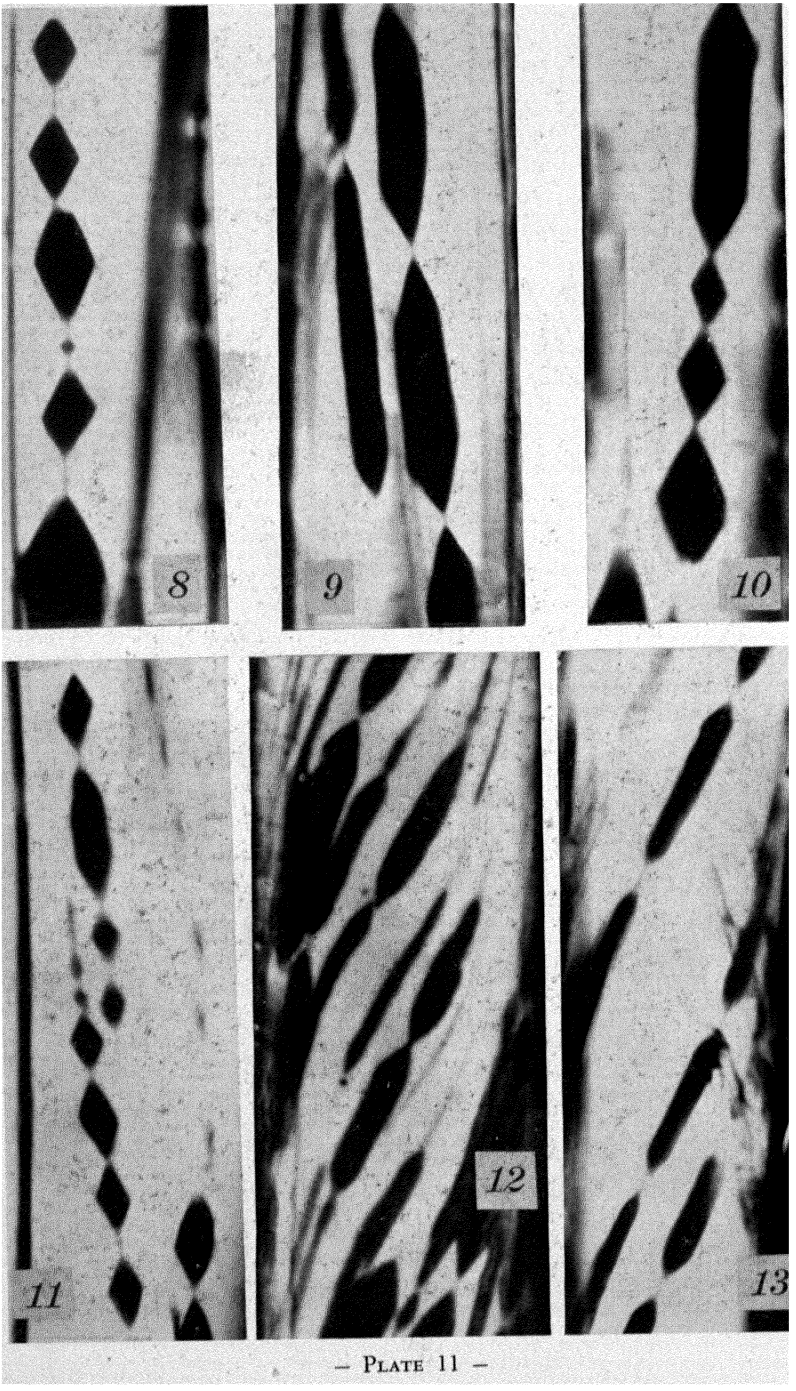

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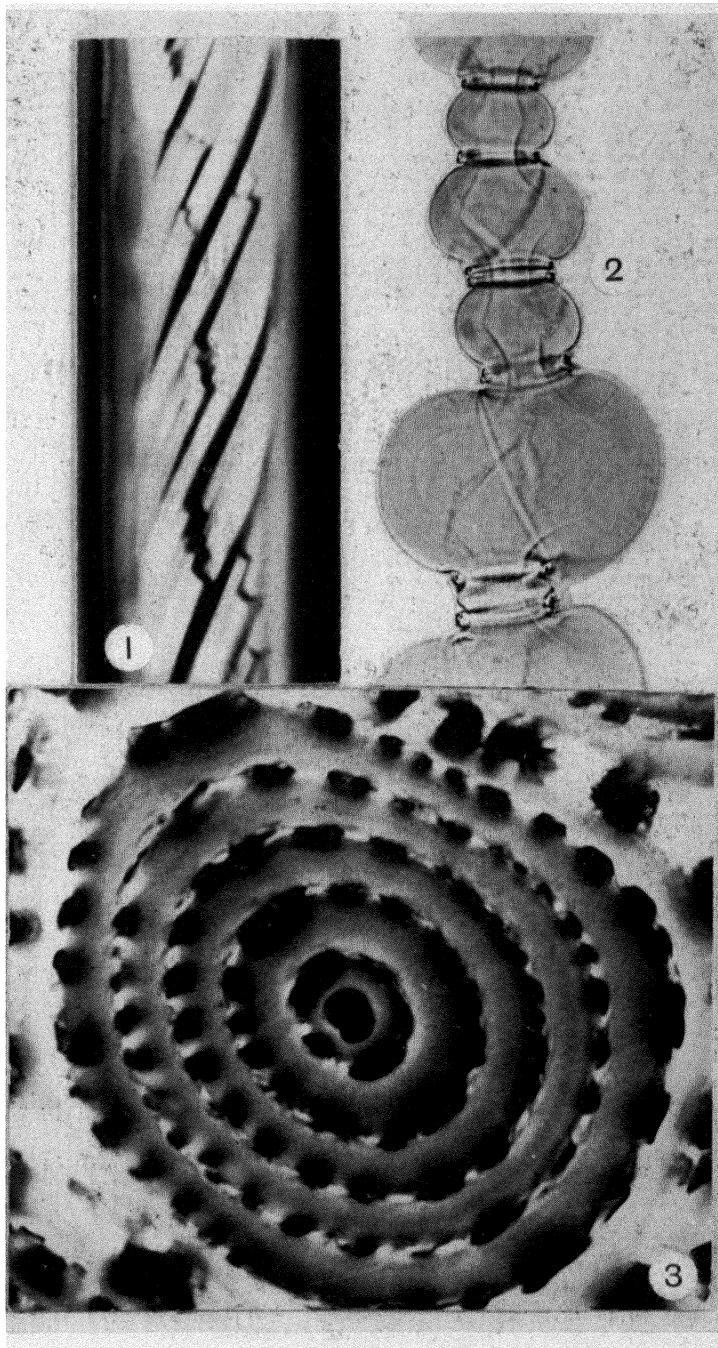

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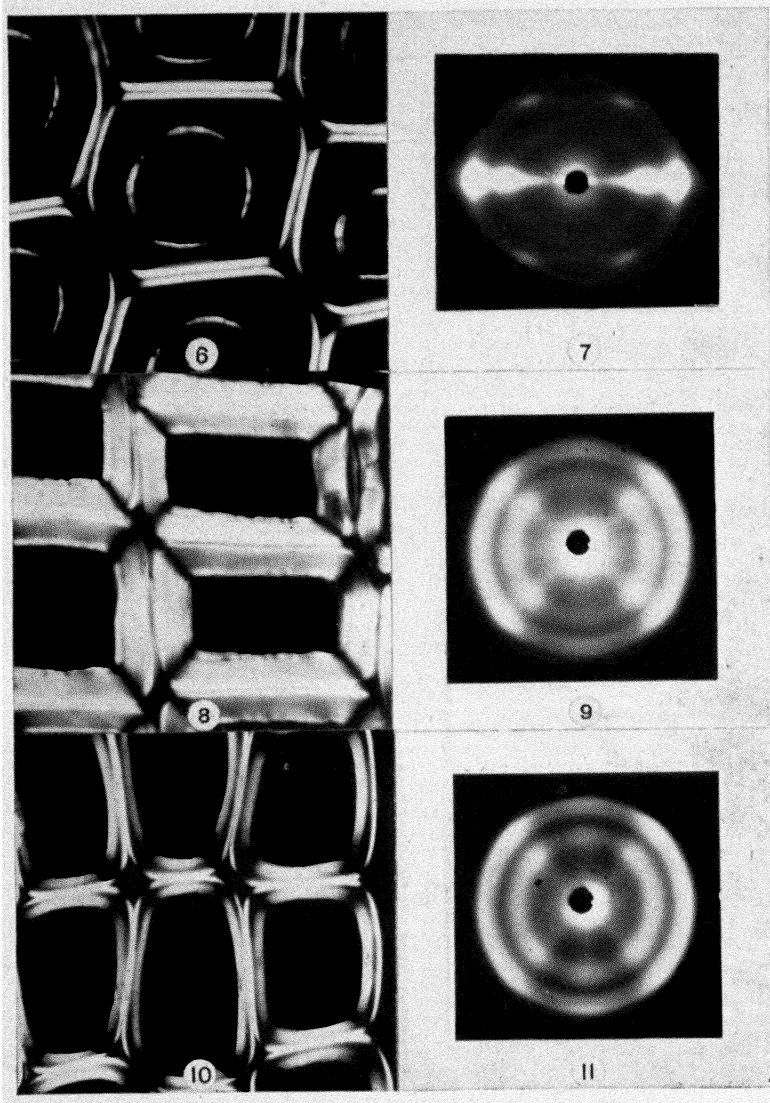


Transverse sections of wood, photographed in polarized light between crossed nicols, and corresponding X-ray diffraction patterns of delignified wood with beam oriented parallel to the long axis of the wood rays.

FIG. 6: *Pinus longifolia,* late wood. × 690.
FIG. 7: *Same,* X-ray diffraction pattern.
FIG. 8: *Sequoia sempervirens,* late wood. × 1030.
FIG. 9: *Same,* X-ray diffraction pattern.
FIG. 10: *Taxodium distichum* "Rotholz." × 690.
FIG. 11: *Same,* X-ray diffraction pattern.

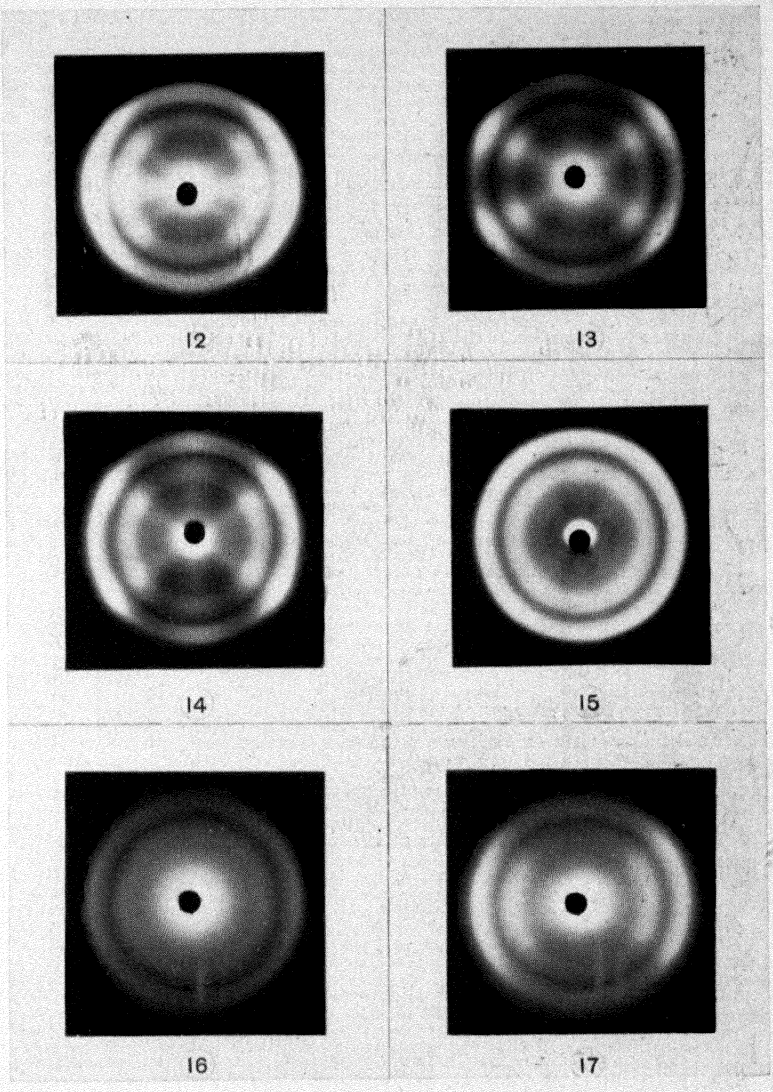


FIG. 12: *Sequoia sempervirens.*— Late wood, X-ray beam perpendicular to the long axis of both the tracheids and the wood rays, *viz.*, tangentially oriented.

FIG. 13: *Same.*— X-ray beam perpendicular to the long axis of the tracheids and at 45° to the long axis of the wood rays, *viz.*, diagonally oriented.

FIG. 14: *Picea rubra.*— "Compression wood," X-ray beam perpendicular to the long axis of the tracheids and parallel to the long axis of the wood rays, *viz.*, radially oriented.

FIG. 15: *Pinus ponderosa.*— "Compression wood," X-ray beam radially oriented.

FIG. 16: *Trochodendron aralioides.*— Early wood, X-ray beam radially oriented.

FIG. 17: *Sequoia sempervirens.*— Early wood, X-ray beam radially oriented.

Plate 15

Fig. 1: *Degeneria vitiensis*.– Stamen re-expanded and cleared in dilute sodium hydroxide, showing sporangia and median and lateral veins. × 23.

Fig. 2: *Same*.– Transverse section of stamen showing four deeply embedded sporangia and four short arcs of endothecium. × 100.

Fig. 3: *Same*.– Transverse section of young conduplicate carpel. × 27.

Fig. 4: *Same*.– Transverse section of carpel at anthesis, showing the development of epidermal papillae on the flaring ventral surfaces. × 27.

Plate 16

Carpels cleared in dilute NaOH and photographed unstained in 95% alcohol.

Fig. 1: *Drimys piperita*.– Two attached carpels, showing stipe and venation of conduplicate lamina. × 20.

Fig. 2: *Drimys macrantha*.– Detached sterile carpel, showing at right pollen attached to stigmatic surface. × 24.

Fig. 3: *Drimys insipida*.– Detached young fruit, showing at left outwardly projecting branches of lateral vein. × 17.

Plate 17

Carpels cleared in dilute NaOH and mounted unstained in diaphane.

Fig. 4: *Pseudowintera axillaris* var. *colorata*.– Two attached carpels, showing lateral view of vascularization. × 24.

Fig. 5: *Pseudowintera axillaris* var. *typica*.– Two attached carpels, showing lateral view of vascularization. × 20.

Fig. 6: *Drimys confertifolia*.– Two attached carpels, showing vascularization and apex of torus. × 20.

Plate 18

Sections of partly re-expanded carpels, stained in Haidenhain's haematoxylin and safranin.

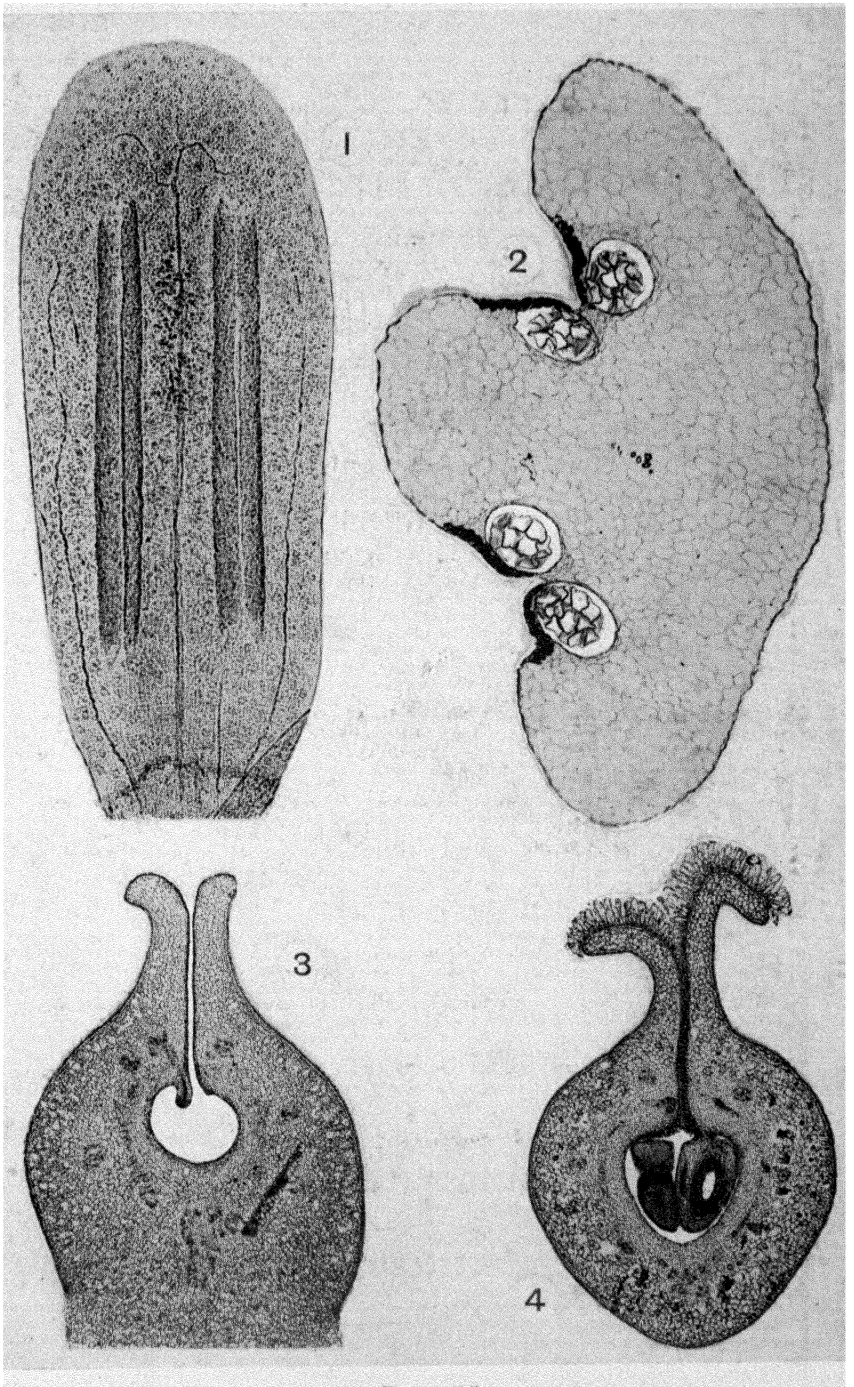
Fig. 12: *Drimys piperita*.– Transverse section of conduplicate carpel, showing stigmatic surfaces and ovules. × 50.

Fig. 13: *Drimys granadensis* var. *mexicana*.– Transverse section of conduplicate, open, upper part of carpel, showing stigmatic surfaces and attachment of ovules. × 50.

Fig. 14: *Same*.– Lower sealed part of carpel, showing internal vestige of cleft and internal papillae. × 50.

Fig. 15: *Bubbia Clemensiae*.– Longitudinal section, showing cleft-like opening, downwardly projecting stigmatic ridges, and ovules. × 24.

Fig. 16: *Zygogynum spathulatum*.– Transverse section of gynaecium, showing parts of three concrescent carpels. × 40.



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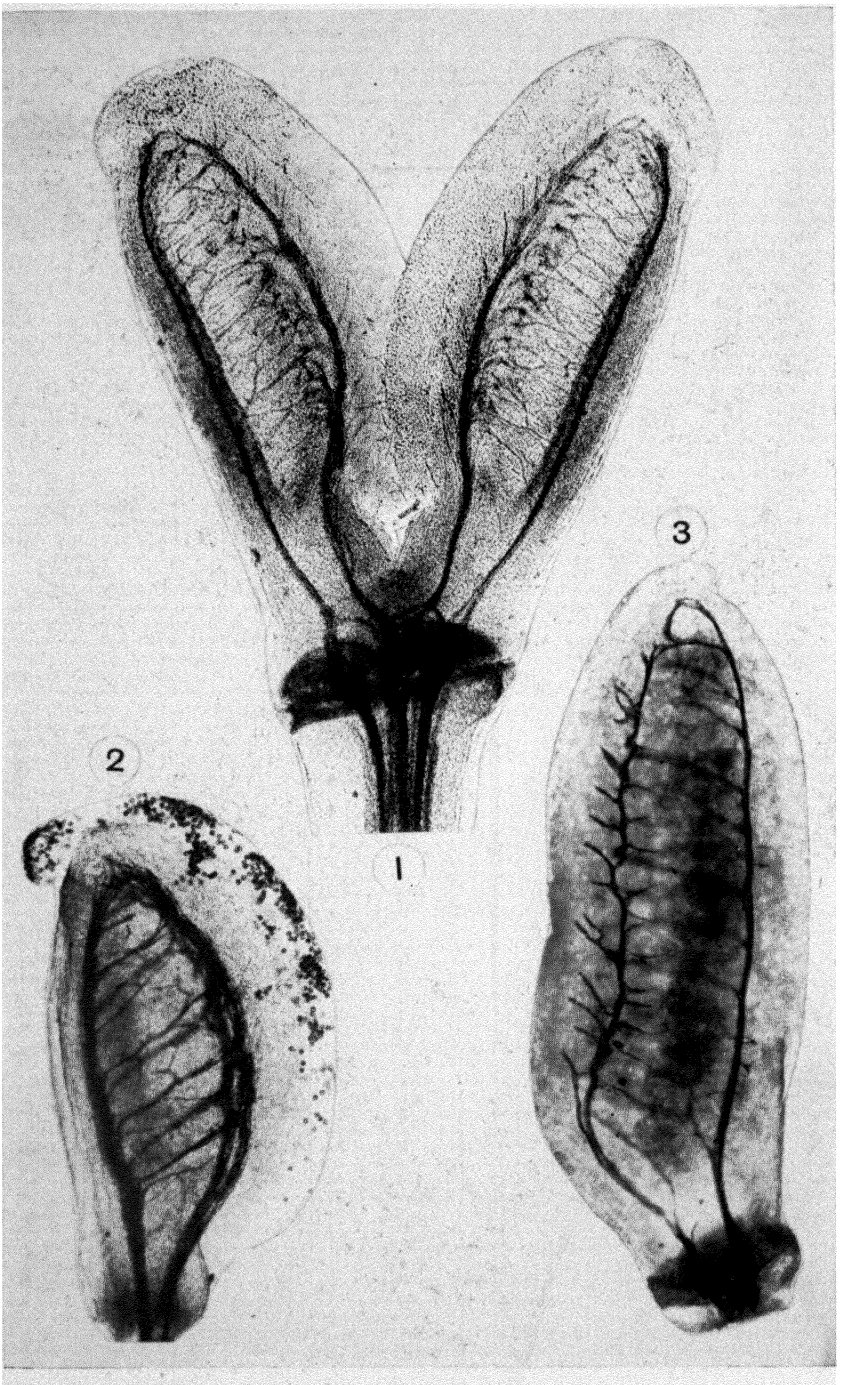

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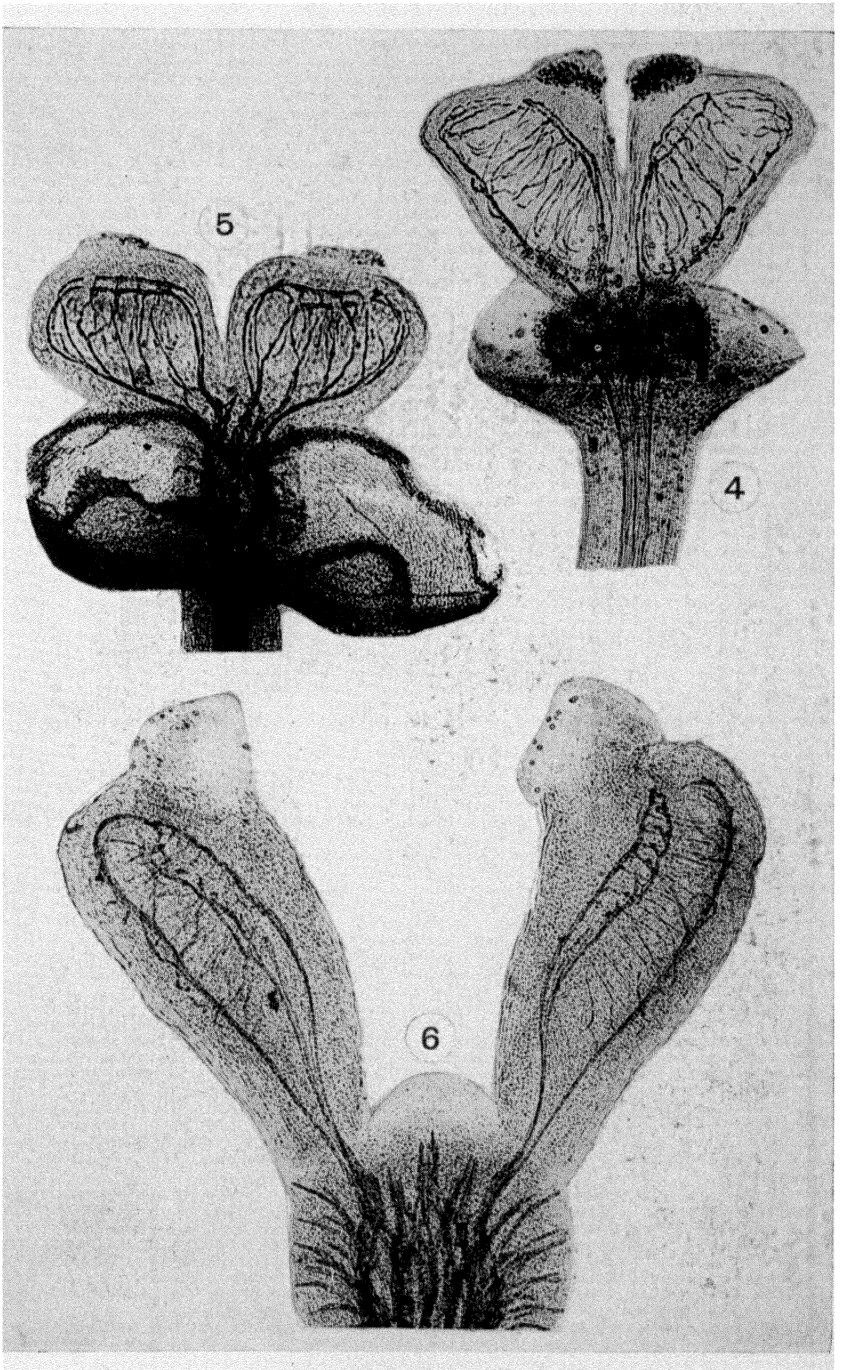

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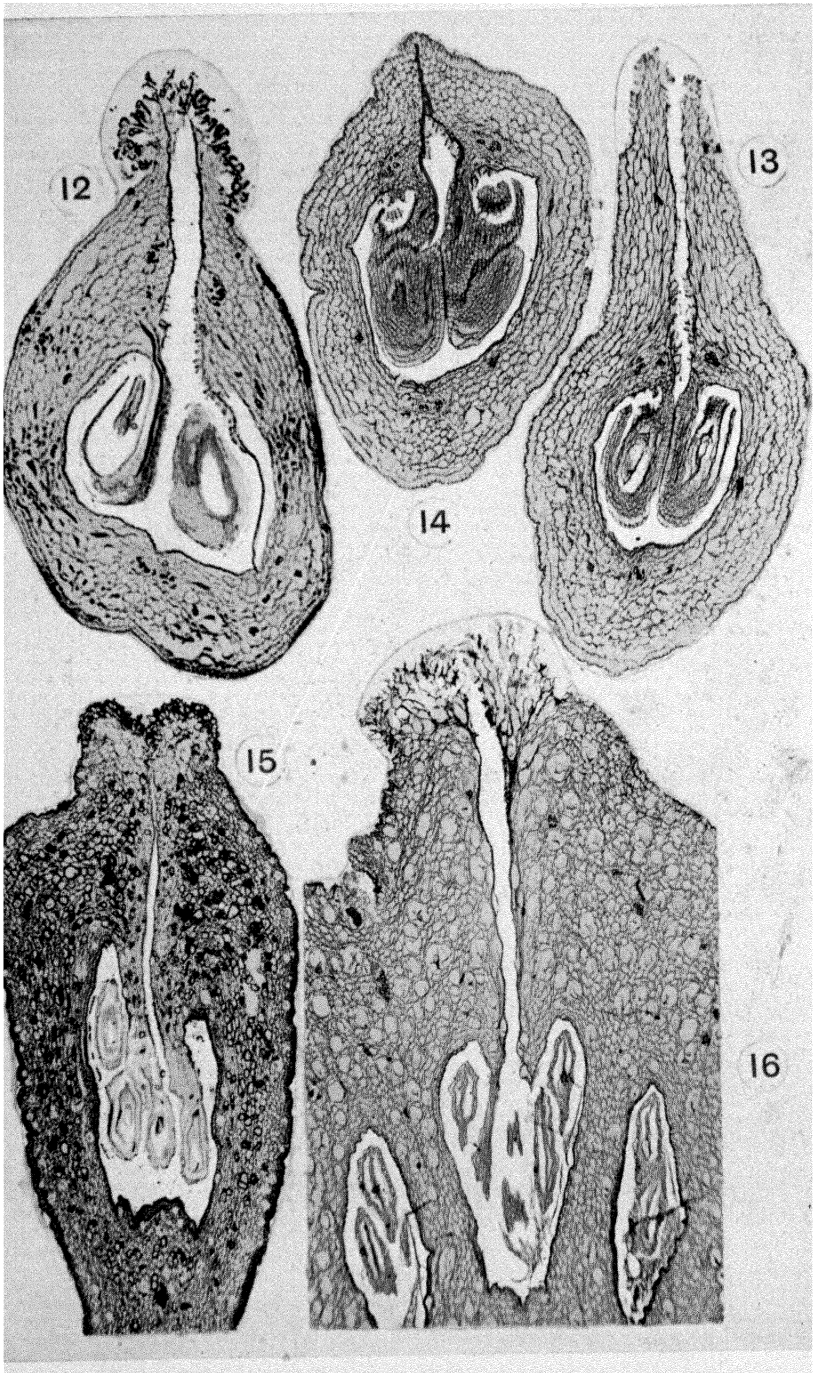


— Plate 18 —

PLATE 20

FIG. 1: *Cecropia angulata.*— Cross section of upper portion of internode, showing "prostoma." × 8.

FIG. 2: *C. sciadophylla* var. *decurrens.*— Cross section of internode, showing "prostoma." × 7.

FIG. 3: *C. angulata.*— Cross section of lower portion of internode, showing formation of secondary wood in "prostoma." × 8.

FIG. 4: *C. angulata.*— Cross section of juvenile plant, showing occlusion of entrance aperture by callus or "stomatome." × 9.

FIG. 5: *C. sciadophylla* var. *decurrens.*— Cross section of nodal diaphragm, showing five layers of three distinct types of tissues. × 15.

FIG. 6: *C. angulata.*— Portion of trichilium, showing food bodies and enveloping mat of hairs. × 34.

FIG. 7: *C. angulata.*— Cross section of nodal diaphragm, showing layers of thin-walled parenchyma. × 9.

FIG. 8: *C. angulata.*— Cross section of nodal diaphragm, showing early stage in construction of a pitlike excavation. × 15.

FIG. 9: *C. angulata.*— Cross section of nodal diaphragm; soft external layers of medullary tissue removed on both sides, and circular perforation cut through horny layer on under side. × 12.

PLATE 21

FIG. 15: *Cedrus Deodara.*— Radial longitudinal section of stem-wood, stained to reveal the primary pit areas, so-called bars of SANIO, pit membranes, and tori. The outlines of the bordered pits coincide with those of the pit membranes. × 300.

FIG. 16: *Keteleeria Davidiana.*— Radial longitudinal section of stem-wood, showing outlines of clustered bordered pits in widely separated primary pit areas. × 300.

FIG. 17: *C. Deodara.*— Radial longitudinal section of the stem-wood, showing uniform thickening of middle lamella between widely spaced primary pit areas. × 300.

FIG. 18: *K. Davidiana.*— Portion of FIG. 16, more highly magnified, showing primary pit area, so-called bars of SANIO, outlines of bordered pits, pit membranes, and tori. × 1480.

FIG. 19: *C. Deodara.*— Portion of FIG. 15, more highly magnified, showing primary pit area, so-called bars of SANIO, pit membrane, and torus. × 1480.

FIGS. 20-24: *K. Davidiana.*— Portions of radial facets of tracheids, stained to reveal the primary pit areas, so-called bars of SANIO, pit membranes, and tori. The outlines of the superimposed bordered pits coincide with those of the pit membranes. × 300.

PLATE 22

FIG. 25: *Cedrus Libani.*— Radial longitudinal section of root-wood, showing triseriate alternating arrangement of pits and entire absence of so-called rims or bars of SANIO. × 360.

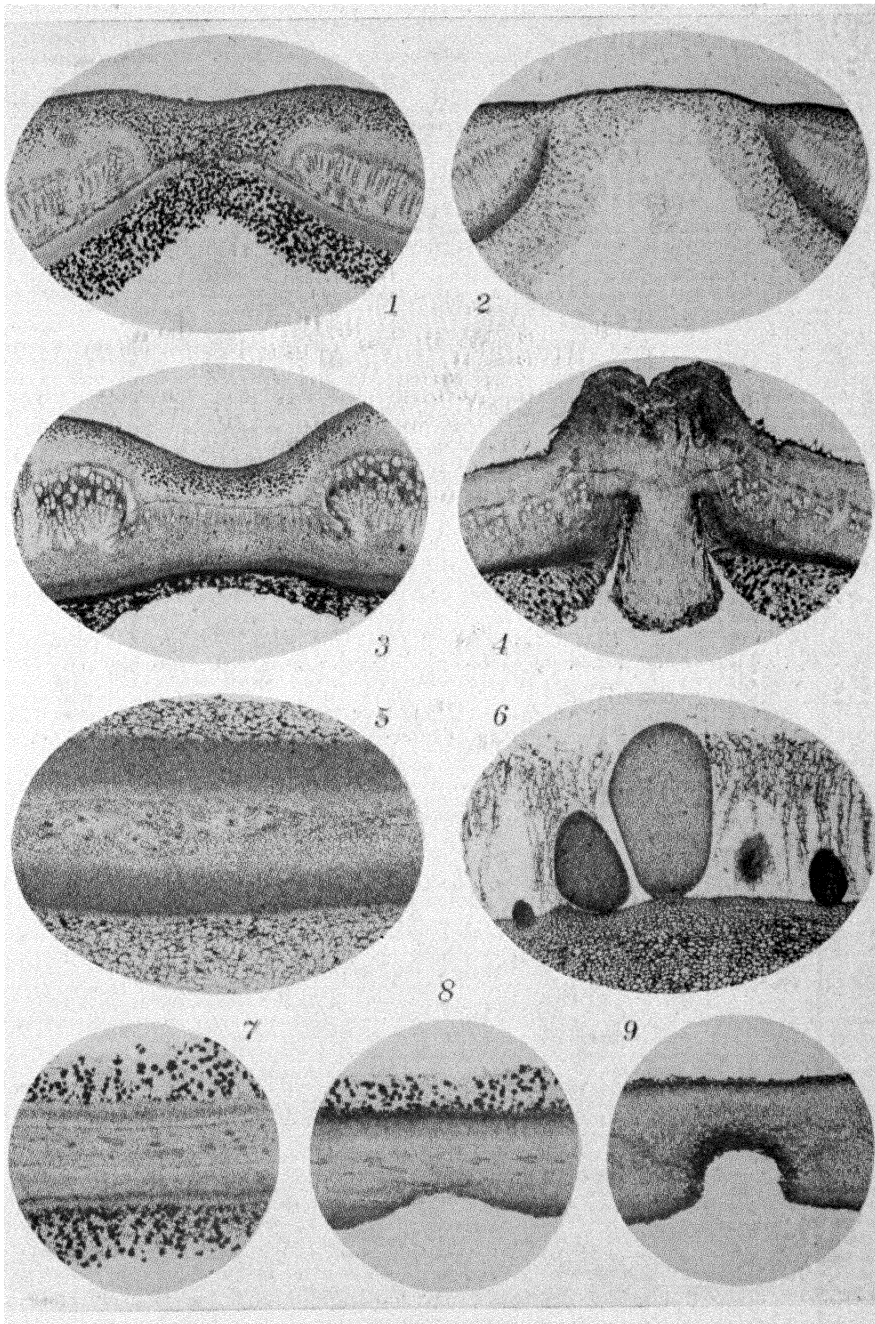
FIG. 26: *C. Libani.*— Radial longitudinal section of root-wood, showing biseriate alternating arrangement of pits. × 360.

FIG. 27: *Cedrus Deodara.*— Radial longitudinal section of stem-wood, showing compressed uniseriate pitting. × 330.

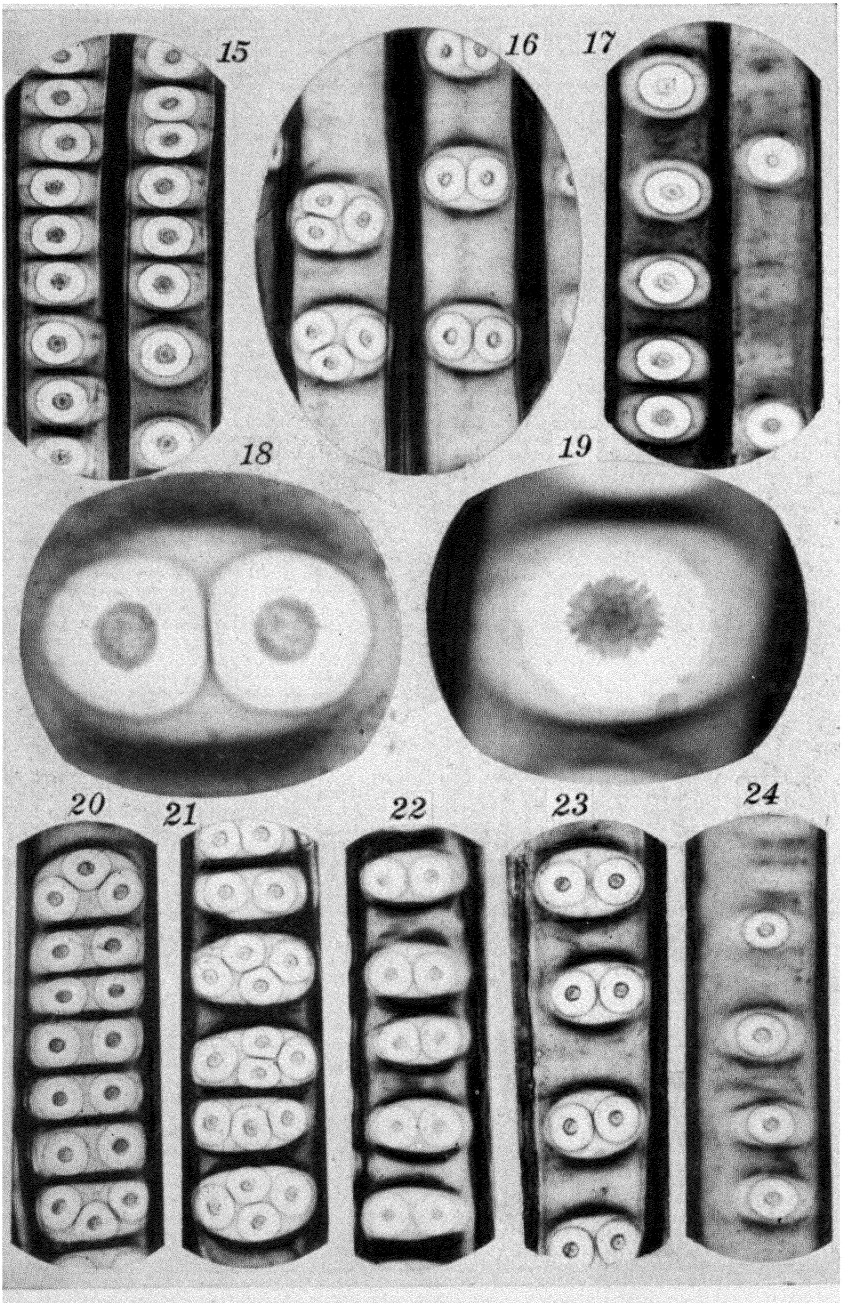
FIG. 28: *C. Libani.*— Radial longitudinal section of root-wood showing widely separated primary pit areas and so-called rims or bars of SANIO. × 360.

FIG. 29: *C. Libani.*— Radial longitudinal section of root-wood, showing clustered, compressed pits in large primary pit areas. × 360.

FIG. 30: *C. Deodara.*— Radial longitudinal section of stem-wood, showing alternating arrangement of pits and absence of so-called rims or bars of SANIO. × 330.



— PLATE 20 —



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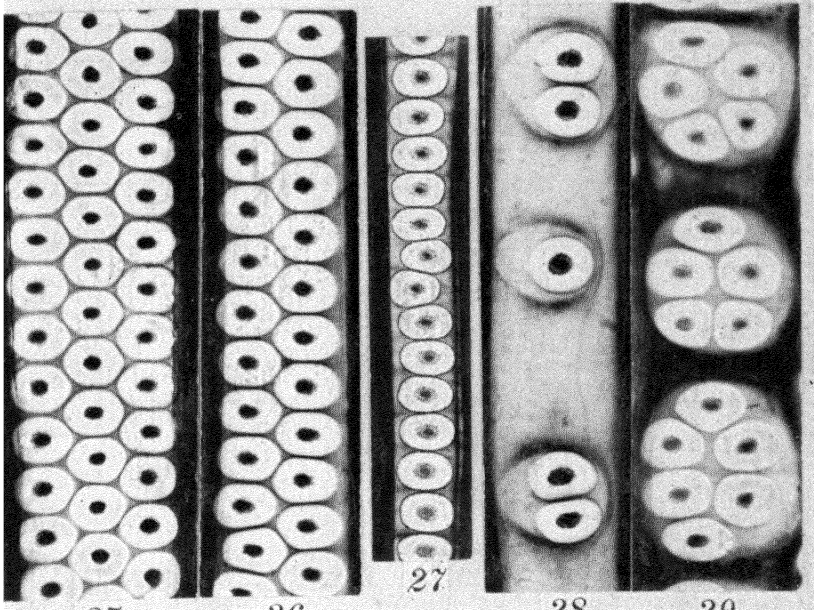

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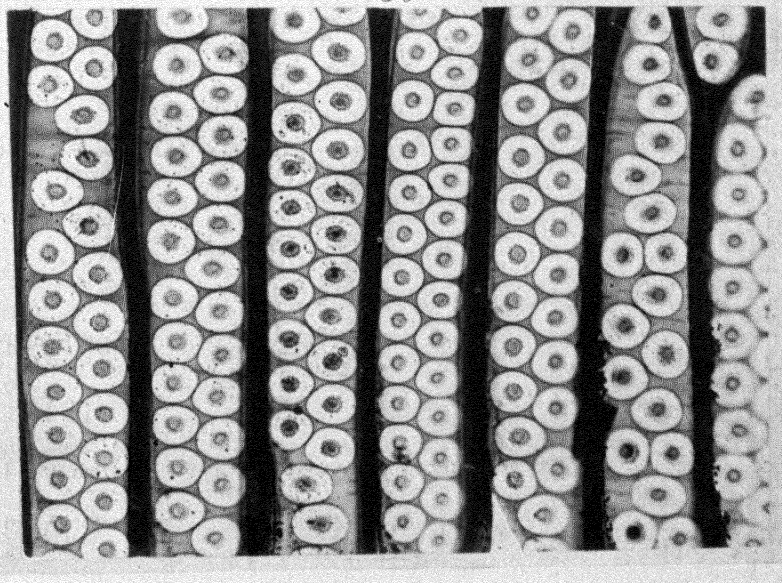


— Plate 22 —

PLATE 23

FIG. 1: Tangential section of hard pine showing sectional view of bordered pits. The tori have been pressed against the left hand orifices by excessive pressure. × 920.

FIG. 2: Tangential section of *Sequoia* showing sectional view of bordered pits. The membrane and torus are seen to occupy a median position between the two arching cell walls. × 1100.

FIG. 3: Tangential section of hard pine showing sectional view of bordered pits. The torus in this case is thin and flexible and has been jammed into the right hand orifice so firmly that it appears bow-shaped. × 920.

FIG. 4: Tangential section of freshly cut green white pine sapwood injected with a carbon mass. The minute carbon particles are seen to penetrate from one cell to another by the bordered pits. × 640.

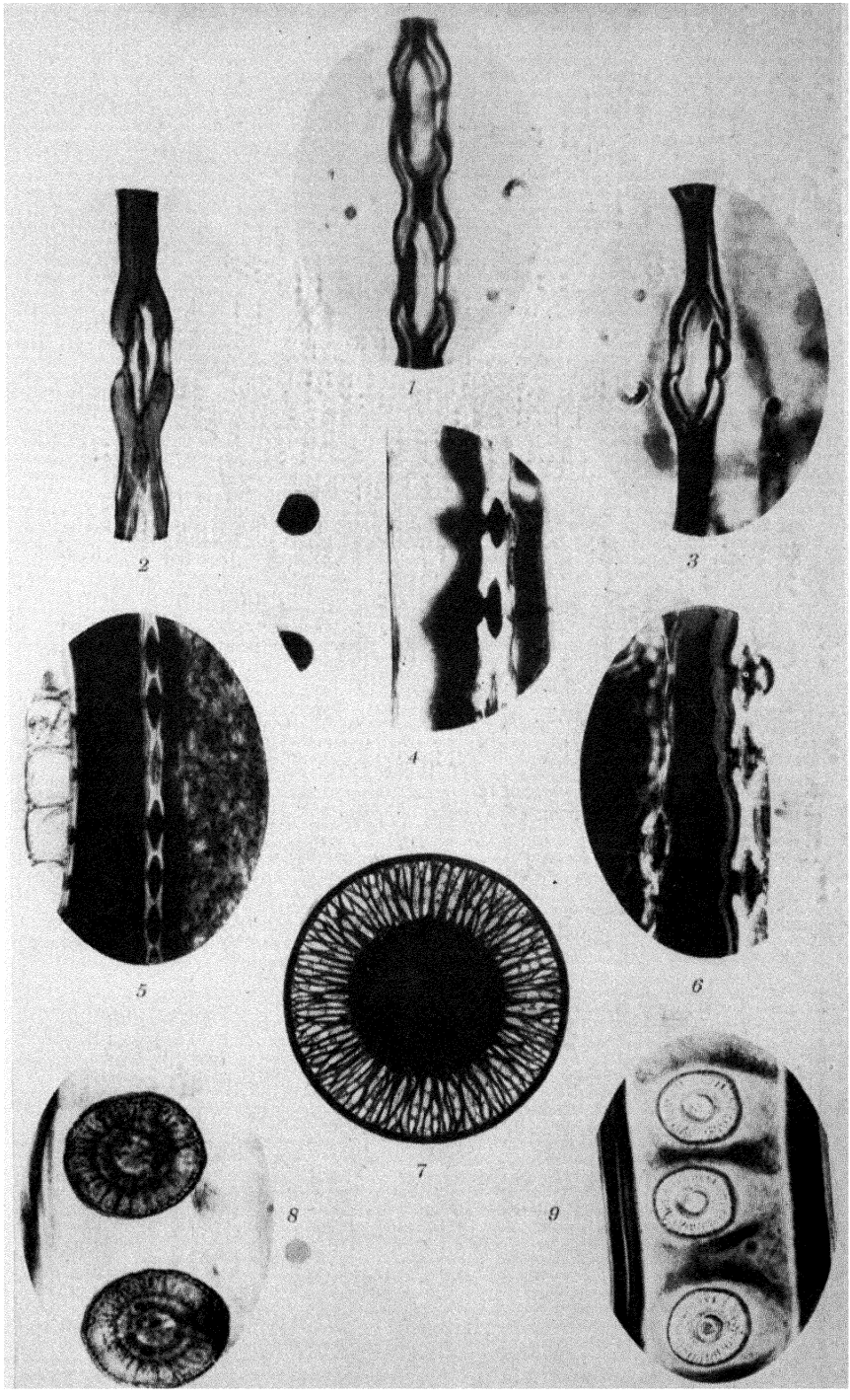
FIG. 5: Tangential section of *Sequoia* heartwood, showing the penetration of carbon mass from one tracheid to another through the numerous bordered pits. × 460.

FIG. 6: Tangential section of the summer wood of a common long-leaf pine paving block. The heavy tar oils are seen to penetrate by means of the bordered pits. × 550.

FIG. 7: Diagrammatic drawing of pit membrane and torus. The perforations are seen to occur in the thinner bands of membrane substance. × 3210.

FIG. 8: Radial section of freshly cut green white pine sapwood treated with a carbon mass. The carbon particles are seen to penetrate the pit membrane in a rim about the torus and in lines radiating outward from it. × 1380.

FIG. 9: Radial section of larch showing large perforations in the pit membranes. × 730.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