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PLANT PHYSIOLOGY DIVISION

THE PLANT CELL WALL

A Topical Study of Architecture, Dynamics, Comparative Chemistry and Technology in a Biological System

by

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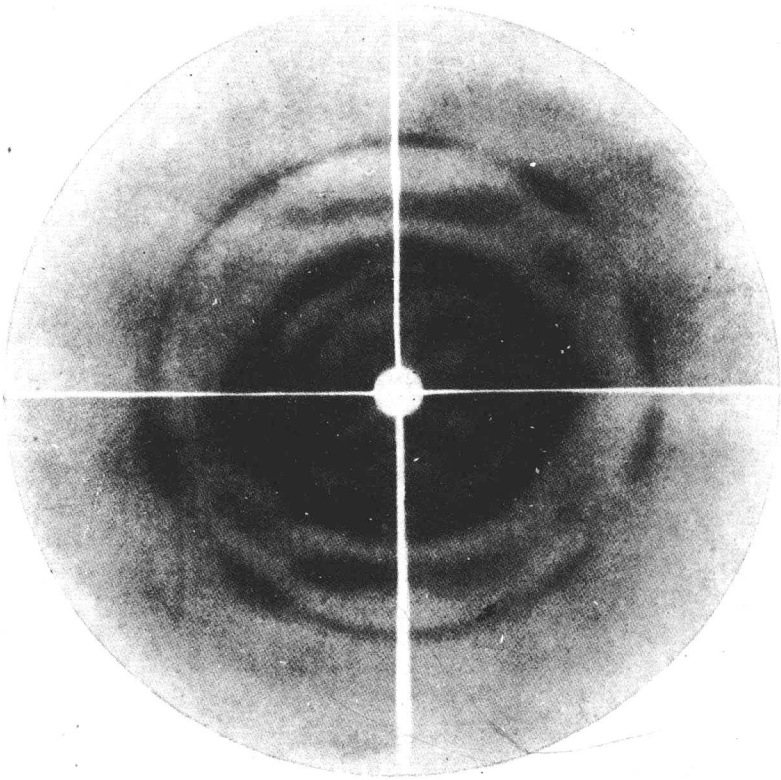

X-ray diagram of hemp fibres before and after delignification.
Upper right and lower left: "Untreated hemp fibres".
Upper left and lover right: "Fibres after lignin extraction".

PREFACE

THE recent great advances in quantitative biology have been made possible by the growth of new concepts and techniques in chemistry, physics, and geology. These fundamental areas, together with medicine, agriculture, and technology have provided the stimulus for the modern inter-disciplinary approach to structure and function in living matter.

Among the more complex biological products, cell walls and intercellular substances are nearly unique in having been subjected to virtually all of the potentially applicable methods of study and analysis. It is indeed a measure of their complexity that we still know so little about these materials, although they have been objects for immunological, geological, colloid, and textile research. Nevertheless, endeavors in these and many other areas have revealed the outlines of a structural–functional system dynamically associated with the living protoplast yet possessed of considerable continuity in the fossil record.

If it were important for no other reason, the study of cell walls and kindred structures would provide an object lesson in the effectiveness of the broad approach. Of course, cell wall research is not of interest solely as an exercise in the unification of scientific methods and ideas. The macromolecular—and often highly structured–products which are manufactured at the protoplastic surface must reflect the interaction between the biochemistry of the cell interior and the physicochemical conditions at the cell boundary. In the constituents of the wall and their arrangement must reside a great body of information about such interactions and the state of the protoplastic surface.

No less important is the contribution which cell wall research can make to the study of evolutionary processes. It is most fortunate that there exist for study comparatively resistant substances such as lignin in plants and bone in animals, so that the proper combination of paleobiochemistry and comparative biochemistry, can

provide new insight into the physiological and biochemical aspects of evolution.

This volume represents an effort to bring together some of this widespread and wide-ranging information, and to a modest extent, to make some attempt at the organization and synthesis of various facts and theories.

Although the results of recent and current research, including the author's studies on lignins and lignification, have been incorporated, this volume is not presented primarily as an advanced research treatise. The future of cell wall research, both in its own right and as an example of a valuable orientation in experimental biology, rests with the present-day students of botany and zoology who are willing to acquire understanding and proficiency in many areas of science and as much with those in chemistry and physics who have latterly become aware of the rich field of investigation that is biology.

To those who made this book possible, I take pleasure in expressing the deepest gratitude. Thanks are due particularly to Professor James Bonner, California Institute of Technology; Professor Arthur W. Galston, Yale University; and Professor David Goddard, University of Pennsylvania; who each in his own way contributed support, encouragement, and stimulating ideas to the author's research on lignification.

Thanks are also due to the students whose loyalty, help and enthusiam lightened many tasks and broadened many ideas: Mr. L. N. Chessin, New York University School of Medicine; Mr. K. Cost, University of Minnesota; Mr. N. Goodman, Brandeis University; Mr. B. LeFevre, Armour and Co., Chicago; Dr. D. Ridgeway, California Institute of Technology; and Mrs. Colin Taylor, University of Rochester.

To his wife and colleague, Barbara Siegel, the author expresses his indebtedness for many years of challenging ideas and constructive criticism.

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Finally, the author is deeply indebted to the Union Carbide Research Institute and the Union Carbide Corporation for their generosity in seeing the manuscript through its gestation period, and to Mrs. J. Hess, Miss M. Wood, Mrs. F. Bouchard, Mrs. B. Clark and Miss G. Plock for their patience and cheerful efforts that guaranteed its safe delivery.

If there are controversial ideas in this monograph, the author is pleased to assume in full the responsibility for their presence and vigorous exposition.

INTRODUCTION: WALLS AND CELLS

WHEN the cell theory and its origins are presented to the student of botany or general biology, reference is almost invariably made to the cellular structure of cork as described in Robert Hooke's *Micrographia*. The existence of well-defined, limiting wall structures has been of great importance in the development of the concepts of biological organization. In the vascular plant body, a multitude of varied patterns of cell and tissue organization underlie a comparatively simple array of organs. The identity of tissues and tissue systems is based upon the cell types present and their arrangement, and is referred in large measure to cell morphology. In many cell types, form becomes fixed during maturation and differentiation in the cell wall. Thus, the death of protoplasts often leaves behind a permanent record of their size, form and arrangement. The stability of the morphological system in vascular plants is illustrated by the cellular and tissue structure which is often discernible in fossils.

Underlying the record of microstructure which the cell wall provides is a vast supply of physicochemical information encompassing the physical structure of polymer aggregates and polymer chains, and the chemistry of polymers, monomers and other small molecules. Therefore, a vital part of cellular economy is reflected in the materials of the cell wall and the physicochemical properties of the protoplastic elements and membranes which regulated their deposition.

The cell wall is a highly functional entity which varies in composition and architecture, internally in accordance with cell–cell and cell–tissue interactions, and, externally in accordance with environmental factors and stresses.

The student of ontogeny, whether concerned with cellular differentiation or morphogenesis in complex organisms, proceeds with confidence that a relationship indeed exists between form and function. The mechanisms of ontogenetic control constitute,

however, one of the great frontier areas of quantitative biology. The nature of the interactions between the genomes of individual cells and biotic, physical, and chemical components of their environment are largely obscure, although the consequences of such interactions are always in evidence.

A study of the cell wall in its several aspects provides one approach to problems of cellular differentiation. In the pursuit of this study, consideration must be given to constitutional and architectural features of cell walls; to the material transformations associated with wall substance and the chemical and physical means for their regulation. Although the cell wall of the vascular plant has been selected as a major subject, a proper biological perspective requires comparative treatment of walls and kindred intercellular systems as they exist among organisms at large. Historical perspective is provided by examination of the phylogenetic aspects of a durable component of the plant wall, lignin. Among organisms, the walls of plant cells, particularly vascular forms, have been studied most extensively. Purely scholarly consideration of plant structure and growth have been reinforced greatly by long-standing economic and technological interests in cell wall derivatives. Accordingly some attention must be given to woods, plastics, fibers, and coal. Such considerations become all the more important when it is recognized that cell wall technology is a function of the fundamental chemistry, physics, and geology of these materials.

Cell Types and Cell Walls in Vascular Plants

The identity of tissue types depends upon the anatomical characteristics of their component cells. Simple tissues contain but one cell type, complex tissues two or more.

Parenchyma, *sclerenchyma*, and *collenchyma* represent simple tissues, parenchyma is the primitive, unspecialized tissue consisting of isodiametric cells with active protoplasts and relatively thin walls. Parenchyma cells are the principle or sole constituent of meristems, pith, cortex, and other tissues. Collenchyma, which forms simple, homogeneous tissue, consists of irregular, elongate

cells with unevenly but heavily-thickened walls. The walls are soft extensible and rich in cellulose and pectin. Collenchyma cells may be recognized by their wall pattern.

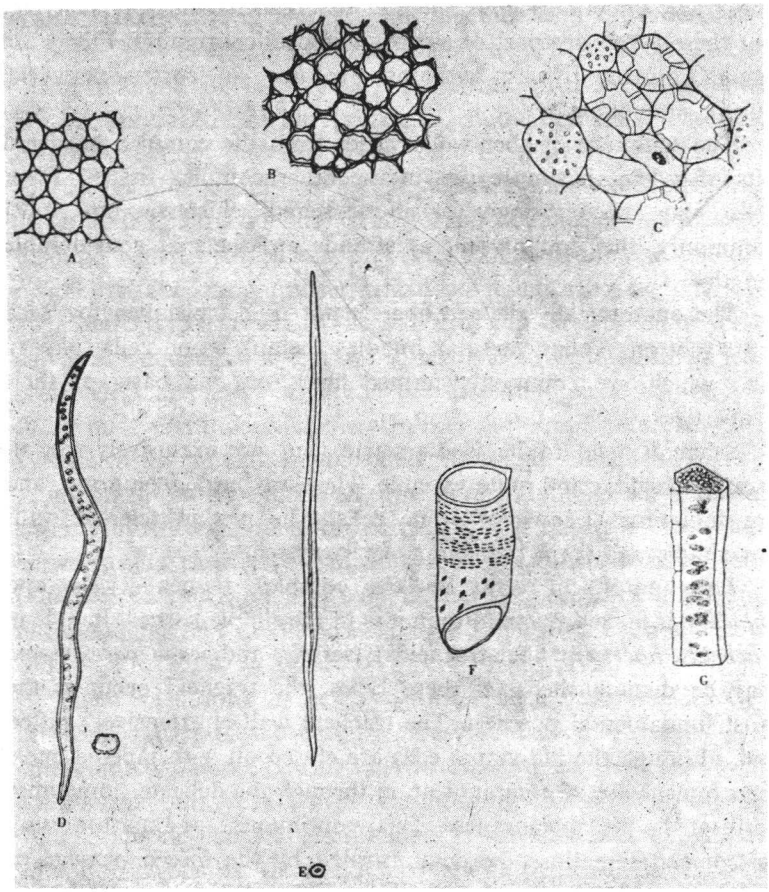

FIG. 1. Cell and wall variations in plant tissues. *A*, thick-walled Pith; *B*, collenchyma; *C*, sclerenchyma, showing sclereids and parenchyma cells; *D*, tracheid; *E*, libriform fibre; *F*, vessel element; *G*, sieve cell with companion cell. (From Eames, A. J. and Mac Daniels, L. H., *An Introduction to Plant Anatomy*, 2nd ed., pp. 83–96. McGraw-Hill, New York, 1947.)

Sclerenchyma, like collenchyma, is a supporting tissue. Unlike collenchyma cells, sclerenchyma cells have thick, hard, lignified walls and are low in water. During maturation their protoplasts degenerate, leaving a non-living sclerenchyma tissue. Sclereids and fibers are sometimes conveniently distinguished in sclerenchyma, but these forms are part of a morphological continuum. Fibers are found in many tissues, but most profusely in cortex, pericycle, xylem, and phloem.

The sculpture of fiber walls varies from the complex bordered pits of xylem to simple pits in the other complex tissues. Fiber cells may occur singly or in scattered clusters, but most commonly, they are present as strands or sheets of considerable length.

The anatomically defined fiber is not to be mistaken for seed hairs (cotton), foliar vascular bundles (hemp), wood cells (paper), etc., which are commonly termed fibers on the basis of their properties.

Sclereids tend to be isodiametric (but not exclusively so), although irregular and quite variable. They are hard, even gritty, and are sometimes known as stone cells. The pits in sclereid walls are simple and form branching cavity systems.

The anatomical array in the complex tissues—*xylem* and *phloem*—is far more varied than it is in the simple tissues. In xylem, *tracheids*, *fibers* (or fiber-tracheids), *vessels*, and *wood parenchyma* may be distinguished. Of these types, the tracheid occupies the most fundamental position. The tracheid wall is extensively pitted and, although the individual cells are closed off with tapered ends, their lumens are in communication through the delicate, sometimes perforated, pit membranes. This continuous, or anastomosing system can sometimes be demonstrated by the forced passage of carbon particles from cell to cell.

The primitive, rather "all-purpose"tracheid is present in some species, but it is often displaced by the apportionment of functions to the other cell types—support to fibers, conduction to vessels, and storage to parenchyma. Vessels possess both pits and end-wall perforations. The pits occur in those regions of the wall where vessels are contiguous. The end walls may contain simple perfora-

tions, scalariform (ladder-shaped), or, less commonly, reticular openings.

Like the pits, end-wall perforations involve highly selective lytic processes whose regulation and chemical nature is unknown. These sculpturings of the cell wall together with other wall modifications, give every appearance of being highly functional changes.

In phloem, as in xylem, parenchyma fiber and sclerid cells are present together with specialized conducting cells. The fundamental structural and functional unit is the *sieve element.* This cell is again distinguished by its wall sculpture—the presence of *sieve areas* and *sieve plates.* The former are clusters of fine pores through which cytoplasmic processes extend from one cell to its neighbors. The latter are perforated communicating regions generally confined to end walls. The pores are 0.5–3.5μ in diameter. Just as vessel elements in xylem are joined end—to-end to form vessels centimeters or meters in length, the sieve tube elements are joined to form a continuous chain. Unlike the vessel elements, living cytoplasm is present in the cells although nuclei are lacking. A modified parenchyma cell, the companion cell, is associated specifically with the sieve cell elements. The nature of the relation between enucleate sieve elements and the nucleated companion cells is not known, but it is evident that they are closely associated in function as they are in position and origin from common mother cells.

These variations in cell type have been reviewed briefly to illustrate a part of the pattern of differentiation. Repeatedly, the condition of the cell wall characterizes these tissues and their component cell types which arise during differentiation.

The developmental plan which is encountered under mesic condition serves as a convenient reference point for the consideration and cell and wall specialization. When the physical environment deviates from these mild, "ordinary" conditions, however, the plant body must undergo suitable modification if it is to survive. In xerophytes, the place of the cell wall in adaptation is well illustrated.

Xeric environments, whether in desert, tundra, or saline regions, select for water-conserving modifications in their plant communi-

ties. Epidermal cells often exhibit the most conspicuous xeric adaptations although these modifications commonly extend into subepidermal layers, and even throughout the plant.

The development of thick cuticles, infiltration of cutin and waxes, and lignification of epidermal cell walls all represent devices for laying a water barrier over the plant surface. Cutinized or lignified hypodermal layers immediately beneath the epidermis are also found among the xerophytes. In some xerophytes, sclerenchyma with its hard, lignified walls is laid down to form insulating sheets or fiber strands.

Exceptional water resistance is found in cork. This tissue is the product of a specialized form of cambial activity. The cork cambium (phellogen) extends files of cells radially outward displacing cortical tissues. Differentiation in these cells entails the intercalation of lipoidal material (suberin) into the cell wall and deposition of lignin upon it. After protoplastic degeneration cork tissue consisting of water impermeable, air-filled cells remains.

The presence of increased amounts of superficial lignin and cutin not only provide water barriers, but may also serve as protective ultraviolet screens in regions of high light intensity.

Under hydric conditions, the problems of insulation, support, and radiation screening are minimized. Hence it is not surprising to find that the epidermis in typical aquatic plants possesses extremely thin cuticles permeable to water and gases, and that stems contain little or no lignin, even in vascular tissues, and no sclerenchyma.

The epidermal cell walls in seed coats sometimes resemble those described in xerophytes. The preservation of dormancy without desiccation for variable periods is often required in seeds. Accordingly, seed adaptations must include adequate, but temporary insulation for the embryo. In addition to cutinization and lignification, the seed coat exhibits a diversity of anatomical and chemical modifications which implicate the cell wall.

The few illustrations of wall and cell variations which have been presented show clearly that cell and cell wall form, function and constitution are indeed regulated by internal and environmental factors alike.

Keeping these concepts in mind, we may now examine the various aspects of cell wall science which must eventually provide a basis for understanding the regulation of cell wall formation by genetic and enviromental control factors.

CONTENTS

CHAPTER 1

CONSTITUTION AND ARCHITECTURE IN THE CELL WALL

I. Analytic Principles and Procedures

The study of cell walls requires the techniques of many classical disciplines and has stimulated the development of new ones as well. The traditional methods of organic chemistry together with newer biochemical methods yield considerable information about the polymeric components of the wall and their constituent subunits. In principle, however, the chemical approach is destructive, and can provide but little direct information about spatial relationships among these many substances. The development of physical methods which allow recognition of architectural elements has been of the utmost importance in the furtherance of cell wall studies. Physical methods are uniquely valuable as a means for probing the cell wall without disrupting it.

A full understanding of cell wall structure will depend upon the continuation of modern trends toward a combined physical–chemical approach.

We will now examine some of these analytic methods and approaches, and their operational basis. The methods and concepts which will be presented are designed to be illustrative rather than exhaustive. Following an inquiry into methodology, the products of these combined investigations will be employed in an effort to construct an integrated picture of the cell wall in its organized state.

Chemical Methods

Analysis of the cell wall by chemical means depends upon successful application of two kinds of technique. First, the several chemical classes represented must be separated from one another