Changes in the Chemical Composition of a Cambial Cell during its Differentiation into Xylem and Phloem Tissue in Trees

1. MAIN COMPONENTS

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Investigations of the changes in the composition of the plant cell during its development can be used as a basis for the study of the mechanism by which the cellular materials are synthesized during secondary thickening of the cell wall. The formation of xylem and phloem tissue in trees has been studied in this way (Allsopp & Misra, 1940; Anderson & Pigman, 1947; Anderson, Wise & Ratliff, 1954; Meier & Wilkie, 1959; Stewart, Amos & Harvey, 1953; Sultze, 1957), and similar investigations of the development of the cell in non-woody tissues have also been made (Fuller, 1958; Jensen & Ashton, 1960; Jermyn & Isherwood, 1956; Whistler & Young, 1960; Wirth, 1946).

However, the percentage composition of whole tissue compared at different stages of growth gives little indication of the changes occurring in a single cell during its development. This information can only be obtained if the weight, in addition to the percentage composition, of a single cell is measured during its differentiation. This paper describes such changes during the development of xylem cells from cambial cells in four species of trees. The percentage composition of the phloem in each species is also reported.

EXPERIMENTAL

Materials

Material for phloem, sapwood and heartwood analyses. Samples were taken from a 30-year old birch (*Betula platyphylla*), a 15-year-old pine (*Pinus ponderosa*), and a 50-year-old sycamore (*Acer pseudoplatanus*) that are growing in the Botanical Gardens, Cambridge, and from a 15-year-old ash (*Fraxinus elatior*) that is growing in Madingley Wood, Cambridge.

A Mattson's increment borer (Pressler borer, J. H. Steward Ltd., Strand, London, W.C. 2) was used to remove four plugs about 3 cm. long from each tree during the period 16–20 November 1959. The samples, which were taken at a height of 4 ft. 3 in. from the ground, were immediately placed in glass-stoppered tubes and frozen to -55° (solid CO_2 and acetone). The samples were cut with a razor blade into sections corresponding to phloem, sapwood and heartwood, the sycamore phloem being further divided into inner and outer portions (Fig. 1).

Material for cambial tissue analyses. Cambial tissue was obtained from ash (*F. elatior*) and sycamore (*A. pseudoplatanus*) saplings (4 years old) grown in Madingley Wood, Cambridge, and from pine (*Pinus sylvestris*) and birch (*Betula* sp.) saplings (4–8 years old) grown in Mildenhall Woods, Thetford. The material was collected during the period 27 July–4 August 1959.

After the outer layers had been stripped from the saplings the soft cambial tissue beneath was gently scraped off and immediately frozen to -55° (solid CO_2 and acetone). Microscopical examination of the material obtained confirmed that it was made up almost completely of cambial cells.

Analytical methods

Drying of samples and preparations. The frozen samples were dried at 100° over P_2O_5 at 0.5 mm. Hg. All pectic substances, holocellulose, hemicellulose and α -cellulose preparations were dried at room temperature over P_2O_5 at 0.1 mm. Hg. Water-extracted material, ethanol- and benzene-extracted material, lignin and material from which the pectic substances had been removed were dried to constant weight in an oven at 105° .

Water content. The decrease in weight of a fresh sample was found after it had been heated at 100° for 3 hr. at 0.5 mm. Hg over P_2O_5 .

Ash. This was obtained from a portion of the dried material which had been maintained at dull red heat (500 – 600°) in a Pt boat in a stream of clean dry air for 2 hr.

Fig. 1. Diagram to show plug taken from the trees and its division into various regions.

Total nitrogen. This was determined by micro-Kjeldahl digestion (Chibnall, Rees & Williams, 1943) followed by distillation and titration and expressed as protein after multiplication by the factor 6.25.

Total phosphorus. This was determined according to Fiske & Subbarow (1925).

Fractionation of the samples (Fig. 2)

Water-soluble material. About 150 mg. of dried material, which had been chopped and passed through a 16-mesh sieve, was extracted three times with distilled water at 100° for 3 hr. After filtration the extracted material was dried and weighed. The extractives were freeze-dried and weighed.

Ethanol- and benzene-soluble material. The water-extracted material was further extracted with ethanol-benzene (1:2, v/v) at 70° for 6 hr. The extractive-free material was dried and weighed. The ethanol and benzene were allowed to evaporate from the extractives and the residue was weighed.

Lignin. Approximately one-quarter of the already extracted material was used for the estimation of lignin by the method of Ritter, Seborg & Mitchell (1932). After treatment with H_2SO_4 , the residue was centrifuged and thoroughly washed, by suspension and recentrifuging, with water. The dried residue was weighed.

Pectic substance. To the remainder of the already extracted material was added 0.2M-EDTA (disodium salt), pH 4.5 (5 ml.). The mixture was shaken in a glass-stoppered

tube by a Microid flask shaker (Griffin and George Ltd., Wembley, Middlesex) at room temperature for 4 hr. After filtration the EDTA solution was dialysed overnight against water. Ethanol was added to the dialysed solution until its concentration was over 80%, and the precipitate that formed was allowed to settle for 24 hr. This precipitate was centrifuged, washed with ethanol, dried and weighed. The dried residual material was weighed.

Holocellulose. The EDTA-extracted material was suspended in 5 ml. of water in a glass-stoppered tube, in a water bath at 75° for 2 hr. Two drops of acetic acid and 30 mg. of $NaClO_2$ were added and after 1 hr. these additions were repeated (Wise, Murphy & D'Addieco, 1946). The holocellulose was washed with water, acetone and ether and the dried residue was weighed.

Hemicellulose and α -cellulose. The holocellulose was fractionated by successive extractions with 5 and 24% (w/v) KOH solution under N_2 at 25° for a total of 4 hr. (Wise *et al.* 1946.) The residue was centrifuged and thoroughly washed with water by suspension and recentrifuging. The washings and the alkaline extracts were combined and neutralized at 4° with cooled acetic acid. The neutralized solution was dialysed for 24 hr. against running tap water followed by distilled water, and the hemicellulose precipitated in the same manner as that described for the pectic substances. The dried material was weighed. The residual α -cellulose was washed with water, acetone and ether, dried and weighed. The yield of α -cellulose was corrected for the amount of ash which it contained.

Fig. 2. Fractionation of the phloem, cambium, sapwood and heartwood samples.

Determination of the weight of a single cell in the cambial, sapwood and heartwood regions of each tree

Sapwood and heartwood. Sapwood and heartwood samples from each tree were extracted with water and ethanol-benzene (1:2, v/v) and then fixed in a dilute chromic acid fixative (Johansen, 1951), dehydrated and embedded in wax. A sledge microtome was used to cut transverse sections approx. 25μ thick. The wax was removed from five sections from each region with xylene and after drying they were weighed (five sections weighed approx. 1 mg.). The sections were stained in 1% (w/v) safranin in 48% ethanol and mounted on a microscope slide. Each transverse section was projected through a light-microscope on a screen, and the total number of cells (about 1000) was counted in a measured area (0.25 mm.^2). This was repeated three times for different areas of each section. The total area of each transverse section was measured (approx. 13 mm.^2) and hence the total number of cells in the five sections could be calculated.

The transverse sections were removed from their mounts, washed in xylene and re-embedded in wax. Longitudinal sections were cut from these blocks, and the width of the specimen, which was measured on a screen after projection through a microscope, gave the thickness of the original transverse section.

The length of a single cell was determined from longitudinal sections (approx. 90μ thick) which were cut from the original blocks. Sections were taken across one annual growth ring. They were dewaxed in xylene and delignified by the method of Wise *et al.* (1946). The sections were gently ground in a mortar with 24% (w/v) KOH (1 ml.); after 1 min. $10N\text{-H}_2\text{SO}_4$ was added until the mixture was neutral (litmus). The fibres were spun down, washed with water and re-centrifuged. They were stained with aqueous 0.5% (w/v) methylene blue, centrifuged and mounted in glycerol on a glass slide. The length of approx. 30 cells was measured in each sample. Microscopic examination of the tissue throughout this treatment showed that no appreciable alteration in the dimensions of the cell occurred.

Distances on the screen for the measurement of length, area and width of the section were calibrated from a standard graticule (Graticules Ltd., London, E.C. 1). The weight of a single cell was given by:

$$\frac{\text{Wt. of the five sections} \times \text{length of cell}}{\text{Total no. of cells in sections} \times \text{thickness of section}}$$

Standard deviations were determined for the total number of cells in a known area and for the length of a cell (Table 2). The standard deviation of the weight of a cell was calculated (Table 1).

Cambium. Cambial tissue from each tree was extracted with water and ethanol-benzene (1:2, v/v). The dried extracted material was weighed and suspended in 0.02M-EDTA (30 ml.) in a polythene beaker which was placed in water in an ultrasonic shaker (Disintegrator system forty; Ultrasonics Industries Inc., Albertson, L. 1, New York). A little detergent (Teepol) was added to the water outside the beaker in order to obtain more efficient propagation of the ultrasonic vibrations into the suspension. After shaking for 1 hr. at room temperature, 75% of the material was present as single cells. This mixture was centrifuged, and the cells were stained with aqueous 0.5% (w/v) methylene

Table 1. *Weight of an extractive-free cell*

Values are given in $\mu\text{mg.} \pm \text{s.d.}$ with number of observations in parentheses.

	Region		
	Cambium	Sapwood	Heartwood
Ash	21.3 ± 0.8 (8)	155 ± 22 (28)	173 ± 18 (28)
Birch	25.9 ± 1.6 (8)	117 ± 14 (30)	125 ± 16 (24)
Sycamore	15.3 ± 1.3 (8)	157 ± 19 (25)	171 ± 28 (30)
Pine	98.0 ± 7.8 (8)	503 ± 79 (28)	430 ± 61 (40)

Table 2. *Length of a cell in each region of each tree*

Values are given in $\text{mm.} \pm \text{s.d.}$ with number of observations in parentheses.

	Region		
	Cambium	Sapwood	Heartwood
Ash	0.71 ± 0.12 (8)	0.98 ± 0.14 (28)	1.08 ± 0.11 (28)
Birch	0.63 ± 0.10 (8)	0.92 ± 0.14 (24)	1.01 ± 0.13 (24)
Sycamore	0.52 ± 0.08 (8)	0.72 ± 0.09 (25)	0.68 ± 0.11 (30)
Pine	0.80 ± 0.13 (8)	1.47 ± 0.23 (28)	1.28 ± 0.21 (40)

blue and re-centrifuged. The cells were made up to a volume of 1 ml. with water.

A known volume of the cells ($5\mu\text{l.}$) was placed on a microscope slide and the total number of cells and fractions of cells in this sample were counted. This was repeated eight times. The weight of a single cell was then calculated (Table 1). The length of a cambial initial was measured (Table 2).

RESULTS

In order that a comparison could be made of the development of the cell in different types of trees, four species were used: *F. elatior*, a ring porous angiosperm, *B. platyphylla* and *A. pseudoplatanus*, diffuse porous angiosperms, and *P. ponderosa*, a gymnosperm. Small quantities of material were taken for the determination of the composition of each region, since this work formed part of an investigation into the seasonal variation in the composition of each region of each tree. Removal of larger quantities over a yearly period would have seriously damaged the trees. The results presented in Tables 3 and 4 are those from samples taken during 1959; material taken at corresponding dates during 1960 gave the same analyses.

In our investigation material from the phloem, cambial, sapwood and heartwood regions of each tree was fractionated into more components than in any similar investigation since that of Stewart *et al.* (1953).

Weight and length of a single extractive-free cell in the cambial, sapwood and heartwood region of each tree. The weights of single cells are shown in Table 1. Cells from all regions of the gymnosperm weighed more than the corresponding cells of the angiosperms, and during differentiation of a cambial cell

into xylem the increase in weight was greatest in the pine and least in the birch. In the angiosperms the mean weight of the sapwood cell was less than that of the heartwood cell, but in pine this was not so.

The lengths of single cells are given in Table 2. In order to account for the variation in length of the cell across an annual growth ring (Bisset, Dads-well & Amos, 1950), the lengths were measured from sections representative of one complete growth

Table 3. *Composition of the samples taken from each tree*

Values are expressed as a percentage of the whole dry material. Water content is expressed as a percentage of the whole fresh material.

		Region of plug				
		Phloem	Cambium	Sapwood	Heartwood	
Ash	Water content	45.4	83.8	28.4	28.9	
	Ash	7.6	10.6	0.5	0.6	
	Protein	1.0	9.4	0.5	0.5	
	Phosphorus	0.55	0.30	0.10	0.05	
	Water-soluble material	35.1	65.3	9.1	6.8	
	Ethanol-benzene-soluble material	2.5	1.9	2.1	0.2	
Birch	Water content	38.2	86.7	49.8	48.6	
	Ash	4.9	8.0	0.3	0.2	
	Protein	3.5	10.6	0.6	0.5	
	Phosphorus	0.11	0.24	0.08	0.04	
	Water-soluble material	18.5	49.6	11.6	3.5	
	Ethanol-benzene-soluble material	8.3	3.7	1.4	1.0	
		Outer Inner				
Sycamore	Water content	38.0	51.5	90.0	43.6	43.9
	Ash	9.8	7.3	17.9	0.6	0.5
	Protein	2.0	9.9	17.2	1.2	0.8
	Phosphorus	0.02	0.05	1.1	0.06	0.03
	Water-soluble material	9.8	15.4	65.6	8.6	7.2
	Ethanol-benzene-soluble material	1.4	1.6	3.7	1.2	0.5
Pine	Water content	57.6	84.9	42.2	48.0	
	Ash	1.3	3.1	0.2	0.2	
	Protein	3.1	7.4	0.6	0.3	
	Phosphorus	0.07	0.15	0.06	0.03	
	Water-soluble material	34.7	61.0	5.7	4.3	
	Ethanol-benzene-soluble material	9.0	5.3	2.5	3.3	

Table 4. *Composition of the samples taken from each tree*

Values are expressed as a percentage of the material already extracted with water and ethanol-benzene (1:2, v/v).

		Region of plug				
	Fraction	Phloem	Cambium	Sapwood	Heartwood	
Ash	Pectic substances	18.0	6.6	1.4	0.5	
	Lignin	23.4	10.0	22.8	22.5	
	α -Cellulose	28.5	42.6	42.4	44.4	
	Hemicellulose	30.2	40.8	32.9	29.8	
Birch	Pectic substances	4.4	18.0	2.6	0.3	
	Lignin	53.1	10.0	23.0	22.5	
	α -Cellulose	22.3	35.2	40.0	43.4	
	Hemicellulose	19.2	36.5	35.0	33.8	
		<div>Outer Inner</div>				
Sycamore	Pectic substances	3.1	6.6	15.0	3.8	1.3
	Lignin	46.9	22.6	2.5	25.3	24.3
	α -Cellulose	29.0	45.0	36.7	40.0	43.0
	Hemicellulose	21.3	25.8	45.0	31.5	31.4
Pine	Pectic substances	10.0	8.5	1.3		1.0
	Lignin	52.8	20.0	30.4		29.5
	α -Cellulose	22.1	36.1	45.0		43.4
	Hemicellulose	15.3	35.0	23.0		25.0

Fig. 3. Weight of each cell-wall component at different stages of development. (a), *F. elatior*; (b), *B. platyphylla*; (c) *A. pseudoplatanus*; (d), *P. ponderosa*. ■, Pectic substances; ●, lignin; ○, α-cellulose; □, hemicellulose. —, Cambial cell to sapwood development; - - -, sapwood cell to heartwood development.

ring. The mean lengths of the cells in each region of the gymnosperm were longer than those in the corresponding regions of the angiosperms. The mean length of a cell increased with age in each species except in the gymnosperm, where a sapwood cell appeared longer than a heartwood cell.

Percentage composition of each region of each tree. The results are given in Tables 3 and 4. In order to compare the composition of the cell walls of the cambium with those of the other regions it is necessary to use the analytical data of the extractive-free material (Table 4), since the total composition included the material of the cell contents in addition to the cell-wall components.

Changes in weight of each cell-wall component during the differentiation of a cambial cell into a xylem cell. This may be calculated from the inform-

ation in Tables 1 and 4. The increase in each component of the cell wall during secondary thickening is illustrated in Fig. 3.

DISCUSSION

It appeared from previous work that secondary thickening of a cambial cell involved the deposition of large amounts of lignin, hemicellulose and α-cellulose, and that little or no further addition was made to the pectic substances. These conclusions were based on the results of histochemical investigations and from comparisons of the percentage composition of the tissue at different stages of development (Allsopp & Misra, 1940; Anderson & Pigman, 1947; Asunmaa & Lange, 1954; Lange, 1954). Allsopp & Misra (1940) stated that the change in relative

composition of the tissue during growth does not give information about the changes in the actual amounts of each component of the cell. Fuller (1958) and Jensen & Ashton (1960) determined the weight of each component in a single cell and found the changes which occurred in their weights during the development of a cell in tissues from root tips. In tissues of wood, Meier & Wilkie (1959) have attempted to show the relative increases in the individual polysaccharide components of the cell wall during thickening by assuming that the weight of the wall was proportional throughout its development to its thickness. No assessment of the possible change in weight because of alteration in length was made. Sultze (1957) assumed that the total amount of araban in the wall did not vary from the initial value found in the cambial cell, and thus the composition of the developing tissue related to this araban content enabled a calculation of the percentage increase of the other components for a fixed amount of araban to be directly correlated to the increase per cell. Again this latter assumption is not entirely satisfactory and can only be justified by direct measurements such as those reported in this investigation (Fig. 3) and that of Thornber & Northcote (1961).

Our results showed that secondary thickening of the cambial cell wall occurred by formation of α -cellulose, hemicellulose and lignin with little change in weight of the pectic substances (Fig. 3). This thickening resulted more from deposition of α -cellulose than hemicellulose, the cambial cell wall having contained equal amounts of these two fractions (Table 4 and Fig. 3). In the angiosperms the increase in weight of the cell wall during secondary thickening which varied for the individual species (birch, 350%; ash, 640%; and sycamore, 930%) was represented by the same relative increase of each component in all three species: α -cellulose, 40–43%; hemicellulose, 30–34%; and lignin, 25–27%. However, in the gymnosperm the increase (410%) was made up by α -cellulose, 47%; hemicellulose, 20%; and lignin 33%.

The α -cellulose fraction of the pine contained a glucomannan (Meier, 1958), which is generally considered to be a component of the hemicellulose. If the amount of the glucomannan is subtracted from the α -cellulose and included in the hemicellulose, then the only difference between the secondary thickening in the angiosperms and the gymnosperm is that in the latter relatively more lignin is formed.

The weight of lignin laid down during the formation of a sapwood cell was very large when expressed as a percentage of the amount present in the cambial cell (680–9300%). This showed that its deposition occurred almost exclusively during secondary thickening. It was also found that the ratio of the increase in weight of lignin to that of

α -cellulose was constant in each species (Fig. 3). This fact lends support to the hypothesis that cellulose acts as a catalytic matrix for the formation of the lignin component (Goodman & Siegel, 1959; Siegel, 1957).

The percentage composition and the carbohydrate composition (Thornber & Northcote, 1961) of the cell-wall components of the cambial cell were very similar in each species (Table 4) and closely resembled those of other cambial tissues and primary cell walls (Bishop, Bayley & Setterfield, 1958; Kivilaan, Beaman & Bandurski, 1959; Northcote, 1958). It was only during differentiation that differences were seen between the species.

During the transition of a sapwood cell into the heartwood in the angiosperms, an increase occurred in the weight of the cell (ash, 11.6%; birch, 6.8% and sycamore, 8.9%). This was largely due to the greater deposition of the α -cellulose component (ash, 71%; birch, 77% and sycamore, 62%) than the lignin and hemicellulose components. In the gymnosperm, the mean weight of a heartwood cell was less than that of a sapwood cell and during the transformation of the sapwood cell into the heartwood a loss occurred in the weight of each component (Fig. 3), the largest of which was that of the α -cellulose. The most significant change in the amount of any component during this transformation in each species was the almost complete absence of the pectic substances in the heartwood. This may be due to a removal of this component from the cell wall, or it may be due to its becoming more firmly bound in the cell wall of the heartwood. If this latter reason is correct there should be an increase in the carbohydrate composition of the other fractions to account for the pectic substances (Thornber & Northcote, 1961).

The percentage composition of the cell wall in the phloem is reported for each species (Tables 3 and 4). These cell walls had a higher percentage of pectic substances than those in the xylem, and in the ash and pine phloem the content of pectic substances was even greater than in the cambial region. The major component in this region was represented by substances which were insoluble in 72% (w/w) sulphuric acid, and the relative amounts of these substances increased with the age of the tissue (Table 4). Because of the large amounts of material other than lignin which may give rise to insoluble products with 72% sulphuric acid in the phloem region (Stewart *et al.* 1953), the values for the Klason lignin obtained for this region are not directly comparable with those for the xylem.

The percentage of materials usually associated with the contents of the cell rather than the cell wall are high in the cambial region, as might be expected. The percentage of these materials is less in the sapwood and even smaller in the heartwood

(Table 3). A single cell in any region of the gymnosperm was five times greater in weight than in the corresponding regions in the angiosperms and contained proportionally smaller percentages of ash, protein and phosphorus. In the xylem of the ash, the amount of water (30 % of the fresh weight) was much smaller than in the xylem of the other trees; Allsopp & Misra (1940) have also reported this.

SUMMARY

1. A quantitative determination has been made of the changes that occurred in the composition of a cambial cell during its differentiation and subsequent maturation in four species of tree, *Acer pseudoplatanus*, *Betula platyphylla*, *Fraxinus elatior* and *Pinus ponderosa*.

2. The weight of α -cellulose, hemicellulose, lignin and pectic substances in the cell wall of a single cell in the cambium, sapwood and heartwood of each tree has been determined.

3. Comparison of the composition of these regions showed that secondary thickening of the cambial cell involved the deposition of α -cellulose, hemicellulose and lignin. This thickening resulted more from α -cellulose than hemicellulose, the cambial cell having equal amounts of each.

4. The amount of pectic substances per cell remained constant during the formation of the sapwood cell, but in the heartwood cell there was an almost complete absence of this fraction.

5. The composition of the cambial cell in each species was very similar.

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2. CARBOHYDRATE CONSTITUENTS OF EACH MAIN COMPONENT

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Thornber & Northcote (1961) have reported the changes in the amounts of the pectic substances, lignin, hemicellulose and α -cellulose components of a cambial cell wall of four species of trees during its differentiation into a sapwood cell and its subsequent maturation into a heartwood cell. This paper

reports the changes in the carbohydrate composition of the pectic substances, hemicellulose and α -cellulose components of a cambial cell wall during the same stages of its development in the same four trees.

The percentage composition of the carbohydrates in these components in the phloem is also reported.