CXLI. BIOCHEMICAL AND HISTOLOGICAL STUDIES ON LIGNIFICATION¹.

PART II. HISTOLOGICAL STUDIES ON THE POLY-SACCHARIDES AND AROMATIC CONSTITUENTS OF THE CELL WALL.

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INTRODUCTION.

THE section of organic chemistry dealing with the carbohydrates is perhaps of more absorbing interest to the plant physiologist than any other, since it deals with the infinite variety of substances composing the tissues of plants. For a long time the botanist had regarded "cell wall" and cellulose as synonymous terms, but as our knowledge of these constituents developed, it became more and more apparent that the cell wall presented such a considerable differentiation of form and substance as to render the classification and relation of these constituents to one another extremely difficult. It is surprising to find that in spite of the enormous advances in our knowledge of the chemical constituents of the cell wall, plant histology has remained more or less empirical and it is not characterised by that degree of exactitude which should be attainable with the chemical knowledge now at our command.

The writer has therefore undertaken these investigations primarily with the object of developing the necessary technique and of furnishing such data as would be helpful in studying the chemical differentiation of the substances comprising cell walls, so as to systematise plant histology on more rational lines.

This has necessarily involved a considerable amount of fundamental work, which should prove of particular interest to those desiring to undertake critical histological work, since at present we do not know exactly the nature of a given constituent stained by a dye, or how the staining capacity of one constituent is affected by its association with another.

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ISOLATION AND PROPERTIES OF THE PRINCIPAL CONSTITUENTS
OF THE CELL WALL.

It will be useful in following the subject under discussion to review briefly the methods adopted by the writer for the isolation and preparation of the principal constituents of the cell walls of plants and their essential characteristics as disclosed by our chemical knowledge.

Celluloses.

In the first group comprising typical cotton cellulose, which is a seed hair, we shall include all fibrous celluloses which constitute the bast of exogenous flowering annuals: *e.g.* the celluloses of flax (*Linum usit.*), hemp (*Cannabis sativa*), China grass (*Rhea* and *Boehmeria* species). Celluloses of this group, when freed from the other associated substances by suitable chemical processes known to be within the limits of resistance of the true cellulose, have not so far been differentiated. They are very resistant to hydrolysing and oxidising agents, and do not possess reactive carbonyl groups.

Closely related to the above group, we have another class of celluloses which are much more widely distributed in the vegetable kingdom. These are differentiated from the first group by containing a slightly higher percentage of oxygen, and by the presence in them of carbonyl and methoxy groups. They appear to be formed from the celluloses of the first group by partial depolymerisation and oxidation.

Among the different types of cellular constituents we have to recognise three distinct types of celluloses, cellulose- α , - β and - γ , possessing different degrees of dispersion and dehydration, and corresponding polymerisation. The essential difference between them, as already mentioned in Part I of these researches, is that the less resistant celluloses, - β and - γ , are soluble in 17.5 % sodium hydroxide solution at the ordinary temperature, and from the alkaline solution cellulose- β is precipitated on acidification with acetic acid, whilst the cellulose- γ remains in solution. The writer has found that this method of separation is not quite sharp for isolating absolutely pure cellulose- α , and has therefore adopted another method for its preparation.

Cellulose- α is prepared by treating cotton wool (5.0 g.) with 100 cc. of 4 % sodium hydroxide at 10 atmospheres pressure for one hour. By this means it is completely freed from cellulose- β and - γ . It is then washed with water and dilute acetic acid, finally washed again thoroughly with distilled water and dried.

Cellulose- β and - γ are prepared from cotton wool by the method of Cross and Bevan [1916].

Cellulose- γ is isolated from the acid filtrate, obtained after filtering off cellulose- β , by submitting it to dialysis to remove mineral matter. It is then concentrated under reduced pressure to a suitable volume and cellulose- γ precipitated by the addition of two volumes of 95 % alcohol. To study their

staining capacity they are best kept suspended in alcohol; a thin film is made by drying a drop of the suspension on a slide.

These celluloses show considerable differences with different stains. (See Table I.)

Hydrocellulose and Oxycellulose. Closely related to the above three cellular constituents we shall next consider their degradation products, which are frequently found in normal cell walls. These degradation products may be divided into two classes: (1) those produced by partial hydrolysis and (2) those produced by oxidation. The first group is typified by hydrocellulose which possesses active carbonyl groups, and shows a very strong adsorptive power for different organic dyes. The writer finds that there are two kinds of hydrocellulose, which, although staining similarly with different dyes, can be differentiated with aqueous iodine in potassium iodide solution. One modification takes immediately a deep blue colour whilst the other is only stained a reddish brown. The latter can be prepared from cotton wool according to Girard's method [1881].

The second modification is prepared by soaking cotton wool with as small a quantity as possible of cold 72 % (by vol.) sulphuric acid and treating almost immediately with 95 % alcohol. The hydrocellulose is then washed free from acid with distilled water and dried in the air. It stains a deep blue with aqueous iodine in potassium iodide solution. (See Table I for staining reactions.)

Under the term "oxycelluloses" we find comprised in the literature a number of compounds of indefinite character which are produced by the action of different oxidising agents on cellulose. These should not be regarded as entities but merely as representing mixtures of oxidised cellulose, hydrocellulose and unchanged cellulose, the difference being only due to the different degrees of surface oxidation of the fibres.

Oxycelluloses possess acidic properties, and also contain active carbonyl groups. There are three types of oxycelluloses, known as α -, β - and γ -oxycellulose, which are differentiated by their behaviour towards dilute sodium hydroxide solution, ammonia and water. α -Oxycellulose is difficultly soluble in alkali and insoluble in ammonia. β -Oxycellulose is soluble in dilute alkali and ammonia, whilst γ -oxycellulose is soluble in hot water, alkali and ammonia solution. α -Oxycellulose was prepared according to the method of Murumow, Sack and Tollens [1901]; β - and γ -oxycelluloses were prepared according to the method of Nastjukoff [1900]. (See Table I for staining reactions.) Oxycellulose may be detected in presence of hydrocellulose and celluloses according to the method of Schwalbe and Becker [1921] and Knagg [1908].

Among the non-fibrous polysaccharide constituents of the cell wall, we shall now consider in turn, mannan, galactan, pectin, amylohemiacellulose, hemicelluloses, gums, starch, lichenin and chitin. This class is characterised by the low resistance of its members towards hydrolytic agents; they are

more or less soluble in dilute alkali. The members are also readily broken down in the plant under the action of its own enzymes, secreted under abnormal conditions. The cell walls of amylaceous seeds especially are composed in part of polysaccharides of this class.

Mannan. Mannans like celluloses also occur in nature in different degrees of polymerisation, but they give mannose instead of glucose on hydrolysis with mineral acids. A variety of mannan termed paramannan which corresponds in many respects with cellulose- α , in that it is very resistant to hydrolytic reagents, is found in coffee beans and *Phytelphas macrocarpa*. It is not acted on appreciably by mineral acids, potassium chlorate and hydrochloric acid, but dissolves in a concentrated hydrochloric acid solution of zinc chloride. Hence it is frequently called mannocellulose. It can be readily separated from the less resistant mannan by boiling it with dilute alkali, in which the latter dissolves. Vegetable ivory, the endosperm of the palm (*Phytelphas macrocarpa*) was selected as the most suitable material for the isolation of mannan. It was boiled in the form of fine shavings with 1 % sodium hydroxide solution and the residue, consisting of mannocellulose, was washed with dilute acetic acid and distilled water, and dried. The less resistant mannans are found in leaves, roots, and stems of many plants, as well as in the cell walls of many fungi.

Mannans form insoluble compounds with copper in alkaline solution and hence are not soluble in Schweitzer's reagent. When they are in association with cellulose they also prevent it from dissolving. The less resistant mannan is isolated according to the method of Salkowski [1894]. With different stains it behaves mostly like gums. (See Table I.)

Galactan. Galactans are strictly analogous to mannans, giving rise to galactose instead of mannose on hydrolysis. They also appear to exist in varying degrees of polymerisation as some of them are not dissolved by boiling with alkali. They also form insoluble copper compounds and behave in most of their properties like the mannans. Galactans are often found in combination with pentosans such as xylan or araban. Galacto-araban is widely disseminated in the cell walls of the cotyledons of many plants, e.g. *Coffea arabica*, *Cocos nucifera* and other palms. Agar-agar of commerce, which is the mucilage from certain genera of the red seaweeds (*Rhodophyceae*), is the most suitable material for the isolation of this constituent. (See Table I for staining reactions.)

Pectins. Pectic substances are closely related to galactan, being derived from it by oxidation processes. In fact they constitute, in more or less intimate association with cellulose, the middle lamella of cell-walls in many tissues, in some only at a certain period of their life history. They occur abundantly in many succulent fruits, and also in fleshy roots and stems.

The most recent investigations of Nanji, Paton and Ling [1925] have cleared up the uncertainty which hitherto existed as to the nature of these substances by devising methods whereby these compounds have been isolated

in pure state, and their nature and relationships to one another determined. (See Table I for staining reactions.)

Amylohemicellulose. Amylohemicellulose is generally associated with starch in the starch-bearing seeds and roots. It gives a blue coloration with iodine in potassium iodide solution, is insoluble in water and is attacked by secretion, but not by translocation, diastase. It is isolated from cereal starches by a method based on this fact, as shown by Ling and Nanji [1925].

It is a white amorphous powder, swelling up in boiling water, and is readily soluble in dilute sodium hydroxide solution. (See Table I for staining reactions.)

Hemicellulose. The hemicellulose constituents of woods sometimes give a dirty brownish blue coloration with iodine in potassium iodide solution. They are sparingly soluble in water, easily in dilute sodium hydroxide solution and are readily hydrolysed by boiling with dilute mineral acids. They are prepared from wood according to the method of O'Dwyer [1923]. (See Table I for staining reactions.)

Gums. Gums are very varied in nature and occur widely distributed among plants, especially trees. They are to be regarded as being derived from the decomposition of pectic substances in the plant. They also consist of polysaccharide acids in combination with hexoses and pentoses, such as galactose, arabinose, and xylose. They are of an acidic character, and occur mostly in nature in the form of salts of potassium, calcium or magnesium. (See Table I for staining reactions.)

Starch. Starch, although not forming a constituent of the cell wall by itself, may often be confused with amylohemicellulose and the hemicelluloses of woods already described, because of the coloration observed with iodine solution in the living cells of the pith, medullary rays and cortex of roots and stems. (See Table I for staining reactions.)

Lichenin. Lichenin, or moss starch, forms the principal constituent of Iceland moss (*Cetraria Islandica*) and Irish Moss (*Chondrus crispus*). It gives a dirty blue coloration with iodine and is insoluble in cold water. It can be best isolated by boiling the commercial dried moss with water, when it swells up, giving a viscous paste which sets to a jelly on cooling. (See Table I for staining reactions.)

Chitin. Chitin has long been recognised as a constituent of the cell-wall of certain fungi, *e.g.* *Boletus edulis*, *Agaricus campestris*, etc. It is very resistant to alkali and this fact is made use of in isolating it from the other constituents. It is insoluble in Schweitzer's reagent, gives a reddish brown stain with iodine in potassium iodide solution, and with a 50 % solution of zinc chloride-iodine it gives a bluish violet coloration. It is best isolated from the shells of the crustacea, lobster or crab, according to the methods of Gilson [1894] or Winterstein [1894, 1895]. When finely powdered and extracted successively with boiling alcohol and ether it is obtained as a white powder. (See Table I for staining reactions.)

Lastly, we have to consider the aromatic constituents of the cell-wall.

The isolation and properties of lignin have already been dealt with in Part I of this work. It may now be mentioned that while the writer was in search of some specific microchemical tests for lignin, it was observed that there exists some close relationship between lignin and resin, as it gives all the reactions of the latter. When dissolved in a little acetic anhydride and mixed with sulphuric acid (sp. gr. 1.53), it gives a deep red colour, changing through green to brown like the resins [Morawski 1889]. When treated with a drop of 1 % solution of vanillin in concentrated sulphuric acid, it gives a deep orange-red colour—violet in some cases—changing after 2–4 hours to reddish brown or bluish violet, a behaviour similar to that of the resins. This view was further confirmed by the similarity in the behaviour of lignin and resin towards different stains. Chemically, resins are not well-defined substances. They are for the most part solid or semi-solid substances of a gummy nature, originating in special cells in the plant. Resinous substances have solubilities and elementary composition similar to those of lignin. There are no good specific reactions for the microscopical detection of resins and essential oils. Resins can therefore only be identified by their physical properties, such as solubility, colour and other characteristics. In the plant resins appear in the liquid form as strongly refractile, homogeneous or vascular-foaming drops, or in solid yellowish brown amorphous masses. Alkannin from alkanna roots, first employed by Muller [1866], stains resins or free lignin and essential oils an intense red colour. Lignin and resins are also deeply stained by malachite green, borax carmin, safranin and ruthenium red.

Procedure followed in the staining experiments.

The period of staining was 2 hours, at the close of which period the excess of the stain was washed off thoroughly first with 90 % alcohol and finally with absolute alcohol and the preparation mounted in cedar wood oil, the cover-glass being ringed round with Canada-balsam. Cedar wood oil was found to be the most suitable general mounting medium as the depth of the stain was not affected. A list of the stains used and their compositions are given below.

- (1) Phloroglucinol—1.0 g. phloroglucinol dissolved in 100 cc. of 12 % hydrochloric acid (1.060).
- (2) Malachite green (alcoholic)—1 g. in 300 cc. of 92 % alcohol.
- (3) Cobalt thiocyanate—One volume of saturated cobalt nitrate solution added to two volumes of saturated ammonium thiocyanate solution.
- (4) Congo red (aqueous)—1 g. in 200 cc.
- (5) Borax carmin (alcoholic)—3.0 g. carmin, 12 g. borax, 480 cc. water and 480 cc. 90 % alcohol.
- (6) Eosin (alcoholic)—1 g. in 300 cc.
- (7) Safranin (alcoholic)—3 g. safranin 500 cc. water and 500 cc. 90 % alcohol.
- (8) Haematoxylin (Delafield)—200 cc. of a saturated solution of ammonium alum, mixed with a solution of 2 g. haematoxylin in 12 cc. absolute alcohol.

The mixture is exposed to light and air for a week. It is filtered and to the filtrate are added 50 cc. of glycerol and 50 cc. of methyl alcohol. It is allowed to stand in sunlight until the full colour develops and is refiltered for use.

(9) Gossypimin (aqueous)—2 g. gossypimin, 900 cc. water and 60 cc. 90 % alcohol.

(10) Aniline blue picrate—3 g. aniline blue, 2 g. picric acid dissolved in a litre of water.

(11) Iodine green (aqueous)—1 % solution.

(12) Ferric thiocyanate (aqueous)—1.0 cc. of saturated ferric chloride to 10 cc. of 10 % ammonium thiocyanate solution.

(13) Ruthenium red (aqueous) 1 in 10,000.

(14) Alkannin (alcoholic)—alkanna roots are extracted with just sufficient amount of 90 % alcohol to cover the roots. The alcohol solution is decanted and filtered for use.

(15) Picro-ponceau (aqueous)—0.1 g. ponceau in 100 cc. of saturated aqueous picric acid.

(16) Orcein (alcoholic)—0.5 % solution in alcohol containing 0.01 % hydrochloric acid.

(17) Vanillin—1.0 g. in 100 cc. of concentrated sulphuric acid.

In the following table the intensity of a stain taken up by a particular constituent is invariably based on the deepest stain obtained with that dye as the standard. It is of course evident that the intensity of the stain would vary according to the density of the material, but so long as due allowance is made for this and the conditions kept as nearly constant as possible, fairly reliable judgment can be given after a little practice as to the intensity of the stain. (See Table I.)

All the results of staining given in Table I hold good for direct staining only. Many of the stains taken up by a constituent can be displaced by another stain having a stronger affinity for the particular constituent. The writer in the course of her work, while trying to differentiate the various constituents of plant tissues by counterstaining has accumulated some useful data, which are epitomised in Table II. Generally the section is first stained with the most permanent primary colour and secondly with the counterstain for a suitable period. Borax carmin and malachite green, aniline blue picrate and gossypimin, haematoxylin and safranin or gossypimin are usually employed.

In addition to these the writer has used a number of pairs of counterstains with sections of timbers with good results for critical differentiation. (See Table III.) Many of these counterstains are especially good for differentiating the middle lamella.

It will be evident from a superficial examination of Table I that most of the stains are not at all specific as they stain a number of different constituents, and this fact has led to many misconceptions. For example, ruthenium red which is generally regarded as a specific stain for pectic sub-

Table I. Showing the depth of stain taken up by different constituents of the cell wall.

	Phloro- glucinol in HCl	Alcoholic maiseite green	Cobalt thio- cyanate	Aqueous Congo red	Alcoholic carmin	Alco- holic eosin	Alco- holic safranine	Haema- toxylin	Aqueous gossyp- min	Aniline blue picrate	Aqueous iodine green	Ferric thio- cyanate	Aqueous ruthenium red	Alcoholic alkannin	Aqueous picro- ponceau	Alcoholic orcein
Cotton wool	-	+2	-	+4	+2	+4	+1	+1	+1	+3	+1	-	-	-	+2	+1
Untreated natural cotton wool	+2	+1	-	+3	-	+3	+3	+1	+3	+3	+1	-	+1	-	+2	+1
Natural cotton wool treated with acid	+1	+2	-	+3	+1	+3	+3	+1	+1	+3	+1	-	-	-	+2	-
Natural cotton wool treated with alkali	-	+2	-	+4	+1	+2	+1	+1	+1	+3	+1	-	-	-	+2	+1
Cellulose- α	-	-	-	+4	-	+1	+1	+1	+1	-	-	-	-	-	-	-
Cellulose- β	-	+1	-	+4	-	+1	+2	+2	+1	+2	+1	-	-	-	+4	-
Cellulose- γ	-	-	-	+4	+1	+2	+2	+2	+4	+2	+2	-	-	-	+1	-
α -Oxycellulose	+1	+2	-	+3	-	+4	+4	+1	+4	-	+2	-	+2	-	+2	-
γ -Oxycellulose	-	-	-	+3	-	+2	+2	+1	+1	-	-	-	+3	-	+1	-
Hydrocellulose	+1	+4	+2	+4	+3	+2	+4	+4	+4	+3	+3	-	-	-	+2	+3
Hemicellulose	-	-	-	+4	+3	+4	+4	+4	-	+1	+3	-	+4	-	+2	-
Gum	+4	+4	-	+3	+3	+4	+4	+4	+4	+3	+4	+3	+4	-	+1	-
Pectinogen	+1	+3	-	+3	+3	+4	+3	+1	+3	+2	+3	-	+4	-	-	+1
Calcium pectate	+1	+3	-	+4	+2	+3	+4	+1	+4	+2	+3	-	+4	-	-	+2
Ferric pectate	-	-	-	+3	+2	+3	+3	+2	+4	+3	+3	+1	+3	-	+4	-
Lignin	-	+3	+4	-	+3	+3	+4	+1	+1	+2	+2	-	+3	-	+1	-
Calcium lignate	-	+3	-	+3	+3	+3	+4	+1	+3	+4	+2	-	+3	-	+1	-
Galactan	-	+4	-	+4	+1	+4	+4	+1	+4	+2	+2	-	+3	-	+1	+2
Starch	-	+4	+3	-	-	+1	+2	-	+3	+2	+4	+3	+1	-	-	-
Mannan	-	+4	-	+4	-	+4	-	+2	-	+4	-	-	-	-	+1	+2
Chitin	-	-	-	-	+1	+3	+1	+2	+1	+3	-	-	-	-	+1	+2
Lichenin	-	+2	-	-	+3	+2	+4	+2	+4	+4	+4	-	+1	-	+3	-
Amylohemiacellulose	-	+3	-	+4	+1	+4	+4	+2	+3	+4	+1	+3	+2	-	+3	+1
Cork	-	+4	+3	+4	+2	+3	-	-	-	+2	+3	+1	-	-	-	-

+1, +2 +3, +4, show increasing intensity of colour, +4 being the strongest.

- Negative.

Table II. *Showing the parts stained by different counterstains.*

- Root.** Malachite green stains: xylem and developing lateral rootlets.
 Borax carmin stains: cortical tissue, endodermis and pith.
 Haematoxylin stains: ground tissue, phloem, cortical tissue, cambium, resin duct and lactiferous vessels.
 Gossypimin stains: xylem, endodermis and starch granules.
 Safranin stains: secondary and primary wood, endodermis, central axis of root and lateral developing rootlets.
- Stem.** Malachite green stains: phloem, xylem elements, traces to first leaf, sclerenchymatous ring, cambiform tissue, spiral vessels, primary vascular bundles, bundle sheath, annular vessels, scalariform vessels, sclerenchyma sheath, nodal diaphragm and secretion cells.
 Borax carmin stains: Primary ground tissue, cambiform tissue, pith, cortical tissue, phloem, primary medullary ray, epidermal tissue, endodermis and nucleated cortical tissue.
 Haematoxylin stains: cortical tissue, primary ground tissue, central axis and medullary ray.
 Gossypimin stains: phloem, xylem, primary medullary ray, cambiform tissue, resin canal, developing branch, thickening band of prosenchyma, starch granules and vascular bundles.
 Safranin stains: xylem elements, phloem, connective tissue and pitted vessels.
 Aniline blue picrate stains: cortical tissue, cambiform tissue, primary ground tissue, central ground tissue and secreting cells of canal.
- Leaf.** Malachite green stains: epidermis, hypodermis, resin duct, xylem, leaf hair and vascular bundle.
 Borax carmin stains: parenchyma, endodermis, pericycle (transfusion tissue), phloem, epidermis, phloem of bundle and primary ground tissue.
 Gossypimin stains: epidermis, sclerenchymatous fibres, xylem elements, stomata, small vascular bundles, cambium, phloem elements and cuticle.
 Aniline blue picrate stains: ground tissue, phloem elements of bundle, palisade layer containing chloroplasts, guard cells of stomata, primary ground tissue, oil gland, epidermis, assimilating tissue, mucilaginous lining of the cell, nucleus and cuticle.

Table III. *Results of different counterstains with sections of Pitch Pine (timber).*

- Phloroglucinol counterstained by malachite green:
 Parts stained by phloroglucinol: autumn wood.
 „ malachite green: spring and summer wood, cell remains, resin canal, medullary ray.
- Congo red counterstained by malachite green:
 Parts stained by Congo red: resin canal.
 „ malachite green: cell wall, cell remains, medullary ray.
- Congo red counterstained by cobalt thiocyanate:
 Parts stained by Congo red: cell remains, medullary ray, resin canal.
 „ cobalt thiocyanate: cell wall.
- Ferric thiocyanate counterstained by malachite green:
 Parts stained by ferric thiocyanate: autumn wood, tracheidal medullary ray.
 „ malachite green: spring and summer wood, cell remains, medullary ray, resin canal.
- Ferric thiocyanate counterstained by aniline blue picrate:
 Parts stained by ferric thiocyanate: tracheidal medullary ray, cell wall, middle lamella.
 „ aniline blue picrate: cell remains, medullary ray and resin canal.
- Phloroglucinol counterstained by aniline blue picrate:
 Parts stained by phloroglucinol: cell wall.
 „ aniline blue picrate: cell remains, medullary ray and resin canal.
- Gossypimin counterstained by malachite green:
 Parts stained by gossypimin: middle lamella.
 „ malachite green: cell wall, cell remains, medullary ray, and resin canal.
- Malachite green counterstained by ponceau:
 Parts stained by malachite green: cell wall, resin ducts, medullary ray.
 „ ponceau: middle lamella.
- Ponceau counterstained by aniline blue picrate:
 Parts stained by ponceau: cell wall, middle lamella.
 „ aniline blue picrate: cell remains, medullary ray, resin canal.
- Alkannin counterstained by aniline blue picrate:
 Parts stained by alkannin: tracheidal medullary ray.
 „ aniline blue picrate: cell remains, primary medullary ray, resin canal.
- Alkannin counterstained by malachite green:
 Parts stained by alkannin: tracheidal medullary ray.
 „ malachite green: cell wall, cell remains, medullary ray, resin canal.

stances and their derivatives is found to stain in addition α -, β - and γ -oxycelluloses, hemicelluloses, gums, galactans, free lignin, mannan and amylo-hemicellulose. Moreover, there are no specific microchemical colour reactions for these constituents so that there is no direct means of differentiating between them even in this manner. On the other hand, many of the constituents can be removed from tissues by specific solvents or suitable chemical treatment and this principle has been employed by the writer in the work now to be described on the microchemical structure of the leaf and stem of *Pinus sylvestris*. Supposing it is desired to localise the pectic substances in different parts of a tissue, and sections of it be treated with specific solvents such as 0.5 % oxalic acid or 0.5 % ammonium oxalate respectively, both the treated and untreated sections being stained with different stains, a negative result will be obtained with some stains in certain parts of the treated sections compared with the untreated ones. Wherever a certain part is not stained after a specific treatment to remove a constituent, whereas the same part is stained in the untreated section, it is possible to say with certainty that those parts which do not stain after treatment must have contained the constituent in question. On the other hand, caution has to be exercised in such observations, for if other parts show no change in their staining capacity after treatment to remove a particular constituent, it need not be concluded that these parts do not contain that constituent. This is because many stains are taken up by constituents other than the one that is removed. These parts are not missed because there are other stains which do not stain the residual cell wall constituents, and by combining the observations of the results of staining of the treated and untreated sections with different stains it is possible to localise different constituents with accuracy. Oxalic acid and ammonium oxalate are only given above as examples, but sections may be submitted to various treatments (*e.g.* enzymes) according to the nature of the constituent under investigation. The value of the results obtained by differential staining will depend largely on the thoroughness with which a constituent is removed by a particular reagent and by the extent to which that reagent is specific in its action. The writer has mainly concentrated on the following treatments, with a view to locate the principal constituents which take part in metabolism. Even if the sections be treated with a reagent which is not specific it is possible to make good deductions by combining the results of staining with those of another treatment. Sections were treated by boiling with 0.5 % ammonium oxalate, 0.5 % ammonium oxalate in 3 % ammonia solution, 4 % sodium hydroxide solution, 3 % hydrochloric acid, or 95 % alcohol. This was usually carried out by heating the sections with the respective reagents in test tubes immersed in a boiling water-bath for 6–8 hours. To ensure complete extraction the supernatant liquid was decanted off every hour and the extraction continued with the fresh reagent. After the extraction was thus completed the sections were washed with hot distilled water and stained as already described.

Throughout the work the sections of the unfixed material were cut with a Leitz hand microtome or by free hand, and in the case of timber with an ordinary carpenter's plane, which gave quite good sections.

The results of observations by the above method of differential staining with sections of the leaf and stem of *Pinus sylvestris* are given in Tables IV and V.

PROBABLE CONSTITUENTS OF DIFFERENT PARTS OF THE LEAF
OF *PINUS SYLVESTRIS* (Table IV).

The epidermis in the leaf of *Pinus sylvestris*, after treatment with ammonia and ammonium oxalate, shows a slight decrease in the intensity on staining with ruthenium red and haematoxylin, indicating that only traces of pectic substances are present. With eosin and haematoxylin, after treatment with sodium hydroxide, a marked change is observed indicating the presence of hemicelluloses. The negative zinc chloride-iodine reaction indicates the absence of true celluloses.

The cell wall of the hypodermal cells gives the cellulose reaction with zinc chloride-iodine, as well as the phloroglucinol reaction, and in this respect it is differentiated from lignocellulose. The cell contents of some of them are stained by alkannin showing the presence of resinous substances. The behaviour with gossypimin and haematoxylin indicates the presence of galactan, but hemicellulose and pectin are absent. The persistence of the staining with ruthenium red after various treatments suggests that the cell wall of these hypodermal cells is composed mainly of an oxidised form of cellulose, such as α -oxycellulose, which is not dissolved by dilute alkali. The contents of these cells also give a strong reaction with benzidine and hydrogen peroxide for oxidases.

Endodermal cells also give the cellulose reaction, contain oxidases, stain persistently like the hypodermal cells with ruthenium red and contain traces of aromatic substances (probably phenolic glucosides) which stain with the vanillin reagent. They are stained by phloroglucinol, especially at the junction of two cells. On treatment with concentrated sulphuric acid the endodermal ring remains intact. Parenchyma cells stain a bluish-green colour with Congo red indicating the presence of carbohydrates of acidic nature such as pectic acid; they also contain oxidases. They do not give the cellulose reaction until these are removed. Vanillin reagent gives a strong coloration in these cells. Their behaviour with eosin, ponceau, iodine green, and gossypimin after different treatments shows that they are principally composed of unoxidised celluloses, cellulose- α and - β , together with pectic substances.

Transfusion tissue like the hypodermal cells stains with phloroglucinol and zinc chloride-iodine. The contents of some cells stain with alkannin and give a strong coloration with vanillin reagent. Results of staining with ruthenium red, iodine green, borax carmin and ponceau show these cells to be composed of α -oxycellulose and neutral pectic substances.

Table IV. *Transverse sections of leaf of Pinus sylvestris.*

	Epi- dermis	Hypo- dermis	Endo- dermis	Paren- chyma	Trans- fusion tissue	Xylem	Phloem	Resin duct	Secre- tory cells
<i>Malachite green:</i>									
Untreated	+4	+2	+2	—	+2	+3	—	—	+3
Ammonium oxalate and ammonium hydroxide	+4	+2	+2	—	+2	+3	—	—	+3
Sodium hydroxide	+4	+3	+3	—	+2	+4	—	—	—
Ammonium oxalate	+4	+3	+3	—	+2	+4	—	—	+3
Alcohol	+4	+2	—	—	—	+4	—	—	—
Hydrochloric acid	+3	+3	+2	—	+2	+3	—	—	—
<i>Borax carmin:</i>									
Untreated	—	+2	—	—	+2	—	+4	+4	+4
Ammonium oxalate and ammonium hydroxide	—	+4	—	+2	—	—	+3	+2	+4
Sodium hydroxide	+2	+3	—	+1	—	—	+4	—	—
Ammonium oxalate	—	+4	—	+2	—	—	+4	+3	+4
Alcohol	—	+3	—	+3	—	—	+4	—	+4
Hydrochloric acid	—	+3	—	+2	—	—	+4	+2	+4
<i>Aniline blue picrate:</i>									
Untreated	—	— (+4)*	— (+4)*	+2	—	—	+4	+2	— (+4)*
Ammonium oxalate and ammonium hydroxide	+1	+1 (+4)*	—	+4	—	+4	—	—	—
Sodium hydroxide	—	+1	—	—	—	—	—	—	—
Ammonium oxalate	—	— (+4)*	—	+4	+2	—	+4	+3	— (+3)*
Alcohol	—	—	—	+4	+1	—	+1	—	—
Hydrochloric acid	—	— (+4)*	—	+4	—	—	+3	+4	— (+3)*
<i>Iodine green:</i>									
Untreated	+3	+2 (+4)*	+3	+2	+3	+4	+4	+4	+2
Ammonium oxalate and ammonium hydroxide	+2	+2	+3	—	+3	+4	—	—	—
Sodium hydroxide	+3	+3	+3	—	+3	+3	—	—	—
Ammonium oxalate	+2	— (+4)*	+3	+1	+1	+2	+1	+1	— (+1)*
Alcohol	+3	+2	+2	+1	+2	+2	+3	—	— (+2)*
Hydrochloric acid	+2	+2	+1	—	+1	+2	—	—	— (+2)*
<i>Ponceau:</i>									
Untreated	+2	+2	+4	+4	+3	+2	+3	+3	+2
Ammonium oxalate and ammonium hydroxide	+2	+2	+3	+4	+3	+2	+3	+4	+1 (+4)*
Sodium hydroxide	+1	+3	+2	+1	+3	+3	—	—	—
Ammonium oxalate	+2	+2	+3	+4	+3	+3	+3	+4	+2
Alcohol	+2	+2	+3	+4	+3	+3	+2	+2	+1
Hydrochloric acid	+2	+2	+3	+4	+3	+3	+2	+3	+2
<i>Ruthenium red:</i>									
Untreated	+1	+3	+3	+3	+3	+3	+4	+3	+4
Ammonium oxalate and ammonium hydroxide	+1	+3	+3	+1	+3	+3	+4	+1	+3 (+4)*
Sodium hydroxide	+1	+3	+3	—	+3	+3	—	—	—
Ammonium oxalate	—	+3	+3	+1	+3	+2	+4	—	+3
Alcohol	+1	+2	+3	+3	+3	+3	+4	+3	+3
Hydrochloric acid	—	+3	+3	—	+3	+2	+3	+2	+4
<i>Alkannin:</i>									
Untreated	—	—	—	—	— (+3)*	—	—	+3	— (+2)*
Ammonium oxalate and ammonium hydroxide	—	—	—	—	—	—	—	—	—
Sodium hydroxide	—	—	—	—	—	—	—	—	—
Ammonium oxalate	—	—	—	—	—	—	—	+2	— (+1)*
Hydrochloric acid	—	(+3)*	—	—	— (+3)*	—	—	+3	— (+2)*
<i>Gossypimin:</i>									
Untreated	+4	+3	+3	+3	+3	+3	+3	+3	+3
Ammonium oxalate and ammonium hydroxide	+4	+2	+3	—	+3	+3	+3	—	—
Sodium hydroxide	+4	—	+3	—	+2	+4	+2	—	—
Ammonium oxalate	+4	+2	+2	—	+	+4	+2	—	—
Alcohol	+4	+2	+1	+1	+1	+2	+4	—	—
Hydrochloric acid	+4	+2	+2	—	+2	+3	+2	—	—

Table IV (continued).

	Epi- dermis	Hypo- dermis	Endo- dermis	Paren- chyma	Trans- fusion tissue	Xylem	Phloem	Resin duct	Secre- tory cells
<i>Zinc chloride-iodine:</i>									
Untreated	—	+4	+4	—	+4	—	+4	Yellow	+4
Ammonium oxalate and ammonium hydroxide	—	+4	—	+4	+4	—	+4	+3	+3
Sodium hydroxide	—	+3	—	+4	+4	—	+4	—	+4
Ammonium oxalate	—	+3	—	+4	+4	—	+4	+3	+4
Alcohol	—	+3	+4	—	+4	—	+4	+4	+4
Hydrochloric acid	—	+3	—†	+4†	+4	—	+4	+4	+4
<i>Vanillin:</i>									
Untreated	—	—	+3	+4	+4	—	+4	+4	+3
Ammonium oxalate and ammonium hydroxide	—	—	—	—	—	—	—	—	—
Sodium hydroxide	—	—	—	—	—	—	—	—	—
Ammonium oxalate	—	—	—	+3	+1	—	+4	+3	+1
Alcohol	—	—	+3	+4	+3	—	+3	+4	+3
Hydrochloric acid	—	—	—	—	+2	—	+3	+4	+2
<i>Haematoxylin:</i>									
Untreated	+2	+1	+3	+1	—	—	+3	+1	— (+3)*
Ammonium oxalate and ammonium hydroxide	+1	+2	+1	—	—	—	+2	—	—
Sodium hydroxide	—	+3	+2	—	+1	+2	—	—	—
Ammonium oxalate	—	+1	+3	+2	+1	+1	+2	—	—
Alcohol	+2	+2	+3	+2	+3	—	+3	—	+2 (+3)*
Hydrochloric acid	—	+2	+3	—	+2	+1	+3	+1	+2
<i>Ferric thiocyanate:</i>									
Untreated	—	—	—	—	—	—	—	—	—
Ammonium oxalate and ammonium hydroxide	—	—	—	—	—	—	—	—	—
Sodium hydroxide	—	—	—	—	—	—	—	—	—
Ammonium oxalate	—	—	—	—	—	—	—	—	—
Alcohol	—	—	—	—	—	—	—	—	—
Hydrochloric acid	—	—	—	—	—	—	—	—	—
<i>Congo red:</i>									
Untreated	—	+1	+2	+1§	+4	+2	+4	+2	+4¶
Ammonium oxalate and ammonium hydroxide	+1	+1 (+3)*	+2	+1§	+3	+2	+4	+2	+3¶
Sodium hydroxide	+2	+2	+3	—	+3	+3	+4	—	+2¶
Ammonium oxalate	—	+2 (blue)	+3	+1	—	+2	+4	+2	+4¶
Alcohol	—	+1	+3	+1	+3	+1	+4	+2	+4¶
Hydrochloric acid	—	+1	+2	+2	+1	+1	+4	+3	+4¶
<i>Eosin:</i>									
Untreated	+3	—	—	+3	—	—	—	+½	—
Ammonium oxalate and ammonium hydroxide	+3	—	—	—	—	+2	—	—	—
Sodium hydroxide	—	—	—	—	—	—	—	—	—
Ammonium oxalate	+3	—	—	—	—	+3	—	—	—
Alcohol	+3	+1	+3	+4	+3	+4	+2	+3	—
Hydrochloric acid	—	—	+3	+4	—	—	+3	+3	—
<i>Phloroglucinol:</i>									
Untreated	—	+4	+4	—	+3	+4	—	—	—
Ammonium oxalate and ammonium hydroxide	—	+2 (m.l.)	+1	—	—	+3	—	—	—
Sodium hydroxide	—	—	—	—	—	+½	—	—	—
Ammonium oxalate	—	+3 (m.l.)	+3	—	+3	+3	—	—	—
Alcohol	—	+4	+4	—	+3	+4	—	—	—
Hydrochloric acid	—	+4	+4	—	+3	+4	—	—	—

* Cell content. † Yellow. ‡ Very dark. § Bluish green. ¶ Bluish black.

— Negative. +1, +2, +3, +4 show increasing intensity of colour, +4 being the strongest.

Table V. Sections of green stems of *Pinus sylvestris*.

	Middle lamella (contents)	Middle lamella (binding layers)	Cell wall A. C.	Cell contents (remains) A. C.	Autumn wood A. C.	Spring and summer wood A. C.	Bordered pits A. C.	Pit (outer ring) A. C.	Pit partition A. C.	Medullary ray (contents) A. C.	Zone sur- rounding medullary ray A. C.	Resin canal A. C.	Primary xylem (remains)
<i>Malachite green:</i>													
Untreated	+4	+4	—	—	+4	+4	+2	×	+1	+3	×	+4	+4
Ammonium oxalate and ammonium hydroxide	+4	+4	+3	+3	+4	+2	×	×	+1	+3	×	+4	+4
Sodium hydroxide	—	—	+3	+3	+4	+2	×	+2	+1	+3	×	+4	+4
Ammonium oxalate	—	—	+3	+3	+4	+2	×	+2	+1	+3	×	+3	+3
Alcohol	—	+4	—	—	—	—	—	—	—	+2	×	+4	+4
<i>Borax carmin:</i>													
Untreated	—	—	—	+3	—	—	×	×	×	+4	+3	+2	+3
Ammonium oxalate and ammonium hydroxide	—	—	—	+3	—	—	×	×	×	+4	+3	+3	+4
Sodium hydroxide	—	—	—	+2	+1	—	×	×	×	—	×	+3	+1 +3
Ammonium oxalate	—	—	—	+2	—	—	×	×	×	+4	×	+3	+4
Alcohol	—	—	—	+1	—	—	—	—	—	+2	×	+3	+4
<i>Ruthenium red:</i>													
Untreated	+4	×	+1	+1	+3	+1	+4	+2	+4	+4	+1	+4	+3
Ammonium oxalate and ammonium hydroxide	—	—	+1	+1	+3	+1	—	—	—	+3	—	+4	+4
Sodium hydroxide	—	—	+2	+2	+3	+2	—	×	×	—	×	—	+4
Ammonium oxalate	—	+2	+1	+1	+3	+1	—	×	×	+4	—	+3	+3
Alcohol	—	—	—	—	+3	—	—	—	—	+3	—	—	+4
<i>Aniline blue picrate:</i>													
Untreated	—	×	+1	+3	+1	+1	+2	×	×	+4	×	—	+4
Ammonium oxalate and ammonium hydroxide	×	×	+4	+3	+4	+4	×	×	×	+4	×	—	+4
Sodium hydroxide	—	—	+3	+2	+4	+3	+4	+3	—	+2	×	+1	+2
Ammonium oxalate	—	—	+1	+1	+1	+1	+1	+1	×	+4	×	—	+4
Alcohol	—	—	—	+1	—	—	—	—	×	+3	×	×	+4
<i>Iodine green:</i>													
Untreated	—	+4	+4	+4	+4	+3	+1	+2	—	+4	×	+4	+4
Ammonium oxalate and ammonium hydroxide	—	+4	+4	+3	+4	+4	+1	+1	×	+4	×	+4	+4
Sodium hydroxide	+4	—	+2	+1	+3	+2	—	×	×	+1	×	+3	+3
Ammonium oxalate	—	+4	+4	+2	+4	+3	+2	+1	×	+4	×	+3	+3
Alcohol	—	+4	+4	+2	+4	+3	+1	+1	—	+4	×	+4	+4
<i>Ponceau:</i>													
Untreated	—	—	+1	—	+1	+1	—	×	×	+4	×	+3	+4
Ammonium oxalate and ammonium hydroxide	—	—	+2	+2	+3	+2	—	×	×	+4	×	+4	+4
Sodium hydroxide	—	—	+1	—	+1	+1	—	×	×	+4	×	—	+4
Ammonium oxalate	—	—	+1	—	+1	+1	—	×	×	+4	×	+3	+4
Alcohol	—	—	+1	—	+1	+1	—	×	×	+2	+1	+3	+4

Haematoxylin:

Untreated	—	+3	—	—	+2	+2	—	—	—	—	—	—	—	—	—	—	+1
Ammonium oxalate and ammonium hydroxide	—	—	—	+1	+1	+2	+2	+2	+2	+1	+1	—	—	—	—	—	+3
Sodium hydroxide	—	+3	+3	+4	+4	+3	+3	+4	+4	+4	+4	+1	×	×	×	×	+4
Ammonium oxalate	—	+1	—	+2	+2	+3	+3	+2	+2	+3	+3	+4	×	×	×	×	+3
Alcohol	—	+2	—	+2	+2	+3	+3	+2	+2	+3	+3	centre	×	×	×	×	+2
												+4	×	×	×	×	+3
												centre	×	×	×	×	+2

Ferric thiocyanate:

[illegible]

Congo red:

[illegible]

Eosin:

Untreated	-	-	-	+3	+1	+1	+3	+3	+3	+1	x	-	+3	+3	-	x	+4	+3
Ammonium oxalate and ammonium hydroxide	-	-	-	+3	+3	+1	+3	+3	+3	+1	x	-	+3	+3	-	x	+3	+4
Sodium hydroxide	-	-	-	+1	+1	+1	+3	+3	+3	+1	x	-	+1	+3	-	x	+3	+1
Ammonium oxalate	-	-	-	+1	+1	+1	+3	+3	+3	+1	x	-	+3	+3	-	x	+4	+3
Alcohol	-	-	-	+2	+2	+2	+3	+3	+3	+1	x	-	+3	+3	-	x	+3	+4

Phloroglucinol:

[illegible]

Alkannin:

[illegible]

A, Longitudinal section. C, Transverse section. — Negative. +1, +2, +3, +4 show increasing intensity of colour, +4 being the strongest. × Not observed.

Xylem cells give a strong phloroglucinol reaction like the true ligno-celluloses and do not give the colour for cellulose with zinc chloride-iodine. They do not contain oxidases, and their general behaviour with different stains suggests the presence of hemicelluloses, pectic substances and α -oxy-cellulose.

Phloem does not give a coloration with phloroglucinol but gives a strong coloration with the vanillin reagent for aromatic substances and with zinc chloride-iodine for cellulose. It is also very active in oxidases. Its staining reactions after treatment with sodium hydroxide indicate the presence of hemicellulose but not of pectic substances.

The resin canal does not give directly the iodine reaction for cellulose until the pectic substances of which it is principally composed are previously removed. The presence of pectic substances is confirmed by its behaviour with eosin, ruthenium red, gossypimin, etc. It is not stained by phloroglucinol, but it gives a deep coloration with the vanillin reagent for aromatic substances. It is also stained by alkannin and gives a strong reaction for oxidases. Its behaviour with borax carmin and Congo red also indicates the presence in small amounts of hemicellulose.

Secretory cells do not stain with phloroglucinol; they give a reaction for cellulose direct, and their contents are stained with alkannin and vanillin reagents. They also give a reaction for oxidases. Their behaviour with ruthenium red, ponceau and borax carmin, shows that these cells are composed principally of cellulose together with hemicelluloses but they do not contain pectic substances. Congo red stains these cells bluish red, indicating that the secretion of these cells is of an acidic character.

PROBABLE CONSTITUENTS OF DIFFERENT PARTS OF THE STEM
OF *PINUS SYLVESTRIS* (Table V)

The middle lamella of many plant tissues can be differentiated with certain stains into three layers, a thin central layer which is bounded on either side with another layer which sometimes stains differently from the central one. Hence the results of staining the middle lamella are given separately in two columns. In the first column are given the results of staining of the central layer (contents). This layer is stained by alkannin and also by the vanillin reagent, thus indicating the presence of resinous substances. It is deeply stained in young stems by ruthenium red but not after treatment with ammonium oxalate, which shows that it is composed of neutral pectic substances. It is not stained by phloroglucinol. The binding layers are not well developed in stems 10-12 years old. Generally, the thin central layer is well marked in the xylem cells close to the cambium layer. Proceeding towards the centre of the stem the central layer almost disappears and is not stained by the majority of colours. In older stems the middle lamella is more strongly differentiated.

The cell wall of the secondary xylem stains deeply with phloroglucinol but curiously it is not stained after the sections are extracted with alcohol. The staining reactions with eosin, iodine green, and ponceau suggest the presence of hemicellulose and of traces of pectin. It is not stained by Congo red and zinc chloride-iodine, but in transverse section a faint blue colour is observed in the layer binding the cell cavity. This suggests that these cell walls are composed of lignocellulose, together with some hemicellulose and a thin coating towards the centre of the cell cavity of free cellulose. Some differences are observed between the autumn and the spring wood. The autumn wood always seems to stain more deeply than the spring wood. Its behaviour with ruthenium red suggests the presence of α -oxycellulose which seems to be absent from the spring wood.

A number of theories have been advanced to explain the difference between the spring and the autumn wood, which lies in the decrease in the diameter of the tracheides and the increase in the thickness of their walls. The writer is of the opinion that the relatively greater thickness of the cell wall in autumn wood may be the result of an increase in the concentration of the nutritive material. On determining the influence of concentration of the nutritive substances on the thickness of the cell wall of yeast it is found that the thickness of the cell wall increases with the concentration of the medium.

Cell remains are stained by zinc chloride-iodine, but not with phloroglucinol. Their staining reactions with ruthenium red, aniline blue picrate, iodine green and Congo red, indicate the absence of pectin and hemicellulose and they are probably composed exclusively of hydrocellulose.

The bordered pit is stained in some respects like the middle lamella by ruthenium red and alkannin.

The medullary ray is stained by alkannin and zinc chloride-iodine, and its staining reactions with ruthenium red, eosin, borax carmin, ferric thiocyanate and Congo red indicate the presence of hemicellulose, pectins and gums. A strong coloration is observed in these cells with the vanillin reagent.

Reactions of the resin canal after different treatments with stains such as alkannin, ponceau and ferric thiocyanate, strongly indicate the presence of hemicellulose, gums and lignin or resin. The presence of lignin or resin is further confirmed with the vanillin reagent.

Primary xylem remains stain deeply with ruthenium red and appear to contain oxycellulose. They are also stained by zinc chloride-iodine but feebly by phloroglucinol.

Oxidases are distributed in all those parts through which migration of food materials is taking place. They differ from the oxidases of the leaf in that they give the blue coloration with guaiacum.

The middle lamella in timber appears to be of different composition from that of the young wood. Pectin substances are absent from the central layer and they are replaced at a later stage by lignin, gums and hemicellulose as may be judged from its reactions with borax carmin, malachite green and

ferric thiocyanate. It is also stained by phloroglucinol and vanillin reagent. Pectin is similarly absent from the binding layers of the middle lamella in timber, and their reactions with stains such as haematoxylin, ferric thiocyanate, Congo red and eosin, show them to be composed of hemicellulose and gums.

Attention may be drawn to the results of staining with some dyes after extraction with alcohol. In some parts after extraction the results are altogether negative. It is probable that traces of resinous or aromatic substances are dispersed over certain plant cells, which are removed by alcohol. This is a matter of importance since alcohol is often used as a fixative and as a preserving medium.

These attempts at differentiating the various constituents of plant tissues by differential staining and microscopical examination are sufficient to show the limitations and the difficulties which are to be confronted in histological work.

SUMMARY.

An attempt has been made to unravel the microchemical structure of plant tissues, and to interpret in chemical terminology the distribution of the different units comprising the cell.

Cellulose- α , - β and - γ , oxycelluloses- α , - β and - γ , hydrocellulose, mannan, galactan, pectin, amylohemcellulose, hemicellulose, gums, starch, lichenin and chitin have been isolated in the purest possible condition and their staining capacities determined.

The relative amounts of the different cellulosic constituents vary widely according to the cultural conditions, general environment and the nature of the plant. Cellulose- β and - γ show a greater affinity for stains than cellulose- α . Oxycelluloses, unlike cellulose- α , - β and - γ and hydrocellulose, are stained by ruthenium red.

Two kinds of hydrocellulose are found which can be differentiated by the coloration given by one of them with iodine in potassium iodide solution.

The microchemical structure of the leaf and stem of pine is investigated by the removal of specific constituents by suitable chemical treatment with solvents such as ammonia, ammonium oxalate, sodium hydroxide, hydrochloric acid and alcohol, followed by differential stainings.

Treatment with ammonium oxalate shows that the middle lamella in young stems of pine is composed of neutral pectic substance whilst in old wood (timber) it is composed of lignin, gums and hemicellulose, pectin being absent.

Extraction of the tissues with alcohol gives inconsistent results.

Resins react in some respects like lignin and are associated with gums in different parts of the same plant.

Lignification sets in very early in the life history of the plant and occurs in ground tissue, fibro-vascular bundles, medulla and cuticularised cells.

The writer in conclusion feels it a great pleasure to express her sincere gratitude to Prof. Arthur R. Ling and Dr Dinshaw R. Nanji for their valuable criticism and for their continuous encouragement and interest in the course of these investigations. Her thanks are also due to Mr J. Harris for supplying her with specimens of different timbers.

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