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Effect of Digestion on Wood Structure

by A. L. M. Bixler

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EFFECT OF DIGESTION ON WOOD STRUCTURE

A Thesis submitted by

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CHAPTER I

INTRODUCTION
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INTRODUCTION

Wood structure has been studied critically for the most part by botanists. Many of these have been more interested in the various types of cells in the plants and their functions than in the nature and structure of the walls and intercellular materials comprising these cells in wood. Wood pulp fiber structure, on the other hand, has been studied almost exclusively by chemists and technicists interested in the commercial usage of these fibers.

Up to the present time there has been practically no work done to relate some of the definitely known features of wood structure with the more commonly accepted theories of pulp fiber structure; by the term fiber structure is meant the structure of wood fibers as isolated by the sulphite, soda, and sulphate pulping processes. In other words, there have been only a few attempts to study wood structure, using the chemicals of these pulping processes as the analytical reagents.

The present work was undertaken in order to study this relationship by following the effects of digestion in calcium bisulphite, sodium hydroxide, and sodium hydroxide-sodium sulphide cooking liquors on the visible structure of several woods.
CHAPTER II

HISTORY
CHAPTER II
HISTORICAL SURVEY

During the past one hundred years the cell wall structures of the various cells in wood have been investigated by botanists and chemists. There has, however, been no general agreement either on the chemical or on the physical composition of the various walls of the xylem or woody tissue.

One important reason for this lack of agreement is probably the fact that the question is approached from two distinct points of view, namely the chemist's and the botanist's, and much of the disagreement lies between these two groups. Heiser (1) gives a very complete review of every phase of fiber structure with an excellent reference list. Kerr and Bailey (2) give a more detailed review of the history of wood structure investigation. Up to about 1835 the botanical investigators had distinguished the cell structure of xylem and other woody tissues and believed they were bound together by a refractive, intercellular substance which differed from the cell walls in its solubilities and other properties. With the discovery of cell division and the consequent new ideas on the origin and growth of cells, the refractive intercellular layer was
designated as "middle lamella". It became quite well agreed that the middle lamella originates between the halves of the split cell-plate and is the first formed membrane or partition wall between young cells. Many botanists believed the walls (cambial cells) of these newly formed cells were composed of simple, very thin, delicate tissue. However, the more critical works of Dipple (3), Sanio (4), Schacht (5), and Strasburger (6) showed that the cambial walls or syllem tissue are not homogenous. Sanio (4) was able to show that the cambial walls of Pinus sylvestris are of considerable thickness and that the walls of adjacent cells are separated by an amorphous intercellular material. Dipple (12) concluded that the walls of the cambium cells were isotropic.

Bailey (7-9) and Kerr and Bailey (2) have shown that the cambial initial is enclosed on all sides with a wall of its own and that these walls in adjacent cells are separated by an intercellular material. These initial or cambial walls retain their identity throughout all conditions of growth, while the intercellular material is plastic and possesses few of the characteristics of a true membrane. As the cell grows, the cambial walls increase in diameter and become thinner,
while the intercellular material is passively molded by these growing walls, and as the cambial walls grow, the intercellular material becomes more and more difficult to distinguish by microscopic methods, except in the corners where three or more cells join.

The cambial walls of trees have been found, in contrast to Dipples' conclusions (12), to be anisotropic. They are composed largely of cellulose and polyuronide and the intercellular material is mainly, if not entirely, of polyuronide composition.

Kerr and Bailey (2) believe the formation of the secondary walls of the mature cell begins after the young cambial cell has attained its full diameter. Others (11-13) are not so specific but agree that the secondary walls are laid down some time after the young cell has begun to grow and increase in diameter. Whatever the case may be, in most wood tissue (fibers, fiber tracheids) these secondary thickenings are very pronounced and take up by far the greatest portion of the wall of an ordinary wood fiber. In a few cases there are what is known as tertiary thickenings (11), (17). These usually take the form of special wrappings on the lumen surface of the secondary wall.
Lignification was found to set in early in the secondary wall formation of all the species studied by Kerr and Bailey (2) and was first noted in the intercellular material and cambial walls. It spreads centripetally through successively formed layers and is very heavy in the cambial walls, though varying in intensity in the secondary layers. The process of lignification tends to obscure the anisotropy of the cambial walls and to mask the staining reactions, so that it is difficult to distinguish the cambial wall from the intercellular material in the mature cell. It has been definitely shown, however, that the cambial walls do persist as individual units of structure in the mature xylem, and that the cells, separated by the isotropic intercellular material or middle lamella, are composed of a cambial wall, anisotropic in cross section, and the secondary wall. The secondary wall is not optically homogenous but has an outer (not the cambial wall) and an inner layer which are anisotropic in cross section, while the bulk of the wall between these two layers is isotropic in cross section but anisotropic in longitudinal section. The optical behavior of these walls in polarized light varies with the micelles' orientation. Walls in which the micelles oriented at right angles to the plane of sectioning are iso-
tropic and those with the micelles parallel to the plane of sectioning are anisotropic, while intermediate orientations produce more or less anisotropy depending on the angle. Finally, Kerr and Bailey (2) conclude that in the mature cell the cambial walls do retain their anisotropy, but that it is masked by heavy lignification, that there is much evidence indicating the original cellulose and pectinides of the cambial cell are not replaced by or transformed into lignin during the tissue differentiation, but that the isotropic intercellular material is composed of lignin and pectic compounds which may be separated by differential solubilities.

As an example of some less critical views, the following may be cited.

Bann and Daniels (13) were unable to detect the cambial walls in the mature cell and believe that the primary walls (cambial walls) and the intercellular material are one and the same thing and that the material between the two secondary walls of adjacent cells is simply their two primary walls fused together.

Jones (14) believes that the original thin, delicate wall of the young cell, somewhat modified and thickened, remains in the mature xylem as the primary cell-wall layer or middle lamella.
Figure 1 is a diagram of a mature wood tracheid according to Kerr and Bailey (2).

A. Diagram of transverse section of a tracheid according to Kerr and Bailey (2).
B. Section of adjacent walls according to Kerr and Bailey (2).

A.

a. Truly isotropic intercellular material.
b. Cambial or primary wall.
c. Outer layer of secondary wall.
d. Central layer of secondary wall.
e. Inner layer of secondary wall.
The isotropic intercellular material is composed of lignin and polyuronides. The cambial wall is cellulose, polyuronides and lignin. The secondary walls are cellulose and lignin. In the outer and inner layers of the secondary walls the fibrils and consequently the micelles are oriented perpendicular to the fiber axis. When such a fiber section (lightly lignified) is treated with 72 per cent sulphuric acid the secondary wall dissolves quite completely, leaving a three layered middle lamella residue consisting of a and b. If, however, the secondary wall is more heavily lignified, as it is in the vessels of hard woods and the fibers and fiber tracheids of soft woods, there is a five layered lignin residue consisting of a, b, and c.

In a more recent work on the structure of the secondary wall, Kerr and Bailey (14) have shown that the secondary wall of normal tracheids, fiber tracheids, and libroform fibers is of a very heterogeneous but firmly coherent structure. They find no evidence that the cellulose matrix of this structure is composed of discrete particles, but that the finer detail of the structure grade down to the limits of microscopic visibility with no such particles in evidence. Any particles that may be formed during disruption or solution of the mem-
brane are merely heterogeneous fragments broken or shredded from the original continuous matrix. The cellulose matrix is composed of two optically different cellulose complexes which vary greatly from plant to plant and even from one fiber to another in the same plant. These two complexes are arranged in various radio-concentric patterns and lignin and other non-cellulosic materials may be deposited in the interstices between the two complexes. In heavily lignified fibers this occurs and sufficient lignin is so deposited that either the cellulose matrix or the lignin system may be dissolved without seriously modifying the structural pattern of the other. Harlow (15) and Freudenberg (16) are able to isolate a similar lignin skeleton.

Kerr and Bailey (14) found at least five different visible types of concentricities in the secondary walls of the various woods they studied, and point out the futility of attempting to homologize all types of fiber in a single structural model.

Dulke (17) presents a somewhat different picture of the structure of the cell of a lignified conifer. Figure 2 is his diagram of a cross section of the mature coniferous xylem.
Figure 2

Transverse section of a tracheid according to Ludtke.

- a. Middle lamella.
- b. Primary lamella.
- c. Secondary lamella or secondary wall.
- d. Tertiary lamella.
- e. Lumen.

The middle lamella is not amorphous but is laminated (Lu) and contains some easily soluble carbohydrates. It is still a question as to whether or not these are chemically combined with lignin, but apparently they form no structural entity. The lignin is confined strictly to the middle lamella. There is no indication given as to the existence of any such structure as the cambial wall. For that matter, this structure is quite exclusive to the work of Kerr and Bailey (2), at least among present day workers in the field of fiber structure.
A structure which lies below the range of the ultramicroscope is attributed to the lignin by Lütke (17), (19). The stratifications existing as part of this structure supposedly run in a longitudinal direction as indicated from the splitting of wood. There are also visible diagonal striations in the middle lamella material which may be seen on portions of the middle lamella clinging to the fibers in incompletely digested sections and chips. These visible striations are oriented at about 90 degrees to the fiber axis.

They are due to what Lewis and Carpenter (19) described as a lignin sheath and Ritter and Childster (20), Soarth, Gibbs and Spier (21), and Freudenberg (16). (22) call the outer layers of fibrils in the secondary wall.

According to Lütke (17) the secondary thickenings are considered as being composed of three layers: 1. primary lamella. 2. secondary lamella or secondary wall. 3. tertiary lamella.

The whole secondary thickening is composed of two substances, cellulose and "Fremdsubstanz" (17), (23). The cellulose is composed of micelles and micelle rows according to the commonly accepted theories of micelles' structure (1), (24). A group of micelle rows or series is the longest structural unit of pure cellulose. It is
about 0.3 microns x 0.5 microns in diameter and length. This unit is contained inside a skin or "Fremdesubstanz" and is called a fibril section, which is just what the name implies, a section of a fibril. The fibrils are in turn covered with "Fremdesubstanz", and the lamellae (17), (26), into which the fibrils of the secondary wall are divided, are also covered with the same substance. The primary and tertiary lamellae are merely the continuous layer of this "Fremdesubstanz" on the fibrils bordering on the middle lamella and the lumen.

As might quite reasonably be expected from such a picture, the "Fremdesubstanz" at certain points forms a continuous diaphragm or membrane across the fiber wall perpendicular to its length. These membranes are known as cross elements and are the units which separate the fibrils into fibril sections. These cross elements account for the constrictions occurring in fiber swelling of the balloon type. The fibril sections are the units which, in Lüdtké's picture (17), correspond to the dermatoesmes of Wiessler (26). Lüdtké (17) absolutely denies the existence of any lateral windings or cellulose fibrils in the outer layers of the secondary thickening and attributes their presence to incomplete removal of the middle lamella.
The existence of such a unit as the cross element is by no means commonly accepted and many workers (2), (16), (27), (29), (30) believe no such units exist or at least are not necessary to explain fiber swelling. On the other hand definite proof of its existence has been claimed by Ludtke (17), Sakostachikoff (30), and Sakostachikoff and Tumarkin (31)(32).

The chemical identity of the "Fremdsubstanz" is still in doubt (17). However, it is quite closely related to lignin, since it stains similarly; yet it is believed not to be identical with the middle lamella lignin. The cross elements (33), which apparently are "Fremdsubstanz", are insoluble in both 94 per cent sulphuric acid and 0.2 per cent chlorine and 0.5 per cent ammonium hydroxide.

The "Fremdsubstanz" effectively isolates the secondary wall cellulose from the lignin, so there is scarcely any question as to the chemical combination of the two, according to Ludtke (17), (23), (34), (35). Ludtke (17) explains the phenomenon of balloon type swelling on the basis of this "Fremdsubstanz", which acts like a semipermeable, elastic, confining membrane.

Dess (36) and Hess and Akim (37), in general, agree with Ludtke's theories. His latter conclusions
are based mainly on X-ray and moving picture studies of swelling. According to him the cellulose fiber is composed of an outer skin with the secondary wall penetrated by an axial concentric system of skin. The fiber is likened to a solid body of water partitioned into cells by the skin system or a tube filled with a colloidal liquid phase surrounded by an elastic membrane.

Hess (36) believes that swelling action entails the penetration of the swelling media through the outer resistant skin; its chemical combination with the inner material, the compound formed dissolving in the excess swelling medium; the continued infiltration of the swelling medium due to osmotic pressure and distending of the membrane skin until it bursts. Under certain conditions streaming particles, due to rupture of one of a number of internal membranes separating the several lamellae, can be seen.

Freudenberg's theory of the structure of a wood fiber agrees more closely with those of Kerr and Bailey and Ritter (14) than do Atkem's. Figure 3 represents Freudenberg's (16), (24) concept of the structure of the fiber walls of a coniferous wood.
Transverse section of tracheid according to Freudenberg.

- **a. Middle lamella.**
- **b. Primary layer with two lamellae.**
- **c. Secondary layer with three lamellae.**
- **d. Tertiary lamella - absent in conifers.**

In general, Freudenberg's (18) picture of a fiber wall is a lot of cellulose fibrils, running at various angles about a linam in the center, and imbedded in a jelly-like matrix of lignin, hemicellulose, and pectin. It resembles a reinforced concrete structure in which the metal reinforcements represent the cellulose fibrils and the concrete, the lignin and hemicellulose-pectin jelly.
The middle lamella is composed of lignin which is impregnated with the jelly-like hemicellulose and pectin. This middle lamella is very, very thin. In fact, it is so thin that it is hard to see under the microscope except in the corners where three or more cells come together. It simply amounts to a layer of embedding matrix between adjacent cell walls. No such unit as the cambial wall has been described. It has no visible lamella and is amorphous and isotropic.

The primary layer consists of two lamellae with the cellulose fibrils running at approximately 70 degrees to the fiber axis. The fibrils in the two lamellae of this layer run in opposite directions and are embedded in the amorphous lignin jelly.

The secondary layer is composed of the same materials as the primary layer but has more lamellae, (the diagram shows four), and makes up the bulk of the fiber wall. The fibrils are here oriented at 0-20 degrees to the fiber axis. This layer contains less lignin than the primary layer and is less dense.

The tertiary lamella lines the lumen and in conifers is absent or so very thin that it is not resolvable with the microscope.

The fibrils of the fiber walls are composed of what Freudenberg (16), (22), calls micelle series. These
series are composed of about sixty to eighty micelles or approximately sixty glucose chains each. In fact, it is intimated that the micelle series is identical with the fibrils. The lignin surrounds each micelle series but penetrates it to only a very slight degree, so that these are the largest units of homogeneous cellulose. The lignin penetrating between the micelle series is of very fine particle size—below microscopic visibility. The cellulose and lignin are probably not chemically combined.

Freudentberg (15), (22), (33), Freudenber, Zocher, and Därr (30), and Fuchs (40), were able to dissolve out the cellulose from a wood section and leave a structural residue of lignin which showed rod double refraction due to the holes left in it by the removal of the micelle series. Harlow (15) isolated such a residue but did not find any rod double refraction, though Gibbs (41) is not convinced that Harlow's lignin isolation was truly lignin at all. Freudentberg (16) bases much of his theory of fibril orientation on the optical behavior of his structural lignin residue. The lignin residue shows that the primary layer is more heavily penetrated with lignin than the secondary layer of the cell wall.

Freudentberg (16) does not contest Gütko's
idea of a "Fremdsubstanz" but simply states that it is not necessary to explain the optical properties of the fibers and lignin residue.

Ritter (42-44) views a wood cellulose fiber as composed of a number of concentric sleeves. It might be likened to a multiple pipe heat exchanger where there are a number of pipes inside each other, each one of smaller diameter than the next outer one. He has detected as many as twelve such concentric sleeves. Scarth, Gibbs, and Spier (21) have detected as many as twenty concentric strata in the summer wood of a spruce tracheid. These sleeves are cemented together with a hemicellulosic material which is of the same refractive index as the fibrils.

Each sleeve consists of compactly wound cellulose fibrils, which are interpenetrated with a hemicellulosic cementing material. In the outer layer of the cell wall the fibrils are wound at nearly 90 degrees to the fiber axis and cause the constrictions observed in ballooning. Ritter (42) (43) emphatically states that it is not the cross elements of Ladtke (17) that cause these constrictions. In fact, he does not grant the existence of such an element, as he has measured fibrils 230 microns long and these were only segments of complete
fibrils, while the cross elements would permit fibril segments not longer than 40 to 60 microns. Ritter (42) attributes transverse fracture of fibers to the presence of slip planes and weakness at pits and bars of Sanio. The fibrils of the inner layers are oriented at from 0 to 30 degrees to the fiber axis, and the fibrils of adjacent lamellae do not alternate in orientation. Anderson (45) disagrees with Ritter and believes the fibrils of adjacent lamellae are inclined in opposite directions.

The fibrils are in turn made up of spindle-shaped units which Ritter (42), (43), (44), (46), and Ritter and Chidiater (20) call fusiform bodies. These units have a preferred orientation of crystals as indicated by their action in polarized light. They are cemented together by the same hemicellulosic medium that holds the fibrils together.

Ritter (42) (46) finds that the fusiform bodies may be broken down to smaller spherical units. These are in a greatly swollen condition, due to their method of isolation and are supposed to have been in an elliptical shape in the fusiform. The spherical particles in the swollen condition measure 0.2 - 0.4 microns in diameter and are birefringent but with ran-
dom orientation of the crystallites.

Wiesner (26) and Farr (48) have described such particles and there seems to be quite general agreement as to their existence. The one notable exception is Kerr and Bailey (14).

Ritter (42), (44), (47), (50), and Ritter and Chidister (20) have isolated the lignin of a wood section by 72 per cent sulphuric acid treatment and find two forms, middle lamella and cell wall lignins. They differ chemically and physically. The cell wall lignin is amorphous, while the middle lamella lignin shows structural characteristics. In fact, Ritter (50) has claimed the existence of fusiform bodies in lignin similar to those of Kürschner (51). Further, Ritter (42), (43), in contrast to Froude (18), believes the cell wall lignin and middle lamella lignin, at least in hardwoods, are not connected as indicated by the lack of sharp points on the middle lamella lignin of the lignin residue and so they are probably not identical. Harlow (15) finds the cell wall lignin more easily soluble than the middle lamella lignin. Whether or not the cell wall lignin is chemically combined with the cellulose is a moot question as far as Ritter (42) is concerned.

The middle lamella was found by Ritter (42)
and Ritter and Chidister (23) to consist chiefly of lignin and to contain no pectic compounds, but it does contain some hemicelluloses. Most of the hemicellulose of the wood is associated directly with the fibrils, fusiform bodies, and spherical units. Ritter (44), (49) has assigned various values to the per cent of lignin of the wood that is found in the middle lamella but finally he simply states that the greater portion is in the middle lamella.

Just what the relation between cell wall lignin and cementing substance is, Ritter never makes clear. The lignin (43) apparently only penetrates the outer laterally wrapped layers of secondary wall. Here it is mixed with the inter-fibrillar cementing substance, the composition of which is not definitely known but which Ritter believes is composed of uronic acids, pentosans, and hexosans. Ritter believes that his interfibrillar cementing material would fit the properties of Lüdtke's "Fremusubstanz".

Seifriz and Rock (52) have dissected a pulp fiber with the micromanipulator and have found it to be composed of fibrils and secondary fibrils which grade down to microscopic visibility in diameter. The structure of the fiber is likened to that of a rope composed of numerous fine strands.
A. J. Bailey (53), working with cotton as well as sulphite and soda pulps, finds definite evidence of fibril structure and the presence of decidedly laterally wrapped fibrils in the outer layers of the secondary walls. He beat the pulps in a ball mill and found that the cross fibrils broke up, forming remarkably uniform fragments with tapered ends and diameters of 3 to 4 microns which he called unit fibrils. The unit fibrils further broke up into smaller very uniformly sized particles, which he designated as cylindricoids, because of their structure. In cross section these particles are circles of the same diameter as the unit fibrils, and are about 3 to 4 microns long. They are believed by the author to be segments of the unit fibrils. A gelatinous material was also noted and identified as cellulose. It is believed to exist in the fiber in conjunction with the cell wall lignin and hemicellulose as a cementing material between the unit fibrils, fibrils, and lamellae.

Carpenter and Lewia (19) have shown very definitely the existence of a lignin sheath encasing very raw Jack pine Kraft fibers. This lignin sheath was insoluble in cuprammonium hydroxide and 72 per cent sulphuric acid, and after treatment in the former it usually appeared as a loosely wound lateral spiral
around the fiber proper, which in this raw state swelled only very little. They concluded that it was a portion of the middle lamella lignin which was not dissolved by the cooking process.

Lewis (54) working mainly with cinematoagraphic swelling of many types of fibers concluded that, in general fibers, exclusive of the lignin sheath, consisted of an outer primary lamella of laterally wound fibrils possibly cemented together with hemicellulose, pentosan or a ligneous material of some sort. This primary lamella was more resistant to solution in cuprammonium hydroxide than were the secondary wall layers and at certain points the fibrils doubled up on themselves or were wound two deep as it were, and here they were stuck together, being at these points, even in quite well cooked fibers, especially resistant to cuprammonium hydroxide. The resistant loops formed in cuprammonium hydroxide at these points may be the cross elements of Läitke although they apparently do not penetrate the secondary wall of the fiber. These resistant places were believed to have a great deal to do with the constrictions formed in balloonin, fibers. Beneath the primary lamella there was evidence of the existence of a semipermeable elastic membrane which made possible the
balloon-like swelling. This membrane was carbohydrate, in fact cellulosic in nature and contained a very high percentage of reducing sugars. When swollen in cuprammonium hydroxide it passed from a more or less firm gelatinous material to a condition in which it appeared to be merely a viscous liquid. It is, however, even in this latter state more resistant to cellulose solvents than the secondary wall and there was some doubt as to whether it did actually dissolve. Even in this apparently very liquid state it acts as a confining membrane to the swelled secondary wall. The exact morphological location of the semipermeable membrane was left in doubt except that it is inside the primary wall and outside the secondary wall.

The secondary wall was encased in this semipermeable membrane and was evidently composed of a number of layers of fibrils, some of which in the outer layers were wound more or less laterally, but most of them in the inner layers were more nearly in the direction of the fiber axis. It was pointed out that it is difficult to tell exactly orientation of the fibrils in the original fiber from their appearance after the fiber has been greatly swollen.
Beating was found to destroy the primary lamella but leave evidence of the semi-permeable elastic membrane for though fibers beaten to a freeness of 30 swelled in cuprammonium hydroxide there was no evidence of constrictions. Oxidation with potassium chlorate and nitric acid eliminated almost entirely any evidence of constricting and membrane material and when swelled in cuprammonium hydroxide showed strong evidence of fibril structure in the secondary walls. Heat ageing apparently embrittled the primary lamella for it broke up easily in cuprammonium hydroxide and the secondary wall swelled with no constrictions and no lateral windings apparent. Over-bleaching was found to destroy evidence of any membrane and though lateral windings were still evident there were no constrictions and no ballooning.

In the foregoing the more important works and viewpoints on wood and fiber structure have been presented as a background to the present problem. The actual history of the immediate problem is very short. Abrams (55) treated microtome sections of a number of woods, chiefly Pinus bungeana, with a number of chemicals, including 1.0 N sodium hydroxide and sodium bisulphite for various times. He found that the 1.0 N
sodium hydroxide first causes the cell walls to swell and striations appear. The resin ducts were acted upon in the early stages of the digestion, while the rays and middle lamella were attacked more gradually. The middle lamella splits away from the cell walls in places as it gradually dissolves, but a portion of it in the corners of the cells persists even through the most drastic treatment. The dilute sodium hydroxide and sodium bisulphite act strongly on the middle lamella and have relatively little effect on the cell walls.

Bailey (56) treated microtome cross sections of Douglas fir with soda, sulphite, and Kraft liquors at 95 degrees C., in Carius tubes of Pyrex glass. The alkaline liquors produced more fragile sections in all soft woods, while the sulphite digestion produced the more fragile sections with cottonwood. From this he concludes that, since the middle lamella is probably more completely removed in the more fragile sections, apparently ease of disintegration and commercially successful pulping do not go hand in hand. The middle lamella of Douglas fir was not completely removed by any of the cooking liquors used, and strap like segments remained in the corners where three or more cells meet. The resistant segment was not soluble in 72 per cent
sulphuric acid but was removed completely by sodium hypochlorite. The ends of these resistant middle lamella segments were found, on removal with sodium hypochlorite, to coincide with hiatus in the secondary walls. It is pointed out that, at commercial temperatures, these results might not be strictly true.

Bailey (57) also noted evidence of these strap-like resistant structures in the middle lamella when he picked it out of a wood section mechanically. He found that the middle lamella tended to cleave either in the center or at its union with the secondary walls, and concluded this indicated definite lack of cohesion at these points. However, he found no evidence of a many-layered middle lamella like that described by Matke (17).

Geragross and Hoffman (53) have digested wood sections with sulphite cooking liquor in a Pyrex glass autoclave, though so far none of the results have been published.
CHAPTER III

MATERIALS AND METHODS
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This problem might be attacked in several ways. The wood could be digested in the form of chips or blocks an inch or two thick, or as thin microtome sections. The use of chips, of course, would most closely approximate commercial conditions in the digester, but either the chip or block method would involve the difficulty of sectioning a very soft, easily macerated material. Such a material could be sectioned by using the proper imbedding methods, but the process would be comparatively tedious and time-consuming, and there would be the ever present danger of altering the appearance of the section despite the greatest care in sectioning.

If, on the other hand, microtome cross sections of the wood are used for digestion, commercial conditions of penetration and liquor to wood ratio would not be even approximated. However, the material (wood) that must be sectioned in this case lends itself most readily to such an operation, and very perfect sections can be cut with a minimum of distortion. Further, the wood receives a minimum of handling, especially after digestion, and the danger of misshaping the various structural parts is negligible. Since it is the purpose of this work simply to
study the effect of the several cooking liquors, and not the effect of any commercial cooking schedules on the structure of the wood, it seems that in this initial study it should be far more important to carry out the digestions under the most critical conditions, rather than to try to approximate commercial conditions. Consequently, it was decided to use thin microtome cross sections for all the digestions. Sections of about 20 microns in thickness were found to work best. Thinner ones were too fragile and much thicker ones showed structural features too poorly.

The method of attack in general was digestion of the microtome cross sections of loblolly pine, spruce, and poplar woods in sulphite, soda, and kraft liquors for varying times at constant liquor concentrations. The digested sections were stained and mounted, and specimens of each digestion photographed. The reactions of other wood sections with various analytical stains and solvents for cellulose, lignin, and polyuronides were noted and photographed.

A. Wood.

Three woods, spruce, loblolly pine, and poplar, were used in this investigation. The wood was taken from four foot sticks of six to eight inches in diameter. It was green and unbarked, and consisted mostly
of sap wood. There was not more than a half-inch core of heart wood in any of the sticks; sap wood was used exclusively in all the work.

Two inch discs were sawed from the logs and split into a convenient size for storage in two quart mason jars in one per cent phenol solution.

B. Sectioning and Preparation of Wood for Digestion.

The three woods were treated in exactly the same manner. The Bausch and Lomb sliding microtome was used for all sectioning. The blocks of wood were cut into splints of convenient size for sectioning, rinsed in water, and 20 micron cross sections cut.

In order to remove any pitch or other alcohol-benzene extractives, the sections were treated in a Soxhlet extractor for two hours with the constant boiling alcohol-benzene mixture (87 per cent benzene - 13 per cent alcohol). They were washed with 35 per cent alcohol and finally with hot distilled water. Until used, they were kept in distilled water.

One kraft cook was carried out on spruce cross sections of 200 microns to 400 microns in thickness. These sections were treated in the same manner as the 20 micron sections.
C. Digestion.

The sulphite digestions were all made in Carlus (50) tubes of Pyrex glass, 7 inches long and 6 mm. inside diameter; the tubes were filled with liquor to a depth of about six inches, leaving one inch air space.

The digestion was carried out in stainless steel autoclaves, fitted with a thermometer well, and heated with gas burners. Each of the glass tubes was placed in half-inch, capped iron pipes, and stood in the autoclave, which was about a quarter filled with water. Three tubes, one for each wood, were digested at a time.

The sulphite liquor was the same strength for all digestions and analyzed 5.6 per cent total sulphur dioxide, 4.5 per cent free sulphur dioxide, and 1.1 per cent combined sulphur dioxide, according to the Palmrose (60) method. The base was C. P. calcium hydroxide. Distilled water was used in making the liquor. Twenty sections with 10 cc. of liquor were used for each digestion, so that there was an excess of liquor and the cook was carried out with liquor at essentially a constant concentration.

All the digestions were made at 150 degrees C. and all cooking times recorded are the time of digestion.
at 150 degrees C. Twelve minutes were required to bring
the autoclave up to temperature. Digestions were made
for 0, 15, 30, 60, minutes, 2 hours, 8 hours, and 10
hours. Since the digestions were carried out in Carius
tubes, no relief was possible and the cooking pressure
corresponds to that of sulphite liquor of the given conzen-
tration at the temperature used (150 degrees C.).

At the end of the digestion the pressure on
the autoclave was released rapidly and the tubes taken
out and quenched in cold water. About five minutes
elapsed from the time the digestions were finished until
the tubes were quenched.

The tubes were opened, the liquor decanted, and
the sections washed with distilled water by decantation
until the wash water tested neutral to litmus. The
sections were kept in distilled water until examined.

The alkaline digestions could not be carried
out in glass tubes because the alkali attacked the glass
and precipitated a gelatinous material on the walls of the
tubes in which the sections became imbedded. Finally
half-inch stainless steel pipe sections, six inches long,
were used. One end was closed permanently with a stain-
less steel pipe cap sealed with ordinary pipe dope. The
other end was closed during digestion with a cap screwed
on loosely. The pipes were conditioned before digesting.
any wood sections in them by digesting the pipes themselves in the cooking liquors until no more iron precipitate formed.

All soda digestions were made with 6 per cent C. P. sodium hydroxide liquor at 175 degrees C. The sulphate digestions were made at 175 degrees C. with 6 per cent liquor, consisting of two thirds C. P. sodium hydroxide and one third C. P. sodium sulphide. Twenty sections and 20 cc. of liquor were used for each digestion. This filled the tubes to about two-thirds of their volume.

The alkaline digestions were carried out in the autoclave that was used for the sulphite cooks. The sulphate and soda digestions were made separately and about four inches of cooking liquor of the same strength as that used inside the tube was placed in the bottom of the autoclave, the tubes standing in this. Fifteen minutes were required to bring the cooks up to 175 degrees C.; the digestion periods were 0, 15, 30, and 60 minutes, respectively. The fictitious pressure caused by air was relieved at about 110 degrees C. and the pressure at which the cooks were made corresponds to the temperature 175 degrees C. The procedure used in blowing down and quenching the sulphite digestion was also used for the alkaline cooks.
The digested sections were washed by decantation with distilled water until they tested neutral to litmus paper, and were kept in distilled water until examined.

D. Microscopic Examination.

Photomicrographs of a typically digested section at each interval were taken. That is, the progress of sulphite digestion, for example, was not followed on a single section by examining it at the end of 30 minutes, 1, 2, and 3 hours of digestion, but by making a series of digestions as described, examining a number of sections from each, and photographing a representative one. The sulphite sections selected for the purpose were stained with 0.5 per cent solution Malachite Green and 0.5 per cent Pontamine Black E by a double staining technique. The section was first stained with Malachite Green for one minute, washed with water, stained with Pontamine Black E for one-half minute, washed with water until no more stain was removed, and mounted in glycerine-gel. It was possible to make mounts that lasted for two or three months. In a few instances the sections were not stained, as the features to be shown were more plainly visible without staining.

The alkaline sections were in most cases stained with potassium ferrocyanide - ferric chloride.
As in the case of the sulphite sections, some of the alkaline sections were not stained. The same mounting medium was used as was used with the sulphite sections.

In order to obtain some visual conception of the chemical composition of the digested sections, a section from each cook was treated on a microscope slide with 72 per cent sulphuric acid and the residue, after standing five minutes in the acid, was photomicrographed. Although the cellulosic portion is not completely removed and hydrolysed in some of the rawer sections, even these sections show what portions of the structure are less resistant to attack by the 72 per cent acid, that is, which parts are most nearly pure cellulose.

Sections from each digestion were further examined for their behavior on swelling in TAPPI cuprammonium hydroxide, 72 per cent sulphuric acid, reaction to cooking stain, Bright stain, C stain, cellulose, lignin, polyuronide and hemicellulose solvents, as well as for their appearance in polarized light. The cooking, Bright, and C stains were applied according to a standard procedure (Griff, 61), which calls for a pH buffer wash before staining. In order to run no risk of altering in any way the appearance of the digested sections to be photomicrographed, these sections were not buffered or
treated with any chemicals except the aqueous stain solutions.

The Kraft digestion of the thick spruce cross section was made like the others. In examining the sections under the microscope, however, they were moderated with a dissecting needle, so that the fiber sections lay on their sides on the slide. In this way the most satisfactory longitudinal view of individual fibers was obtained. This is, of course, not a true microtome tangential or radial section of the fiber but such sections if they were thin enough for good visual results were badly distorted by the digestion and it was impossible to find a satisfactory field for photomicrographing. The sections were thin enough so that there were no whole fibers but only fiber segments with one or both ends cut off squarely, and by studying their swelling behavior, the missing link, so to speak, between the swelling of 20 micron cross sections and the swelling of whole fibers was obtained. These sections were also studied for their solubility in cellulose solvents, G stain reactions, and behavior in polarized light.

Some of the lignin solvents used in studying the digested sections were the commercially used bleaching agents: calcium hypochlorite liquor, and chlorine followed by a dilute alkali wash. In order to make this study more
complete, sections of unbleached spruce wood were treated with these chemicals, as well as Cross and Bevan and holocellulose reagents (Institute Standard Methods Number 13 and Number 14), and the results photographed.

5. Yield and Lignin Determinations.

In order to make the visual results of the digestions more understandable, quantitative yield and lignin determinations were made on each wood for each liquor used at the time when the intercellular material was just completely removed (as judged by visual observation). These times were 2 hours for sulphite, 30 minutes for soda, and 15 minutes for Kraft digestions for all woods. These digestions were made on alcohol-benzene-extracted 20 micron cross sections in exactly the same manner as the cooks for visual inspection.

The yield determinations were made on 60 to 70 milligrams samples of air dry sections. The sections were air dried to facilitate the weighing, which on the micro-chemical balance was difficult with very moist samples, and to insure more accurate sampling. The digested sections were washed by decantation, as was done in the case of the other cooks, until the supernatant liquid was neutral to litmus. In order to insure the
the removal of sodium hydroxide from the cellulose in
the alkaline digestions, the digested sections were
washed with 5 per cent hydrochloric acid after most of
the alkali had been removed by washing with distilled
water and then were washed again with distilled water to
neutrality to litmus.

In order to avoid loss, filtration was not
used to separate the digested sections, but the distilled
water was decanted off as completely as possible without
losing any of the sections and the remainder evaporated
in an oven at 105 degrees C. The yield values are re-
ported on the oven-dry basis. All the determinations
were run in duplicate and checked within a reasonable
degree of accuracy (See Appendix, Table III).

The ash content of the digested sections
from three control spruce sulphite digestions was not
more than 0.4 per cent of the oven-dry weight of the di-
gested sections. It is believed that with the acid wash
of the alkali-digested sections, the ash content of these
will not run much, if any, over this value. Consequently,
since the purpose of these determinations is not extreme
accuracy or a critical study of quantitative yield and lignin
results, the ash content of the yields was not determined
and hence is neglected in the yield and lignin values re-
ported. The micro balance used for these determinations
was capable of an accuracy of 0.002 milligram.

The lignin determinations were made on the total weight of the digested sections from each yield determination, according to the Institute Standard Method Number 13, with the modification that two cc. of 72 per cent sulphuric acid were used in every case. The 72 per cent sulphuric acid treatment of the digested sections was made in the same containers in which the yields were weighed, so as to eliminate any error of loss in transferring the material.

The method was standardized by making a determination on duplicate 50 mg. alcohol-benzene extracted samples of spruce wood meal of known lignin content as determined by the Institute Standard Method Number 13. The results are shown in Table I.

Table I.

Result of the Calibration of the Lignin Method

<table>
<thead>
<tr>
<th>Wt. Sample</th>
<th>Wt. Lignin</th>
<th>% Lignin</th>
<th>% Lignin by Micro-method</th>
<th>TAPPI M-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>43.593 mg</td>
<td>11.847 mg</td>
<td>27.15%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.597 &quot;</td>
<td>12.969 &quot;</td>
<td>27.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>27.07%</td>
<td>27.62%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This value determined by D. Brown.
CHAPTER IV

RESULTS AND DISCUSSION
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RESULTS AND DISCUSSION

In the following discussion these definitions will be followed (see Figures 4, 5, and 6):

1. All that portion of the fiber wall inside the cambial wall will be referred to as secondary wall.

2. The narrow, lightly stained band surrounding the secondary wall will be referred to as the cambial or primary wall.

3. The material between adjacent cambial walls will be referred to as intercellular material.

Figures 4, 5, and 6 are cross sections of spruce, pine, and poplar, respectively, untreated except for alcohol-benzene extraction. They are stained with aqueous phlorogluucinol acidified with sulphuric acid. In the soft woods the inner portions of the secondary walls stain light pink, the outer portions of the secondary walls, especially in the corners, stain a much deeper red color, while the intercellular material stains a very deep red. Close examination of Figures 4 and 5 reveals a narrow lighter staining band of material about each cell. This band is most distinctly visible at the corners and stains about the same color as the inner part of the secondary wall. These bands are brightly birefringent in polarized
light and are apparently the cambial or primary walls. Kerr and Bailey (2) describe. In the case of poplar (Figure 6), the entire secondary wall stains a uniform pink, with only the intercellular material being deep red and showing as chunks in the corners where three or more cells meet and showing as fine lines between these points. The rays and vessel walls stain a uniform deep red color. The cambial walls cannot be distinguished in the untreated poplar sections except by polarized light, though as will be shown later, they do stand out after long digestion.

The pink phloroglucinol color is commonly accepted as an indication of the presence of lignin. On this basis, the untreated soft woods show a highly lignified material between the cells and in the outer portions of the cell walls and a relatively less heavily lignified cambial wall and inner portion of secondary wall (Figures 4 and 5).

The hard wood, on this same basis, shows a rather uniformly, relatively lightly lignified secondary wall and heavily lignified intercellular material, rays, and vessel walls. The two figures of soft woods do not show any rays and resin ducts but the rays, as will be shown later, are heavily lignified, while the resin ducts are somewhat less heavily lignified.
Figure 4
Untreated Spruce 20 Micron Cross Section. Stained with Phloroglucinol.

Figure 5
Untreated Loblolly Pine 20 Micron Cross Section. Stained with Phloroglucinol.
Figure 6
Untreated Poplar 20 Micron Cross Section. Stained with Phloroglucinol.
A. Sulphite Digestion.

1. Visual Results.

Figures 7, 8, 9, and 10 show visually the results of sulphite digestion on spruce wood. Figure 7 is stained with the Malachite Green-Black 3 double stain and the material looks much like the undigested wood sections, showing that there has been very little attack that can be detected visibly up to this point. The Black 3 does not take at all on this section and the Malachite Green accounts for all the staining.

At the point in the digestion represented by Figure 9 there has been considerable attack on the intercellular material. This is most plainly seen in the center of the photograph; the intercellular material is almost completely removed, except in the corners where three or more cells meet. Here there are chunks of deeply stained material which have not been removed. The secondary walls stain very lightly and uniformly, though there are darker patches along the edges in places where the Malachite Green takes more heavily. The rays also stain considerably lighter in Figure 9 than in Figure 7.

At two hours digestion (Figure 9) Malachite Green, though it stains the cell walls, is almost completely washed out, while Black 3 is fixed more permanent-
ly and is not washed out. The intercellular material has been completely removed, and there has been considerably more attack on the secondary wall beneath the cambial walls. The mere fact that the cambial walls stand out from the secondary walls (Figure 9) does not necessarily indicate that a certain amount of the secondary wall has been removed from this region. It might possibly be caused by the cambial wall being loosened from the secondary wall through dissolving of a slight amount of cementing material, so to speak, and a simultaneous shrinkage of the secondary wall due to removal of material more or less uniformly from all parts of this wall, or by simultaneous stretching of the cambial wall, or both. However, in the light of the fact that both phloroglucinol and Malachite Green staining indicate relatively great concentrations of lignin in the outer portions of the secondary wall and, as will be shown later, that the outer portions of the secondary wall are much less soluble in 72 per cent sulphuric acid than the inner portions, it seems most probable that the attack on the secondary wall is concentrated in the outer portions and that these portions may be spoken of as actually having been removed; thus, the gap between the cambial wall and secondary wall is mainly due to removal of the portion
Figure 7
Spruce Sulphite 20 Micron Cross Section. Digested $\frac{1}{2}$ Hr. at 150° C. Stained with Malachite Green-Black E.

Figure 8
Spruce Sulphite 20 Micron Cross Section. Digested 1 Hr. at 150° C. Stained with Malachite Green-Black E.
of secondary wall which occupied this space. Figure 9
represents an approximate commercial yield (Table II), as
will be shown later.

Figure 10 representing 8 hours digestion, shows
that extreme conditions of cooking have relatively little
more effect on the visible structure of spruce than the
2 hour digestion. In general, the cambial walls of the
section digested for eight hours stand out from the sec-
ondary walls and the cells are a little less closely
packed than the sections digested for only two hours,
but the effect is relatively small compared to that
produced by the first two hours.

Figures 11, 12, and 13 are the lignin resid-
dues, as determined by 72 per cent sulphuric acid, of
the digestions represented by Figures 7, 8, and 9. The
structure of the lignin residue from the thirty-minute
digestion is quite complete. The intercellular material
remains as a semi-complete web or network, and the chunks
in the corners and the thinner portions of the material
connecting them are plainly visible. The inner portions
of the secondary wall have swelled and dispersed quite
completely, leaving only the outer portions resistant to
the acid. These outer portions of the secondary wall
Figure 9
Spruce Sulphite 20 Micron Cross Section. Digested 2 Hrs. at 150° C. Stained with Malachite Green-Black E.

Figure 10
Spruce Sulphite 20 Micron Cross Section. Digested 5 Hrs. at 150° C. Stained with Malachite Green-Black E.
are apparently most resistant at the radial ends and in the corners. This result would seem to confirm the contention that the gap showing between the cambial and secondary walls in the two-hour digested section is due mainly to removal of material from the outer portion of the secondary wall. Apparently the cambial walls have been dissolved in Figure 11 for there is definitely a space between the intercellular material and the more resistant outer portions of the secondary wall from which the acid has removed something. This space was occupied by the cambial wall, (Figure 7), which is strongly birefringent; when the action of the acid on such a section is observed in polarized light, it gradually loses its birefringence, and becomes entirely dark. The section shown in Figure 11 is completely dark in polarized light and completely soluble in chlorine and ammonia.

From Figure 12, showing the lignin residue corresponding to Figure 3, it is apparent that the added half-hour digestion has had considerable effect. The lignin residue no longer retains its structural entity, for considerable portions of it have been removed, and only isolated parts remain. The long string in the left
Figure 11
72% Sulphuric Acid Residue. Spruce Sulphite, $\frac{1}{2}$ Hr. at 150° C.

Figure 12
72% Sulphuric Acid Residue. Spruce Sulphite, 1 Hr. at 150° C.
Figure 13
72% Sulphuric Acid Residue. Spruce Sulphite, 2 Hrs. at 150° C.
of the photomicrograph is the residue of a ray, which still retains some semblance of structure. The residue in Figure 12 is completely soluble in chlorine and 10 per cent ammonium hydroxide.

The 2-hour digested sections are practically completely soluble in 72 per cent sulphuric acid (Figure 13). The intercellular material and cell wall lignin have been completely removed by the digestion, and the remaining secondary and cambial walls are apparently quite completely carbohydrate in nature, for they are completely soluble in the 72 per cent acid. The only residue is the medullary ray cells, which still contain enough sulphuric acid-resistant material to possess considerable structure in the lignin residue. The eight-hour digested sections are completely soluble in 72 per cent sulphuric acid, not even the fragment of a ray remaining.

Figures 14, 15, 16, and 17 show the progress of sulphite digestion of loblolly pine. The results are practically the same as those observed for spruce. At thirty minutes, there has been very little or no visible effect; at sixty minutes, the intercellular material has been attacked considerably, and though there has been some attack beneath the cambial walls, it is substantially less than with spruce. Two hours digestion (Figure 16)
Figure 14
Pine Sulphite 20 Micron Cross Section. Digested 3/4 Hr. at 150° C. Stained with Malachite Green-Black E.

Figure 15
Pine Sulphite 20 Micron Cross Section. Digested 1 Hr. at 150° C. Stained with Malachite Green-Black E. E Filter.
Figure 16
Pine Sulphite 20 Micron Cross Section. Digested 2 Hrs. at 150⁰ C. Stained with Malachite Green-Black E.

Figure 17
Pine Sulphite 20 Micron Cross Section. Digested 10 Hrs. at 150⁰ C. Unstained.
removes the intercellular material completely, and there is considerable attack on the secondary wall. The cambial walls, however, do not stand out as distinctly as in the case of spruce, indicating the removal of relatively less secondary wall material. The 30 and 60 minute sections took up the Malachite Green stain more strongly; the 2-hour sections took Black 8 more strongly. The section digested for 10 hours is unstained and shows very clearly that, even though the cambial walls are not evident in figure 13 and only indefinitely so in figures 14 and 15 there are cambial walls in nature pine, that they are not removed by sulphite digestion, but that considerable of the secondary wall is removed after 10 hours of digestion. One feature concerning these cambial walls, which though it is distinctly noticeable in the 3-hour spruce digestion (figure 10), is much more spectacularly so in the 10-hour pine digestion, is that, while there is apparently considerable material removed from the secondary wall on the radial ends, there seems to be nothing removed on the tangential sides, and, in fact, here the cambial walls and secondary walls appear to be fused together, even after 10-hour digestion.
The 72 per cent sulphuric acid residue, figures 18 and 19, corresponding to the 30 and 60 minute digestions, are similar to those of spruce. At 30 minutes the lignin residue presents a rather complete structure. The carbohydrates are undoubtedly not completely dispersed in Figure 18, primarily because the lignin residue did not allow thorough enough contact with the acid. The figure, however, does show which portions of the structure are most resistant. There is quite a complete web of intercellular material and considerable of the outer zone of the secondary walls which are resistant to the acid, while the cambial walls are apparently quite completely dispersed, and the inner zone of the secondary walls are greatly swelled, filling the lumen, though only partially dispersed. The section shown in Figure 18 is completely dark in polarized light, but is not completely soluble in chlorine and ammonium hydroxide.

At one hour digestion, the lignin residue no longer retains its original structural form but disintegrates to small chunks and pieces (figure 19). As was true with spruce, the rays show more resistance than the other structures. The material shown in Figure 19 is completely soluble in chlorine and ammonium hydroxide.
Figure 18
72% Sulphuric Acid Residue. Pine Sulphite, $\frac{1}{2}$ Hr. at 150° C.

Figure 19
72% Sulphuric Acid Residue. Pine Sulphite, 1 Hr. at 150° C.
The lignin residue from the 2-hour pine digestion presents exactly the same appearance as that of the 2-hour spruce digestion, so that it was not photographed. The 10-hour pine digested sections are completely soluble in 72 per cent sulfuric acid.

Sulphite digestion affects poplar in much the same way it does spruce and pine, except that attack on the secondary wall is not discernible, visually at least. Thirty minutes' digestion shows very little attack, as Figure 20 looks much like the undigested wood Figure 6.

At 60 minutes, Figure 21, there are still pieces of intercellular material in the corners, but they have been reduced in size considerably, and in some places between these pieces the thin ribbon of this material has been removed. The secondary walls take neither Malachite Green nor Black 3 stains very heavily, but the vessel walls, rays, and intercellular material remaining, stain very deeply with Malachite Green.

At 2 hours, Figure 22, the intercellular material has apparently been completely removed. This section was not stained, for staining seemed to obscure the fact that the intercellular material was re-
Figure 20
Poplar Sulphite 20 Micron Cross Section. Digested $\frac{1}{2}$ Hr. at $150^\circ$ C. Stained with Malachite Green-Black E.

Figure 21
Poplar Sulphite 20 Micron Cross Section. Digested 1 Hr. at $150^\circ$ C. Stained with Malachite Green-Black E.
Figure 22
Poplar Sulphite 20 Micron Cross Section. Digested 2 Hrs. at 150°C. Unstained.

Figure 23
Poplar Sulphite 20 Micron Cross Section. Digested 8 Hrs. at 150°C. Stained with Malachite Green-Congo Red.
moved or at least was transparent. Staining lowered the contrast, so that this was not so distinctly discernable in photomicrographs. This fact leads to the belief that the intercellular material has been gelatinized or rendered transparent, yet that there is still something there that takes the stain. This same effect was also noticed at times in the case of the soft woods and will be discussed later.

Eight hours' digestion shows very little more effect than is shown by 2 hours. Figure 23 is stained with cooking stain. The Malachite Green takes quite strongly on the rays, and to a lesser degree on the vessel walls. It, however, does not stain the fiber walls at all, and the Congo Red stains them a light pink. From the photograph on the cambial wall cannot be distinguished, but it does exist as can be shown by raising and lowering the focus of the microscope and from the fact that there is an outer strongly birefringent band around each secondary wall.

In Figure 24, the 30-minute poplar subchite digestion, some parts of the lignin residue are quite dark and others are light. This is not due to staining out to the focus. Those parts of the structure which are dark are in focus, while those which are light are out of focus. The intercellular material shows a quite complete
Figure 24
72% Sulphuric Acid Residue. Poplar Sulphite, 
\( \frac{1}{2} \) Hr. at 150° C.

Figure 25
72% Sulphuric Acid Residue. Poplar Sulphite, 
1 Hr. at 150° C.
Figure 26
72% Sulphuric Acid Residue. Poplar Sulphite, 2 Hrs. at 150° C.
retention of its structure, but the secondary walls have apparently quite completely dispersed. This fact would substantiate the previously noted staining effect with phloroglucinol, indicating a more uniform and lesser degree of lignification in the poplar secondary wall than in the corresponding pine and spruce walls. The dark wide bands are two rays which are quite resistant at this stage of digestion.

Figure 25 shows that practically everything in a 1-hour digested section but the rays and a few pieces of intercellular material are soluble in 72 per cent sulphuric acid.

The small fragments of light colored material in Figure 25 are this remaining intercellular material, and are light colored because they are not in exact focus. At 2 hours, only the rays remain insoluble in 72 per cent sulphuric acid, and the smaller particles of material in Figure 26 are portions of rays which, not being as resistant as the others, have broken up. The 6 and 10 hour digestions gave sections which were completely soluble in 72 per cent sulphuric acid.

2. Microchemical Analysis

To this point the physical effects of sulphite digestion have been chiefly considered, but before the
picture of digestion can no even approximately complete, the effects on the chemical constituents of the various units of structure must be studied. In the discussion of the untreated wood cross sections a brief picture, based on phloroglucinol staining of the lignin distribution in the various structural units, was presented. Further, it has become quite generally agreed that the intercellular material is non-cellulosic, and that the cambial walls and secondary walls are mainly cellulosic in nature. This picture of the chemical composition of the cross sections will be used as a basis for the following discussion.

In the discussions of the chemical constituents of the variously digested sections the following analytical definitions will be used:

1. Lignin stains — phloroglucinol, aniline hydrochloride, zinc chloroiodide, and iodine-sulphuric acid.

2. Cellulose stains — zinc chloroiodide, iodine-sulphuric acid.

3. Lignin solvents — chlorine and 10 per cent ammonium hydroxide, and chlorine and sodium sulphite. The latter dissolves both lignin and part of the hemicelluloses. By hemicelluloses is meant the
aggregate of intimately associated pentoses hexoses and polyuronides in the wood. This aggregate of hemi-celluloses is not homogeneous in its behavior to solvents. Parts of it are soluble in water and other parts resist all known cellulose solvents. 4. Cellulose solvents - 72 per cent sulphuric acid and cuprammonium hydroxide. The former dissolves cellulose and polyuronides.

5. Polyuronide solvents - 5 per cent ammonium oxalate, and dilute acid, followed by dilute alkali.

6. Hemicellulose solvents - dilute acids, followed by dilute alkali.

In digestion up to and including one hour, the intercellular material of none of the woods is completely removed, as can be seen from figures 3, 15, and 21. It gives the typical lignin reactions to stains and solvents and does not dissolve in cellulose solvents. It was noted that the residual intercellular material in the 30-minute and one-hour digestions was very much more easily removed by the lignin solvents than it was in the undisgested wood. This, of course, might be expected, since there is not as much material to remove, but it seems quite possible that the sulphite liquor has reacted with the re-
main ing material in such a way as to make it more easily soluble in the lignin solvents. This same effect was also noted with the commercial lignin solvents, chlorine and sodium hydroxide, and calcium hypochlorite. All these lignin solvents apparently completely remove the residual intercellular material after partial sulphite digestion.

In some cases, especially with pine and spruce, where sections had been digested 2 hours and longer, there seems to be a gelatinous material remaining in the intercellular spaces, and in the spaces between the cambial and secondary walls. It was impossible to stain unmistakably this material in any way, and so it is very difficult to prove conclusively its existence or non-existence. It was not possible to macerate such a section with either polymernide or hemicellulose solvents, and the lignin solvents produced a more fragile section, yet it could not be called maceration. By maceration is meant the removal, dissolving, or altering of the intercellular material in such a way that the cells have no tendency to remain in position in the section, but simply float apart. The only solvents that would produce maceration were
sulphuric acid and cuprammonium hydroxide of sufficient strength to swell the cell walls. With these two reagents, as the secondary walls swelled, filling the lumen, the cells floated apart, possibly indicating a solution of some material which had held them together. The fact that the residual intercellular material, if there is any such, is removed only by the cellulose solvents, may indicate that it is carbohydrate in nature and is of a higher degree of polymerization than the polyuronide or hemicellulose solvents will attack.

Thirty minute and one hour digestions do not remove all of the ligninous material from the secondary walls, which is as would be expected from the visual inspection. Up to this point these walls show lignin reactions to stains, though these reactions are quite weak in the one hour digestions. The inner portion of the secondary walls is completely soluble in cellulose solvents, but the outer portion is not, and the residue of this outer portion left after treatment with the cellulose solvents dissolves completely in lignin solvents. Further, if the secondary walls are delignified before treatment with cellulose solvents both the inner and outer portions are then completely soluble in these reagents. Chlorine and sodium hydroxide and calcium
hypochlorite also dissolved this ligneous portion of
the secondary wall, so that the walls were then com-
pletely soluble in the cellulose solvents. The second-
ary walls in the 30-minute digested sections of spruce
and pine give a positive Maule test, indicating the
presence of lignin, but the one hour digestion of spruce
and pine showed only a weak yellow color in the second-
ary walls instead of the characteristic pink, showing
that after one hour the lignin has been practically
all removed from these walls.

The 30-minute poplar digestions reacted
only weakly yellow to the Maule test, as might be ex-
pected since the secondary walls showed weaker lignin
reactions in the untreated wood. The lignin stains
produced not only weak reactions on these poplar sec-
tions, but stained the walls very uniformly. However,
treatment with 72 per cent sulphuric acid and cuprammoni-
um hydroxide showed a resistant outer portion to the
secondary wall.

At one hour digestion, the poplar and, at
2 hours, the spruce and pine give only the faintest
reactions to all the lignin stains and solvents but
the potassium ferricyanide – ferric chloride stain,
which stained the poplar and the inner portion of the
pine and spruce secondary walls a light, uniform blue
and the corners of the spruce and pine walls a quite
dee blue. Phloroglucinol stained these corners pale
yellow, not touching the rest of the wall. The sec-
ondary walls of these sections were, at first glance,
quite completely soluble in cellulose solvents, though
if the solution were carried out slowly with diluted
solvents the outer portions, especially the radial
ends, of the pine and spruce could be seen to be more
resistant, than these inner portions and left a very
small amount of fibre residue. There was apparently
sufficient lignin in the pine and spruce one hour and
poplar 30-minute digestions to mask the blue color of
the iodine stains for cellulose. The two-hour spruce
and pine and one-hour poplar showed for the first time
the typical blue reaction of iodine for cellulose.

The secondary walls of the 2-hour poplar
digestion show lignin reactions with potassium ferri-
cyanide - ferric chloride only and stained a very weak
blue. The 10-hour digested sections of all the woods
give no lignin reactions with solvents or stains,
stain blue with iodine, and dissolve completely in cellu-
lose solvents.

The cambial walls even in the most lignified sections behave like cellulose. They are brightly birefringent; they give very faint reactions to lignin stains even on the 30-minute digestions; they are soluble in 72 per cent sulphuric acid after 30-minute digestions, (Figure 11), and are apparently soluble even in a section which was digested only for the 12 minutes required to bring the autoclave up to 150 degrees C. At least they lose their birefringence completely when the section is treated with 72 per cent sulphuric acid. The most drastic digestions do not remove them, (Figures 10 and 17), and they are not soluble in chlorine and 10 per cent ammonium hydroxide, chlorine and sodium sulphite, chlorine and sodium hydroxide, or calcium hypochlorite.

The rays in the soft woods and the vessel walls and rays in the poplar are the most resistant units. Even at 2 hours digestion the lignin residue of the rays retains its structure. The vessel walls are not as resistant as this, and are completely soluble in 72 per cent sulphuric acid after 2 hours digestion. At 10 hours, however, the rays are completely soluble. The residues left in 72 per cent sulphuric
acid from the rawer cooks are completely soluble in lignin solvents.

The manner in which the cells in the digested cross sections dissolve in 72 per cent sulphuric acid and cuprammonium hydroxide is quite unique and distinctive. The secondary walls are affected first, and in the 30-minute and one-hour digested sections swell, filling the lumen; then, being restricted from swelling outwardly by either the cambial wall or the outer zone of heavily lignified material, the swollen wall goes out of one end or the other but never both ends of the restricting outer shell. As the wall goes out, it turns inside out, so that the part that originally bordered the lumen is now outside, and the more heavily lignified band and cambial wall are inside. The swelling continues, and the secondary wall elongates and becomes thinner, until the outer portion disappears and the inner portion leaves a residue which is soluble in chlorine and ammonium hydroxide. Figures 66, 67, 68 show three steps in this swelling action. They are pictures of a Kraft digested cell, but the rawer sulphite sections swell in the same way. The cambial walls of sections digested for 2 hours are no longer resistant enough to the solvents to restrict the secondary walls from swelling outward. Neither
Figure 27
Spruce Soda 20 Micron Cross Section. Digested \( \frac{1}{2} \) Hr. at 175\(^\circ\) C. Stained with Potassium Ferricyanide-Ferric Chloride.

Figure 28
Spruce Soda 20 Micron Cross Section. Digested \( \frac{1}{2} \) Hr. at 175\(^\circ\) C. Stained with Potassium Ferricyanide-Ferric Chloride.
Figure 29
Spruce Soda 20 Micron Cross Section. Digested 1 hr. at 175° C. Stained with C Stain.
do the secondary walls cohere as strongly but break up and melt like pieces of snow on a hot stove, without actually swelling a great deal. The cambial walls dissolve and the dissolving fragments of secondary wall float out unhindered, (Figure 78).

B. Soda Digestion.

1. Visual Results.

Figures 27, 28, 29, show the effects of soda liquor digestion on spruce. Up to 15 minutes there has been only very little visible attack, though in a few places the intercellular material has been removed (Figure 27). The secondary walls have swelled considerably, and the cambial walls have become wrinkled.

Thirty minutes digestion removes practically all the intercellular material and considerable of the outer lignified portion of the secondary wall (Figure 28). The secondary walls are swollen to about the same degree as in the 15-minute digestion, and the cambial walls are very wrinkled. The cells are much more closely packed than in the sulphite digested sections which showed a corresponding degree of attack on the intercellular material at 2 hours digestion. The swelling of the secondary walls and wrinkling of cambial walls
is distinctly characteristic of soda digestion.

Sixty minutes of digestion removes a great deal more material from the outer portion of the secondary walls. The secondary walls do not appear to be swollen nearly so much, but this may be due to the fact that they are much thinner, because of the removal of much of the outer lignified band. The cambial walls are very much wrinkled, and the cells are extremely closely packed, so that the wrinkled cambial walls fill up most of the space that had been occupied by the intercellular material and the outer lignified layer of secondary wall.

A section digested for 15 minutes in soda liquor and then treated with 72 per cent sulphuric acid is shown in Figure 30. It is quite similar to that of a thirty-minute sulphite digestion. The intercellular material leaves quite a complete web; the outer portions of the secondary walls are resistant, while the inner portions are attacked, and dispersed by the acid, and the cambial walls are apparently dissolved, for the section shown in Figure 30 is completely dark in polarized light.

Thirty minutes digestion renders the lignin residue entirely structureless (Figure 31).
There are a very few small particles of intercellular material left, but by far the greater part of the residue is from the secondary walls; in fact, it still holds the shape of the walls. Soda liquor digestion is remarkably different from sulphite liquor digestion in that at the point where in both cases the intercellular material is all dissolved or at least gelatinized and rendered transparent and soluble in cellulose solvents, the secondary walls of a section digested in the former (Figures 26 and 31) still contain much 72 per cent sulphuric acid-insoluble material, while the secondary walls of a section digested in the latter (Figure 9 and 13) are practically completely soluble.

The residue in both Figures 30 and 31 is completely soluble in 72 per cent sulphuric acid after a treatment with chlorine and 10 per cent ammonia, indicating that it is lignin. This would seem to indicate that maceration or pulping action can be accomplished by means of soda liquor with much less attack on the actual fiber walls (secondary) than with sulphite liquor. Now, if the lignin remaining in the secondary wall after the maceration with the soda liquor can be removed with less degradation to the cell wall than sulphite digestion
Figure 30
72% Sulphuric Acid Residue. Spruce Soda, ½ Hr. at 175° C.

Figure 31
72% Sulphuric Acid Residue. Spruce Soda, ½ Hr. at 175° C.
produces, then, as bleaching with chlorine and ammonia would seem to indicate possible, a fiber of greater strength and with other properties just as good might be produced by some such treatment. These considerations also apply to kraft digestion.

Figures 32, 33, and 34 show that pine digests very much like spruce with soda liquor. The cambial walls do not stand out so plainly, but they are distinctly wrinkled, and the secondary walls are much swelled.

The lignin residue of the fifteen minute digested section shown in Figure 35, does not retain its structure nearly as well as the spruce did. This is not an isolated case but seems to be typical of the alkali digested sections. The spruce soda digestion does not show this appearance, which can be explained by the fact that this particular field was chosen near the center of a large section, and the reinforcement of adjacent cells prevented the disruption of any one cell, while on the other hand, the pine field was chosen nearer the edge of the section, where the swelling pressures are not balanced. The kraft digestions show just the opposite effects on spruce and pine for this same reason. This behavior is distinctly characteristic of alkaline
Figure 32
Pine Soda 20 Micron Cross Section. Digested $\frac{1}{2}$ Hr. at 175° C. Stained with Potassium Ferricyanide-Ferric Chloride.

Figure 33
Pine Soda 20 Micron Cross Section. Digested $\frac{1}{2}$ Hr. at 175° C. Stained with Potassium Ferricyanide-Ferric Chloride.
Figure 34
Pine Soda 20 Micron Cross Section. Digested 1 Hr.
at 175° C. Stained with G Stain.
Figure 35
72% Sulphuric Acid Residue. Pine Soda, \( \frac{1}{2} \) Hr. at 175°C.

Figure 36
72% Sulphuric Acid Residue. Pine Soda, \( \frac{3}{4} \) Hr. at 175°C.
digested sections, for the sulphite digestions did not show nearly the same tendency to disrupt the lignin structure, even near the edges of the sections. Hence, though the immediate conclusion is that the secondary walls probably develop a greater swelling force after alkaline digestion than after sulphite digestion, it might indicate that the secondary walls of the alkali digested sections possess a greater toughness and gelatinizing ability, which might conceivably be true also in beater hydration or swelling; thus it might be expected that alkaline pulps would lend themselves to stronger sheet information than sulphite pulps. The 30-minute digested sections break up on sulphuric acid treatment, and though there is considerable insoluble material, the sections do not retain their structure.

The intercellular material of poplar is removed more rapidly than that of the soft woods by soda digestion, just as it is by sulphite digestion. There is very little visible attack at the point where the digestion is just brought to 175 degrees C. (Figure 37), but at 15 minutes digestion (Figure 36), the intercellular material is completely removed. Further digestion up to half an hour has very little more visible effect.
Figure 37
Poplar Soda 20 Micron Cross Section. Digested 0 Hr. at 175° C. Stained with Potassium Ferricyanide-Ferric Chloride.

Figure 38
Poplar Soda 20 Micron Cross Section. Digested \( \frac{1}{4} \) Hr. at 175° C. Stained with C Stain.
Figure 39
Poplar Soda 20 Micron Cross Section. Digested $\frac{1}{3}$ Hr. at $175^\circ$ C. Unstained.
Figure 40
72% Sulphuric Acid Residue. Poplar Soda, 0 Hr. at 1750 °C.

Figure 41.
72% Sulphuric Acid Residue. Poplar Soda, 1/2 Hr. at 1750 °C.
The zero digestion, when dissolved in 72 per cent sulphuric acid, leaves a quite complete intercellular lignin residue (Figure 40). The secondary walls dissolve almost completely, and though there is an outer, more resistant portion it does not retain its structure, but disintegrates in sulphuric acid leaving a residue of very fine particles, indicating that it is probably not so heavily lignified as the soft woods. The 15-minute digested sections leave no structural lignin residue (Figure 41). The residues shown in Figures 40 and 41 are both completely soluble in chlorine and 10 per cent ammonium.

2. Microchemical Analysis.

The question of completeness of removal of the intercellular material by soda digestion is no more settled than with sulphite digestion. It would seem that there must be some material or force existing between the cells, holding them together, for even though the intercellular material appears to have been removed as far as its being visible is concerned, the cells do not float apart when digested in soda liquor, but remain in place. If there is a material remaining between the cells after digestion, it is impossible to stain it, and treatments with dilute acids and alkalis,
ammonium oxalate, or lignin solvents did not remove it, or at least did not produce maceration. As in the case of sulphite digested sections, the only solvents producing maceration were cuprammonium hydroxide and sulphuric acid of sufficient strength to swell the secondary walls. Maceration was not prevented by the pressure of the cover glass, for it was shielded up with a strand of glass wool under either side, so that it did not touch the section.

The material removable from the secondary wall by soda liquor digestion is lignous just as with sulphite liquor for the outer portion of the walls of raw sections where this material is evidently concentrated reacts positively to lignin stains, dissolves in lignin solvents, and resists cellulose solvents.

As might be expected from the visual examination, the 15-minute soft wood and 0-minute poplar sections show strong reactions to lignin stains. The Mäule reaction is positive, and removes considerable of the outer portion of the secondary walls, after which the sections including the rays are completely soluble in cuprammonium hydroxide and 72 per cent sulphuric acid. Though the section, treated in this way, is completely soluble in cellulose solvents, the amount of material removed from the secondary walls is considerably less
than in the sulphite or soda digestion. The rays of the 15-minute soda digested sections are resistant to cellulose solvents.

The secondary walls of the one-hour digested sections of spruce and pine stain uniformly green with potassium ferricyanide - ferric chloride but react negatively to the other lignin stains. The whole reaction is negative and, as far as is visibly discernible, removes nothing from the secondary walls, though the sections are somewhat more fragile after this treatment. The sections, except for a few fragments of rays, are completely soluble in cellulose solvents.

The cambial walls are not appreciably attacked by soda digestion and behave much like those digested with sulphite liquor. They are soluble in cellulose solvents even in the 15-minute digested sections. The most extreme digestion (1-hour) does not appreciably attack them visibly, except for the wrinkling effect. They are sufficiently resistant to the cellulose solvents to restrict untoward swelling of the secondary walls, even at one hour of digestion, until those have turned inside out as shown by figures 66, 67, and 68 and described on page 69. In this respect they differentiate soda from sulphite digestion. Whereas in the former they remain relatively resistant to cellulose solvents, even when the digestion
has proceeded to the extent of removing most of the secondary wall lignin, in sulphite sections digested to the same extent they are more easily disrupted by cellulose solvents. The precise cause of this difference is not known, but it would seem to be an important factor in pulps prepared by these two processes.

3. Kraft Digestion

1. Visual Results.

Figures 42, 43, and 44 show the effects of kraft digestion on spruce sections. The action is much like that of soda digestion but proceeded somewhat more rapidly. While soda digestion required 30 minutes to remove completely the intercellular material of spruce and pine, kraft digestion apparently removed it quite completely in 15 minutes. No difference in rate of attack was noticeable between the soda and kraft digestions of poplar. The cambial walls are not wrinkled as much at the shorter digestion times as with the corresponding soda digestions, but appear about the same at 60 minutes. Fifteen-minute digestion removes practically all of the intercellular material with relatively little attack on the secondary walls, and the outer heavily lignified portion, which stains dark blue to the potassium ferricyanide-
Figure 42
Spruce Kraft 20 Micron Cross Section. Digested $\frac{1}{4}$ Hr. at 175°C. Stained with Malachite Green. F Filter.

Figure 43
Spruce Kraft 20 Micron Cross Section. Digested $\frac{1}{2}$ Hr. at 175°C. Stained with Potassium Ferricyanide-Ferric Chloride.
Figure 44
Spruce Kraft 20 Micron Cross Section. Digested 1 Hr. at 175° C. Stained with C Stain.
ferric chloride stain (Figure 42), is still present.

At 30 minutes practically all the material that is removable from the secondary walls by the digestion has been removed and the walls stain uniformly light blue with potassium ferricyanide - ferric chloride. One-hour digestion has relatively little more effect than 30 minutes. As was noted in the soda digestions the cells are more closely packed after the intercellular material has been removed than they were in the sulphite digestions. At all stages of digestion the secondary walls are considerably swollen.

The results of pine digestions are very similar to the spruce (Figures 45, 46, 47). Figure 45 does not show very definitely that the intercellular material has been removed completely, except in the upper center of the photomicrograph. The reason for this will be explained later. The cells are closely packed and even in the 30-minute digestions the intercellular spaces are occupied largely by the cambial walls.

The 15-minute section was stained with potassium ferricyanide - ferric chloride and indicates that, though the intercellular material has been removed, the outer lignified part of the secondary wall has been attacked to only a slight degree. Figure 46 is stained
Figure 45
Pine Kraft 20 Micron Cross Section. Digested $\frac{1}{2}$ Hr. at $175^\circ$ C. Stained with Potassium Ferricyanide - Ferric Chloride.

Figure 46
Pine Kraft 20 Micron Cross Section. Digested $\frac{3}{4}$ Hr. at $175^\circ$ C. Stained with Potassium Ferricyanide - Ferric Chloride. A Filter.
Figure 47
Pine Kraft 20 Micron Cross Section. Digested 1 Hr. at 175° C. Stained with Black E.
with the same stain and would indicate that considerable but not all of this outer portion has been removed.

The 60-minute section is stained with Black S and took the stain quite strongly on both the secondary and cambial walls. The outer portion of the secondary wall has been severely attacked and the cambial walls stand out distinctly, and are wrinkled.

The poplar digestions (figures 46, 49, 50) are very similar to those of soda. The intercellular material is not visibly attacked at just bringing the digestion up to temperature, but is completely removed at 15 minutes. The difference between 15 and 30 minutes digesting is visibly very small.

The 72 per cent sulphuric acid-insoluble residue of spruce (Figure 51) and pine (Figure 52) of the 15-minute digestions are very similar to those of soda. The inner portion of the secondary walls in the lower right of figure 11 have not completely dispersed, and the outer resistant portion is evident. Where the secondary walls have dispersed more completely, the structure has been considerably disrupted. At no place is there any intercellular material evident (Figure 11). The pine lignin residue (Figure 52), shows the presence of a great deal of resistant secondary wall material,
Figure 48
Poplar Kraft 20 Micron Cross Section. Digested 0 Hr. at 175° C. Stained with Potassium Ferricyanide - Ferric Chloride.

Figure 49
Poplar Kraft 20 Micron Cross Section. Digested \( \frac{1}{2} \) Hr. at 175° C. Unstained.
Figure 50
Poplar Kraft 20 Micron Cross Section. Digested 3/4 Hr. at 175°C. Stained with Potassium Ferricyanide - Ferric Chloride.
Figure 51
72% Sulphuric Acid Residue. Spruce Kraft, $\frac{1}{3}$ Hr. at 175°C.

Figure 52
72% Sulphuric Acid Residue. Pine Kraft, $\frac{1}{2}$ Hr. at 175°C.
Figure 53
72% Sulphuric Acid Residue. Poplar Kraft, 0 Hr. at 175° C.
and an incomplete web of intercellular material. This particular field is not exactly typical of these digestions, for the intercellular material was more completely removed than the photomicrograph would lead one to believe. It is an example of what was noted throughout all this work, namely, that there will be certain sections or certain parts of some one section that apparently are not as completely digested as the remainder. It seems most probably that this is due to differences in the wood for the only other possible cause, poor contact with the liquor, seems highly improbable under the circumstances. Figure 53 shows the lignin residue of the poplar kraft section just brought up to 175 degrees C. It is very similar to the corresponding soda residue, though the intercellular web is somewhat less complete.

The 30-minute digestions of spruce and pine and the 15-minute digestion of poplar are completely soluble in 72 per cent sulphuric acid except for very small traces of ray material.

Kraft digestion exhibits the same semi-selective effect on the soft woods as is shown by soda digestion and removes the intercellular material almost completely before very greatly attacking the outer layer of the secondary walls.
2. Microchemical Analysis.

Lignin stains indicate the spruce and pine 15-minute digestions contain considerable lignin in the secondary walls. These sections after treatment with lignin solvents are completely soluble in cellulose solvents. Further, the residue not soluble in cellulose solvents before delignification is completely soluble in chlorine and 10 per cent ammonium hydroxide, leaving little doubt as to its ligneous nature. The 30 and 60-minute digestions apparently remove all lignin for the sections are completely soluble in 72 per cent sulphuric acid. Even the ray material is almost completely soluble.

The cambial walls behave very similarly to those digested by the straight saw liquor. They do not show positive reactions to lignin stains, even in the rawest sections, and are not noticeably attacked in the longest digestions. Even at one hour digestion, they are sufficiently resistant to restrict the swelling of the secondary wall in cellulose solvents, though eventually they are completely soluble in the reagents.

Though the intercellular material of spruce and pine was rendered transparent by 15-minute and 30-minute kraft digestions and described earlier as having been removed, there are some indications that it was
stained with potassium ferricyanide - ferric chloride. This effect is noticeable in Figures 42, 43, 45, and 46. A study of the photomicrographs would serve to indicate merely that the intercellular material was not completely removed. As was stated before, this apparently is not true, since there is no intercellular residue left after the treatment with 72 per cent sulphuric acid. It would seem that the intercellular material has been gelatinized or rendered transparent, but not removed or at least not completely so. This gelatinous material appears not only in the intercellular spaces but in the space between the secondary and combial walls. It seems to stain a very light blue with the above-mentioned stain, while the ungelatinized, untransparent intercellular and peripheral secondary wall materials stain a very deep blue, almost black. All this does not show up as definitely as it might on the photomicrographs for in a single one of these the important factor of variable focus is missing, and further, it is difficult to reproduce on one photographic plate all those distinctions which the eye can detect.

The presence of this gelatinous material was more noticeable in the Kraft than either the sulphite or the soda digestions, and may, if actually existent, be
a factor in the strength of kraft papers. Attempts were made to more definitely show the presence or absence of this gelatinous material by means of India ink staining methods, but the results were no more definite than with the other stains. No further evidence could be obtained with solvents and the results are just as described under sulphite and soda digestions. Cellulose solvents indicated the absence of any lignin in the gelatinized intercellular material, and that it is probably of a carbohydrate nature.

One point that must be kept in mind in this consideration of the presence of a gelatinous material is that at 450 diameters those 20 micron sections are of considerable apparent thickness, that in those soda and kraft sections where the supposed presence of the gelatinous material was most noticeable the cambial walls were considerably mercerized and wrinkled, and since the digested sections are quite fragile it is entirely possible that the apparent presence of this gelatinous material is an optical illusion produced by the wrinkled cambial wall folding over in places to give the effect of a transparent material in the intercellular spaces and between the cambial and secondary walls. The absolute existence of this material is, then, a moot question,
but it is believed that the above facts do furnish a tentative evidence of its existence.

The intercellular lignin is not removed in the poplar zero digestion as shown by lignin stains and solvents which latter remove or dissolve it. The secondary walls stain with lignin stains and gave a positive Wäule test. The 15-minute poplar sections show only very weak lignin reactions to stains and a negative Wäule test, and are completely soluble in cellulosic solvents.

3. Quantitative Yield and Lignin Determination.

The results of the yield and lignin determinations are shown in Table II. The sulphite yields are in the range of commercial digestion, though those of soda and kraft are perhaps a little below what might be considered an average commercial yield. However, they are close enough to allow the photomicrographs of the sections corresponding to these yields to be considered as approximate pictures of what has happened to the wood structure during commercial digestion.

Though the sulphite yields and lignin contents of the 2-hour digested sections are somewhere
near those of commercial practice, the manner in which such sections swell in sulphuric acid would seem to indicate that there is a more drastic action on both the cambial and secondary walls of thin cross sections than there is when whole fibers are digested and the secondary walls are protected by the cambial walls from direct contact with the cooking liquor. Ordinary unbleached sulphite pulps will swell and balloon very distinctly. The thin cross sections after digestion break up as shown in Figure 1 in a manner that would tend to indicate more or less fibrillation and very little typical swelling action like that necessary to produce ballooning.

The lignin contents of these yields are also within or very close to those usually found in unbleached commercially digested woods. A lignin content equal to that found in the sulphite digestions represents a good average of commercial unbleached sulphite pulp, that of the kraft digestions a rather raw pulp yet within the usual commercial range, and that of the soda digestions also a rather raw pulp.

These quantitative lignin results are in good agreement with the conclusions drawn from the microchemical analysis of the digestions, where there was found considerably more 72 per cent sulphuric acid-in-
soluble material in the alkaline digestions than in the sulphite digested sections when both were digested to the point where the intercellular material was just completely removed. These results indicate that kraft digestion not only attacks the intercellular material of soft woods faster than soda liquor but also that by the time the intercellular material is all removed, the former has also removed more of the cell wall lignin (72 per cent sulphuric acid-insoluble) than the latter. A comparison of the 30-minute soda and 15-minute kraft lignin residues verifies this.

With poplar the case is somewhat different and, though kraft and sulphite digestions act at about the same rate on poplar as on pine and spruce, soda digestion apparently removes both the intercellular material and the cell wall lignin at a greater rate than kraft digestion, for though the yield is higher in the case of the soda digested poplar sections, the lignin content is lower than that of the kraft digested poplar sections.

This may be summed up by saying that sulphite digestion does not differentiate very definitely between the intercellular material (lignin) and the outer, heavily lignified portion of the secondary walls, but attacks
both at about the same rate in thin cross sections. Soda and sulphate liquors, on the other hand, do make a differentiation in this respect and remove the intercellular material much more rapidly than they do the outer band of the secondary walls. This does not mean, however, that the lignin percentage in the alkali-digested sections represents quantitatively the total amount of lignin in the cell walls for, though the intercellular lignin is removed more rapidly, there is a simultaneous attack on the secondary walls.

Figure 54 is the cross section of a spruce chip after it has been digested in a commercial Mitscherlich sulphite digester. The section was taken about a quarter of an inch from the end of the chip. In order to section it, it was dehydrated with carbon tetrachloride and imbedded in paraffin, which, after sectioning, was washed out with carbon tetrachloride. This treatment, though it may distort the residual wood structure, should have no other visual effect. The photomicrograph distinctly shows that commercial digesting of this type removes the intercellular material quite completely.

The cambial walls, though not as distinct as
in the digested cross-sections, are plainly evident, but it is difficult to determine whether or not much of the material in the outer layer of the secondary walls has been removed. The probability, however, is that it has been removed to quite a large extent, but that the vigorous embedding manipulations have so distorted the cambial walls that they do not stand out from the secondary walls as direct evidence of this fact, as they do in digested cross-sections. Figure 54 then furnishes further proof that the results observed in digesting thin cross sections approximate those obtained when thicker sections (chips) are digested.
Table II.

Yield and Lignin Determinations

<table>
<thead>
<tr>
<th>Wood</th>
<th>Digesting Liquor</th>
<th>Time</th>
<th>% Yield</th>
<th>% Lignin</th>
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<tr>
<td>Spruce</td>
<td>Sulphite</td>
<td>2 hrs.</td>
<td>45.5</td>
<td>2.7</td>
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<tr>
<td>Pine</td>
<td></td>
<td></td>
<td>47.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Poplar</td>
<td></td>
<td></td>
<td>45.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Spruce</td>
<td>Soda</td>
<td>30 min.</td>
<td>47.0</td>
<td>10.5</td>
</tr>
<tr>
<td>Pine</td>
<td></td>
<td></td>
<td>45.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Poplar</td>
<td></td>
<td></td>
<td>43.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Spruce</td>
<td>Kraft</td>
<td>15 min.</td>
<td>46.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Pine</td>
<td></td>
<td></td>
<td>44.6</td>
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</tr>
<tr>
<td>Poplar</td>
<td></td>
<td></td>
<td>39.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Figure 54

E. Bleaching Action.

1. Action on Previously Digested Cross Sections.

The study of bleaching was carried out almost entirely on spruce, since more natural details are easily visible with this than the other woods. Figures 55 and 56 show the effects of bleaching on a sulphite section such as shown in Figure 7. The section in Figure 55 has been bleached 4 hours in calcium hypochlorite and that in Figure 56 has been bleached with chlorine and sodium hydroxide until the dark brown color produced when the chlorinated section is washed with alkali no longer appears. The intercellular material has been removed by both bleachings quite as completely as continued digestion would remove it, but the secondary wall has not been attacked quite so drastically as is the case with digestion; the bleached sections have had all the lignin removed from them, for they do not stain with lignin stains, do stain with cellulose stains, show negative Mäule reactions and are completely soluble in cuprammonium hydroxide and 72 per cent sulphuric acid. Upon dissolving in the cellulose solvents, even when the action is caused to proceed slowly, there is no evidence of any resistant outer layer in the secondary wall.
Figure 55
20 Micron Spruce Cross Section Digested in Sulphite Liquor for ½ Hr. at 150° C. and Bleached with Calcium Hypochlorite for 4 Hrs.

Figure 56
20 Micron Spruce Cross Section Digested in Sulphite Liquor for ½ Hr. at 150° C. and Bleached with Chlorine and Sodium Hydroxide.
In effect, then, the action of both these bleaching agents is to delignify with somewhat less visible attack on the secondary walls than that produced by the action of sulphite digestion.

The effects of bleaching on sections digested in either soda or kraft liquor are very much the same as those observed on sulphite digested sections. There is, however, a difference in the way the chlorine-bleached and calcium hypochlorite-bleached sections dissolve in 72 per cent sulphuric acid when they have been subjected to extremely drastic treatment with these chemicals.

Figure 57 is a spruce kraft section that has been digested for 30 minutes and bleached over night in calcium hypochlorite liquor. Figure 58 is a similarly digested spruce kraft section that has been bleached with chlorine and ammonium hydroxide with twelve treatments of each of these chemicals, each treatment being very thorough. The photomicrographs were taken as the two sections were dissolving in 72 per cent sulphuric acid. In the chlorine-bleached section the cambial wall is resistant enough to restrict the swelling secondary wall, while in the calcium hypochlorite-bleached section the cambial wall has evidently been dissolved almost immediately, and the secondary walls swell outward.
Figure 57
20 Micron Spruce Cross Section Digested in Kraft Liquor for \( \frac{1}{2} \) Hr. at 175° C. and Bleached with Ca\(\text{cium Hypochlorite for 4 Hrs.}, \) Dissolving in Sulphuric Acid.

Figure 58
20 Micron Spruce Cross Section Digested in Kraft Liquor for \( \frac{1}{2} \) Hr. at 175° C. and Bleached with Chlorine and Sodium Hydroxide, Dissolving in Sulphuric Acid.
Both of these sections are finally completely soluble in the acid. Though the chlorine bleach was carried out using an ammonium hydroxide wash, sodium hydroxide shows the same results. All this would seem to indicate that calcium hypochlorite over-bleaching has a more harmful effect on the cambial wall than chlorine over-bleaching, which might lead to the conclusion that, even though swelling does not show it, under less drastic conditions in order to produce the same degree of lignin removal, hypochlorite bleaching would tend to weaken the cambial wall more than chlorine bleaching. It is a quite commonly accepted fact that chlorine bleaching leaves a pulp with greater strength properties than hypochlorite bleaching, so that, in the light of the above facts, the loss in strength due to this latter type of bleaching may be due to its weakening effect on the cambial walls.


In order to make the study of bleaching more complete, sections of alcohol-benzene extracted but undigested spruce were treated with calcium hypochlorite, chlorine and sodium hydroxide, chlorine and 10 per cent
ammonium hydroxide chlorine and sodium sulphite, and
chlorine and alcohol-pyridine. The chlorine treatments
were continued until the characteristic colors produced
on adding the washing solutions no longer appeared.
The hypochlorite treatment was continued for three days.

Calcium hypochlorite affects the wood in a
manner which is distinctly different from any of the
chlorination treatments, (Figures 60, 61, 62, 63).
Whereas, in all cases, the latter removed the inter-
cellular material to a greater or less degree, and
caused the cambial walls of adjacent cells to stand
apart, appearing distinctly as being a unit of struc-
ture of each individual cell, the hypochlorite (Figure
69), though it apparently removes more of the outer
layer of the secondary wall than any of the chlorine
bleaches except perhaps the chlorine-sodium hydroxide,
does not remove the material that is between adjacent
cambial walls, and apparently even draws them closer
together in the corners where in the wood (Figure 4)
they stand apart, and causes them to have the appearance
of a unit intercellular structure. These walls are re-
solved or separated so that the section then has very
much the appearance of Figure 61, by treatment with 10
Chlorine and Sodium Sulphite. Unstained.

Figure 62

20 Micron Spruce Wood Cross Section Bleached with
Ammonium Hydroxide. Unstained.
Figure 59
20 Micron Spruce Wood Cross Section Bleached in Calcium Hypochlorite for 3 Days. Stained with Phloroglucinol.

Figure 60
20 Micron Spruce Wood Cross Section Bleached in Chlorine and Sodium Hydroxide. Stained with C Stain.
Figure 63
20 Micron Spruce Wood Cross Section Bleached with Chlorine and Alcohol - Pyridine. Stained with Black W.
per cent hydrochloric acid and 10 per cent ammonium hydroxide, 5 per cent ammonium oxalate, and 2 per cent sodium hydroxide. These solvents apparently removed the binding material between the walls completely. Hot water removed this material partially and cuprammonium hydroxide and 72 per cent sulphuric acid removed it completely, as the first stage in dissolving the sections which were completely soluble in those cellulose solvents. 10 per cent hydrochloric acid or 10 per cent ammonium hydroxide alone and cold water had not the slightest effect. This evidence would seem to indicate that this binding material is hemicellulosic in nature and consequently it appears that calcium hypochlorite bleach liquor does not dissolve the hemicelluloses as completely as do any of the chlorine bleaching treatments.

Methenium red does not stain this hypochlorite-bleached section at all. Potassium ferricyanide - ferric chloride stains the intercellular material and cambial walls, as well as the secondary walls, a pale green. C stain and phloroglucinol stain the binding intercellular material a fairly deep yellow. This is most plainly seen in the corners, where three or more cambial walls meet. The section shown in Figure 59 was stained with phloroglucinol; in this, these deeply stained
corners are very definitely shown. The cell walls stained only a very pale yellow with this stain. It is not believed that the potassium ferricyanide - ferric chloride stain reaction is particularly significant, since it will stain even a quite thoroughly digested and bleached section this same pale green color. It has been noted in some of the earlier work that on sections where digestion has removed a considerable portion of the secondary wall lignin the remaining portion of wall containing non-cellulosic material gives a yellow color with phloroglucinol. On the basis of this and the above facts, it would seem that the secondary walls also contain material similar to this binding intercellular material. There is the chance, also, that this binding material may be related to the gelatinous material noted in the kraft digestions.

As previously pointed out, all the chlorination treatments apparently remove the intercellular material and, though the sections are very fragile, they are not macerated. At least, the cellular walls do not remain fused together, and, if there is any intercellular material left, it was not possible to stain it.

Chlorine and 4 per cent sodium hydroxide produces a section that looks much like a soda kraft-digested section. It does not stain with lignin stains, stains
blue with iodine, and is completely soluble in cellulose solvents with a type of swelling action similar to that of a chlorine-bleached kraft-digested section. C stain colors the secondary and cambial walls a pale violet color.

The chlorine and ammonium hydroxide-bleached section is very much like the chlorine and sodium hydroxide-bleached one, but the walls are not wrinkled or mercerized like those of the latter. The binding and intercellular material has apparently been removed and the cell walls attacked. Lignin stains react negatively, iodine gives a blue color, and the sections are completely soluble in cellulose solvents. The C stain color is a violet, similar to that of the chlorine sodium hydroxide-bleached sections, and both cambial and secondary walls are colored the same. According to Kerr and Bailey (2) chlorine and ammonium hydroxide is simply a delignifying treatment and does not dissolve polyuronides. However, as was pointed out before, it apparently does have a more drastic effect on the middle lamella than hypochlorite liquor. It would seem then, that, if the chlorine and ammonium hydroxide treatment does not remove the polyuronides, some of the other hemicellulosic materials are removed by this treatment and that they are
responsible to a large degree for the binding together of the cambial walls in a hypochlorite-treated section. In any case it may be said that hemicellulosic material is probably responsible for this binding together of the cambial walls of the hypochlorite-treated wood sections and that hypochlorite treatment has a lesser solvent power for this material than chlorine and ammonium hydroxide.

The chlorine and sodium sulphite-bleached or Cross and Bevan cellulose section looks much like that bleached with chlorine and ammonium hydroxide. The intercellular material has been removed completely, and the cell wall attacked, though not as drastically as the hypochlorite, chlorine and sodium hydroxide or chlorine and ammonium hydroxide attack this portion. That is, the Cross and Bevan treatment does not remove as much of the secondary wall as the other treatments. However, this section is completely soluble in cellulose solvents, and, according to the chloro-lucinol reaction, is completely delignified. C stain gives no color with the Cross and Bevan cellulose.

The holocellulose looks very much like the Cross and Bevan cellulose, but there is apparently some of the intercellular material remaining, even though the
treatment was carried out until there was no more color reaction. It may be that more prolonged treatment, since it is a very mild one, would remove this intercellular material more completely. The residual intercellular material is transparent but does not stain, and is not affected by any but cellulose solvents of swelling strength. The outer portion of the secondary walls is attacked to about the same extent as those of the Cross and Bevan cellulose. Iodine stains holocellulose cell walls blue. C stain colors the inner portions of the secondary walls very light yellow, and the outer portions and cambial walls a deeper yellow.

In general, then, it may be said that all these bleaching treatments completely remove the lignin (72 per cent sulphuric acid-insoluble material) from spruce wood; that the Cross and Bevan and holocellulose treatments are the mildest; the chlorine-ammonium hydroxide and chlorine-sodium hydroxide treatments are very similar, except for the mercerization caused by sodium hydroxide, and remove more of the secondary wall material than the holocellulose and Cross and Bevan cellulose treatments; and that, though the calcium hypochlorite probably attacks the cell wall most strongly, it does not remove the intercellular material and leaves the
cambial walls fused together. All these bleaching reagents produce very fragile sections from both undigested and digested cross sections but not complete maceration, indicating that, though the intercellular material is apparently removed as far as is visibly discernable in all but the hypochlorite sections, possibly there is some material remaining between the cells which, though gelatinized and transparent, holds them in place. Bailey (63) has isolated the middle lamella of Douglas fir mechanically and found about 14 per cent of pentosans in it. It may be that this material is not completely removed by the bleaching treatments, especially in the undigested hypochlorite-treated wood and that it with other hemicellulosic material prevents the maceration of these sections.

The visual and microchemical results of the digestions and bleaching treatments on the thin cross sections are largely in support of the picture of wood structure presented on the basis of staining reactions at the beginning of this discussion. In cross section the woods consist of cells composed of a secondary wall, which in soft woods consists of two distinct zones, a thin primary wall and an isotropic material.
The secondary walls composing the bulk of the fibers are mostly cellulose and lignin, though there are no doubt other constituents present; this work has to do primarily with the former two. The lignin is definitely concentrated in the outer layer, as is shown by its staining reactions, its solubility in lignin solvents and cooking and bleach liquors. The inner layer is mostly cellulose, since it resists lignin solvents and in digested and delignified sections stains blue with iodine and dissolves in cuprammonium hydroxide and 72 per cent sulphuric acid. The secondary wall as a whole is isotropic in cross section and anisotropic in longitudinal section.

The primary walls, identified as the cambial walls of Kerr and Bailey (2), though probably containing other materials in the untreated wood, are mainly cellulose. They resist the action of lignin solvents, cooking liquor and bleach liquor. In delignified and digested sections they are completely soluble in 72 per cent sulphuric acid and cuprammonium hydroxide and stain blue with iodine. They are strongly anisotropic in cross section and isotropic in longitudinal view. On the radial ends of the soft wood cells these walls are separated from the cellulosic inner portion of the secondary
wall by heavily lignified bands of secondary wall material. These bands of the secondary walls are mainly restricted to the radial ends, and on the tangential sides the cambial walls are in direct contact with the cellulosic portions of the secondary walls; apparently the two are very intimately joined at these two points, for they are not separated even by prolonged treatments of cooking liquors, bleaching agents, cellulose or lignin solvents.

The intercellular material is composed mainly of lignin, as is indicated from its stain reactions, and solubilities. It is apparently not completely soluble in lignin solvents, and delignified sections could be macerated only with cellulose solvents. From this fact it would seem that the portion of the intercellular material not soluble in lignin solvents consists of hemicelluloses.

F. Fiber Structure.

This section will deal with the so-called grosser units of fiber structure and the properties of fibers related to them. No attempt has been made to study the finer fibril and micelle structure, the present work being confined to the more easily visible units.
It has been definitely shown that the cambial walls not only exist in mature wood cells as definite structural units, but that none of the commercial digesting liquors or bleaching agents remove them, and that they are definitely a part of a pulp fiber. There is a heavily lignified outer band in the secondary walls of wood fibers, which it is attacked and may be completely removed by commercial cooking liquors and, though not so completely removed, is delignified by commercial bleaching materials.

It has frequently been mentioned that the cambial walls in cross section are strongly birefringent. Figure 64 is a cross section of a 2-hour spruce sulphite digestion in polarized light between crossed nicols. The very bright bands are the cambial walls; the shadowy outlines of the dark secondary walls can also be seen.

The 10-hour poplar sulphite section (Figure 65) shows unmistakably the cambial walls of poplar. They, too, are birefringent. Poplar exhibits the same swelling behavior as spruce and pine.

It has been more or less assumed up to now that the cambial walls are the units that restrict the outward swelling of the secondary walls when these are treated with 72 per cent sulphuric acid and cuprammonium
Figure 64
20 Micron Spruce Cross Section Digested in Sulphite Liquor for 2 Hrs. at 150° C. Stained with Congo Red. Taken in Polarized Light between Crossed Nicols.

Figure 65
20 Micron Poplar Cross Section Digested in Sulphite Liquor for 10 Hrs. at 150° C.
hydroxide. There is, of course, the possibility that the outer lignified band of the secondary wall causes, or at least is partially responsible for this restricting action. This possibility, however, is eliminated when the restricting action is observed even in sections where this band has been completely removed, as it has been in one-hour kraft digestions. This type of swelling occurs even in a one-hour kraft digested section that has been bleached with chlorine and ammonium hydroxide. The swelling action was followed on a section treated in this manner in polarized light, and, as long as the cells remained upright, even though the secondary walls were oozing out the ends, the brightly refringent cambial walls remained intact with unchanged perimeter, though they were rounded out by the swelling secondary wall.

Figures 66, 67, and 68 show three steps in the swelling of a one-hour spruce kraft digested cell with cuprammonium hydroxide. In Figure 66 the secondary wall has swelled, filling the lumen, and is just in the process of oozing out the end of the restricting cambial wall. Another cell has already turned inside out, and the collapsed cambial wall is adhering to
Figure 68
Cross Section of Single Cell Turned Inside Out and
Greatly Swelled in Cuprammonium Hydroxide. Spruce
Kraft 20 Micron Cross Section Digested for 1 Hr.
at 175° C.
Figure 66
Cross Section of Single Cell Swelling in Cuprammonium Hydroxide. Spruce Kraft 20 Micron Cross Section Digested for 1 Hr. at 175° C.

Figure 67
Cross Section of Single Cell Turned Inside Out by Swelling in Cuprammonium Hydroxide. Spruce Kraft 20 Micron Cross Section Digested for 1 Hr. at 175° C.
one side of the greatly swelled secondary wall. Figure 67 shows the cell first described just after it has turned inside out. The secondary wall is greatly swelled and is outside the cambial wall which is still adhering to the secondary wall all the way around. Figure 68 shows the same cell after further treatment with cuprammonia. The secondary wall has now broken loose from the cambial wall on all but two points at opposite sides and has become greatly thinned and elongated. This thinning and elongation of the secondary wall continues until it disappears into solution along with the cambial wall. Both are completely soluble. This series of photomicrographs, and the fact that the cambial walls restrict swelling, shows one important difference between these and the secondary walls. The former do not swell, and the latter do swell in cellulose solvents, even though, according to all evidence of stains and solvents, both are practically 100 per cent cellulose. It is probable however, that there are small amounts of pentoses present, for digestion probably does not remove all the hemicellulosic material.

In order to further study these characteristics, photomicrographs, figures 69 and 70, were taken.
Figure 69 shows two summer wood cells. The one has been stretched with the micromanipulator by inserting the tools in the lumen and spreading them. The cambial wall breaks and is seen clinging to the secondary wall which stretches like a rubber band but does not snap back when the tension is released. Here again it is shown that the secondary wall and cambial wall differ, in that the secondary wall may be greatly stretched mechanically, while the cambial wall cannot. The secondary wall will not elongate indefinitely, but in Figure 69 is stretched nearly to the breaking point. Both cells in Figure 69 were originally the same size. Figure 70 shows the same cell as Figure 69 in polarized light. The bright spot is the position of the cambial wall residue.

Figures 71 and 72 are sections of fibers digested as thick cross sections in kraft liquor for one hour. The fiber section (Figure 71) is stained with C stain and shows the cambial wall in longitudinal view as the lighter staining band on each side. The two black bands just inside these are the secondary walls and the lighter gray strip in the center is the lumen. Figure 72 is a similar fiber section in polarized light. The secondary wall is bright, though not uniformly so, and the cambial wall, which is not birefringent in
Figure 69
Cross Section of Two Single Cells, One Stretched with Micromanipulator. Spruce Kraft 20 Micron Section Digested for 1 Hr. at 175°C.

Figure 70
Same as Figure 69 but Taken in Polarized Light.
Figure 71
Section of a Fiber from a Spruce Kraft 200 Micron Cross Section Digested for 1 Hr. at 175° C. Stained with C. Stain.

Figure 72
Section of a Fiber from a Spruce Kraft 200 Micron Cross Section Digested for 1 Hr. at 175° C. Taken in Polarized Light.
longitudinal view, and which in ordinary light shows up just as in Figure 71, is not visible. The significance of the nonuniformity of the birefringence of the secondary walls in Figure 72 is not definitely understood.

Figure 73 is a fiber section, like that in Figure 71, swelling in cuprammonium hydroxide. The secondary wall is coming out at the one end of the cambial wall, which has been rolled back and is wrinkled or folded. Figure 74 is the same in polarized light. In this case the swollen crown is not visible. There is also a piece of the cambial wall peeling back, like that shown in Figure 75; this also is not visible in polarized light.

In Figure 75 the cambial wall ruptured and the secondary wall is swelling outward where there is no restricting cambial wall. In some cases the cambial wall does not rupture, as shown in Figure 75 and, as the secondary wall oozes out the end, it rolls back and gradually dissolves, until the whole section is soluble. As long as the cambial wall behaves like this and remains intact, there is no swelling, except at the end. In other cases, as shown in Figure 75, the cambial wall splits longitudinally and rolls back, and the secondary walls swell laterally over the region where this has happened. If the cambial wall splits or ruptures at
Figure 73
Section of a Fiber Swelling in Cuprammonium Hydroxide. From a Spruce Kraft 200 Micron Cross Section Digested for 1 hr. at 175° C.

Figure 74
Section of a Fiber Swelling in Cuprammonium Hydroxide. Taken in Polarized Light. From a Spruce Kraft 200 Micron Cross Section Digested for 1 hr. at 175° C.
Figure 75
Section of a Fiber Swelling in Cuprammonium Hydroxide. From a Spruce Kraft 200 Micron Cross Section Digested for 1 Hr. at 175° C.

Figure 76
Section of a Fiber Swelling in Cuprammonium Hydroxide. From a Spruce Kraft 400 Micron Cross Section Digested for 1 Hr. at 175° C.
some intermediate point, the secondary wall swells laterally at this point and folds the cambial wall back in both directions; when this occurs at two different points the cambial wall may be folded back from these points until a constriction like that in Figure 76 is formed. When two such constrictions form close enough together, the swollen secondary wall between them shows a balloon-like appearance. This is the familiar balloon-type swelling, in which the cambial wall has been definitely identified as the cause of the constrictions. The wrinkled cambial wall shows very clearly to the right of the balloon in Figure 76.

Up to this point all the evidence presented would seem to indicate that the cambial wall is simply a continuous sheath encasing the secondary wall. However in some cases when the 400 micron craft sections were treated with cuprammonium hydroxide instead of folding back or splitting as previously described, the cambial wall untwists from the secondary wall and in this untwisted form looks very similar to the lignin sheath of Carpenter and Lewis (12). However, though the lignin sheath of these two workers was insoluble in 72 per cent sulphuric acid as well as in cuprammonium hydroxide, this untwisted form of what was apparently the cambial wall
is completely and quickly soluble in cuprammonium hydroxide. This is as might be expected, since it was observed on a one-hour kraft digested section, which sections are completely soluble in 72 per cent sulphuric acid. Hence, these two structures are apparently not identical. The secondary walls of the fiber sections, in which the cambial walls apparently behave in this manner, swelled laterally in cuprammonium hydroxide with no evidence of constrictions. It is believed that this behavior of the cambial walls furnishes some evidence that they may not be a continuous sheath of cellulose, but that they consist of laterally wrapped fibrils or bands, closely wound and cemented together with some non-cellulosic material so as to form the appearance of a continuous sheath.

From the above described behavior of the cambial walls it would seem that the outer, laterally wrapped fibrils of Ritter, Lewis, A. J. Bailey, and others may be associated with these walls, and in fact, these walls may be composed of such fibrils. If this be the case and with the fact in mind that these walls are definitely not soluble or disrupted even on very drastic digestion with any of the cooking liquors or bleaching liquors, Lüdtke's claim that these lateral outer wrappings are
merely a portion of the middle lamella or intercellular material, which is soluble in thoroughly digested fibers, seems unfounded.

As far as this work is concerned, there was no evidence whatsoever of fibrillation in the secondary wall; this would seem to indicate that the secondary wall has a structure similar to that attributed to it by Kerr and Bailey (2). However, it must be remembered that, before fibrillation can be observed in these walls, they must be subjected to very drastic acid and oxidation treatments and none of these sections were so treated. The results of such treatment on these sections would probably be very interesting.

In all of the swelling work on 20 micron cross sections the secondary walls always oozed out of one or the other end of the cambial wall but never both. Now, if such a thing as Lüdtke's cross elements exists, it would seem that either they exist only in the secondary walls and do not connect with the cambial walls, or that, if they do connect with the cambial walls in at least a few cases, the secondary walls should have oozed out of both ends of the cambial walls simultaneously. Consequently this evidence must be taken as opposing the existence of such a unit as the cross element, or at least
that it is probably confined to the secondary wall and
does not connect with the cambial walls.

The manner in which kraft and sulphite diges-
ted sections dissolve in 72 per cent sulphuric acid is
distinctive. The cambial walls of kraft sections (Figure
77) restrict outward swelling of the secondary walls
until both dissolve, and the secondary walls swell and
fill the lumen, tending to ooze out of the ends of the
restricting cambial walls. The cambial walls of 2-hour
sulphite sections (Figure 78) dissolve quickly and allow
the pieces of secondary walls, which swell very little
but simply break up, to float free. Apparently the acid
treatment of sulphite digestion makes the cambial walls
less resistant to and the secondary walls less tenacious
in 72 per cent sulphuric acid. Dilute cuprammonium
hydroxide causes the 2-hour sulphite sections to swell
more like the kraft section (Figure 77).

At none of the various stages of the digestions
is there any definite evidence of cleavage of the inter-
cellular material either in the center or at the cambial
walls like that described by Bailey (57). Neither is there
any evidence of a resistant strap-like structure (58),
nor lignin sheath (13). This does not mean that there
are no such structures but simply that these treatments
of the wood did not show them. As the digestion progres-
Figure 77
20 Micron Spruce Cross Section Digested in Kraft Liquor for 1 Hr. at 175°C. Dissolving in Sulphuric Acid.

Figure 78
20 Micron Spruce Cross Section Digested in Sulphite Liquor for 2 Hrs. at 150°C. Dissolving in Sulphuric Acid.
as the intercellular material first dissolves at the thin places between the pieces in the corners. The pieces in the corners apparently dissolve more slowly or at least are the last portions to dissolve or solvate. From these experiments, however, it can hardly be concluded that these pieces are more resistant material than the other parts of the intercellular material but that their slower solution is most probably due merely to the fact that they are greater masses of material exposing relatively less surface to the attack of the cooking liquor.

G. C stain Reactions.

The color charts (pages 136-40) show the approximate Ridgway colors produced by C stain on the variously treated sections. These colors are not to be taken as exact matches of the colors produced on the fiber walls but as approximations, to give a clearer conception of the colors actually observed than could be given by verbal description. The colors were all obtained on spruce sections.

Up to 2 hours' digestion C stain colors sulphite fiber walls a uniform yellow; after 2 hours' digestion the inner portions of the secondary walls
and cambial walls are colorless, and the outer portions of the secondary walls are yellow. Hence, at this point C stain for the first time distinguishes non-uniformity of lignification in the secondary walls. C stain does not color the 10-hour sulphite digestions at all.

The secondary walls of 30-minute sulphite digested sections, bleached with either calcium hypochlorite or chlorine, are colorless but the cambial walls stain a very light pink in C stain. The cambial walls then would seem to be distinguished from the secondary walls by C stain, the former being responsible, to a great degree at least, for the pink color of bleached sulphite pulp.

The unbleached kraft and soda digestions pass from the yellows in the rower sections over to lavenders and yellow brown colors with the more thoroughly digested sections. The secondary walls in the well-cooked sections are yellow brown and the cambial walls tend more toward the violets and lavenders. Here again the C stain reactions are a little different on two walls.

Bleaching the alkaline-digested sections has a slight but definite effect on the C stain reactions. The greatest effect is in the secondary walls, where the color passes from the yellow browns to the blue and red.
browns. The change in the cambial walls is somewhat less marked except in the raw sections, where the change is from yellow to violet. The trend of change in the more thoroughly digested sections is mostly a lightening of the color. It would appear that the bleached soda cambial walls are considerably greener in color than those of the kraft-digested sections. Different types of bleaching chemicals produce slightly different shades but the general trend is the same if the bleaching is not carried out for too long a time. A 24-hour calcium hypochlorite bleach produces a section that will not stain at all with C stain, though extended bleaching with chlorine and ammonium hydroxide or chlorine and sodium hydroxide does not materially alter the violet colors produced with a more moderate treatment.

A 48-hour calcium hypochlorite bleach treatment of a commercial unbleached kraft pulp did not remove its power to be stained, though the color was somewhat redder than that produced by a 4-hour bleach. This same pulp when bleached with chlorine and 4 per cent sodium hydroxide produced the typical bleached kraft violet shade. When further treated with 10 per cent sodium hydroxide, the C stain color was
somewhat more red.

Calcium hypochlorite, chlorine and ammonium hydroxide, and chlorine and sodium hydroxide bleachings on undigested but extracted wood all gave sections staining lavender or violet, whereas the unbleached wood stains yellow.

It would seem to follow from these experiments that sulphite digestion does not produce any characteristic change in the fiber that may be detected by C stain; it simply removes the material already present in the wood, that produces the yellow color with C stain. On the other hand, alkaline digestion does seem to produce a characteristic change in the fiber which is detected by C stain and which bleaching apparently does not materially alter, except in the case of a long hypochlorite bleach.

The three bleaching mediums, calcium hypochlorite, chlorine and ammonium hydroxide, and chlorine and sodium hydroxide, all produce a characteristic change in the fibers which is detected by C stain. In part the C stain reactions of the bleached, undigested wood are quite similar to those of the alkali-digested bleached sections. The cambial walls of the former are very nearly the same shade but a little lighter in color than those
of the latter. The secondary walls in the two cases are considerably different. Apparently, then, alkaline digestion has a somewhat different effect on the secondary walls, at least as far as C staining is concerned, than it does on the cambial walls. Here, again, it is shown that the cambial and secondary walls, though both apparently cellulose, possess somewhat different properties.

Calcium hypochlorite removed the C stain characteristics from soda and kraft digested sections after 17 hours' bleaching. Undigested but extracted wood sections even though they were bleached for three days still retained definite C staining characteristics. It appears, then, that alkaline digestion so alters the constituents in the fibers, that are responsible for the C stain reactions, that these constituents are more easily soluble in or removed by calcium hypochlorite after than before alkaline digestion.

As was pointed out before, Cross and Sevan cellulose does not stain at all with C stain and in holocellulose only the outer portions of the secondary walls stain a light yellow.

Apparently C staining reactions cannot be correlated with the removal of lignin from the wood as
attested to by the wide variety of colors obtained when the lignin is removed by different agents. Since the alkaline digestions and bleaching agents, where relatively strong alkalines are used, produce fiber walls with staining characteristics distinctly different from those of the untreated wood, it seems reasonable to conclude, at least tentatively, that the alkalies are the materials which produce the characteristic changes that are responsible for the C stain reaction in the fiber walls.

Further, since sulphite digestion and chlorine bleaching treatments, where no or relatively weak alkalies are used, produce sections which do not stain at all with C stain, it would seem that the action of these chemicals is merely to remove the materials which, present in the untreated wood, produce the yellow C stain reaction.

The action of sulphite digestion apparently not only merely removes the power of C stain to color the fiber walls but, in walls where this power is not completely removed, and which still stain yellow, the materials responsible for the C stain colors must have been altered. Both hypochlorite and chlorine bleaching, instead of producing fiber walls which show the color reactions produced when undigested sections are bleached
with these reagents remove entirely the power of the secondary walls of the digested sections to stain, and cause the cambial walls to stain a weak pink. The action of alkaline digestion is apparently different, and not only does C stain produce a characteristic color even in thoroughly digested alkaline sections but only hypochlorite bleaching seems able to remove the C staining power from the secondary as well as the cambial walls. Chlorine bleaching alters the colors produced but the walls still stain.

These observations, in general, would seem to confirm the value of C stain for use in fiber analysis for it is apparently quite sensitive in detecting even rather small differences in treatment given to wood and this covers a wide variety of treatments. The two major units of fiber structure, secondary walls and cambial walls, behave differently toward C stain; the colors observed on pulp fibers is a composite of the different colors produced on these two units of structure. Usually the outer layer of the fiber, where the view is through more than two thicknesses of the secondary wall, is more nearly the color of the secondary wall and the lumen, the color of the secondary wall is very markedly modified by the color of the cambial wall.
COLOR CHART I.

C Stain Colors on Digested Spruce Wood Cross Sections

<table>
<thead>
<tr>
<th>Secondary Wall</th>
<th>Cambial Wall</th>
<th>Intercellular Material</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Untreated Wood

Sulphite Digested
15 Minutes

10 hours  Colorless  Colorless  Removed

Soda Digested

30 Minutes  Removed

1 Hour  Removed

Kraft Digested

30 Minutes  Removed

1 Hour  Removed
COLOR CHART II.

C Stain Colors on Digested-Bleached Spruce Wood Cross Sections

<table>
<thead>
<tr>
<th></th>
<th>Secondary Wall</th>
<th>Cambial Wall</th>
<th>Intercellular Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphite Digested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH Bleached</td>
<td>Colorless</td>
<td>Very pale</td>
<td>Removed pink</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH Bleached</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Calcium hypochlorite Bleached (24 Hours)</td>
<td>Colorless</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Soda Digested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH Bleached</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cl-NaOH Bleached</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Calcium hypochlorite Bleached (24 Hours)</td>
<td>Colorless</td>
<td>Colorless</td>
<td></td>
</tr>
<tr>
<td>Kraft Digested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH Bleached</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cl-NaOH Bleached</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Calcium hypochlorite Bleached (24 Hours)</td>
<td>Colorless</td>
<td>Colorless</td>
<td></td>
</tr>
</tbody>
</table>
**COLOR CHART III.**

**C Stain Colors on Undigested Bleached Spruce Wood Cross Sections**

<table>
<thead>
<tr>
<th>Secondary Wall</th>
<th>Cambial Wall</th>
<th>Intercellular Material</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Calcium Hypochlorite Bleach (3 days)**
- **Calcium Hypochlorite Bleach. Washed with 10% HCl-10% NH OH**
- **Cl-NaOH Bleached**
- **Cl-NH OH Bleached**
- **Cl-Ma SO Bleached**
- **Cl-Alcohol-Pyridine Bleached**
CHAPTER V

DISCUSSION OF FIBER STRUCTURE AND ITS POSSIBLE SIGNIFICANCE TO SOME COMMERCIAL OPERATIONS
CHAPTER V.

DISCUSSION OF FIBER STRUCTURE AND ITS POSSIBLE SIGNIFICANCE TO SOME COMMERCIAL OPERATIONS

The following section is not intended to be direct conclusion from experimental fact but merely to be an hypothetical discussion of some of the intimations that the experimental facts might imply.

The presence of the cambial walls with their somewhat distinctive properties as a unit of fiber structure may serve to explain and support some of the existing evidence on beating action (commercial hydration). As was shown these walls swell very little in the strongest swelling agents, and it might be concluded from this behavior that they hydrate and gelatinize relatively little on beating. One function of beating, then, should be to break these walls down, or possibly to fibrillate them so that the more gelatinous and consequently more easily hydrosoluble secondary walls may be exposed to contact with each other and set to form cohesive bonds between fibers when they are felted in a sheet of paper. It is realized that sheet strength has been correlated with other factors but this does not eliminate the possibility of this factor playing an important part in the phenomenon of sheet strength.
The cambial walls of kraft digested sections are very much wrinkled and the friction between two fibers with such walls might be supposed to be relatively great in comparison with the smooth surfaces apparently presented by sulphite digested fibers. This might account to some extent for the well known strength inherent in kraft pulp.

The processing of a glassine pulp may involve the almost complete removal of the cambial walls from the fibers so that the secondary walls may form an almost continuous sheet of gelatinous material. The tiny white specks observed in uncolored glassines might possibly be the remnants of the cambial walls.

The strength and rigidity of individual fibers may be dependent to a great extent on the cambial walls for they are relatively less gelatinous than the secondary walls and are not easily stretched out of shape without breaking while the secondary walls are more or less plastic and may be molded and distorted permanently from their original shape and size without breaking, as stretching with the micro-manipulator shows.

Sheet strength might also be tied up with the gelatinous inter and intracellular material noted in the digested sections. Even when commercially cooked
chips are macerated this material may clinging to the walls and be the natural pentosan or hemicellulosic material that has been quite definitely correlated with the development of sheet strength on beating. (62). There is no doubt that there is much pentosan in the intercellular material (63), and there probably is in the outer layer of the secondary wall too. The fact that this gelatinous material is more evident and apparently present in greater abundance in the kraft digested than sulphite digested sections might be another cause for the superior strength of kraft pulps.

Digestion has been found to attack and de-lignify the intercellular material much faster than it does the outer ligneous layer of the secondary wall. There is considerable lignin in this layer even after the intercellular material has been rendered completely soluble in 72 per cent sulphuric acid. A comparison of the cooking progress photomicrographs with the quantitative yield and lignin results shows this very distinctly. This effect might reasonably be supposed to be accentuated in whole fibers where the liquor contact with the secondary walls is probably not nearly so good as it is with the thin cross sections. These considerations lead to the conclusion that average commercial pulping prob-
ably delignifies the intercellular material completely and that what lignin there is left in the pulp after digestion is in the secondary walls. Hence the function of commercial pulping may be described as a removal or delignification of the intercellular material with some accompanying attack on the secondary walls, more or less depending on the cooking schedule and the digesting liquor used, while to bleaching may be ascribed the function of removing or dissolving the secondary wall lignin left after digestion.

Reversion in color of bleached pulp might conceivably be tied up with these cambial walls since it seems that bleaching action must consist of the penetration through these walls of the bleaching liquor, dissolving of the lignin and the diffusing out of the colored bleach residue. These diffusing actions might be relatively slow and unless sufficient time is allowed for outward diffusion it might be incomplete, leaving the colored residues inside the cambial walls. Of course, the penetration of the cambial walls may be facilitated by the presence of the pits which no doubt are related quite definitely with these walls (see Figure 10).

Another consideration is the evidence of the presence of fibrillar structure in the cambial walls which, if
true, may admit of the presence of zones of noncellulosic material from which the lignin or other cementing materials might have been removed, providing another point of entry rather than direct diffusion through the cambial walls. However, it is doubtful if any such cracks exist in these walls even after thorough cooking and bleaching, for none of the sections gave any indication of their presence, and when they did show on the 400 micron sections, it was only after treatment with cellulose solvents.

The presence of relatively great amounts of lignin in the outer layer of the secondary walls suggests that here, if anywhere in the secondary walls, lignin and carbohydrates are chemically combined, though the mere concentration of relatively great quantities of lignin here is, of course, no a priori reason for such combination.

Despite the fact that most workers in the field of fiber structure have believed that there is a semipermeable membrane of some sort encasing the secondary walls it seems doubtful that such a membrane is necessary to explain ballooning action of fibers. The fact that the swollen secondary wall forming the balloons does not disperse might, it seems, be just as well attri-
buted to cohesive or surface tension forces. There was no visible evidence of any such membrane in any of the digested cross sections, but it might have been impossible to stain or see it, for it is supposedly very thin and transparent, and those workers who have actually isolated and seen it used dark field illumination. If, however, there is such a membrane it no doubt lies inside the cambial wall, and it seems most probably that it is associated with this wall, for, in view of the heavily lignified outer portion of the secondary wall and the fact that there is no definite plane of cleavage between the outer heavily lignified and the inner cellulosic portion of this wall, it is difficult to understand how the membrane would be associated with it and what function it might possibly have in the plant, if associated with it. It appears that it would be more reasonable to believe that the membrane in the mature xylem existed originally as a lining of the lumen of the cambial initial and as such would most likely in the mature xylem be associated with the cambial wall.
The following are some ideas for future work that were suggested by this problem:

1. More exhaustive study of the possible fibrillar structure of and effect of various chemicals on the structure of both the cambial and secondary walls of digested cross sections.

2. Isolation by either or both chemical and mechanical means and study of the chemical properties of the cambial and secondary wall.

3. Development of a cell whereby digestion can be carried out on thin sections under the microscope at commercial temperatures and pressures.

4. More exhaustive study of the nature and properties of the gelatinous inter and intracellular materials observed in digested and bleached sections.

5. Continuation of the study of the stain reactions on both cross and longitudinal digested and bleached sections.
CHAPTER VI

SUMMARY

The effect of digestion on 20 micron cross sections of spruce, pine, and poplar woods in sulphite, soda, and kraft cooking liquors and the effects of hypochlorite and chlorine bleaching on these and undigested wood sections have been studied. The digestions were carried out under approximately commercial conditions of temperatures and liquor strengths. Numerous photomicrographs have been taken of the variously treated sections, which have been studied for their reactions to a number of analytical stains and solvents, as well as to swelling in cellulose solvents.

Kerr and Bailey's picture (2) of wood structure, consisting of intercellular material, cambial walls, and secondary walls, has been largely verified as the results of the digestions. The cooking liquors quickly attack and remove or gelatinize the intercellular material and attack the outer portion of the secondary walls of the soft woods, causing a gap to appear between the cambial and secondary walls. The intercellular material as well as the outer band of the secondary wall of the soft woods apparently consists mainly of lignin, for they not only dissolve in cooking liquors
but react positively to lignin stains and solvents and do not dissolve in the cellulose solvents. This ligneous material can apparently be completely removed by all the cooking liquors, though there is some evidence of an intercellular and intracellular gelatinous, transparent material, remaining after digestion. This is most apparent in the kraft-digested sections.

The visual examinations as well as the microchemical reactions and the results of the quantitative yield of the digested sections and lignin contents of these yields indicate that soda and kraft liquors act very rapidly on the intercellular material of the soft woods and remove the secondary wall lignin relatively much more slowly, while sulphite digestion, by the time the intercellular material has been removed, has also removed most of the removable secondary wall material. Alkaline digestion, therefore, shows a more or less selective action and removes most of the intercellular material before greatly attacking the secondary wall. The actions of kraft and soda digestion on soft woods are very closely the same, except that kraft liquor acts a little more rapidly.

The effects of digestion on poplar are not so obvious visually, but the results of microchemical reactions and of the quantitative yield and lignin
determination show that the effects are in general the same as with the soft woods, except that in this case soda digestion has a more rapid action than does kraft digestion. The secondary walls of poplar are apparently less heavily and more uniformly lignified than the secondary walls of the soft woods, and the secondary walls of the former do not have a distinct band of heavily lignified material around the outside.

The cambial walls have been found to be composed mostly of cellulose, for they are not visibly attacked and persist intact through the most drastic digestions and bleaching treatments. In a digested section they react positively to cellulose stains and solvents, though in the wood they probably contain lignin and polymers. Consequently, all pulp fibers, at least those from the woods studied (and probably many more, for Kerr and Bailey (2) found the cambial walls existing in the xylem cells of a great number of woods), have the cambial wall as a definite part of their structure.

Bleaching closely parallels the action of digestion and they both apparently attack the same structures. In fact, bleaching, though probably it delignifies more completely, removes less material from
the secondary wall than digestion. The cross sections
of the Mitscherlich cup and the rapidity with which
digestion attacks the intercellular material would seem
to indicate that to commercial digestion, though it
does attack the secondary wall to a considerable degree,
may be attributed the functions of removing the inter-
cellular material and producing pulping action and macera-
tion, whereas to bleaching may be attributed the function
of removing the residual lignin from the secondary walls.

Swelling experiments show that sulphite di-
gestion has a more harmful effect on the walls of the
20 micron cross sections than alkaline digestion, which
does not weaken the cambial walls. The secondary walls
of sections digested in sulphite liquor for 2 hours or
more, on dissolving in sulphuric acid, break up into
pieces and melt away, rather than swelling. The cambial
walls dissolve very quickly and the pieces of secondary
wall float out unrestricted. On the other hand when al-
kaline digested sections are treated with sulphuric acid
the secondary walls swell and come out one or the other
end of the restricting cambial wall.

Swelling experiments on digested, bleached
cross sections indicate that hypochlorite bleaching has
a more harmful effect on the cambial walls than do the
chlorine bleachings. The cambial walls of the hypochlorite-bleached sections dissolve very quickly in cellulose solvents and allow the secondary walls to swell laterally, while after chlorine bleaching the cambial walls resist cellulose solvents long enough for the secondary walls to swell and fill the lumen and begin to ooze out the ends of the restricting cambial walls.

The presence of the cambial wall as a definite portion of pulp fiber structure has been shown and the recognition of its presence and properties may lead to a better understanding of many facts concerning the behavior of wood pulp fibers. Though nearly all cellulose in a digested section, it is somewhat different from the secondary wall. It does not swell in cellulose solvents, though it does finally dissolve. It will not stretch mechanically, though the secondary wall will stretch to several times its original perimeter before rupturing.

When the secondary walls of digested sections are treated with cellulose solvents, the cambial walls restrict their swelling, and the former finally ooze out one or the other end of the cambial walls, turning each cell inside out. In thicker sections, where the cell segments are lying on their sides, cellulose solvents
cause the cambial walls to peel off or fold back, allowing the secondary walls to swell laterally over the portions of the segment where this has occurred and restricting lateral swelling where the cambial wall remains intact. On the longer segments, the cambial wall has been seen to break in two places somewhat apart from each other and the secondary wall, swelling outward at these places, rolls the cambial wall back until it forms a narrow constriction typical of balloon-type swelling. In other cases, working with similar sections, the cambial wall was seen to apparently unwind from the secondary wall, which swelled laterally over the region where this occurred. The unwinding cambial wall presented an appearance very much like the lignin sheath described by Carpenter and Lewis (12) with the difference, however, that it is apparently soluble in cuprammonium hydroxide. There is evidence that the cambial walls are of fibrillar structure rather than a continuous sheath.

No evidence of fibrill structure, as it is commonly depicted, was observed in the secondary wall of any of the sections. However, none of the sections were treated as drastically as is apparently necessary to show up fibrillation. The cambial walls are very definitely associated with the constrictions occurring in balloonning fibers, and it seems quite probable that the usually des-
cribed laterally wound fibrils in the outer layers of
the fiber are confined largely to these walls.

G stain reactions have been studied on the
variously digested and bleached sections. G stain re-
actions are found to be quite sensitive to small dif-
ferences in treatments of the sections and their value
for analytical work is confirmed. The action of sul-
phite liquor and Cross and Sevan cellulose treatments
seems to be merely to remove the G staining power of
the cell walls: the action of alkaline digestion as
well as hypochlorite and chlorine bleaching agents
apparently so alters the material in the wood that is
responsible for the G stain color of the untreated wood
that other colors than those produced in the untreated
wood are produced in the treated sections.

Bleaching sulphite-digested sections with
chlorine removes the G staining power from the second-
ary walls and causes the cambial walls to stain pink.
Bleaching with chlorine causes the secondary walls of
the alkaline-digested sections to pass from the yellow-
s and chocolate browns toward the violets. Hypochlorite
bleaching, though it does not remove the G staining
power from undigested wood sections does remove the G
staining power from sulphite or alkaline-digested sec-
tions.
The cambial walls of any of the variously treated sections stain differently than the secondary walls, so that the C stain colors produced in pulp fibers lying flat on a slide with the light passing through both walls are a composite of the colors produced in the individual walls.
CHAPTER VII

CONCLUSIONS
CHAPTER VII

CONCLUSIONS

1. The sulphite, soda, and kraft digestions attacked the intercellular material of the 20 micron cross sections of the spruce, pine, and poplar woods and completely removed or gelatinized it.

2. The sulphite, soda, and kraft digestions attacked the secondary walls of the 20 micron cross sections of the spruce and pine wood and removed or gelatinized a considerable portion of the outer layer of these walls, especially on the radial ends, and caused the cambial walls to stand out from them. The interior portions of the secondary walls were not visibly attacked by the digestions. The secondary walls of the 20 micron cross sections of the poplar wood digested in sulphite, soda, and kraft liquors were not visibly attacked, even with the most prolonged digestions, though prolonged digestion did loosen the cambial walls from the secondary walls.

3. The sulphite, soda, and kraft digestions did not materially attack the cambial walls of the 20 micron cross sections of the spruce, pine, and poplar woods; these persisted through the most prolonged digestions and bleaching treatments and completely encase
the secondary walls in the digested sections.

4. Kraft and soda digestion had a semi-selective action on the 20 micron cross sections of spruce and pine; the attack on the intercellular material was nearly completed before any very great visible attack was made on the secondary wall. Sulphite digestion did not show this selective action on the 20 micron cross sections of the spruce and pine, and the attack on the intercellular material and the secondary walls proceeded very nearly simultaneously.

5. The swelling action in sulphite acid of the 20 micron cross sections of spruce-digested in sulphite, soda, and kraft liquors indicated that sulphite digestion had a more drastic effect on both the cambial and secondary walls than did either the soda or kraft digestions.

6. The actions of the various bleaching treatments on the digested and undigested 20 micron cross sections of the spruce, pine, and poplar woods were similar to one another and closely paralleled those of the digestions but were a little more specific in that, though they practically completely delignified the sections, they made a somewhat less apparent visible attack on the secondary walls than did the digestions.
8. It is believed that there is some evidence to indicate that neither digestion nor bleaching actually completely removes the intercellular material and the outer layer of the secondary walls from the 20 micron cross sections of spruce, pine, and poplar, but that it is partially dissolved with the effect of delignification and that the undissolved part remains in place as a transparent and apparently gelatinous material.

9. The swelling action in sulphuric acid of 20 micron cross sections of spruce wood digested in kraft liquor, and bleached with calcium hypochlorite, and chlorine and sodium hydroxide, indicated that the former bleaching treatment had a more drastic effect on the cambial walls than the latter.

10. Kerr and Bailey's picture (2) of mature wood structure was in general verified on the spruce, pine, and poplar cross sections. The intercellular material was mostly lignin, and was amorphous, and isotropic. The cambial walls in the wood were mostly cellulose and were birefringent in cross section. The secondary walls of the spruce and pine consisted of two parts, the outer part just under the cambial walls, which is mostly lignin, and the inner portion, comprising most of the secondary wall, which is mainly cellulose and is isotropic in cross section.
different colors of these two walls.
CHAPTER VIII

REFERENCES CITED
REFERENCES CITED


   Quoted from Kerr and Bailey (2).

   Quoted from Kerr and Bailey (2).

   Quoted from Kerr and Bailey (2).

   Quoted from Kerr and Bailey (2).


   Quoted from Kerr and Bailey (2).

    Quoted from Kerr and Bailey (2).


34. Lüdtke, M., Ber., 61: 465-70 (1928).


40. Fuchs, W., Biochem. Z., 192: 165-6 (1928).


CHAPTER IX

APPENDIX
Table III.

Yield and Lignin determinations

<table>
<thead>
<tr>
<th>Wood</th>
<th>Digesting Liquor</th>
<th>Time</th>
<th>% Yield</th>
<th>% Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce</td>
<td>Sulphite</td>
<td>2 hrs.</td>
<td>45.6</td>
<td>2.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>2 hrs.</td>
<td>46.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Pine</td>
<td>&quot;</td>
<td>2 hrs.</td>
<td>50.5</td>
<td>2.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>2 hrs.</td>
<td>44.7</td>
<td>---</td>
</tr>
<tr>
<td>Poplar</td>
<td>&quot;</td>
<td>2 hrs.</td>
<td>44.8</td>
<td>1.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>2 hrs.</td>
<td>45.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Spruce</td>
<td>Soda</td>
<td>30 min.</td>
<td>46.0</td>
<td>10.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>30 min.</td>
<td>48.0</td>
<td>10.7</td>
</tr>
<tr>
<td>Pine</td>
<td>&quot;</td>
<td>30 min.</td>
<td>45.3</td>
<td>10.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>30 min.</td>
<td>46.3</td>
<td>10.4</td>
</tr>
<tr>
<td>Poplar</td>
<td>&quot;</td>
<td>30 min.</td>
<td>43.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Spruce</td>
<td>Kraft</td>
<td>15 min.</td>
<td>46.8</td>
<td>3.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15 min.</td>
<td>46.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Pine</td>
<td>&quot;</td>
<td>15 min.</td>
<td>46.9</td>
<td>3.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15 min.</td>
<td>42.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Poplar</td>
<td>&quot;</td>
<td>15 min.</td>
<td>36.9</td>
<td>7.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15 min.</td>
<td>40.0</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Table IV.

**Ridgeway Numbers Corresponding to C Stain Colors**

in Color Charts I, II.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Secondary Wall</th>
<th>Cambial Wall</th>
<th>Intercellular Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphite-Digested 15 min.</td>
<td>25YG-Yb</td>
<td>25YG-Yb</td>
<td>25YG-Yb</td>
</tr>
<tr>
<td>10 hrs.</td>
<td>Colorless</td>
<td>Colorless</td>
<td>Colorless</td>
</tr>
<tr>
<td>Soda-Digested 30 min.</td>
<td>21'Y-Yb</td>
<td>21'0-Yb</td>
<td>Removed</td>
</tr>
<tr>
<td>1 hr.</td>
<td>17'0'O-Yb</td>
<td>61'0'VR-Yb</td>
<td></td>
</tr>
<tr>
<td>Kraft-Digested 30 min.</td>
<td>13'0'C-Y-O</td>
<td>61'0'VR-V</td>
<td></td>
</tr>
<tr>
<td>1 hr.</td>
<td>17'0'C-Y</td>
<td>61'0'VR-Vb</td>
<td></td>
</tr>
<tr>
<td>Sulphite-Digested 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl-NH OH Bleached</td>
<td>Colorless</td>
<td>Very pale pink</td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium Hypochlorite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached (24 hrs.)</td>
<td>Colorless</td>
<td>Colorless Removed</td>
<td></td>
</tr>
<tr>
<td>Soda-Digested 15 min.</td>
<td>17'0'C-Yd</td>
<td>45'0'Blue,f</td>
<td></td>
</tr>
<tr>
<td>Cl-NH OH Bleached</td>
<td>17'0'C-Yd</td>
<td>49'0'Blue,f</td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH</td>
<td>17'0'C-Yd</td>
<td>49'0'Blue,f</td>
<td></td>
</tr>
<tr>
<td>Calcium Hypochlorite</td>
<td>Colorless</td>
<td>Colorless</td>
<td></td>
</tr>
<tr>
<td>Bleached (24 hrs.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kraft-Digested 30 min.</td>
<td>17'0'C-Yb</td>
<td>57'0'VR-Vb</td>
<td></td>
</tr>
<tr>
<td>Cl-NH OH Bleached</td>
<td>13'0'C-Y-Ob</td>
<td>61'0'VR-Vb</td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH</td>
<td>13'0'C-Y-Ob</td>
<td>61'0'VR-Vb</td>
<td></td>
</tr>
<tr>
<td>Calcium Hypochlorite</td>
<td>Colorless</td>
<td>Colorless</td>
<td></td>
</tr>
<tr>
<td>Bleached (24 hrs.)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table V.

Ridgway Numbers Corresponding to O Stain Colors in Color Chart III.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Secondary Wall</th>
<th>Cambial Wall</th>
<th>Intercellular Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undigested-Bleached Calcium Hypochlorite (3 days)</td>
<td>61''''VR-Vb</td>
<td>23, Yellow, d</td>
<td></td>
</tr>
<tr>
<td>Calcium Hypochlorite (3 days) Washed with 10% HCl-10% NaOH</td>
<td>61''''VR-Vb</td>
<td>61''''VR-Vb Removed</td>
<td></td>
</tr>
<tr>
<td>Cl-Na OH</td>
<td>65''''RR-Vf</td>
<td>65''''RR-Vf</td>
<td></td>
</tr>
<tr>
<td>Cl-NaOH</td>
<td>57''''VS-Vf</td>
<td>57''''VS-Vf</td>
<td></td>
</tr>
<tr>
<td>Cl-Na SO</td>
<td>Colorless</td>
<td>Colorless</td>
<td></td>
</tr>
<tr>
<td>Cl-Alcohol-Pyridine</td>
<td>25'YG-Yd</td>
<td>23, Yellow, d</td>
<td></td>
</tr>
</tbody>
</table>